IDENTIFICATION OF LEAD COMPOUNDS WITH ANTI-TUBERCULOSIS ACTIVITY IN INDIGENOUS SPICES OF KERALA

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(2009-09-121)

M.Sc. (INTEGRATED) BIOTECHNOLOGY

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

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THESIS

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2014

DECLARATION

I hereby declare that this thesis entitled **"IDENTIFICATION OF LEAD COMPOUNDS WITH ANTI-TUBERCULOSIS ACTIVITY IN INDIGENOUS SPICES OF KERALA"** 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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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
ºC	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

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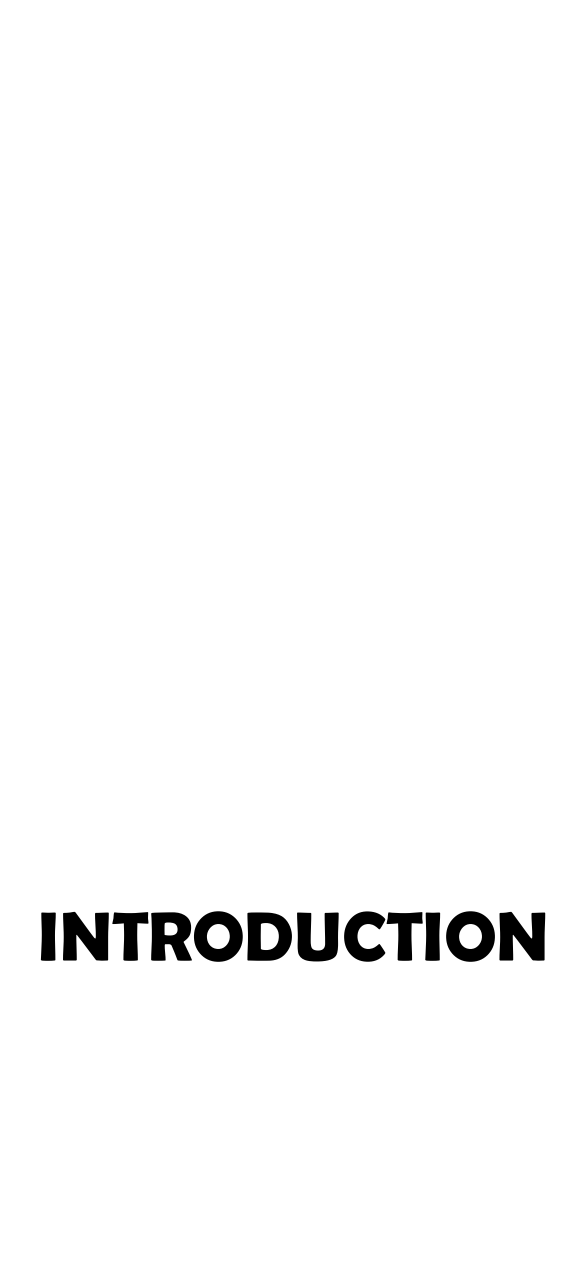


INTRODUCTION

1. INTRODUCTION

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) was declared a global health emergency almost 20 years ago. Yet it continues to be the leading cause of death from a single bacterial pathogen (Gandy and Zumla, 2002). Resistance and persistence are the major issues in TB control (Sacchettini *et al.,* 2008). The World Health Organization (WHO) recently estimated that globally 440000 new cases of multidrug-resistant TB (MDR-TB), i.e., resistance to the most efficacious frontline drugs, isoniazid and rifampicin, have been reporting every year. 'Extensively drug-resistant' TB (XDR-TB), which shows additional resistance to fluoroquinolones and an injectable drug

(kanamycin, capreomycin or amikacin), is also increasing. Patients infected with drug-resistant strains of MTB are prone to treatment failure, thus posing a risk of disseminating resistant strains in their communities (Espinal *et al.,* 2000). Persistence of infection despite extensive chemotherapy is the second major issue in the control of TB. While drug-susceptible TB requires 6–9 months of combination therapy to achieve cure, MDR-TB and XDR-TB treatments take years. Although a few new candidates have recently entered early clinical development, there remains an urgent medical need for the discovery and development of new anti-mycobacterial drugs with new mechanisms of action to keep drug resistance at bay and develop faster-acting regimens to reduce treatment duration and hence increase compliance (Koul *et al.,* 2011).

Second half of the last decade marked drastic change in the drug discovery processes especially antibacterial drug development strategies. Approaches to target selection, composition and diversity of chemical libraries as well as screening strategies were changed. The apparently rational genomics approach to antibacterial drug discovery initiated in the late 1990s (high-throughput screening of genetically essential targets) has been replaced by a diverse set of discovery strategies, including going back to classical phenotypic whole-cell screens, revisiting old clinically validated targets, and re-evaluating old abandoned compounds and drugs (Brotz and Sass, 2010). *De novo* target-based

antibacterial drug discovery has so far not been successful, explaining the current trend to move back to whole-cell screens and old targets and drugs. However, cell-based lead finding and optimization, although feasible, is a black box approach, excluding the use of several modern medicinal chemistry approaches, such as structure-based design and fragment-based screening. Going back to old targets and compounds is useful and pragmatic but might not yield sufficient compounds with new mechanisms of action to feed the pipelines. Therefore, there is clearly a need to reconnect modern genome biology with drug discovery to provide the basis for rational, target-based programmes that can make full use of modern lead finding and optimization tools (Dick and Young, 2011).

Emerging themes in target identification are chemical target validation with a tool, compound *in vitro* and genetic target validation via silencing in animal models. Another interesting approach is the dissection of the extended mechanism of action of existing cidal antibacterials for the identification of compound-induced intracellular bacterial cell death pathways (Kohanski *et al.,* 2007). In addition to these probe-driven 'throw a spanner in the works' approaches, systems biology approaches, incorporating bioinformatics and computational modelling of biological networks, are becoming more capable of the prediction of potential candidate targets that can be validated experimentally. While the reductionist approach has been, and will continue to be, an essential part of biological research, the increasing amounts of data on genomics and physiology of various organisms generated by high-throughput technology has effectively brought an end to 'naive reductionism' and has led to the birth of systems biology. The key contribution of systems biology is the elucidation of emergent properties resulting from a comprehensive analysis of complex large-scale biological interaction networks. In order to integrate various forms of experimental and literature data for the construction and analysis of biological network models, the use of computational tools and bioinformatics is indispensable. This leads to a concomitant trend of rapid development of *in silico* methods for data and model analyses. In the field of drug discovery, many researchers have proposed novel computational methodologies to aid various

stages of drug discovery, including target identification, drug design and lead optimization.

Right from the start of civilization, plants have been used as a source of valuable medicine and structurally diverse chemical compounds for therapeutic and industrial use. Medicinal plants are the most important source of life saving drugs for majority of world's population. The World Health Organization has estimated that more than 80% of the world's population in developing countries depends on herbal medicine for basic healthcare needs. Herbs have played significant role in many ancient traditional system of medicine in Asia such as the Ayurveda, Unani and Chinese medicine. Today the herbal products symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Blind dependence to the synthetics becomes lower and people are returning to the natural with hope of safety and low cost. The present investigation aimed to find out potential lead molecules with anti- tuberculosis activity in selected traditional spice varieties of Kerala *viz. Eleteria cardamomum*, *Curcuma longa* and *Zingiber officinale*, which have been used for the treatment of lung diseases including tuberculosis in Indian traditional system of medicine. However, its efficacy and mode of molecular mechanism of drug activity was not so far scientifically demonstrated. Therefore, its efficacy under *in vitro* condition was also tested. The results insight to the discovery of novel drugs against tuberculosis.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 TUBERCULOSIS

Tuberculosis (TB) is caused by bacteria, *Mycobacterium tuberculosis* (MTB). It is a curable and preventable disease that most often affects the lungs. TB is transmitted from person to person, by people with pulmonary (lung) TB who releases MTB into the air through coughing, sneezing or spitting. A person needs to inhale only a few of these germs to become infected. About one-third of the world's population has latent TB, which means people have been infected by TB bacteria but are not (yet) ill with disease and cannot transmit the disease. TB is one of the most ancient diseases, which still remains as a world's infectious killer particularly among the tropical developing countries like India. TB is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent (WHO., 2013). Worldwide, one-third of the total population is infected; 9 million are ill and nearly 1.5 million people die from TB each year (American lung association, 2013). At least one-third of people living with HIV worldwide in 2012 are infected with TB bacteria, although not yet ill with active TB. People living with HIV and infected with TB are 30 times more likely to develop active TB disease than people without HIV (WHO., 2013). In each year nearly 2 million people in India develop TB, of which 0.87 million are infectious case. The estimate indicates that annually around 3,30,000 Indians die due to TB. The historical background of TB and its impacts in global and national level are well reviewed by several authors (Chakraborty, 2004: Sandhu, 2011).

2.2 TREATMENT SYSTEM

Live Bacille Calmette-Guerin (BCG) vaccine derived from the TB vaccine developed in 1921 remains the only vaccine against TB (WHO., 2013). Active, drug-sensitive TB disease is treated with a standard six-month course of four antimicrobial drugs that are provided with information, supervision and support to the patient by a health worker or trained volunteer. Without such supervision and support, treatment adherence can be difficult and the disease can spread. The vast

majority of TB cases can be cured when medicines are provided and taken properly. The treatment consists of taking more than one medication for a duration of 3 to 6 months. Isoniazid, Rifampin, Ethambutol and Pyrazinamide are some of the medications used to treat TB. Since 1995, over 56 million people have been successfully treated and an estimated 22 million lives saved through use of Directly Observed Treatment, Short-Course (DOTS) and the Stop TB Strategy recommended by WHO. The World Health Organization (WHO) has recently promoted the DOTS strategy as an effective intervention that will lead to reduce tuberculosis transmission and decreasing the number 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).

2.3 PROBLEMS RELATED TO PRESENT TREATMENT SYSTEM

The drugs available for the treatment of TB were discovered in a period of two decades (1944, 1965). The primary or frontline drugs in the treatment of TB are Rifampicin, Isoniazid, Ethambutol, Streptomycin and Pyrazinamide. When these drugs fail, the patients will be treated with second-line drugs such as Kanamycin, Amikacin, Cycloserine and Para-amino salicylic acid. These drugs should be administrated by the patient for a minimum period of six months to 2 years.

Mycobacterium 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). Co-infection of TB and HIV is a problem since the emergence of HIV/AIDS in 1980s. Combined treatment of TB and HIV involves a high pill count with associated adherence problems, overlapping toxicity profiles of the anti -retroviral and anti- TB drugs, drug interaction between rifampin (antituberculosis drug) and the anti-retroviral protease inhibitors, and the risk of immune reconstitution syndrome. Despite the

flaws with and growing resistance to current TB treatments, no new TB drugs have been developed in nearly 50 years.

2.4 NEED FOR NEW DRUGS

Reasons for developing new tuberculosis drugs are: (1) to improve current treatment of active tuberculosis by shortening the total duration of treatment or by providing for more widely spaced intermittent therapy; (2) to improve the treatment of multidrug-resistant tuberculosis (MDR-TB), and (3) to provide more effective treatment of latent tuberculosis infection (LTBI) in low-incidence countries where this intervention is a component of the control strategy. Of these, the first is most compelling. In the light of these, there is an urgent need to find novel, faster and better drugs to defeat TB.

2.5 NEW DRUG DEVELOPMENT CURRENT STATUS

Following nearly 3 decades of neglect, there is now renewed interest in the development of new drugs for the treatment and prevention of TB (O'Brien and Nunn, 2001). Globally, WHO and several International agencies have been promoting R & D for the discovery of new drug for combating drug resistant TB. In India Council of Scientific and Industrial Research (CSIR) has already initiated R & D in this line and promoting India's Open Source Drug Discovery Network (OSDD) for the discovery of anti-tuberculosis drugs. However, pharmaceutical companies are not shown much interest for investing on the discovery of new anti-tuberculosis drugs due to several constraints. The research is expensive, slow and difficult and requires specialized facilities for handling MTB. There are few animal models that closely mimic the human TB disease. Development time of any anti-TB drug will be long. In fact, clinical trials will require the minimum six-month therapy with a follow-up period of one year or more. In addition, it is hard to demonstrate obvious benefit of new anti-TB agents over pre-existing drug, since clinical trials involve multidrug combination therapy using high effective ordinary anti-TB drugs. Finally, there is the perceived lack of commercial return to companies engaged in the development

of new anti-TB drugs, because over 95 percent of TB cases worldwide are in developing countries.

Recently, several small scale biotech companies and many large pharmaceutical companies, such as GlaxoSmithKline (Brentford, United Kingdom), AstraZeneca (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), Fluoroquinolones (Clinical Phase II) and TBA-354 (Preclinical trial) (Susan, 2012).

Recently, significant progress has been made in reinvigorating the almost non-existent pipeline of novel agents for the treatment of tuberculosis and in re-establishing the infrastructure for the conduct of clinical trials of new tuberculosis drugs and treatment regimens. Recent studies of long-acting rifamycin derivatives and potent fluoroquinolone antibiotics are leading to improved regimens for the treatment of active and latent tuberculosis. A number of other compounds in late preclinical and early clinical development showed great promise. The rapid increase in knowledge of mycobacterial pathogenesis is leading to the identification of new drug targets, including

those believed to play a role in latent infection or in the phenomenon of persistence. A major challenge will be to sustain and increase funding for continued developmental and clinical work if the promise of tuberculosis elimination, or at least significant lessening of the global tuberculosis epidemic, is to be achieved (O'Brien and Spigelman, 2005).

2.6 PLANT AS A SOURCE OF MEDICINE

The use of natural products with therapeutic properties is as ancient as human civilisation and, for a long time, mineral, plant and animal products were the main sources of drugs (Pasquale, 1984). The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new modus vivendi of the industrialised western societies, in which drugs from natural resources were considered either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value.

The discovery of antibiotics, Pencillin from pencillium fungus and its impacts especially in the antiinfection therapy during the Second World War was a best example to the drug discovery from microbes. Higher plants are also producing innumerable number of chemical molecules in accordance with the needs and challenges of the plant environment and most of these have therapeutic value. Currently about 25% of the drugs prescribed worldwide are derived from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organisation (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from

plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum.* It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin (Yue-Zhong Shu, 1998). The vast majority of these cannot yet be synthesised economically and are still obtained from wild or cultivated plants. 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 muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies (Williamson *et al*., 1996).

In recent years, 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). This interest in drugs of plant origin is due to several reasons, such as, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorised by legal authorities dealing with efficacy and safety procedures, and many published papers point to the lack of quality in the production, trade and prescription of phytomedicinal products.

The NCI (National Cancer Institute, USA) 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. Of the estimated 250,000–500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use (Payne et al., 1991). Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumour activity, but even these were not screened for other pharmacological activities (Hamburger and Hostettman, 1991).

2.7 ANTIMICROBIAL PROPERTIES OF PLANTS

The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments (Nagesh and Shanthamma, 2009). Extracts of plants were used for the treatment of various diseases and this forms the basis for all Indian systems of medicine. However, this area is not much developed when compared to modern system of medicine, mainly because of the lack of scientific documentation in this field. Mostly the pharmacological activity of medicinal plants resides in its secondary metabolites which are comparatively smaller molecules in contrast to the primary metabolites such as proteins, carbohydrates and lipids. These natural products provide clues to synthesize new structural types of antimicrobial and antifungal chemicals that are relatively safe to man (Kalimuthu *et al*., 2010).

The effect of plant extracts on bacteria has been studied by a large number of researchers in different parts of the world (Reddy *et al*., 2001; Ateb and Erdo, 2003). Agarry *et al*., (2005) have shown the potent antimicrobial activities of the gel and leaf of *Aloe vera* against a wide range of bacteria and fungi. Bearberry and cranberry juice have been used to treat urinary infections while plant

species such as lemon balm, garlic and tea tree are described as broad-spectrum antimicrobial agents (Rios and Recio,2005). Mathabe *et al*., (2006) reported that methanol, ethanol, acetone and hot water extracts from different plant parts (leaves, roots, bark and stem rhizome), of *Indigofera daleoides*, *Punica granatum*, *Syzygium cordatum*, *Gymnosporia senegalensis*, *Ozoroa insignis*, *Elephantorrhiza elephantina*, *Elephantorrhiza burkei*, *Ximenia caffra*, *Schotia brachypetala* and *Spirostachys africana* showed remarkable antibacterial activity against *Vibro cholera*, *Escherichia coli* and *Staphylococcus aureus*, *Shigella* species and *Salmonella typhi.* The methanol extracts of forty nine different plant extracts were screened for antifungal activity, out of which forty three plant extracts exhibited varying degrees of inhibitory activity against the fungi (Varaprasad *et al*., 2009). Antibacterial activities of aqueous and methanol extracts of some medicinal plants reported by Girish and Satish, (2008) against gram positive human pathogenic bacteria showed the methanol extracts had wider range of activity on these organisms than the aqueous extracts, which indicates that the methanol extracts of all selected plants may contain the active components.

2.8 MEDICINAL PLANTS AND TRADITIONAL SYSTEM OF TREATMENT IN INDIA

India is known for its traditional systems of treatments such as Ayurveda, Siddha, and Unani. Treatment systems are well mentioned in the ancient Vedas and other scriptures. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India (Subhose, 2005). The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. The alternative medicines in the traditional systems are derived from herbs, minerals, and organic matter, while for the preparation of herbal drugs only medicinal plants are used. Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. Pandey *et al.* (2013) said that in India, about 70 percent of rural population depends on the traditional Ayurvedic system of medicine. In the Western countries,

approximately 40 per cent of people are using the herbal medicine for the treatment of various diseases. This interest in traditional medicines is growing rapidly due to the attention being given to it by the Governmental agencies and different NGO's comprising of general public and researchers as well as the increased side effects, adverse drug reactions, and cost factor of the modern medicines (Pandey *et al.,* 2013).

India is the largest producer of medicinal plants. There are currently about 250,000 registered medical practitioners of the Ayurvedic 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 treatments includes 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).

2.9 INDIGENOUS PLANTS AND TRADITIONAL KNOWLEDGE

Traditional knowledge (TK) is the know-how, skills and practices that are developed, sustained and passed on generation to generation within a community, often forming part of its cultural or spiritual identity (http://www.wipo.int/tk/en/tk/). Indigenous plants includes plants that have developed, occur naturally, or existed for many years in an area. 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 ethnobotany 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,

ethnobotany as “the study of the relationship which exists between people of primitive societies and their plant environment”. In India plants have been used by tribals and local people for curing of various diseases. 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). The safety and efficacy data are available for even fewer herbs, their extracts and active ingredients and the preparation containing them. Tropical and subtropical Africa contains between 40–45,000 species of plant with a potential for development and out of which 5,000 species are used medicinally (Van Wyk, 2008).

The former Prime Ministers of India Jawaharlal Nehru (1950) and Indira Ghandi (1973) advocated the integration of the best of indigenous medicine with modern medicine. The Government established a Central Council of Indian Medicine, a statutory body with a mandate to ensure conformity of standards of education and regulation of practice in respect to the traditional systems. Arab medicine was introduced into India by Arab and Persian settlers. 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. They were influenced by the Ayurvedic System and local Indian practitioners (Borins, 1987). 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 confirmed 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.

2.10 AGRICULTURAL CROPS AS NEUTRACEUTICALS

Due to risk of toxicity or adverse effect of drugs, consumers are turning massively to food supplements to improve health where pharmaceutical fails. This resulted in a world-wide nutraceuticals revolution (Rohan *et al.,* 2011). The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, founder and Chairman of the Foundation for Innovation in Medicine (FIM), Cranford, NJ. According to DeFelice, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease"(Brower, 1998). Presently over 470 nutraceutical and functional food products are available with documented health benefits (Rajat *et al*., 2012). Many of these new products that are being promoted to treat various diseases find their origin in the plant kingdom. This is an obvious choice as many plants produce secondary compounds as alkaloids to protect themselves from infections and these constituents may be useful in the management of human infection. Many of the phytomedicines are the typical examples. The original idea in these concepts goes back three thousand years ago. Hippocrates (460-377 BC), the well –recognized father of modern medicine, stated "Let food be thy medicine and medicine be thy food" to predict the relationship between appropriate foods for health and their therapeutic benefits (Bagchi, 2006). Tomato, guava, papaya, watermelon (Lycopene), Corn, avocado, spinach (Lutin), Carrots, various fruits and vegetables (β-Carotene), Turmeric (curcumin), and Citrus fruits (Flavonones) were the major nutraceutical crops (Rajat *et al.,* 2012).

2.11 NEED TO VALIDATE TRADITIONAL KNOWLEDGE

It is well demonstrated that plants are the best source of anti-microbial compounds. Since time immemorial several herbal remedies have been used against tuberculosis in the traditional systems of treatment especially in India and in African countries. But the efficacies of these herbal formulations are not scientifically validated due to several reasons such as lack of efficient screening methods, high expense slow and difficulties in executing the experimental works,

lack of model organism for testing etc. Turnbull (2009) states that "if there is to be a future for us all, it depends on treating the planet and the totality of its environmental and cultural resources as a commons to be shared and sustained. In recent years the significance of traditional knowledge has been explored tremendously in view of its value to biotechnology, particularly the pharmaceutical, phytomedicinal, nutriceutical, and herbal sectors. 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). More recent research continues to validate the importance of an ethnobotanically targeted approach to the initial discovery of therapeutics (Lewis *et al.,* 1999; Schuster, 2001). Such research draws on the traditional knowledge of local and indigenous communities who have custodian of such resources, thereby allowing a targeted testing of specific plants for specific purposes. Assessing the worth of drugs obtained from traditional sources both now and in the past is difficult. A few recent examples, however, provide a commonality of independent estimates in the billions. In the last decade, Japanese or Kampo traditional drug sales reached \$56 billion annually (Okada, 1996). Others estimated that only one-eighth of the pharmaceutically important drugs have been discovered in the rain forests globally. If, as described above, as many as three-quarters of plant-derived drugs used today are of traditional origin, then in this single ecosystem, such discoveries could generate a total value of \$110 billion (Mendelsohn and Balick, 1995).

Thus, considering its significance to the global economy and health, it is clear that traditional knowledge should be protected, and a part of the value generated from its protection should be transferred back to the authors of this knowledge, i.e., the indigenous people.

Scientific evaluation and demonstration of the efficacy of traditional knowledge attained prima face importance due to the following reasons.

- To prevent bio-piracy.
- To aid more acceptance to our herbal products in the global market.
- To discover novel drugs against emerging killer diseases.
- Disease having multifactorial causation herbal drugs are more advisable.
- Natural products/plant based drugs are safe, cause less or no side effects, readily available and economic.
- Natural resources can be utilized sustainably and economically.

2.12 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.

2.12.1 High throughput screening

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). 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). Target validation, assay development, secondary screening, ADME/Tox, and lead optimization are among the areas in which there is an increasing use of HTS technologies. It is becoming fully integrated within drug discovery, both upstream and downstream, which includes increasing use of cell-based assays and high-content screening (HCS) technologies to achieve more physiologically relevant results and to find higher quality leads. 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).

2.12.2 *In silico* screening

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.* (2007) 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.* (2007). 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.* (2007).

2.12.3 *In vitro* screening

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). In most cases part of the tissue is removed from freshly killed or anaesthetized animal and suspended in a chamber containing warmed oxygenated physiological salt solution (Hill and Rang, 2009) for the *in vitro* studies of a drug candidate. A major drawback of *in vitro* system is that each cell type is studied in isolation, whereas in the human body, there might be multiple organ interactions that are critical to drug toxicity (Albert, 2005). The pharmaceutical efficacy of a receptor-ligand complex, i.e., the property that determines whether it is a full agonist, a partial agonist, or an antagonist, often depends on the type of assay used (Kenakin, 1999). This may have an important bearing on the selection of possible drug molecule.

2.12.4 *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: Kerns *et al.,* 2008: Pelkonen *et al.,* 2011 and Garcia-Alcover *et al.,* 2012). *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.

2.13 VIRTUAL SCREENING

Virtual screening uses computer-based methods to discover new ligands on the basis of biological structures (Shoichet, 2004). 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. 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); Schneide (2010); Lavecchia and Giovanni (2013).

2.13.1 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.*, 2006). AccordingtoKitchen *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 was explained by Mihasan, (2012).

2.14 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 tools (Abraham *et al.,* 2014). 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 (Shafer, 1976). The theory was first developed by Arthur P. Dempster and Glenn Shafer (Fine, 1977).

2.15 SELECTED SPICES

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

2.15.1 Turmeric- *Curcuma longa* L.

Turmeric has been put to use as a foodstuff, cosmetic, and medicine. A comprehensive information on the plant such as its origin, consumption, composition, etc. and various properties including antioxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, antimicrobial, cardiovascular effects and gastrointestinal effects etc. of *C. longa* was well reviewed by Akram *et al.* (2010) and Prasad and Aggarwal (2011). The adverse reactions of *C. longa* was given in detailed by Gupta *et al.* (1980), Brinker (1998) and Sandeep *et al.* (2010). As

turmeric is a natural botanical product, it is not patentable (Royal Botanical Gardens, 2012). *C. longa* has been used traditionally for thousands of years as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its supposed antimicrobial property (Chaturvedi, 2009).

2.15.2 Ginger- *Zingiber officinale* Roscoe

Zingiber officinale is extensively used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines. In India the fresh and dried roots were measured distinct medicinal products. The antimicrobial activity of ginger using its methanolic and ethanolic extract against various human pathogens was determined by broth dilution method (Mustafa *et al.,* 1999). Al-Amin *et al.* (2006) well explained about Anti-diabetic and hypolipidaemic properties of ginger. Anti-cancer properties, anti-inflammatory response, antinauseant and antiemetic properties of ginger were well described by Suthar *et al.* (2003). Surh *et al.* (1998) had reviewed the mutagenic studies of ginger and ginger constituents. They noted that in one study an ethanolic extract of the rhizome showed mutagenic activity. The most notable chemical constituents in ginger are the so-called "pungent principles", the gingerols, which give ginger its characteristic aroma. Also present are volatile oils, other oleoresin compounds, and starches, proteins, and fats (Bradley, 1992: Pedersen, 1994).

2.15.3 Cardamom- *Eleteria cardamomum* (L.) Maton

The plant *E. cardamomum* of the Zingiberaceae family is one of the world's very ancient and expensive spices (Lwasa and Bwowe, 2007), mainly grown in Sri Lanka and South India. The seeds of their ripe fruits are used medicinally, as a spice, and also as a flavouring agent in curries, coffee and cakes, particularly in the Arab countries. The seeds contain essential oil in concentration of about 4% of dry weight. The main compound is 1, 8-cineole (representing 50% or more), with smaller amounts of α-terpineol, borneol, camphor, limonene, α-terpenyl acetate, and α-pinene (Miyazawa and Kameoka, 1975; Agaoglu *et al.,*

2005). Indian cardamom is low in fat and high in protein, iron, and vitamins B and C. Cardamom seeds, with their sweet and spicy aroma, are used in aromatherapy to stimulate energy (Lawrence, 1979; Kaushik, 1988). Korikontimath *et al.* (1999) and Wyk and Wink 2004 said that it also acts as Ayurvedic aphrodisiac and remedy in case of digestive problems, asthma, bronchitis, and urinary complaints and several other human ailments. The antimicrobial activities of *E. cardamomum* extracts was well explained by Harborne *et al.* (1975). According to Derg, 2005 antimicrobial activity assays indicated that cardamom seed had inhibitory activity on *Mycobacterium smegmatis, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Micrococcus luteus,* and *Candida albicans*; however no inhibitory activity was observed against *Pseudomonas aeruginosa.*

2.16 *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).

2.17 TARGET PROTEIN

An enzyme, decaprenylphosphoryl-β-D-ribose 2-epimerase (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.,* 2011). 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, AutoDock Vina (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).

2.18 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

To validate anti-tuberculosis activity and identification of lead compounds in indigenous spices of Kerala, most common and widely used spices belonging to the family Zingiberaceae *viz*, *Eleteria cardamomum*, *Curcuma longa* and *Zingiber officinale* were selected for the study.

3.1 SELECTED SPICE VARIETIES

3.1.1 *Curcuma longa* L.

Curcuma longa, commonly known as 'Turmeric' (Plate 1A and 1B) is a tropical rhizomatous perennial herb and can be grown on different types of soil under irrigated and rainfed conditions. This spice is native to Asia and India. The main turmeric growing States in India are Kerala, Andhra Pradesh, Maharashtra, Orissa, Tamilnadu and Karnataka. Nearly 220 phytochemicals were isolated and identified from *C. longa.*

3.1.2 *Zingiber officinale* Roscoe

Zingiber officinale (Ginger) (Plate 1C and 1D) is a biennial or perennial reed-like herb, grown for the pungent, spicy underground stems or rhizomes. It is a tropical plant adapted for cultivation even in regions of subtropical climate such as the high ranges. Nearly 200 phytochemicals were so far reported from *Z. officinale.*

3.1.3 *Eleteria cardamomum* (L.) Maton

Eleteria cardamomum (Cardamom) (Plate 1E and 1F) is a rhizomatous herb of India having aromatic seeds. The habitat of *E. cardamomum* is the evergreen forests of the Western Ghats. It is grown in areas where the annual rainfall ranges from 1500-4000 mm with a temperature range of 10-35 ºC. Nearly 60 phytochemicals were reported from the seed of *E. cardamomum.*

A) Rhizome of *Curcuma longa* L. B) *Curcuma longa* Plant

C) Rhizome of *Zingiber officinale* Roscoe D) *Zingiber officinale* Plant

E) Seeds of *Eleteria cardamomum* (L.) Maton F) *Eleteria cardamomum* Plant

Plate1. Selected spice varities

3.2 *IN SILICO* SCREENING

3.2.1 Source of phytochemicals 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 a database which provides information on large collection of phytochemicals from different plant species. Advanced search facility in the database enables us to search phytochemicals under four different categories. In the category 'Plant search' options such as 'Details a particular plant', 'High concentration chemicals', 'Chemicals with one activity', 'Ethnobotanical uses' and list of chemicals and activities for a plant are provided. In the category 'Chemical search' provides the options, 'Plants with a chosen chemical', 'Activities of chosen chemical', 'List of activities' and plants for a chemical and list of common activities (synergies) for a list of chemicals. Plants with a specific activity, search for plants with several activities, chemicals with a specific activity, Lethal Dose (LD) information for a chemical, search for plants/chemicals with one or more activities and search for plants/chemicals with a super-activity are under the category 'Activity searches'. Similarly, ethnobotany searches include ethnobotanical uses for a particular plant and plants with a particular ethnobotanical use. The database is available on the URL: www.ars-grin.gov/duke

3.2.3 PubChem

PubChem is an open access database which provides information about chemical molecules and their activities against biological systems. The database is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is a part of the National Institutes of Health (NIH), United States. The users can freely access millions of compound structures and descriptive datasets. The PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds.

PubChem consists of three dynamically growing primary databases.

1. Compound databases, as on August 2014, 31 million entries, contains pure and characterized chemical compounds.

2. Substance databases, August 2014, 75 million entries, contains also mixtures, extracts, complexes and uncharacterized substances.

3. Bioassay databases, August 2014, bioactivity results from 1644 high-throughput screening programs with several million values.

PubChem is available in the URL: https://pubchem.ncbi.nlm.nih.gov

3.2.4 ChemSpider

ChemSpider is an open access chemical structure database providing fast access to over 30 million structures, properties and associated information. By integrating and linking compounds from more than 470 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

3.2.5 Dictionary of Natural Products

Dictionary of Natural Products is a structured database holding information on chemical substances. It includes descriptive and numerical data on chemical, physical and biological properties of compounds; systematic and common names of compounds; literature references; structure diagrams and associated connection tables. Dictionary of Natural Products is available on the URL: www.dnp.chemnetbase.com

3.2.6 ChemSketch

ACD/ChemSketch is a chemically intelligent drawing interface that allows you to draw almost any chemical structure including organics, organometallics, polymers, and markush structures. It can be used to produce professional looking structures and diagrams for reports and publications. ChemSketch is available for download from the URL: www.chemsketch.xtremedownload.com

3.2.7 CORINA

CORINA is a fast and powerful three dimensional (3D) structure generator tool for small and medium sized, typically drug-like molecules. It's robustness, comprehensiveness, speed and performance makes CORINA a perfect application to convert large chemical datasets or databases from 2D to 3D structures. CORINA is available on the URL: www.molecular-networks.com/onlinedemos/ corina_demo

3.3 TARGET MOLECULE SELECTION (PROTEIN)

3.1 *Mycobacterium tuberculosis* DprE1 protein

Mycobacterium tuberculosis contains several proteins and peptides with enzymatic and non-enzymatic activities. Decaprenylphosphoryl-β-D-ribose

epimerase (DprE1) (Plate 2) which plays a key role in the synthesis of arabinan, a major component in bacterial cell wall and responsible for virulence in bacteria, was selected as the target molecule for *in silico* screening. The three dimensional structure of DprE1 (4FDO; Riccardi *et al.,* 2013) was retrieved from Protein Data Bank (PDB id 4FDO).

3.4 SOURCE OF TARGET MOLECULE

3.4.1 The Protein Data Bank (PDB)

The Protein Data Bank (PDB) is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data typically obtained by X-ray crystallography or Nuclear Magnetic Resonance (NMR) spectroscopy and submitted by biologists and biochemists from around the world are freely accessible on internet *via.* the websites of its member organizations (PDBe, PDBj and RCSB). The PDB is a key resource in the area of structural biology. Most major scientific journals and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived databases that categorize the data differently. URL: www.rcsb.org

3.4.2 PDBsum

The PDBsum is a pictorial database that provides at-a-glance an overview of the contents of each 3D structure deposited in the Protein Data Bank. The PDBsum shows the molecule(s) that make up the structure (*i.e.* protein chains, DNA, ligands and metal ions) and schematic diagrams of their interactions. It helps to identify the location of ligand binding sites on a protein, the fundamental process in computer aided drug designing. URL: http://www.ebi.ac.uk/pdbsum/

3.5 DOCKING TOOLS

The selected phytochemicals (ligands) were docked against the target protein using the tool AutoDock for finding out the molecules having more

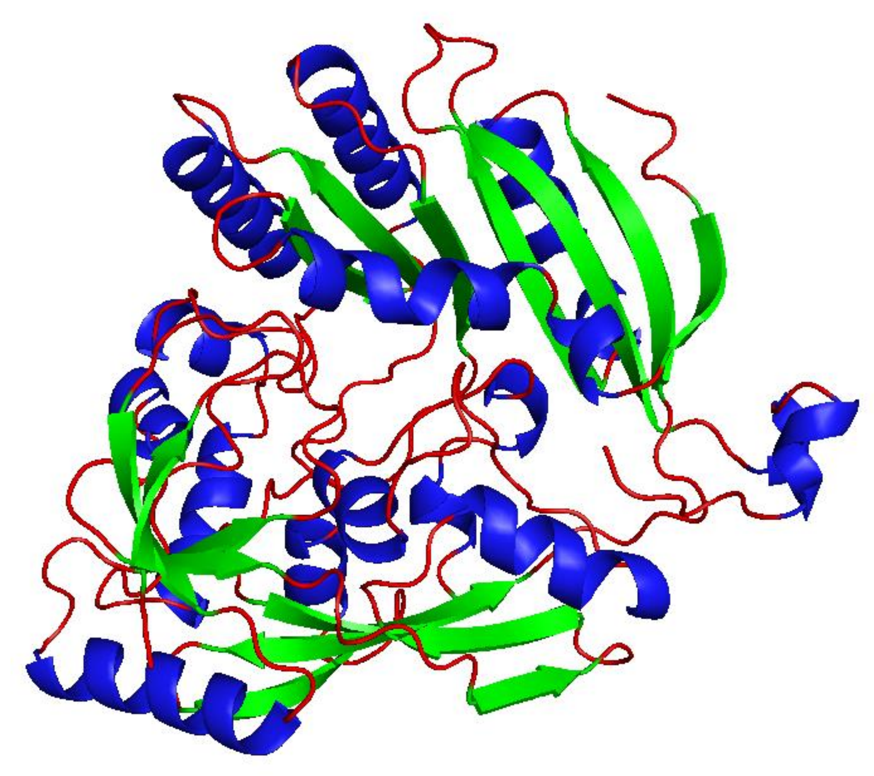


Plate 2. 3-D structure of Decaprenylphosphoryl-β-D-ribose epimerase retrieved from PDB

binding affinity towards the target and to calculate free energy of binding after forming the target-ligand complex. The molecules with free energy of binding ≤5 Kcal mol^{-1} were further docked with other docking tools such as iGEMDOCK, HEX Server, FireDock and SwissDock to reduce the error in selection of true hits. The scores obtained were statistically analyzed following Dempster-Shafer Theory (DST) and identified best leads.

3.5.1 AutoDock

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 4.2 and AutoDock Vina. AutoDock 4.2 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. AutoDock 4.2 is open access and is available under the GNU General Public License.

AutoDock has applications in: X-ray crystallography, Structure-based drug design, Lead optimization, Virtual screening (HTS), Combinatorial library design; Protein-protein docking, Chemical mechanism studies. AutoDock is freely downloadable from the URL: www.autodock.scripps.edu/

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 a free service with no login or registration requirement and it is an online application and docked result 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 webserver 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 EADock DSS 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 MOLECULAR DOCKING USING AutoDock

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 centered 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 ≤-5 Kcal mol^{-1} were considered as hit molecules and they were further analysed by Lipinski's rule of Five.

The result obtained after AutoDock were analysed 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 *IN VITRO* STUDIES

3.6.1 Plant materials and preparation of extracts

The mature seeds of *Eleteria cardamomum* were collected from the cultivated field at Regional Agricultural Research Station (RARS) Pambadumpara, Idukki district and mature rhizomes of *Zingiber officianale* and *Curcuma longa* were collected from Agriculture College Vellayani, Thiruvananthapuram. The samples were air dried at room temperature and powdered using a mixer grinder (Preethi Pvt. Ltd.). Each powdered sample was extracted with 99% ethanol using a Soxhlet apparatus for 6-8 hours. The extracts were concentrated to dryness under reduced pressure using a rotary vacuum evaporator. Five gram each sample was sent to Central for Research in Tuberculosis Institute, Chennai for further *in vitro* analysis.

3.6.2 Anti-tubercular Assay

Anti-mycobacterial activity of the three plant extracts were evaluated by Luciferase reporter phage (LRP) assay against Standard strain of *M. tuberculosis* H37RV at three different concentrations (25, 250 and 500 µg/mL). A compound is considered as an anti-tubercular agent if fifty percent reduction in relative lights units (RLU) is observed when compared to the control using luminometer (Karthik *et al*., 2011).

3.6.3 Microbial strain for anti-*Mycobacterium tuberculosis* Assays

Standard strain of *M. tuberculosis* H37RV maintained at National Institute for Research in Tuberculosis, Chennai was used for the anti-mycobacterial assay.

3.6.4 Luciferase reporter phage (LRP) assay

Standard strain H37RV was grown in Middlebrook 7H9 complete medium 12 with and without seed extracts of *Eleteria cardamomum*, rhizome extracts of *Zingiber officianale* and *Curcuma longa* respectively for 3 days at 37°C. Luciferase Reporter Phage Assay was done using concentrations of 25, 250 and 500 µg/mL of each plant extract. 50 µL bacterial suspension equivalent to MacFarlands No. 2 standard was added to 400 µL of G7H9 with and without the test compound. For each sample, two drug-free controls and three drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µL of the high titer Luciferase reporter phage (phAE129) and 40 µL of 0.1 M $CaCl_2$ were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 µL of the mixture was taken from each tube into a luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10 S of integration in the Luminometer. Duplicate readings were recorded for each sample and the mean was calculated.

The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000 (Siva Kumar *et al*., 2007).

RESULTS

4. **RESULTS**

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, Plate 2). It consists of 481 amino acids, 26 β sheets, 20 α helices, 293 H-bonds.

4.1.2 Detection of active site

The active site of the target protein (DprE1) 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 (Plate 3).

4.1.3 Ligand preparation

The phytochemicals retrieved from the open access databases were depicted in Appendix I, II, III. The structures of phytochemicals created using the tool ChemSketch were shown in figure 1.

4.2 DOCKING

4.2.1 Phytochemicals from *Curcuma longa* and DprE1 protein

A total of 211 chemical molecules present in *Curcuma longa* were docked with DprE1 using the tool AutoDock. The docked molecules having free energy of binding $\geq {}^{-}5$ kcal mol^{-1} were selected as hit molecules. Of the 211 molecules, 101 showed free energy of binding less than $^{-}5$ kcal mol^{-1}. Therefore, five molecules which showed top least free energy of binding were selected as hit molecules for further studies.

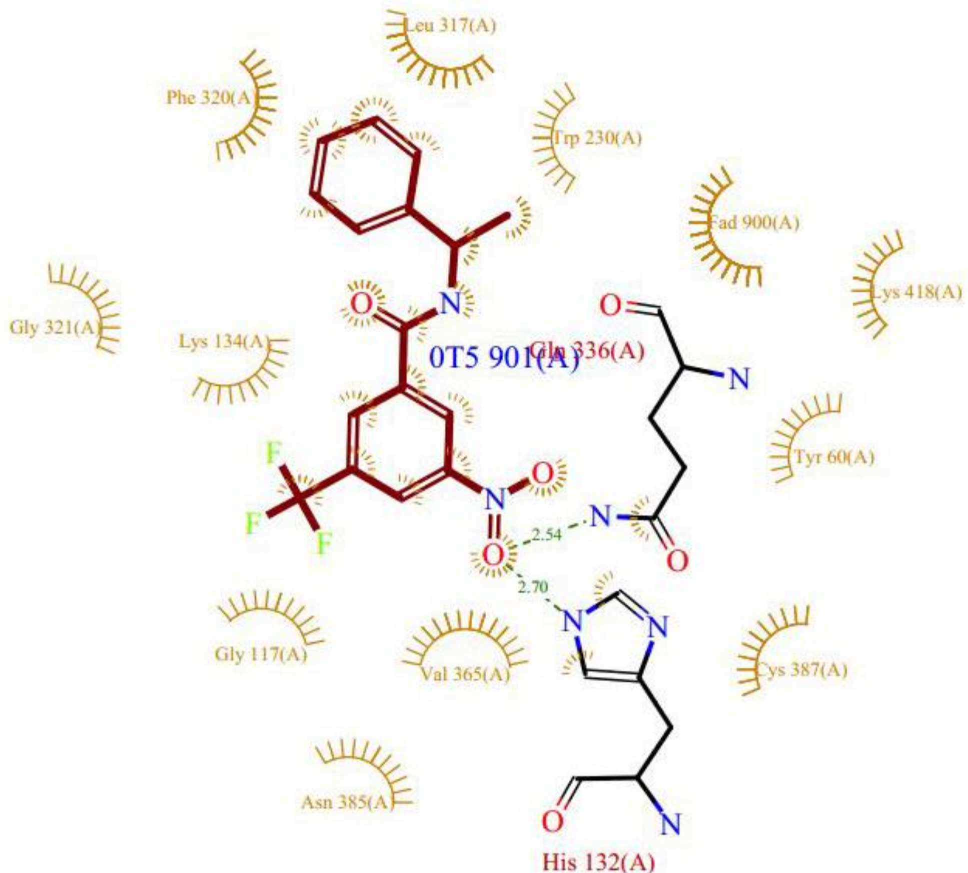


Plate 3. The active site of the target protein (DprE1) detected using the tool PDBsum.

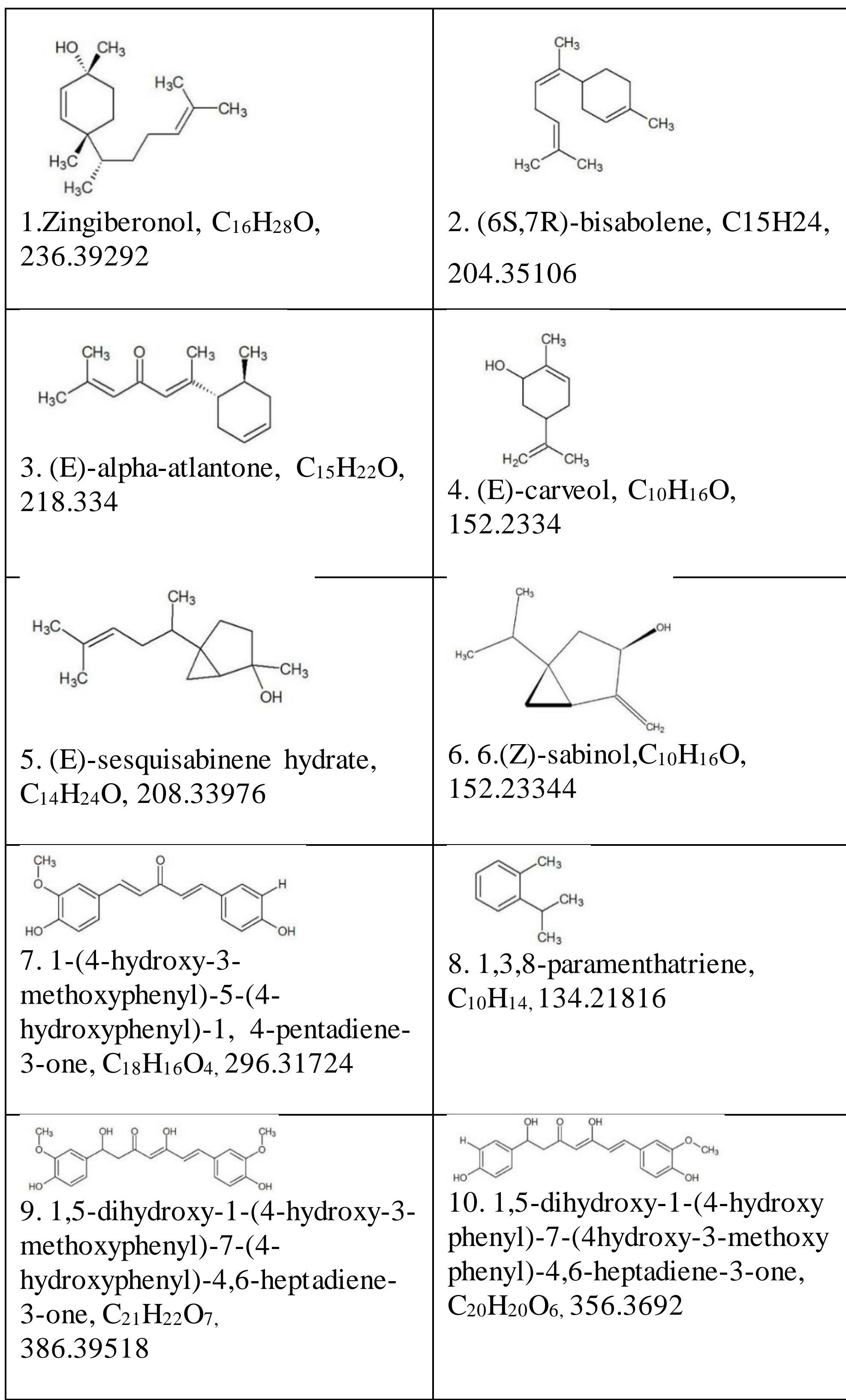


Figure 1. The structures of phytochemicals created using the tool ChemSketch with Molecular Formula and Formula Weight

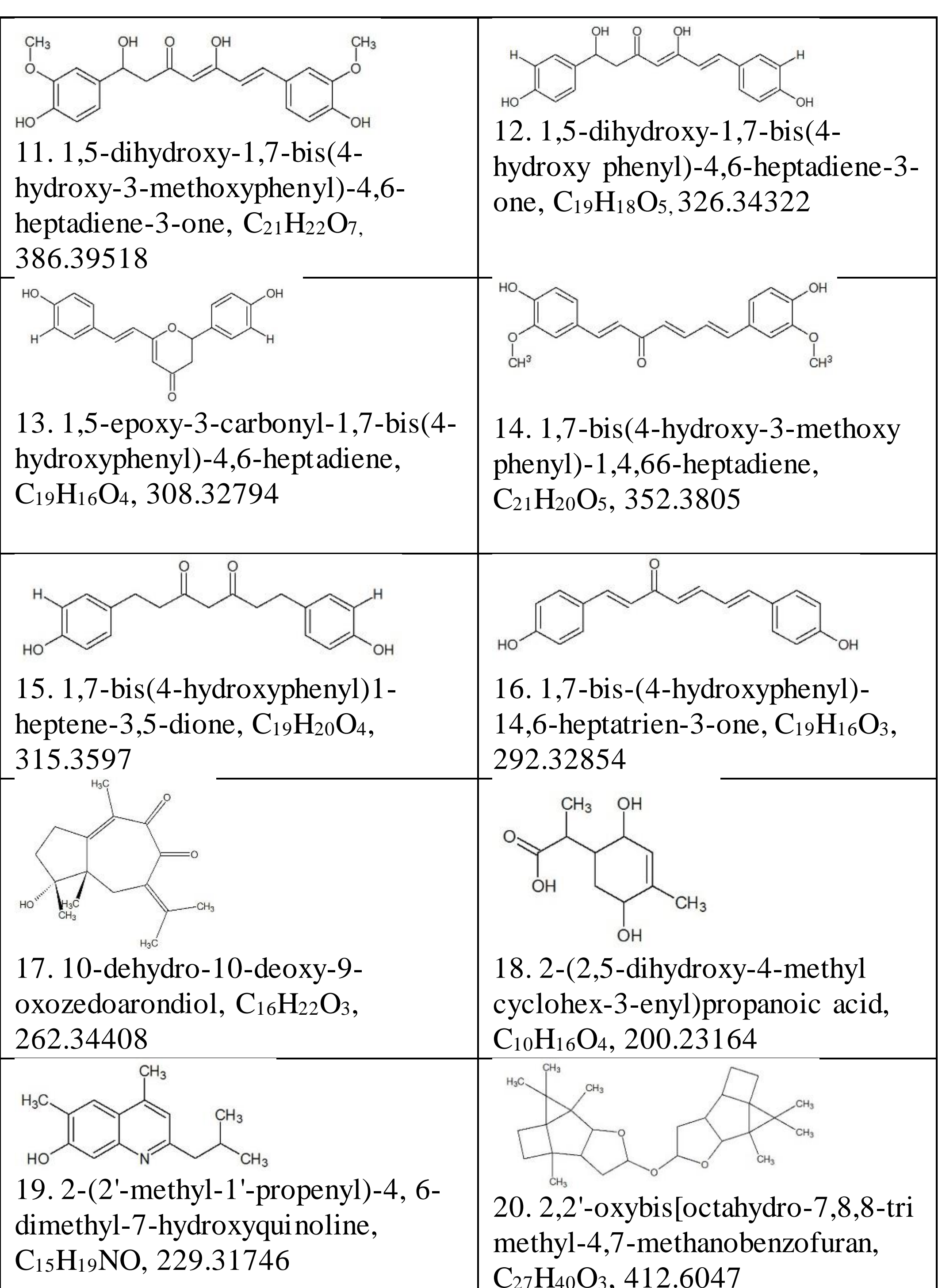


Figure 1. Continued

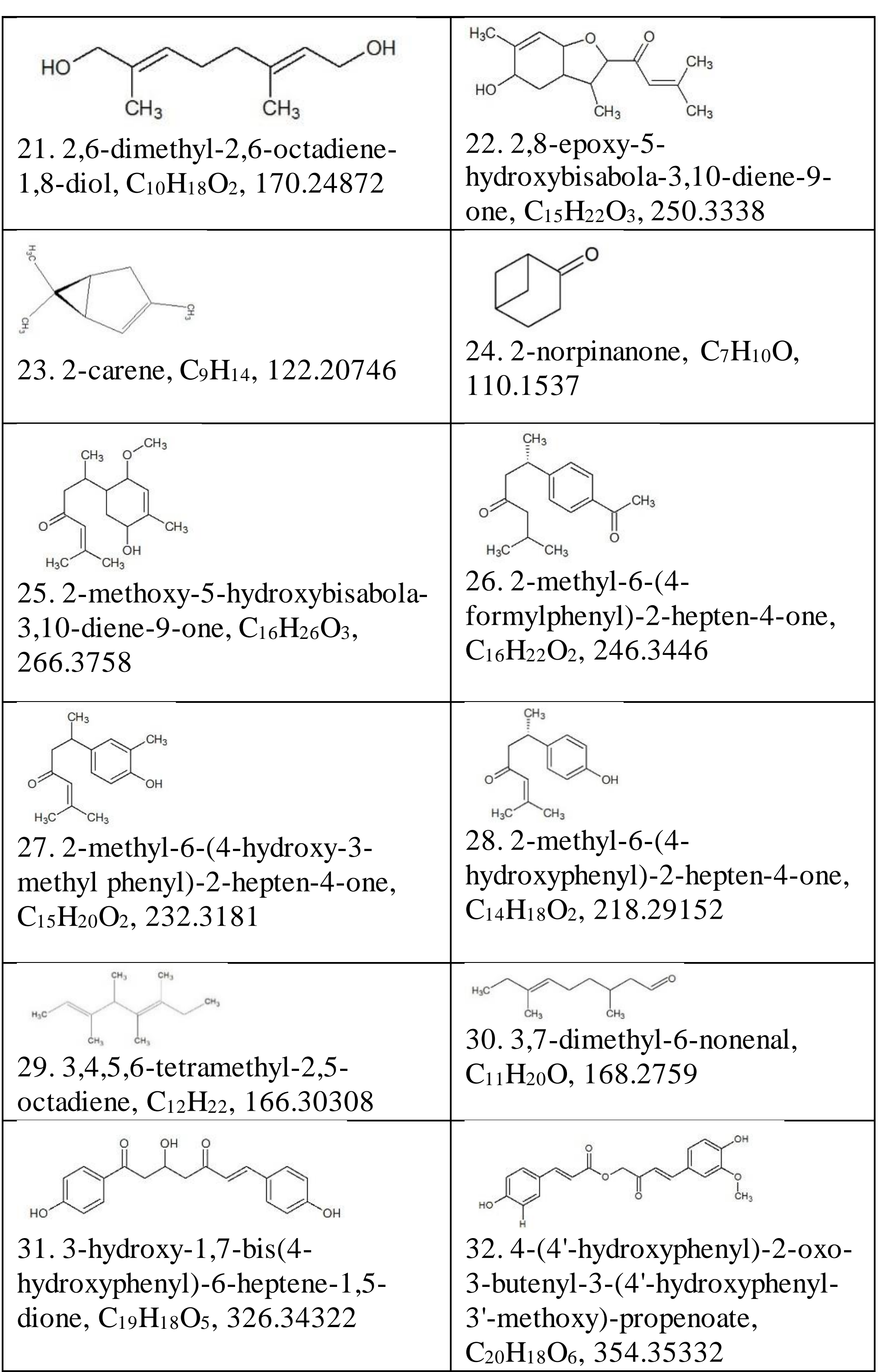


Figure 1. Continued

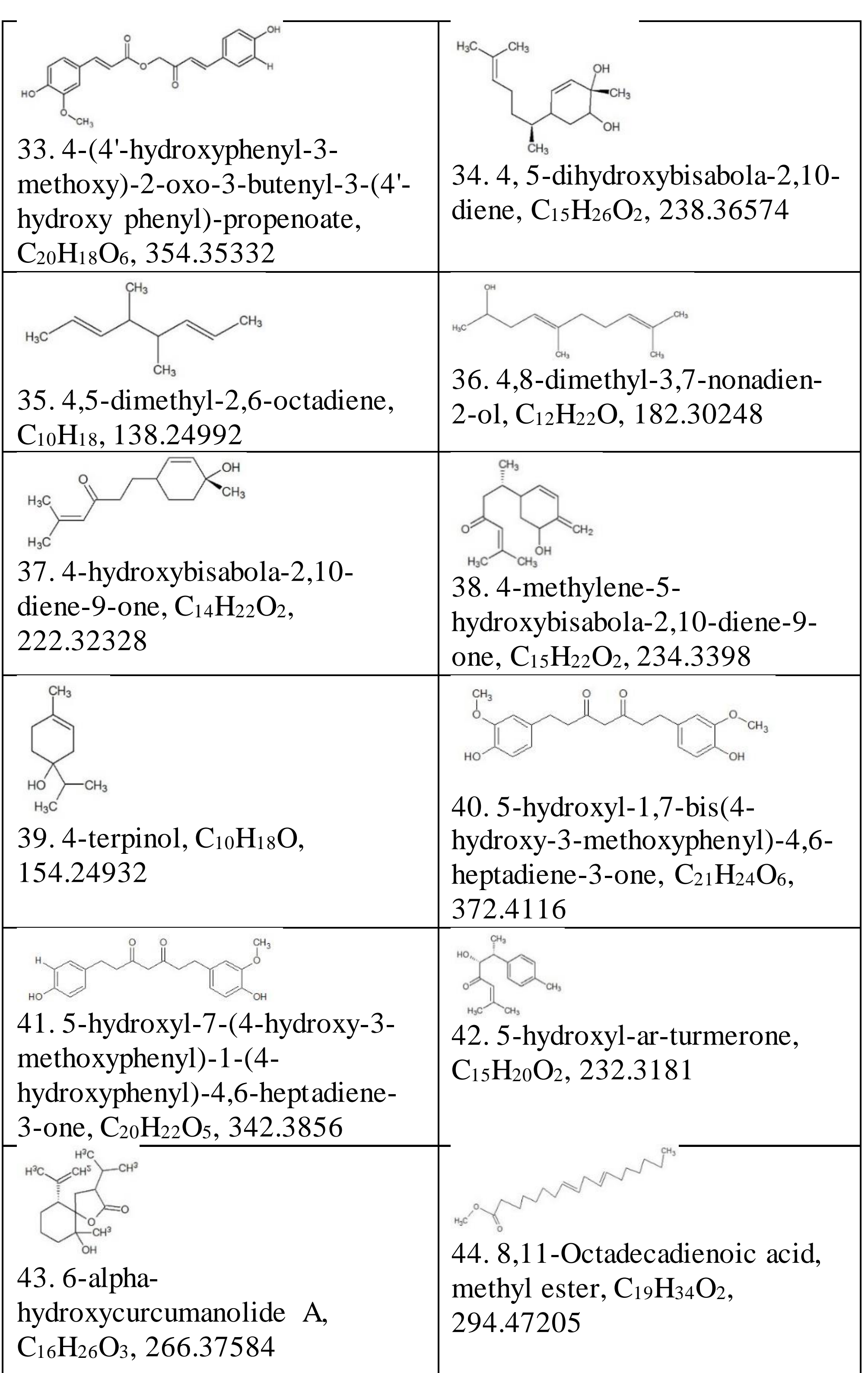


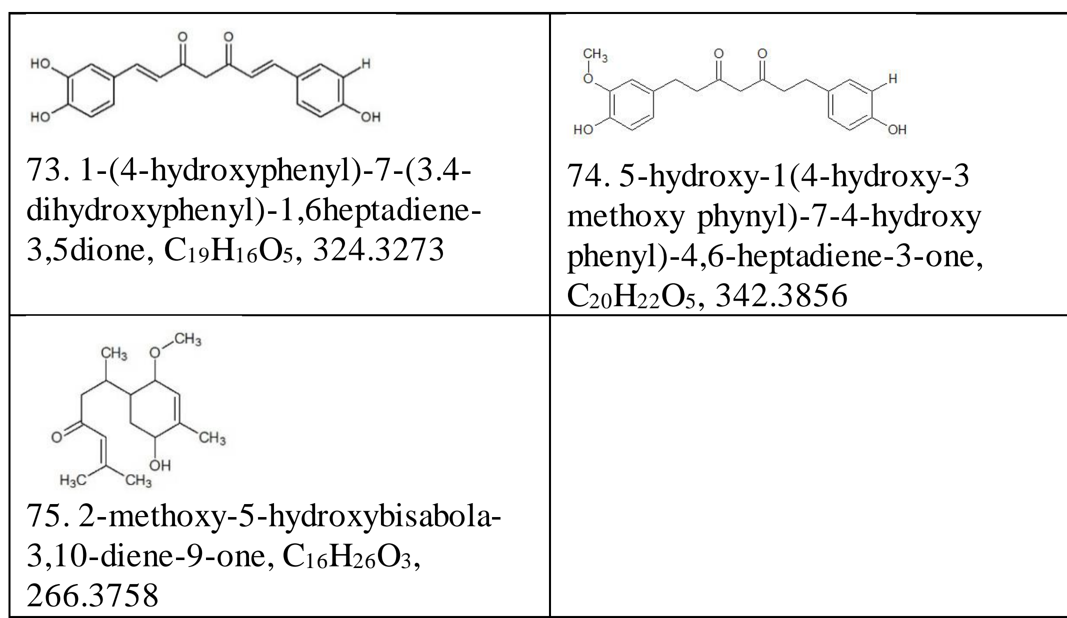
Figure 1. Continued

45. Alpha-oxobisabolene, $C_{15}H_{24}O$, 220.35046	46. Alpha-turmerone, $C_{15}H_{22}O$, 218.33458
47. ar-turmerol, $C_{15}H_{22}O$, 218.33458	48. Benzene, 1-methyl-4-(1-methyl propyl), $C_{11}H_{16}$, 148.24474
49. Bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene), $C_{16}H_{28}$, 220.39352	50.Bisabola-3,10-diene-2-one, $C_{15}H_{24}O$, 220.3504
51. Bisabolone-9-one, $C_{15}H_{22}O_2$, 234.33398	52. BisacuroneC, $C_{14}H_{22}O_3$,238.32268
53. Cis-ocimene, $C_{10}H_{16}$, 136.23404	54. R-citronellene, $C_{11}H_{20}$, 152.2765
55. Curculonone C, $C_{14}H_{22}O_2$, 222.3232	56. Curculonone D, $C_{16}H_{30}O_2$, 254.4082
57. Curcuma-J, $C_{15}H_{20}O_2$, 232.3181	58. Curcuma-L, $C_{15}H_{26}O$, 222.3664

Figure 1. Continued

59. Cyclocurcumin, $C_{21}H_{20}O_6$, 368.37	60. Dehydrocurcumene, $C_{15}H_{24}$, 204.35106
61. Epiprocurcumenol, $C_{15}H_{22}O_2$ 234.3339	62. Iso-artemisia ketone, $C_{10}H_{18}O$, 154.24932
63. m-cymene, $C_{10}H_{14}$, 134.2181	64. Phellandrol, $C_{10}H_{16}O$, 152.2334
65. Piperitone epoxide, $C_9H_{14}O_2$ 154.2062	66. p-mentha-1,4(8)-dien, $C_{10}H_{16}$, 136.2340
67. p-meth-8-en-2-one, $C_9H_{14}O$, 138.2068	68. Sylvestrene, $C_{10}H_{16}$, 136.2340
69. Trans-ocimene, $C_{10}H_{16}$, 136.234	70. Turmeronol A, $C_{15}H_{20}O_2$, 232.318
71.Turmeronol B, $C_{15}H_{20}O_2$, 232.318	72.1-(4-hydroxy-3-methoxyphenyl)-7-(3.4-dihydroxyphenyl)-1,6heptadiene-3,5dione, $C_{20}H_{18}O_6$, 354.3533

Figure 1. Continued



73. 1-(4-hydroxyphenyl)-7-(3.4-dihydroxyphenyl)-1,6heptadiene-3,5dione, $C_{19}H_{16}O_5$, 324.3273	74. 5-hydroxy-1(4-hydroxy-3 methoxy phynyl)-7-4-hydroxy phenyl)-4,6-heptadiene-3-one, $C_{20}H_{22}O_5$, 342.3856
75. 2-methoxy-5-hydroxybisabola-3,10-diene-9-one, $C_{16}H_{26}O_3$, 266.3758	

Figure 1. Continued

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) were shown in the docked structures between the target protein and top ranked five molecules with least free energy of binding (Plate 4).

4.2.2 Phytochemicals from *Zingiber officinale* and DprE1 protein

A total of 183 chemical molecules present in *Zingiber officinale* were docked with DprE1. Of the 183 molecules, 63 of them showed free energy of binding less than $^{-}5$ kcal mol^{-1}. The five molecules which 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) were shown in the docked structures between the target protein and top ranked hit molecules (Plate 5).

4.2.3 Phytochemicals from *Eleteria cardamomum* and DprE1 protein

A total of 54 chemical molecules present in *Eleteria cardamomum* were docked with DprE1 and the results showed that out of 54 chemical molecules 22 of them showed binding energy less than $^{-}5$ 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) and the docked structures between the target protein and top ranked hit molecules were shown in (Plate 6).

4.4 IDENTIFICATION OF LEAD MOLECULES THROUGH RANK SUM TECHNIQUE

Five top ranked hit molecules with least free energy of binding were again docked with DprE1 using different docking tools such as Hex server, iGEMDOCK, FireDock and SwissDock. The docked results were analysed following rank sum techniques (DST) and the comparative results were shown in table 1.

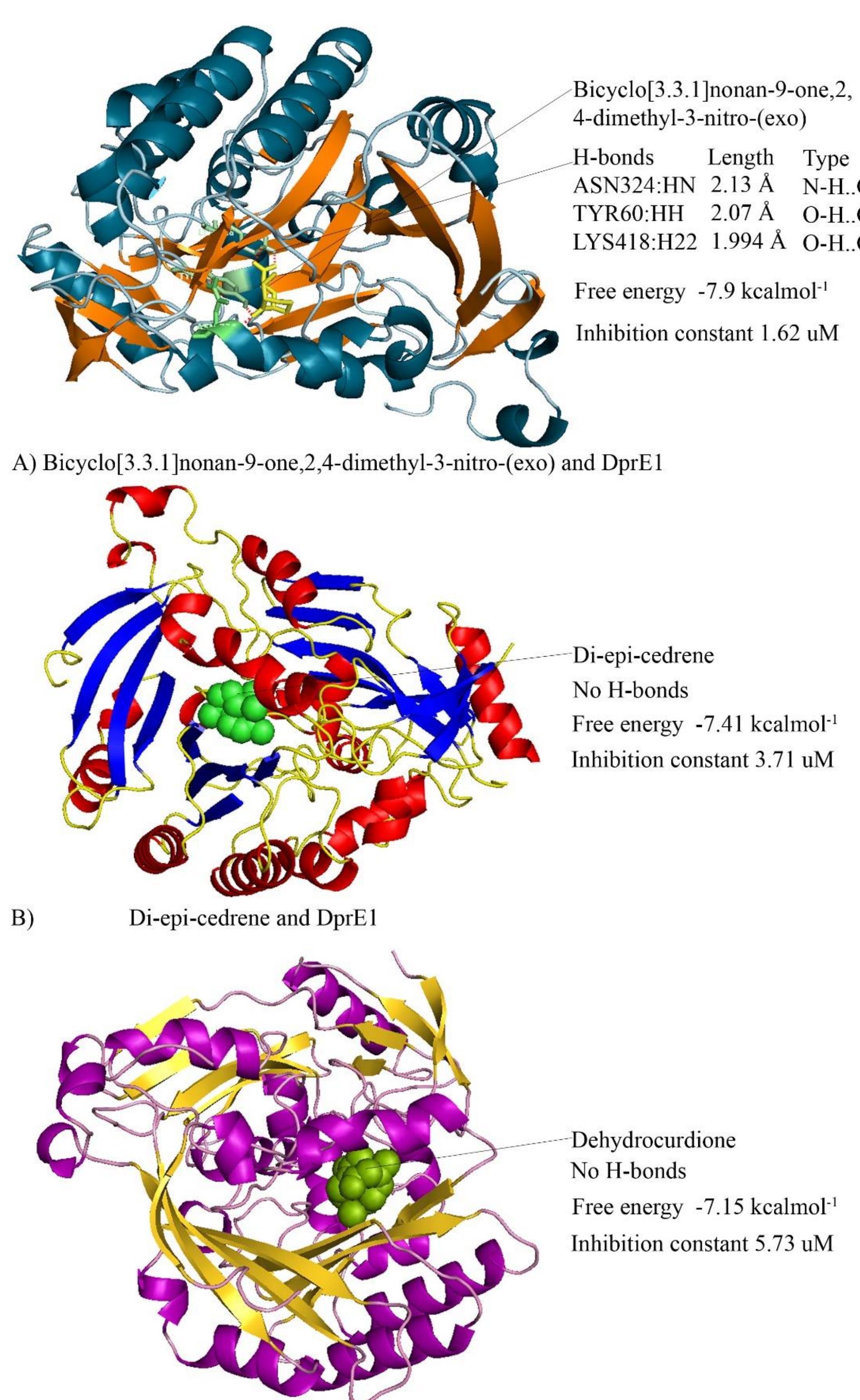
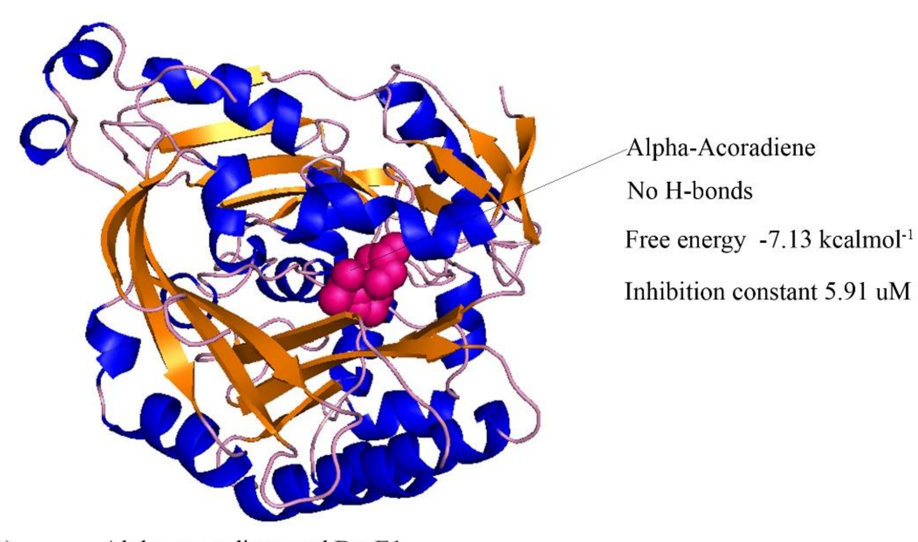
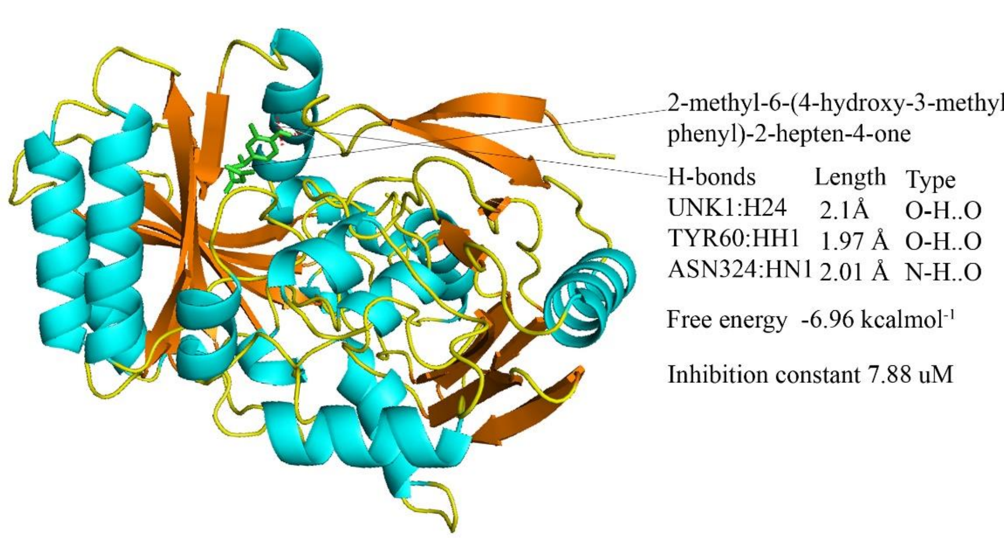


Plate 4. Docked structures between the target protein and top ranked five molecules of *C. longa* with least free energy of binding.



D) Alpha-acoradiene and DprE1



E) 2-Methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one and DprE1

Plate 4. Continued

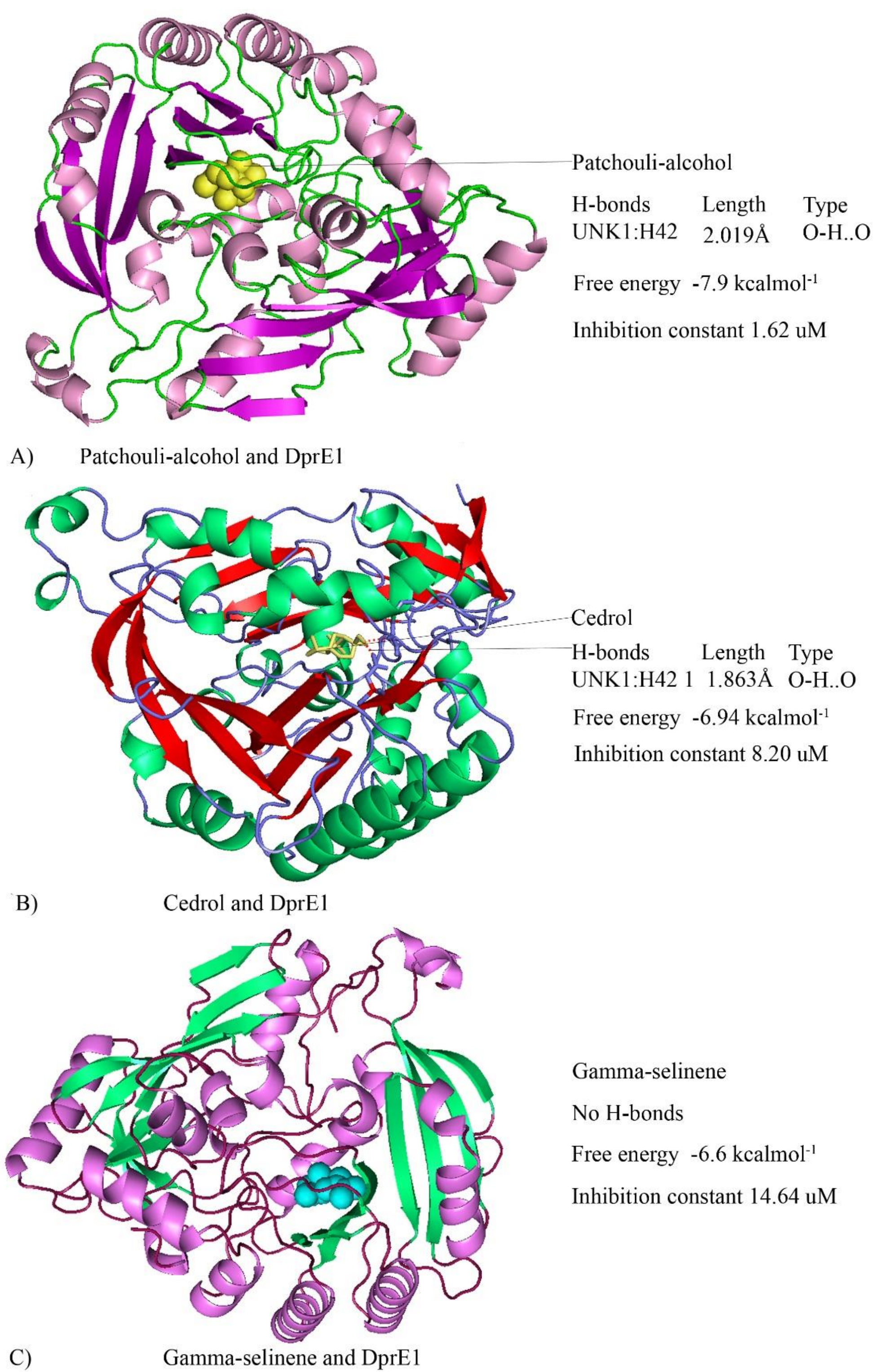
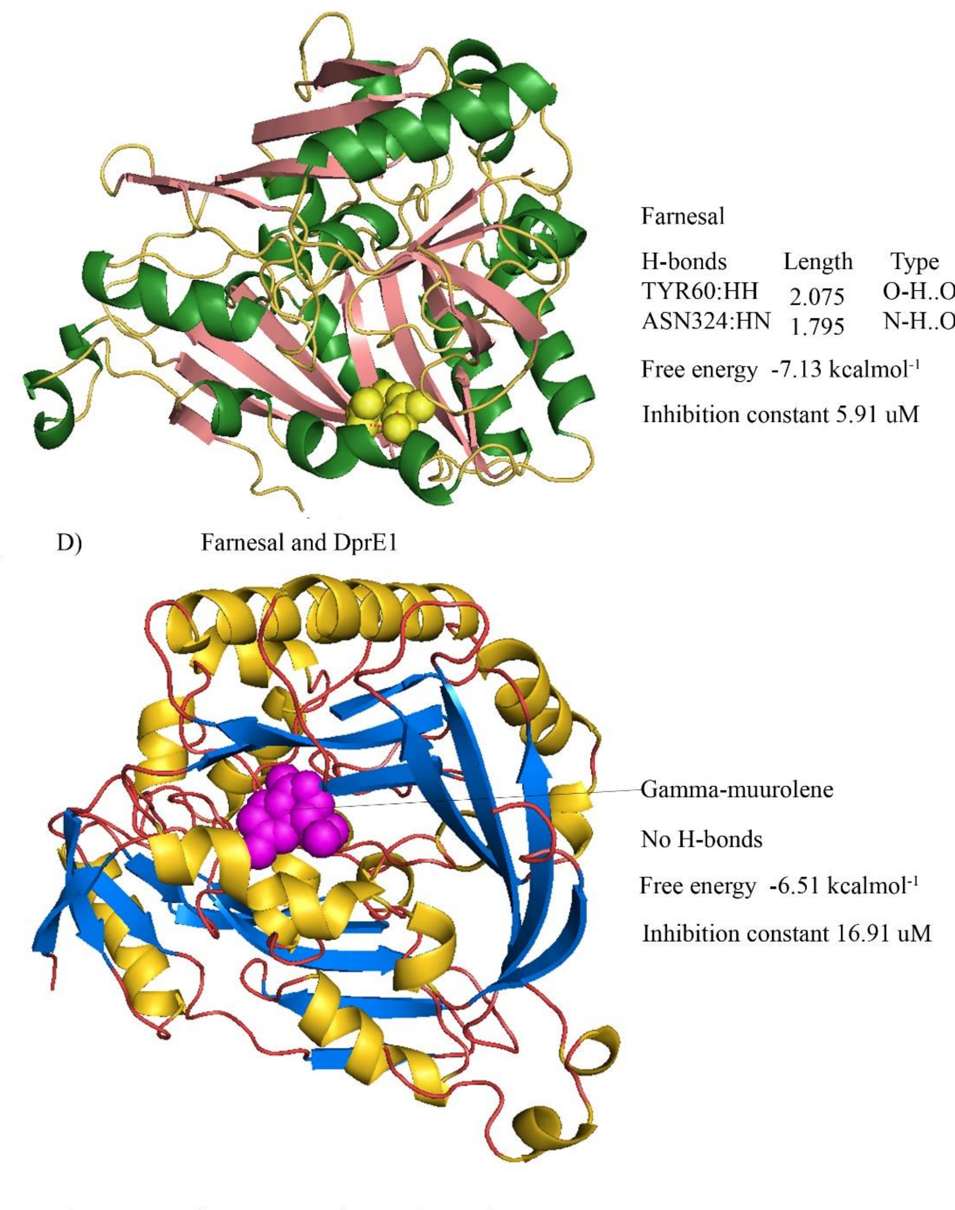


Plate 5. Docked structures between the target protein and top ranked hit molecules of *Z. officinale*



D) Farnesal and DprE1

E) Gamma-muurolene and DprE1

Plate 5. Continued

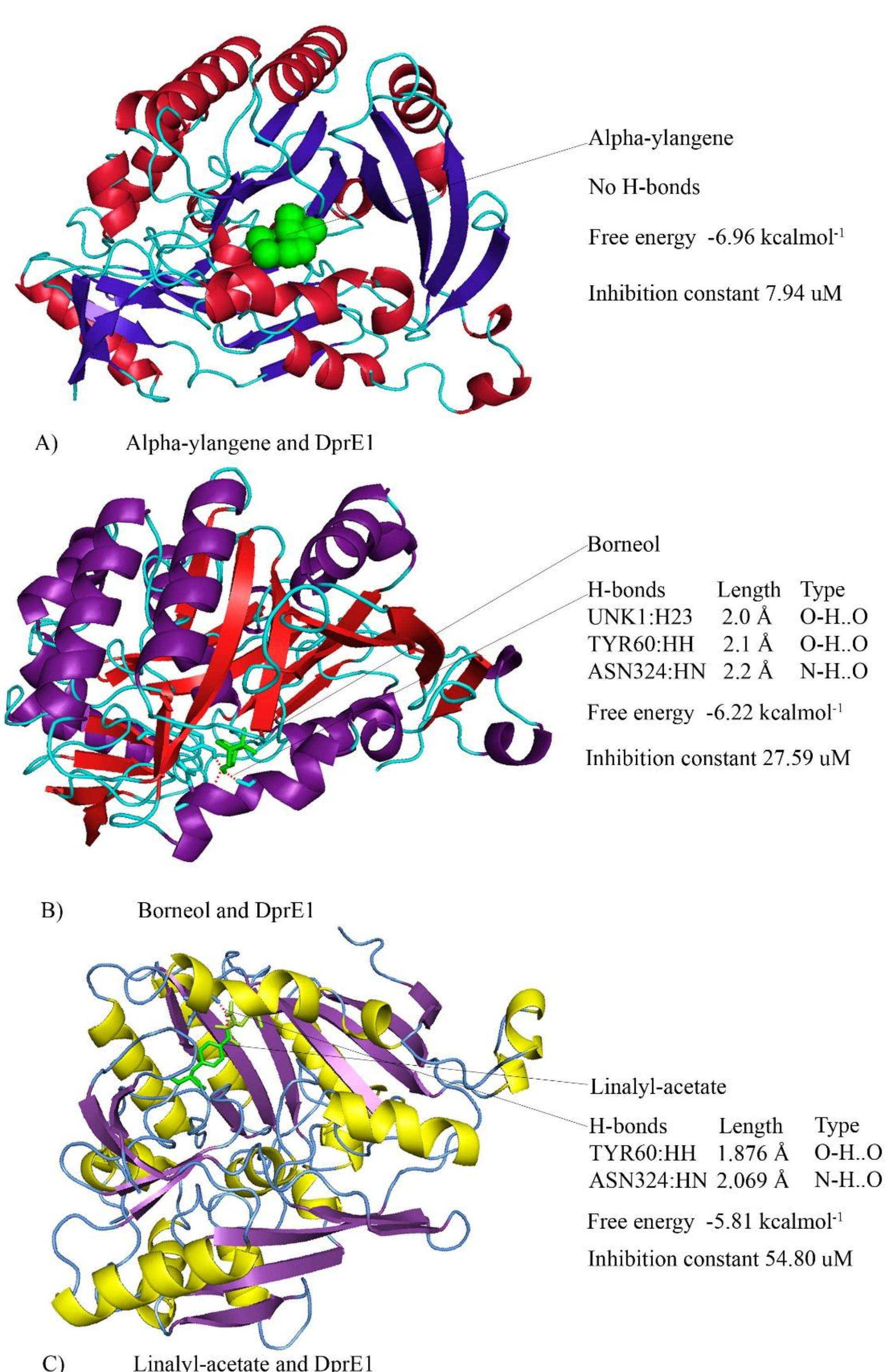
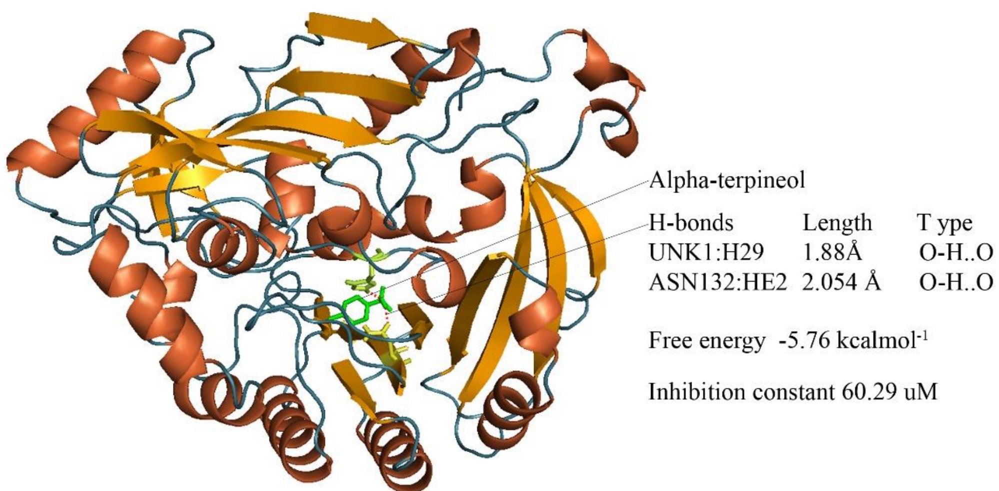
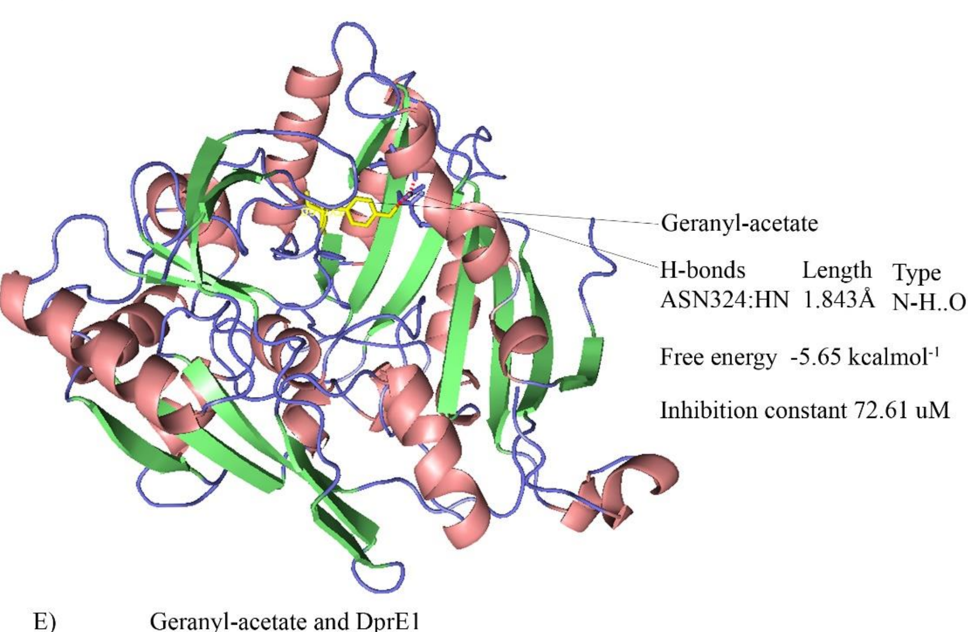


Plate 6. Docked structures between the target protein and top ranked hit molecules of *E. cardamomum*



D) Alpha-terpineol and DprE1



E) Geranyl-acetate and DprE1

Plate 6. Conti

Table 1. Docking result using various softwares and their ranksum value using DST tool

Plants	Phytochemicals	AutoDock (kcal mol^{-1})	Hex Server (kcal mol^{-1})	iGEMDOCK (kcal mol^{-1})	FireDock (kcal mol^{-1})	SwissDock (kcal mol^{-1})	Total Rank
Eleteria cardamomum	Alpha-ylangene	-6.96 (4)	-167.87 (2)	-74.6339 (4)	-38.41 (4)	-7.26 (3)	17
	Borneol	-6.22 (2)	-139.6 (1)	-55.6345 (1)	-32.58 (2)	-6.68 (1)	7
	Linalyl-acetate	-5.81 (1)	-178.78 (3)	-77.7693 (4)	-34.35 (3)	-7.11 (2)	13
	Alpha-terpineol	-5.76 (1)	-152.92 (1)	-65.3031 (2)	-29.51 (1)	-6.65 (1)	6
	Geranyl-acetate	-5.65 (1)	-206.3 (4)	-75.5682 (4)	-35.94 (4)	-7.82 (4)	17
Zingiber officinale	Patchouli-alcohol	-7.13 (4)	-156.13 (1)	-68.1285(1)	-36.61 (1)	-6.80 (1)	8
	Cedrol	-6.94 (3)	-177.72 (2)	-76.014 (3)	-38.99 (2)	-7.02 (1)	11
	Gamma-selinene	-6.60 (1)	-193.60 (3)	-78.0978(4)	-41.20 (3)	-7.21 (2)	13
	Farnesal	-6.53 (1)	-222.38 (4)	-79.0607(4)	-41.83 (3)	-7.75 (4)	16
	Gamma-muurolene	-6.51 (1)	-183.41 (2)	-74.9549(3)	-43.38 (4)	-7.23 (2)	12
Curcuma longa	Bicyclo[3.3.1]nonan-9-one,2,4-dimethyl-3-nitro-(exo)	-7.9 (4)	-148.91(1)	-86.6165(4)	-34.51(2)	-6.83 (1)	12
	Di-epi-cedrene	-7.41 (2)	-172.28(2)	-69.3682(1)	-40.38(4)	-7.02 (1)	10
	Dehydrocurdione	-7.15 (1)	-171.4(1)	-82.9958(4)	-39.84(4)	-6.76 (1)	11
	alpha-Acoradiene	-7.13 (1)	-189.42(2)	-72.3062(1)	-31.25(1)	-7.28 (2)	7
	2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one	-6.93 (1)	-240.79(4)	-84.6381(4)	-41.65(4)	-8.45 (4)	17

Three molecules were selected as leads by rank sum technique (DST). They were Alpha-ylangene from *E. cardamomum*, Farnesal from *Z. officinale* and 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one from *C. longa* respectively. The 3-D structures of these molecules were given in Plate 7.

4.5 IN VITRO SCREENING

4.5.1 Luciferase reporter phage (LRP) assay

The result of the anti-tubercular activity by Luciferase reporter phage assay is presented in Table 2 against H37Rv a standard strain of *Mycobacterium tuberculosis*. The percentage of inhibition in RLU was calculated using following formula

$$\text{Percentage of inhibition in RLU} = \frac{\text{Control sample OD} - \text{test OD}}{\text{Control OD}} \times 100$$

The results revealed that all the three plants have potential antitubercular activity in the order of merit *Zingiber officinale* rank first *Eleteria cardamomum rank* second and *Curcuma longa* rank third respectively.

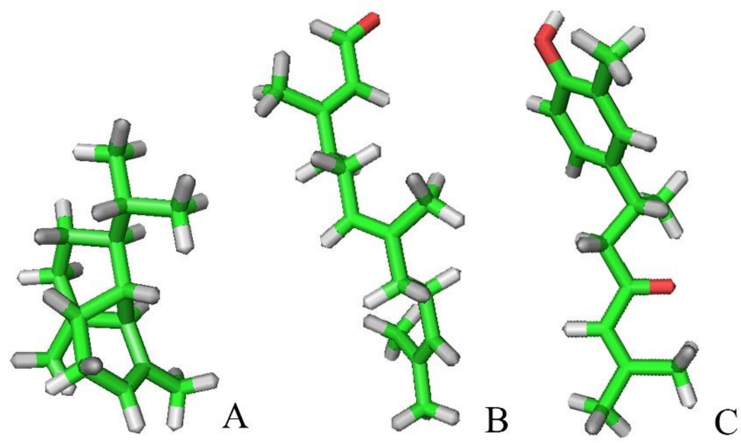


Plate 7. 3-D structures of lead molecules

A) Alpha-Ylangene B) Farnesal

B) 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one

Table 2: Anti-tubercular activity of ethanolic extracts of *Curcuma longa, Zingiber officinale,* and *Eleteria cardamomum* against standard strain of *M. tuberculosis* H37Rv

SI. No.	Plant name and part	Optical Density (% Reduction in RLU)		
		25 µg/ml	250 µg/ml	500 µg/ml
1	*Curcuma longa* Rhizome	123406 (15.30%)	44183 (69.67%)	42162 (71.06 %)
2	*Zingiber officinale* - Rhizome	97432 (33.13%)	38199 (73.78%)	28325 (80.56%)
3	*Eleteria cardamomum* Seeds	91169 (37.43%)	39515 (72.88%)	36698 (74.8%)

*Average control OD 145712

DISCUSSION

5. DISCUSSION

Tuberculosis is an ancient infectious killer disease particularly among the tropical developing countries like India. Although several drugs have been discovered for the treatment of TB during the period from 1944 to1965 due to the emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) strains of *Mycobacterium tuberculosis* those drugs are now ineffective (Almeida *et al.,* 2011). The current treatment system has also encountered with several inherent problems such as (1) the patient has to take several antibiotics for long term (months/years), (2) cause serious side effects especially in patients with immunodeficiency disorders, (3) 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) and (4) Co-infection of TB and HIV. Combined treatment of TB and HIV involves a high pill count with associated adherence problems, overlapping toxicity profiles of the anti-retroviral and anti-TB drugs, drug interaction between rifampin and the anti-retroviral protease inhibitors and the risk of immune reconstitution syndrome. Despite the flaws with and growing resistance to current TB treatments, no new TB drugs have been developed in nearly 50 years. In the light of these, there is an urgent need to find novel, faster and better drugs to defeat TB.

It is well acknowledged fact that drugs derived from natural products are better than synthetic drugs. Plants are producing innumerable number of chemical molecules (secondary metabolites) in response to needs and overcome challenges of the plant environment and most of them have therapeutic activity. It is estimated that more than 4.0 million plants have been used as source of medication but only 5-15 percentage of them had been scientifically investigated and the total number of natural products produced by plants has been estimated over 500,000. It implies that plants are highly sophisticated natural chemical factories where a large variety of chemical compounds are manufactured with great precision and ease from simple raw materials. Unlike synthetic compounds plant derived secondary compounds are synthesized within the living system in

accordance with environmental and other stimuli including the attack of pathogens/ diseases and therefore it may be safe and cause less or no side effects. It was also noted that unlike modern medicine Ayurvedic/herbal drugs contains combinations of therapeutic molecules which can act simultaneously at a number of target and therefore such compound drugs are more effective to treat disease caused by multifactorial causation than modern drugs which act on single target. Since time immemorial several herbal remedies have been used against tuberculosis in the traditional systems of treatment especially in India and in African countries. In India, TB has been mentioned in the *Vedas* and the old *Ayurvedic* scriptures. In Ayurveda, tuberculosis is known as *Rajayakshma,* the king of disease. It is also known as *Yakshma*, *Shosha* and *Kshaya.* Several herbal remedies have been described in Ayurvedic literatures for the treatment of various types of TB and numerous herbal preparations which have been used by the local people against TB are to be documented in literature. However, the efficacies of these herbal formulations are not scientifically validated due to several reasons such as lack of efficient screening methods, high expense, slow and difficulties in executing the experimental works, lack of model organism for testing etc. (Nisha *et al.,* 2014).

Selection of right materials is *prima face* importance for the successful completion of any R & D venture. 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 agroclimatic 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 natural products with high value therapeutic activity. The State Kerala is popularly known as God's Own Country. Kerala has many unique varieties of plant species having high therapeutic value. Of these, globally accepted indigenous spice varieties attained special attention. The spices of Kerala have been used in the traditional systems of medicine for curing many ailments particularly diseases affected to human respiratory system. Of these, the selected spices for present study *viz. Eleteria cardamomum*, *Curcuma longa* and *Zingiber officinale* have been got popularity in

all over the world. (Sirirugsa, 1999; Bhowmik *et al.,* 2009; Kumar *et al.,* 2013;)

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). Recent research reveals that dietary spices in their minute quantities has an immense influence on the human health by their antioxidative, chemopreventive, antimutagenic, anti-inflammatory, immune modulatory effects on cells and a wide range of beneficial effects on human health by the action of gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems (Kochhar, 2008; Lampe, 2003; Kretchmer 1994; Kohlmeier *et al*., 1995; Hendrich *et al*., 1994; Rao 2003; John, 2001). In these back drops, the present investigation was carried out to scientifically demonstrate the efficacy of antituberculosis activity in the three selected indigenous spices of Kerala.

The discovery of innovative leads with potential interaction to specific target is of central importance to the early-stage of drug discovery. This is conventionally achieved by wet-lab high-throughput screening (HTS), an established technology adopted by pharmaceutical industry. On the other hand, the high cost and low hit rate associated with HTS have stimulated the development of computational alternatives, namely Bioinformatics tools (Cheng *et al*., 2012). The bioinformatics methods now referred as *in silico* screening methods need not require the live raw material; high investments and this method provide clear molecular level theoretical insights about the mode of action and accordingly detect the exact lead molecule for further investigation. Among the *in silico* screening methods docking is widely applied one in practice. It can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to elucidate fundamental process. Several docking tools are currently available as open source on the web and commercially, which are developed based on different sampling algorithms and scoring functions, all

are well reviewed by many authors (Meng *et al*., 2011; Kitchen *et al.,* 2004). Of these, AutoDock is the widely used tool for docking and it was illustrated by Mihasan (2012). Therefore, in the present study for preliminary screening the AutoDock tool was used.

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). DPA is a precursor of mycobacterial cell wall content arabinan which is the sole known donor substrate for a series of membrane embedded arabinosyltransferases. Inhibition of the activity of DprE1 leads to the inhibition of the synthesis of arabinan (Lucarelli *et al*., 2010). Based on the forgoing information the DprE1 was used as receptor molecules. The X-ray crystallographic 3 D structure of the protein was downloaded from Protein Data Bank, which is a major source of open access authenticated protein structure database. Visualization of the 3 D structure 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 therapeutic activity and following such methods (Nisha *et al.,* 2013; Nisha *et al.,* 2014; Rini *et al*., 2014) the ligand molecules were prepared for the present study. 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. It is well acknowledged that ChemSketch is an open access user-friendly tool provides extensive task duty requirements in drawing, 3D spectral information, physical chemical properties and customer programming

(Zhenjiang *et al.,* 2004). Similarly, CORINA is an open access tool for the generation of 3-D structures of small molecules and the same software application was used here as followed by Nisha *et al*. (2014). Generally, in order to avoid the unwanted docking exercise the small molecules will be analysed based on the conventional Lipinski's rule of Five (Lipinski *et al*., 1997) which predicts potential pharmacological activity such as Absorption, Distribution, Metabolism and Excretion (ADME). But recently many authors have cited that natural products are exception to the Lipinski's rule of five (Boldi, 2006; Ganesan, 2008). Therefore, in the present study such exercise was excluded. However, considering the limitation in AutoDock tool, compounds having molecular weight more than 600 Da were not included for docking. As a general principle the docked structures having ΔG bind less than $^{-}5$ kcal mol^{-1} were selected as best hits or promising lead molecules. Accordingly out of 211 compounds screened in *Curcuma longa* 101, out of 183 compounds screened in *Zingiber officinale* 63 and out of 54 compounds screened in *Eleteria cardamomum* 22 of them showed free energy of binding $\geq {}^{-}5$ kcal mol^{-1} and noted as hit molecules. Perusal of the structures of the small molecules revealed that many compounds belongs to same categories with almost same functional group/pharmacophore properties and it may be a reason to list out more number of hit molecules with moderate free energy of binding. The docked results of the top ranked five hit molecules in *C. longa* (Plate 4) showed free energy of binding between -6.96 and -7.9 kcal mol^{-1} and among the five top ranked hit molecules only two hit molecules showed three H-bonds each. The H-bond length between donor and acceptor atom range was in between 1.97 Å and 2.1 Å and the bond types include O-H..O and N-H..O . Similarly in *Z. officianale* the free energy of binding among the top ranked five hit molecules was range between $^{-}6.6$ and $^{-}7.9$ kcal mol^{-1}. Of these, two hydrogen bonds were occurred between the target and the hit molecule Farnesal (-7.13 kcal mol^{-1}), one H-bond each was observed between the target and Cedrol (-6.94 kcal mol^{-1}) and Patchouli alcohol (-7.9 kcal mol^{-1}) respectively. The H-bond length between donor and acceptor atom range was in between 1.7 Å and 2.01 Å and the bond types include O-H..O and N-H..O. In *Eleteria cardamomum* the hit

molecules have free energy of binding range between $^-5.65$ and $^-6.96$ kcal mol^{-1} and among the five hit molecules four of them having H-bonds. In the order of merit, Borneol having three, Linalyl acetate having two, Alpha terpineol having two, and Geranyl acetate having one H-bonds respectively. The H-bond length between donor and acceptor atom range was in between 1.8 Å and 2.2 Å and the bond types include O-H..O and N-H..O, hence it can be inferred that the bonds formed were too strong. However, in order to nullify the errors in lead identification the top ranked hit molecules were again docked using the docking tools such as Hex server, iGEMDOCK, FireDock and SwissDock. The docked results were statistically analysed following DST and Zhang rule and the selected lead molecules *viz.* 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one (*C. longa),* Alpha-ylangene (*E. cardamomum*) and Farnesal (*Z. officinale)* derived from each plant species with parameters were recorded (Table 2). Among the three plants, the best lead molecule 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one was obtained from *Curcuma longa* (three H-bonds, free energy -6.96Kcal mol^{-1}). The significance of applying DST and Zhang's rule for lead identification was well described (Rao *et al.,* 2013).

The major problem with *in silico* screening is that many, and in some cases the vast majority of the compounds that are predicted to be active are in fact not active when screened experimentally. There are two theories proposed on the reasons for this phenomenon. Some researches argue that docking can usually generate good poses of a ligand in an active site, however, scoring functions are generally not successful at correctly ranking either ligands or poses (Warren *et al*., 2006). Others have pointed out that docking programs do not always generate correct poses and that the highest ranked pose for a given ligand is often incorrect. Scoring functions would therefore function much better at ranking if docking programs did not produce so many incorrect poses for each compound (Kontoyianni *et al.,* 2005; Perola, 2006). Regardless of whether it is the docking programs or the scoring functions that are at fault, the issue is that virtual screening can generate an enormous number of false positives – compounds that are scored highly *in silico* but do not actually bind to the target *in vivo* or *in vitro*.

These false positive can also be blamed to some extent for false negatives. In the case where true positives are scored relatively poorly (and perhaps even eliminated) because of spuriously high-scoring false positive that are ranked ahead of them. Several methods have been suggested to eliminate false positive and they are well reviewed (Peach and Nicklaus, 2009).

In order to confirm the results obtained through *in silico* screening the ethanol extracts of each plant has been screened by *in vitro* method. The lead compounds identified from the three selected plants were belonging to the category of mono-terpenoids and in general the solvent of mono terpenoides is ethanol. Therefore, the extracts were prepared in ethanol. The crude ethanolic extract may affect the *in vitro* results. But considering the difficulty to procure the high value low volume phytochemicals and other limitations the author has used crude extract for a preliminary test. As reported earlier (Papitha *et al.,* 2013) the standard strain of *M. tuberculosis* H37RV maintained at National Institute for Research in Tuberculosis, Chennai was used for the anti mycobacterial assay. The Luciferase reporter phage assay methodology is rapid, inexpensive and less laborious for high throughput screening of compounds for their anti-mycobacterial activity compared to BATEC methodology which is costly, cumbersome and uses radioactive reagents. The *in vitro* assay results revealed that all the three plants have potential antitubercular activity. In the order of merit *Zingiber officinale* rank first *Eleteria cardamomum* rank second and *Curcuma longa* rank third respectively. The results insights the possibility of novel drugs from these plants. Further studies including series of *in vitro* tests using single isolated compounds from these plants and clinical studies are to be essential.

SUMMARY

6. SUMMARY

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is the second worldwide killer infectious disease. Globally, it kills 1.4 million people every year and in India around 30,000 people die due to TB. The standard therapy for the treatment of tuberculosis is the administration of several antibiotics, which have several limitations including long term treatment, side effects to the patients, emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) mutants and adverse effect to immune system in patents co-infection with HIV.

In the aforementioned circumstances, discovery of novel faster, cheaper and better drug is become the need of the hour. Perusal of the literature revealed that several herbal remedies have been used against tuberculosis in the traditional systems of treatment especially in India and in African countries. The Gods Own Country, Kerala is blessed with globally accepted unique spices which have been used in the traditional systems of medicine for curing many ailments particularly diseases affected to human respiratory system. But the efficacies of these herbal formulations are not scientifically validated due to several reasons such as lack of efficient screening methods, high expense, slow and difficulties in executing the experimental works, lack of model organism for testing etc. In these backdrops, in the present study the phytomolecules reported from three indigenous spice varieties of Kerala *viz. Elettaria cardamomum, Curcuma longa* and *Zingiber officinale* were screened through *in silico* and *in vitro* methods.

For *in silico* screening 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). The active site of the target protein (DprE1) was detected using the tool PDBsum.

Information regarding the chemical molecules (phytochemicals) reported in the selected spices were collected through literature survey and databases. The canonical

SMILES of the phytochemicals were retrieved from chemical databases such as PubChem, Chemspider and Dictionary of Natural products. Total 448 phytochemicals (*Curcuma longa* – 211, *Zingiber officinale* – 183 and - *Eleteria cardamomum* – 54) were screened. Out of 448 phytochemicals structures of 373 (*Curcuma longa*-137, *Zingiber officinale*-182 and *Eleteria cardamomum*-0) were retrieved from databases and remaining compounds structures were created using Chemsketch. The three dimensional structures (3D) of all phytochemicals were created using the tool CORINA.

All selected phytochemicals were docked into the binding site of DprE1 using the docking tool, AutoDock 4.2. As a general principle the docked structures having ΔG bind less than $^{-}5$ kcal mol^{-1} were selected as best hits or promising lead molecules. Out of 211 compounds screened in *Curcuma longa* 101, out of 183 compounds screened in *Zingiber officinale* 63 and out of 54 compounds screened in *Eleteria cardamomum* 22 of them showed free energy of binding ≥ $^{-}5$ kcal mol^{-1} and noted as hit molecules and these molecules were further analysed by Lipinski's rule of Five.

In order to nullify the errors in lead identification the top ranked hit molecules were again docked using the docking tools Hex server, iGEMDOCK, FireDock and SwissDock. The docked results were statistically analysed following DST and Zhang rule and selected the top ranked molecules from each plant *viz.* 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one from *C. longa,* Alpha-ylangene from *E. cardamomum* and Farnesal from *Z. officinale* as lead molecules.

For *in vitro* screening mature seeds of *Eleteria cardamomum* were collected from the cultivated field at Regional Agricultural Research Station (RARS) Pambadumpara, Idukki district and mature rhizomes of *Zingiber officianale* and *Curcuma longa* were collected from Agriculture College Vellayani, Thiruvananthapuram. The samples were air dried at room temperature and powdered

and extracted with 99% ethnol using a Soxhlet apparatus for 6-8 hours. The extracts were concentrated to dryness using a rotary vacuum evaporator.

Anti-mycobacterial activity of the three plant extracts were evaluated by Luciferase reporter phage (LRP) assay against standard strain of *M. tuberculosis* H37RV at three different concentrations (25, 250 and 500 µg/mL). The results revealed that all the three plants have potential antitubercular activity. In the order of merit *Zingiber officinale* rank first (% reduction in RLU at 25 µg/ml 33.13%, 250 µg/ml 73.78% and 500 µg/ml 80.56%) *Eleteria cardamomum* rank second (% reduction in RLU at 25 µg/ml 37.43%, 250 µg/ml 72.88 % and 500 µg/ml 74.8%) and *Curcuma longa* rank third (% reduction in RLU at 25 µg/ml 15.30%, 250 µg/ml 69.67% and 500 µg/ml 71.06%) respectively. The results substantiate the traditional use of these three spice varieties against tuberculosis. Further R & D focusing on the lead molecules will lead to the discovery of novel anti-tuberculosis drug with desirable qualities.

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7. REFERENCES

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APPENDICES

APPENDIX I

Phytochemicals selected from *Curcuma longa* with molecular formula and weight

Sl.no	Phytochemicals	Molecular formula	Molecular Weight (Da)
1.	1,2,3-Trimethyl-cyclopent-2-enyl-methanol	$C_9H_{16}O$	140.22274
2.	(4S-5S)-germacrone-4-5-epoxide	$C_{15}H_{22}O_2$	234.33398
3.	(6S,7R)-bisabolene	$C_{15}H_{24}$	204.3510
4.	(E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one	$C_{11}H_{12}O_3$	192.21
5.	(E)-carveol	$C_{10}H_{16}O$	152.2334
6.	(E)-alpha-atlantone	$C_{15}H_{22}O$	218.3345
7.	(E)-sesquisabinenehydrate	$C_{14}H_{24}O$	208.3397
8.	(E-E-E)-3-7-11-15-Tetramethylhexadeca-1-3-6-10-14-pentaene	$C_{20}H_{32}$	272.46808
9.	(Z)-beta-Farnesene	$C_{15}H_{24}$	204.35106
10.	(Z)_sabinol	$C_{10}H_{16}O$	152.2334
11.	1-(3-Cyclopentylpropyl)-2-4-dimethylbenzene	$C_{16}H_{24}$	216.36176
12.	1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1-4-pentadiene-3-one	$C_{18}H_{16}O_4$	296.3172
13.	1-3-8-paramenthatriene	$C_{10}H_{14}$	134.2181
14.	1-(4-hydroxy-3-methoxyphynyl)-7-(3-4-dihydroxyphenyl)-1-6heptadiene-3-5dione	$C_{20}H_{18}O_6$	232.3181
15.	1-(4-hydroxyphynyl)-7-(3-4-dihydroxyphenyl)-1-6heptadiene-3-5dione	$C_{19}H_{16}O_5$	324.32734
16.	1-4-dimethyl-2-2-methylpropyl)-benzene	$C_{12}H_{18}$	162.27132
17.	1-5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E-4E)-1-4-dien-3-one	$C_{19}H_{18}O_5$	326.343201
18.	1-5-Bis(4-hydroxyphenyl)penta-1-4-dien-3-one	$C_{17}H_{14}O_3$	266.29126
19.	1-5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4-6-heptadiene-3-one	$C_{21}H_{22}O_7$	386.3951
20.	1-5-dihydroxy-1-7-bis(4-hydroxy-3-methoxyphenyl)-4-6-heptadiene-3-one	$C_{19}H_{18}O_5$	326.3432
21.	1-5-dihydroxy-1-7-bis(4-hydroxyphenyl)-4-6-heptadiene-3-one	$C_{21}H_{22}O_7$	386.3951
22.	1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene	$C_{19}H_{18}O_5$	326.3432
23.	1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,66-heptadiene	$C_{21}H_{20}O_5$	352.3805
24.	1,7-bis(4-hydroxyphenyl)1-heptene-3,5-dione	$C_{19}H_{20}O_4$	312.3597
25.	1,7-bis-(4-hydroxyphenyl)-14,6-heptatrien-3-one	$C_{19}H_{16}O_3$	292.3285

26.	1,8-cineole	$C_{10}H_{18}O$	154.24932
27.	1,10-dehydro-10-deoxy-9-oxozedoarondiol	$C_{16}H_{22}O_3$	262.34408
28.	1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4hydroxy-3-methoxyphenyl)-4-6-heptadiene-3-one	$C_{10}H_{16}O_4$	200.2316
29.	1-epi-cubenol	$C_{15}H_{26}O$	222.36634
30.	2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic-acid	$C_{10}H_{16}O_4$	200.23164
31.	2-(2'-methyl-1'-propenyl)-4,6-dimethyl-7-hydroxyquinoline	$C_{15}H_{19}O$	229.3174
32.	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428.73328
33.	2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran	$C_{27}H_{40}O_3$	412.6047
34.	2,3,5-Trimethylfuran	$C_7H_{10}O$	110.1537
35.	2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one	$C_9H_{12}O_2$	152.19038
36.	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene	$C_{20}H_{32}$	272.46808
37.	2,6-dimethyl-2,6-octadiene-1,8-diol	$C_{10}H_{18}O_2$	170.2487
38.	2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde	$C_{15}H_{24}O$	220.35046
39.	2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one	$C_{15}H_{22}O_3$	250.3333
40.	2-Bornanol	$C_{11}H_{20}O$	168.2759
41.	2-carene	C_9H_{14}	122.2074
42.	2-methoxy-5-hydroxybisabola-3,10-diene-9-one	$C_{16}H_{26}O_3$	266.3758
43.	2-methyl-6-(4-formylphenyl)-2-hepten-4-one	$C_{16}H_{22}O_2$	246.3446
44.	2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one	$C_{15}H_{20}O_2$	232.3181
45.	2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one	$C_{14}H_{18}O_2$	218.2915
46.	2-hydroxymethylanthraquinone	$C_{15}H_{10}O_3$	238.2381
47.	2-norpinanone	$C_7H_{10}O$	110.1537
48.	3,3,5-trimethylcyclohexanolacetate	$C_{11}H_{20}O_2$	184.275299
49.	3,4,5,6-tetramethyl-2,5-octadiene	$C_{12}H_{22}$	166.3030
50.	3,7-dimethyl-6-nonenal	$C_{11}H_{20}O$	168.2759
51.	3-bornanone	$C_{10}H_{16}O$	152.23344
52.	3-hydroxy-1,7-bis(4-hydroxyphenyl)-6-heptene-1,5-dione	$C_{19}H_{18}O_5$	326.3432
53.	4-(4'-hydroxyphenyl-3-methoxy)-2-oxo-3-butenyl-3-(4'-hydroxyphenyl)-propenoate	$C_{20}H_{18}O_2$	355.3533
54.	4,5-dihydroxybisabola-2,10-diene	$C_{15}H_{26}O_6$	238.3657
55.	4,5-dimethyl-2,6-octadiene	$C_{10}H_{18}$	138.24992
56.	4,8-dimethyl-3,7-nonadien-2-ol	$C_{12}H22O$	182.3024
57.	4-(4'-hydroxyphenyl)-2-oxo-3-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate	$C_{20}H_{18}O_6$	355.3534
58.	4-hydroxybisabola-2,10-diene-9-one	$C_{14}H_{22}O_2$	222.32328

59.	4-methylene-5-hydroxybisabola-2,10-diene-9-one	$C_{15}H_{22}O_2$	234.33398
60.	4-terpinol	$C_{10}H_{18}O$	154.2493
61.	5-hydroxy-1(4-hydroxy-3methoxyphynyl)-7-4-hydroxyphenyl)-4,6-heptadiene-3-one	$C_{21}H_{24}O_6$	372.41166
62.	5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	$C_{20}H_{22}O_5$	324.3856
63.	5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one	$C_{20}H_{22}O_5$	324.3856
64.	5-hydroxyl-ar-turmerone	$C_{15}H_{20}O_2$	232.3181
65.	6-alpha-hydroxycurcumanolidea	$C_{16}H_{26}O_3$	266.3758
66.	7-epi-sesquithujene	$C_{15}H_{24}$	204.351105
67.	8,11-octadecadienoicacidmethylester	$C_{19}H_{34}O_2$	294.47206
68.	20-oxopregn-16-en-12-ylacetate	$C_{23}H_{34}O_3$	358.51426
69.	Adoxal	$C_{14}H_{26}O$	210.35564
70.	Alpha-acoradiene	$C_{15}H_{24}$	204.35106
71.	Alpha-atlantone	$C_{15}H_{22}O$	218.33458
72.	Alpha-curcumene	$C_{15}H_{22}$	202.33518
73.	Alpha-humulene	$C_{15}H_{24}$	204.35106
74.	Alpha-oxobisabolene	$C_{15}H_{24}O$	220.35046
75.	Alpha-santalene	$C_{15}H_{24}$	204.35106
76.	Alpha-santalol	$C_{15}H_{24}O$	220.35046
77.	Alpha-terpinene	$C_{10}H_{16}$	136.233994
78.	Alpha-turmerone	$C_{15}H_{22}O$	218.33458
79.	Alpha-bisabolol	$C_{15}H_{26}O$	222.36634
80.	Alpha-pinene	$C_{10}H_{16}$	136.23404
81.	Alpha-terpineol	$C_{10}H_{18}O$	154.24932
82.	Alpha-zingiberene	$C_{15}H_{24}$	204.35106
83.	Ar-turmerone	$C_{15}H_{22}O$	218.3358
84.	Arabinose	$C_5H_{10}O_5$	150.1299
85.	Aristolene	$C_{15}H_{24}$	204.35106
86.	Ar-turmerol	$C_{15}H_{20}O$	216.3187
87.	Ascaridole	$C_{10}H_{16}O_2$	168.232803
88.	Azulene	$C_{10}H_8$	128.17052
89.	Benzene,1-methyl-4-(1-methylpropyl)	$C_{11}H_{16}$	148.2447
90.	Beta,beta-dimethylstyrene	$C_{10}H_{12}$	132.20228
91.	Beta-terpinene	$C_{10}H_{16}$	136.233994
92.	Beta-atlantone	$C_{15}H_{22}O$	218.33458
93.	Beta-santalene	$C_{15}H_{24}$	204.35110
94.	Beta-turmerone	$C_{15}H_{22}O$	218.33458
95.	Beta-carotene	$C_{40}H_{56}$	536.87264
96.	Beta-curcumene	$C_{15}H_{24}$	204.35106
97.	Beta-pinene	$C_{10}H_{16}$	136.23404
98.	Beta-sesquiphellandrene	$C_{15}H_{24}$	204.35106
99.	Bicyclo[3.3.1]nonan-9-one,2,4-dimethyl-3-nitro-(exo)	$C_{11}H_{17}NO_3$	211.25758
100.	Bicyclo[7.2.0]undecane,10,10-dimethyl-2,6-bis(methylene)	$C_{16}H_{28}$	220.39352
101.	Bisabola-3,10-diene-2-one	$C_{15}H_{24}O$	220.35046
102.	Bisabolene	$C_{15}H_{24}$	204.35106

103.	Bisabolone-9-one	$C_{15}H_{22}O_2$	234.33398
104.	Bisacumol	$C_{15}H_{22}O$	218.334595
105.	Bisacurone a	$C_{15}H_{24}O_3$	252.349304
106.	Bisacurone b	$C_{15}H_{24}O_3$	252.349304
107.	Bisacurone c	$C_{14}H_{22}O_3$	238.32268
108.	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	308.32794
109.	Caffeic acid	$C_9H_8O_4$	180.15742
110.	Calebin a	$C_{21}H_{20}O_7$	384.379303
111.	Capric acid	$C_{10}H_{20}O_2$	172.2646
112.	Carvacrol	$C_{10}H_{14}O$	150.21756
113.	Carvone	$C_{10}H_{14}O$	150.21756
114.	Caryophyllene	$C_{15}H_{24}$	204.35106
115.	Caryophyllene oxide	$C_{15}H_{24}O$	220.35046
116.	Cinnamic acid	$C_9H_8O_2$	148.15862
117.	Cis-ocimene	$C_{10}H_{16}$	136.23404
118.	Citronellylpentanoate	$C_{14}H_{26}O_2$	226.35504
119.	Corymbolone	$C_{15}H_{24}O_2$	236.34986
120.	Cubebene	$C_{15}H_{24}$	204.351105
121.	Cuminyl alcohol	$C_{10}H_{14}O$	150.21756
122.	Curculonone c	$C_{14}H_{22}O_2$	222.32328
123.	Curculonone d	$C_{16}H_{30}O_2$	254.4082
124.	Curcuma-j	$C_{15}H_{20}O_2$	232.3181
125.	Curcuma-l	$C_{15}H_{26}O$	222.36634
126.	Curcumanolide d	$C_{15}H_{22}O_3$	250.33338
127.	Curcumenol	$C_{15}H_{22}O_2$	234.33398
128.	Curcumenone	$C_{15}H_{22}O_2$	234.33398
129.	Curcumin	$C_{21}H_{20}O_6$	368.3799
130.	Curcuphenol	$C_{15}H_{22}O$	218.33458
131.	Curdione	$C_{15}H_{24}O_2$	236.34986
132.	Curlone	$C_{15}H_{22}O$	218.33458
133.	Curzerenone	$C_{15}H_{18}O_2$	230.30222
134.	Cyclocurcumin	$C_{21}H_{20}O_6$	368.3799
135.	Cyclohexyl formate	$C_7H_{12}O_2$	128.16898
136.	D-alpha phellandrene	$C_{10}H_{16}$	136.23404
137.	D-camphene	$C_{10}H_{16}$	136.23404
138.	D-camphor	$C_{10}H_{16}O$	152.23344
139.	Dehydrocurcumene	$C_{15}H_{24}$	204.35106
140.	Dehydrocurdione	$C_{15}H_{22}O_2$	234.33398
141.	Dicumylperoxide	$C_{18}H_{22}O_2$	270.36608
142.	Di-epi-cedrene	$C_{15}H_{24}$	204.35106
143.	Dihydro-ar-turmerone	$C_{15}H_{22}O$	218.33460
144.	Dihydro-curcumin	$C_{21}H_{22}O_6$	370.39578
145.	Dihydroturmerone	$C_{15}H_{22}O$	218.33460
146.	D-sabinene	$C_{10}H_{16}$	136.23404
147.	E-chrysanthenyl acetate	$C_{12}H_{18}O_2$	194.27012
148.	E-ferulicacid	$C_{10}H_{10}O_4$	194.184
149.	Epiprocurcumenol	$C_{15}H_{22}O_2$	234.33398
150.	Eudesma-3,7(11)-diene	$C_{15}H_{24}$	204.35106
151.	Eugenol	$C_{10}H_{12}O_2$	164.20108
152.	Farnesyl acetone	$C_{18}H_{30}O$	262.4302
153.	Ferulic acid	$C_{10}H_{10}O_4$	194.184

154.	Gamma-bisabolene	$C_{15}H_{24}$	204.35106
155.	Gamma-curcumene	$C_{15}H_{22}$	202.33518
156.	Gamma-elemene	$C_{15}H_{24}$	204.35106
157.	Gamma-gurjunenepoxide	$C_{15}H_{24}O$	220.35046
158.	Gamma-terpinene	$C_{10}H_{16}$	136.23404
159.	Gamma-terpineol	$C_{10}H_{18}O$	154.24932
160.	Geranic acid	$C_{10}H_{16}O_2$	168.232803
161.	Geranyl linalool	$C_{20}H_{34}O$	290.48336
162.	Germacrene d	$C_{15}H_{24}$	204.35106
163.	Germacrone	$C_{15}H_{22}O$	218.33458
164.	Gitoxigenin	$C_{23}H_{34}O_5$	390.51306
165.	Guaiacol	$C_7H_8O_2$	124.13722
166.	Hexadecane-1,2-diol	$C_{16}H_{34}O_2$	258.43996
167.	Himachalene	$C_{15}H_{24}$	204.35106
168.	Hop-17(21)-en-3beta-ol	$C_{30}H_{50}O$	426.717407
169.	Hopenone-i	$C_{30}H_{48}O$	424.701508
170.	Iso-artemisiaketone	$C_{10}H_{28}O$	154.24932
171.	Isoborneol	$C_{10}H_{18}O$	154.24932
172.	Junipercamphor	C_{15} H_{26} O	222.37142
173.	L-alpha-curcumene	$C_{15}H_{22}$	202.33518
174.	Limonene	$C_{10}H_{16}$	136.23404
175.	Linalool	$C_{10}H_{18}O$	154.24932
176.	M- cymene	$C_{10}H_{14}$	134.21816
177.	Menthofuran	$C_{10}H_{14}O$	150.21756
178.	Menthol	$C_{10}H_{20}O$	156.2652
179.	Methyl eugenol	$C_{11}H_{14}O_2$	178.227707
180.	Monodemethoxy curcumin	$C_{20}H_{18}O_5$	338.35392
181.	Nerolidylpropionate	$C_{18}H_{30}O_2$	278.4296
182.	O-coumaric-acid	$C_9H_8O_3$	164.15802
183.	O-cymene	$C_{10}H_{14}$	134.21816
184.	P-coumaricacid	$C_9H_8O_3$	164.15802
185.	P-meth-8-en-2-one	$C_9H_{14}O$	138.20686
186.	P-mentha-1,4(8)-dien	$C_{10}H_{16}$	136.23404
187.	Nerolidol	$C_{15}H_{26}O$	222.36634
188.	P-methoxycinnamic acid	$C_{10}H_{10}O_3$	178.1846
189.	P-methylacetophenone	$C_9H_{10}O$	134.1751
190.	P-cymene	$C_{10}H_{14}$	134.21816
191.	Phellandrol	$C_{10}H_{16}O$	152.23344
192.	Piperitone	$C_{10}H_{16}O$	152.23344
193.	Piperitone-epoxide	$C_9H_{14}O_2$	154.20626
194.	Protocatechuic-acid	$C_7H_6O_4$	154.12014
195.	Pyrazolo[1,5-a]pyridine,3,3a,4,7-tetrahydro-3,3-dimethyl-(3as)	$C_9H_{14}N_2$	150.22086
196.	R-citronellene	$C_{11}H_{20}$	152.2765
197.	Stigmasterol	$C_{29}H_{48}O$	412.69082
198.	Sylvestrene	$C_{10}H_{16}$	136.23404
199.	Syringic-acid	$C_9H_{10}O_5$	198.1727
200.	Teresantalol	$C_{10}H_{16}O$	152.23344
201.	Tetrahydrocurcumin	$C_{21}H_{24}O_6$	372.411713
202.	Thymol	$C_{10}H_{14}O$	150.21756

202.	Thymol	$C_{10}H_{14}O$	150.21756
203.	Trans-ocimene	$C_{10}H_{16}$	136.23404
204.	Turmerone	$C_{15}H_{20}O$	216.3187
205.	Turmeronol a	$C_{15}H_{20}O_2$	232.3181
206.	Turmeronol b	$C_{15}H_{20}O_2$	232.3181
207.	Vanillic-acid	$C_8H_8O_4$	168.14672
208.	Vanillin	$C_8H_8O_3$	152.14732
209.	Z-alpha-bergamotene	$C_{15}H_{24}$	204.35106
210.	Z-cinerone	$C_{10}H_{14}O$	150.21756
211.	Z-ferulicacid	$C_{10}H_{10}O_4$	194.184

APPENDIX II

Phytochemicals selected from *Zingiber officinale* with molecular formula and weight

Sl.no.	Phytochemicals	Molecular formula	Molecular weight (Da)
1.	1,8-cineole	$C_{10}H_{18}O$	154.24932
2.	1-(4-hydroxy-3-methoxyphenyl)-3,5-diaceloxyoctane	$C_{19}H_{28}O_6$	352.4219
3.	1-(4-hydroxy-3-methoxyphenyl)-3,5-octanediol	$C_{19}H_{28}O_6$	352.42202
4.	10-dehydrogingerdione	$C_{21}H_{30}O_4$	346.4605
5.	10-epizonarene	$C_{15}H_{24}$	204.35106
6.	10-gingediol	$C_{21}H_{36}O_4$	352.50814
7.	10-gingerdione	$C_{21}H_{32}O_4$	348.47641
8.	10-gingerol	$C_{21}H_{34}O_4$	350.49231
9.	10-shogaol	$C_{21}H_{32}O_3$	332.47698
10.	12-gingerol	$C_{23}H_{38}O_4$	378.545
11.	14-gingerol	$C_{25}H_{42}O_4$	406.599
12.	16-gingerol	$C_{27}H_{46}O_4$	434.652
13.	2,2,4-trimethylheptane	$C_{10}H_{22}$	142.2816
14.	3-phenylbenzaldehyde	$C_{13}H_{10}O$	182.2179
15.	4-gingerol	$C_{15}H_{22}O_4$	266.333
16.	4-phenylbenzaldehyde	$C_{13}H_{10}O$	182.2179
17.	6-dehydrogingerdione	$C_{17}H_{22}O_4$	290.35418
18.	6-gingediol	$C_{17}H_{28}O_4$	296.402
19.	6-gingerdione	$C_{17}H_{24}O_4$	292.37006
20.	6-gingerol	$C_{17}H_{26}O_4$	294.38594
21.	6-methyl-5-hepten-2-one	$C_8H_{14}O$	126.2
22.	6-methylgingediacetate	$C_{22}H_{34}O_6$	394.50176
23.	6-methylgingediol	$C_{18}H_{30}O_4$	310.4284
24.	6-paradol	$C_{17}H_{26}O_3$	278.38654
25.	6-shogaol	$C_{17}H_{24}O_3$	276.37066
26.	7-gingerol	$C_{18}H_{28}O_4$	308.413
27.	8-gingediol	$C_{19}H_{32}O_4$	324.45498
28.	8-gingerol	$C_{19}H_{30}O_4$	322.4391
29.	8-shogaol	$C_{19}H_{28}O_3$	304.42382
30.	9-gingerol	$C_{20}H_{32}O_4$	336.466
31.	9-oxo-nerolidol	$C_{15}H_{24}O_2$	236.350
32.	Allo-aromadendrene	$C_{15}H_{24}$	204.35106
33.	Alpha-cadinene	$C_{15}H_{24}$	204.35106
34.	Alpha_cadinol	$C_{15}H_{26}O$	222.36634
35.	Alpha-copaene	$C_{15}H_{24}$	204.3511
36.	Alpha_curcumene	$C_{15}H_{22}$	202.33518

37.	Alpha-farnesene	$C_{15}H_{24}$	204.35106
38.	Alpha-linolenic acid	$C_{18}H_{30}O_2$	278.4295
39.	Alpha-muurolene	$C_{15}H_{24}$	204.35106
40.	Alpha-phellandrene	$C_{10}H_{16}$	136.23404
41.	Alpha_pinene	$C_{10}H_{16}$	136.23404
42.	Alphaselinene	$C_{15}H_{24}$	204.35106
43.	Alpha_terpinene	$C_{10}H_{16}$	136.23404
44.	Alpha_terpineol	$C_{10}H_{18}O$	154.24932
45.	Alpha_zingiberene	$C_{15}H_{24}$	204.35106
46.	Ar_curcumene	$C_{15}H_{22}$	202.33518
47.	Aromadendrene	$C_{15}H_{24}$	204.35106
48.	Beta-bisabolol	$C_{15}H_{26}O$	222.36634
49.	Beta_ionone	$C_{13}H_{20}O$	192.2973
50.	Beta_bisabolene	$C_{15}H_{24}$	204.35106
51.	Beta-carotene	$C_{40}H_{56}$	536.87264
52.	Beta-caryophyllene	$C_{15}H_{24}$	204.35106
53.	Beta-elemene	$C_{15}H_{24}$	204.35106
54.	Beta-eudesmol	$C_{15}H_{26}O$	222.36634
55.	Beta-himachalene	$C_{15}H_{24}$	204.35106
56.	Beta-myrcene	$C_{10}H_{16}$	136.23404
57.	Beta-phellandrene	$C_{10}H_{16}$	136.23404
58.	Beta-pinene	$C_{10}H_{16}$	136.23404
59.	Beta-selinene	$C_{15}H_{24}$	204.35106
60.	Beta-sesquiphellandrene	$C_{15}H_{24}$	204.35106
61.	Beta-sitosterol	$C_{29}H_{50}O$	414.7067
62.	Beta-thujone	$C_{10}H_{16}O$	152.23344
63.	Bornyl-acetate	$C_{12}H_{20}O_2$	196.286
64.	Caffeic-acid	$C_9H_8O_4$	180.15742
65.	Calamenene	$C_{15}H_{22}$	202.33518
66.	Camphene	$C_{10}H_{16}$	136.23404
67.	Camphenehydrate	$C_{10}H_{18}O$	154.24932
68.	Camphor	$C_{10}H_{16}O$	152.23344
69.	Capric-acid	$C_{10}H_{20}O_2$	172.2646
70.	Caprilic-acid	$C_8H_{16}O_2$	144.21144
71.	Capsaicin	$C_{18}H_{27}NO_3$	305.41188
72.	Caryophyllene	$C_{15}H_{24}$	204.35106
73.	Cedrol	$C_{15}H_{26}O$	222.36634
74.	Chavicol	$C_9H_{10}O$	134.1751
75.	Chlorogenic-acd	$C_{16}H_{18}O_9$	354.30872
76.	Citral	$C_{10}H_{16}O$	156.24932
77.	Citronellal	$C_{10}H_{18}O$	154.24932
78.	Citronellol	$C_{10}H_{20}O$	156.2652
79.	Citronellyl-acetate	$C_{12}H_{22}O_2$	198.30188
80.	3-carene	$C_{10}H_{16}$	136.23404
81.	Cumene	C_9H_{12}	120.19158
82.	Curcumin	$C_{21}H_{20}O_6$	368.3799
83.	D-borneol	$C_{10}H_{18}O$	154.24932
84.	Decanal	$C_{10}H_{20}O$	156.2652
85.	Decanaldehyde	$C_{10}H_{20}O$	156.2652
86.	Dehydrozingerone	$C_{11}H_{12}O_3$	192.21118
87.	Delphinidin	$C_{15}H_{11}O_7$	303.24364

88.	Delta-3-carene	$C_{10}H_{16}$	136.23404
89.	Delta-cadinene	$C_{15}H_{24}$	204.35106
90.	Di-o-methylhexahydro-Curcumin	$C_{23}H_{28}O_6$	400.46482
91.	Diethyl-sulfide	$C_4H_{10}S$	90.1872
92.	Dedecanoic-acid	$C_{10}H_{20}O_2$	172.2646
93.	Elemol	$C_{15}H_{26}O$	222.36634
94.	Ethyl-isopropyl-sulfide	$C_5H_{12}S$	104.21378
95.	Ethyl myristate	$C_{16}H_{32}O_2$	256.42408
96.	Farnesal	$C_{15}H_{24}O$	220.35046
97.	Farnesene	$C_{15}H_{24}$	204.35106
98.	Farnesol	$C_{15}H_{26}O$	222.36634
99.	Ferulic acid	$C_{10}H_{10}O_4$	194.184
100.	Furaldehyde	$C_5H_4O_2$	96.08406
101.	Furanogermenone	$C_{15}H_{20}O_2$	232.3181
102.	Gadoleic-acid	$C_{20}H_{38}O_2$	310.51452
103.	Galanolactone	$C_{20}H_{30}O_3$	318.4504
104.	Gamma-eudesmol	$C_{15}H_{26}O$	222.36634
105.	Gamma-muurolene	$C_{15}H_{24}$	204.35106
106.	Gamma-selinene	$C_{15}H_{24}$	204.35106
107.	Gamma-amino butericacid	$C_4H_9NO_2$	103.11976
108.	Gamma-terpinene	$C_{10}H_{16}$	136.23404
109.	Geraniol	$C_{10}H_{18}O$	154.24932
110.	Geranyl-acetate	$C_{12}H_{20}O_2$	196.286
111.	Gingediacetate	$C_{21}H_{32}O_6$	380.47518
112.	Gigerenone a	$C_{21}H_{24}O_5$	356.41226
113.	Gigerenone b	$C_{22}H_{26}O_6$	386.43824
114.	Gigerenone c	$C_{20}H_{22}O_4$	326.38628
115.	Gingerone	$C_{11}H_{14}O_3$	194.22706
116.	Glyoxal	$C_2H_2O_2$	58.03608
117.	Heptan-2-ol	$C_7H_{16}O$	116.20134
118.	Heptan-2-one	$C_7H_{14}O$	114.18546
119.	Hexahydrocurcumin	$C_{21}H_{26}O_6$	374.42754
120.	Humulene	$C_{15}H_{24}$	204.35106
121.	Isoeugenyl-methyl-ether	$C_{11}H_{14}O_2$	178.22766
122.	Isogingerenone	$C_{22}H_{26}O_6$	386.43824
123.	Isovalerylaldehyde	$C_5H_{10}O$	86.1323
124.	Kaempferol	$C_{15}H_{10}O_6$	286.2363
125.	Limonene	$C_{10}H_{16}$	136.23404
126.	Linalool	$C_{10}H_{18}O$	154.24932
127.	Menthol-acetate	$C_{12}H_{22}O_2$	198.30188
128.	Methyl-6-gingerol	$C_{18}H_{28}O_4$	308.41252
129.	Methyl-isobutyl-ketone	$C_6H_{12}O$	100.15888
130.	Methyl-nonyl-ketone	$C_{11}H_{22}O$	170.29178
131.	Methyl-8-gingerol	$C_{20}H_{32}O_4$	336.466
132.	Methyl-allylsulfide	C_4H_8S	88.17132
133.	Methylcaprylate	$C_9H_{18}O_2$	158.23802
134.	Methylglyoxal	$C_3H_4O_2$	72.06266
135.	Methyl-heptenone	$C_8H_{14}O$	126.19616
136.	Myrcene	$C_{10}H_{16}$	136.23404
137.	Myricetin	$C_{15}H_{10}O_8$	318.2351
138.	Myristic-acid	$C_{14}H_{28}O_2$	228.37092

139.	Myrtenal	$C_{10}H_{14}O$	150.21756
140.	N-butyraldehyde	C_4H_8O	72.10572
141.	N-heptane	C_7H_{16}	100.20194
142.	N-nonanol	$C_9H_{20}O$	144.2545
143.	N-propanol	C_3H_8O	60.09502
144.	Neo-isopulegol	$C_{10}H_{18}O$	154.24932
145.	Neral	$C_{10}H_{16}O$	152.23344
146.	Nerol	$C_{10}H_{18}O$	154.25
147.	Nerolidol	$C_{15}H_{26}O$	222.36634
148.	N-nonane	C_9H_{20}	128.2551
149.	N-octane	C_8H_{18}	114.22852
150.	Nonan-2-one	$C_9H_{18}O$	142.23862
151.	Nonanal	$C_9H_{18}O$	142.23862
152.	Octan-1-al	$C_8H_{16}O$	128.212
153.	Oleic-acid	$C_{18}H_{34}O_2$	282.46136
154.	P-coumaric acid	$C_9H_8O_3$	164.15802
155.	P-cymen-8-ol	$C_{10}H_{14}O$	150.21756
156.	P-hydroxybenzoic-acid	$C_7H_6O_3$	138.12074
157.	Paradol	$C_{17}H_{26}O_3$	278.38654
158.	Patchouli-alcohol	$C_{15}H_{26}O$	222.36634
159.	O-cymene	$C_{10}H_{14}$	134.21816
160.	Pentosan	$C_{15}H_{26}O_{13}$	414.35914
161.	Perillene	$C_{10}H_{14}O$	150.21756
162.	Phosphatidic-acid	$C_5H_9O_8P$	228.093922
163.	Pipecolic-acid	$C_6H_{11}NO_2$	129.15704
164.	Propionaldehyde	C_3H_6O	58.07914
165.	Quercetin	$C_{15}H_{10}O_7$	302.2357
166.	Raffinose	$C_{18}H_{32}O_{16}$	504.43708
167.	Rosefuran	$C_{10}H_{14}O$	150.21756
168.	Sabinene	$C_{10}H_{16}$	136.23404
169.	Selina-3,7(11)-diene	$C_{15}H_{24}$	204.351
170.	Sesquithujene	$C_{15}H_{24}$	204.35106
171.	Terpinene-4-ol	$C_{10}H_{18}O$	154.24932
172.	Terpinolene	$C_{10}H_{16}$	136.23404
173.	Tricyclene	$C_{10}H_{16}$	136.23404
174.	Undecan-2-ol	$C_{11}H_{24}O$	172.30766
175.	Undecan-2-one	$C_{11}H_{22}O$	170.29178
176.	Valine	$C_5H_{11}NO_2$	117.14634
177.	Vanillic-acid	$C_8H_8O_4$	168.14672
178.	Vanillin	$C_8H_8O_3$	152.14732
179.	Xanthorrhizol	$C_{15}H_{22}O$	218.33458
180.	Zingerone	$C_{11}H_{14}O_3$	194.22706
181.	Zingiberol	$C_{16}H_{28}O$	236.39292
182.	Zingiberonol	$C_{16}H_{28}O$	263.39292
183.	Zonarene	$C_{15}H_{24}$	204.35106

APPENDIX III

Phytochemicals selected from *Eleteria cardamomum* with molecular formula and weight

Sl. no	Phytochemicals	Molecular formula	Molecular weight (Da)
1	1,8-cineole	$C_{10}H_{18}O$	154.24932
2	Alpha-copaene	$C_{15}H_{24}$	204.35106
3	Alpha-phellandrene	$C_{10}H_{16}$	136.23404
4	Alpha-pinene	$C_{10}H_{16}$	136.233994
5	Alpha-terpinene	$C_{10}H_{16}$	136.233994
6	Alpha-terpineol	$C_{10}H_{18}O$	154.24932
7	Alpha-terpinylacetate	$C_{12}H_{20}O_2$	196.286
8	Alpha-thujene	$C_{15}H_{24}$	204.35106
9	Alpha-ylangene	$C_{10}H_{16}$	136.23404
10	Beta-pinene	$C_{10}H_{16}$	136.23404
11	Beta-sitostenone	$C_{29}H_{48}O$	412.69082
12	Beta-sitosterol	$C_{29}H_{50}O$	414.7067
13	Bisabolene	$C_{15}H_{24}$	204.35106
14	Borneol	$C_{10}H_{18}O$	154.24932
15	Campesterol	$C_{28}H_{48}O$	400.68012
16	Camphene	$C_{10}H_{16}$	136.23404
17	Camphor	$C_{10}H_{16}O$	152.23344
18	Capric acid	$C_{10}H_{20}O_2$	172.2646
19	Citronellal	$C_{10}H_{18}O$	154.24932
20	Citronellic-acid	$C_{10}H_{18}O_2$	170.24872
21	Citronellol	$C_{10}H_{20}O$	156.2652
22	Desmosterol	$C_{27}H_{44}O$	384.63766
23	Dodecanoicacid	$C_{12}H_{24}O_2$	200.31776
24	Eugenylacerate	$C_{12}H_{14}O_3$	206.23776
25	Gamma-terpinene	$C_{10}H_{16}$	136.23404
26	Geranic-acid	$C_{10}H_{16}O_2$	168.23284
27	Geraniol	$C_{10}H_{18}O$	154.24932
28	Geranyl-acetate	$C_{12}H_{20}O_2$	196.286
29	Heptanoic acid	$C_7H_{14}O_2$	130.18486
30	Hexanoic acid	$C_6H_{12}O_2$	116.15828
31	Limonene	$C_{12}H_{20}O_2$	196.286
32	Linalool	$C_{28}H_{58}NO_7P$	551.736382
33	Linalyl-acetate	$C_{12}H_{20}O_2$	196.286
34	Lysophosphatidylcholine	$C_{28}H_{58}NO_7P$	551.736382
35	Methyl-heptenone	$C_8H_{14}O$	126.2
36	Myrcene	$C_{10}H_{16}$	136.23404

37	Nerol	$C_{10}H_{18}O$	154.25
38	Nerolidol	$C_{15}H_{26}O$	222.36634
39	Neryl-acetate	$C_{12}H_{20}O_2$	196.286
40	N-hentriacontane	$C_{31}H_{64}$	436.83986
41	N-heptacosane	$C_{27}H_{56}$	380.73354
42	N_nonacosane	$C_{29}H_{60}$	408.7867
43	N-pentacosane	$C_{25}H_{52}$	352.68038
44	N-tricosane	$C_{23}H_{48}$	324.62722
45	N-tritriacontane	$C_{33}H_{68}$	464.89302
46	Oleic-acid	$C_{18}H_{34}O_2$	282.46136
47	Palmitic-acid	$C_{16}H_{32}O_2$	256.42408
48	P-cymene	$C_{10}H_{14}$	134.21816
49	Perillic acid	$C_{10}H_{14}O_2$	166.21696
50	Phosphatidylethanolamine	$C_7H_{14}NO_8P$	271.161722
51	Phytol	$C_{20}H_{40}O$	296.531
52	Sabinene	$C_{10}H_{16}$	136.23404
53	Stigmasterol	$C_{29}H_{48}O$	412.69082
54	Terpinene-4-ol	$C_{10}H_{18}O$	154.24932

ABSTRACT

IDENTIFICATION OF LEAD COMPOUNDS WITH ANTI-TUBERCULOSIS ACTIVITY IN INDIGENOUS SPICES OF KERALA

by

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Abstract of the thesis

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ABSTRACT

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is the second worldwide killer infectious disease and it kills annually 1.4 million people globally and 30,000 people in India. Although drugs are available to treat tuberculosis they have several limitations including long term treatment, side effects, emergence of multidrug-resistant (MDR) and extensively drug resistant (XDR) mutants and adverse effect to immune system in patients co-infection with HIV. Therefore, discovery of novel faster, cheaper and better drug is become the need of the hour. Since time immemorial several herbal remedies have been used against tuberculosis in the traditional systems of treatment especially in India and in African countries.

The indigenous spices of Kerala are well known for its use to treat human respiratory system. But its efficacies and mode of action are seldom investigated. In the present study the phytomolecules reported from *Elettaria cardamomum, Curcuma longa* and *Zingiber officinale* were screened through *in silico* and *in vitro* methods. For *in silico* screening Decaprenylphosphoryl-beta-D-ribose epimerase (DprE1), an enzyme responsible for the synthesis of arabinan, the virulent factor in *M., tuberculosis* was selected as the target molecule. The 3-D structure of the molecule was retrieved from PDB (PDB id 4FDO). 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. The canonical SMILES of the phytochemicals were retrieved from open access chemical databases and 3D structures were created using CORINA. Total 448 phytochemicals (*C. longa* – 211, *Z. officinale* – 183 and *E. cardamomum* – 54) were screened. Out of 448 phytochemicals structures of 373 (*C. longa* –137, *Z. officinale* –182 and *E. cardamomum* –0) were retrieved from databases and remaining compound's structures were created using Chemsketch. All selected phytochemicals were docked into the binding site of DprE1 using the tool, AutoDock 4.2. The docked structures having ΔG less than $^{-}5$ kcal mol^{-1} were selected as best hit molecules. Out of 211 compounds screened in *C. longa* 101, out of 183 compounds screened in *Z. officinale* 63 and out of 54 compounds screened in *E. cardamomum* 22 of them showed free energy of binding $\geq$ $^{-}5$ kcal mol^{-1} and these molecules were further analysed by Lipinski's rule of Five. To nullify the errors in

lead identification the top ranked hit molecules were again docked using the tools Hex server, iGEMDOCK, FireDock and SwissDock. The docked results were statistically analysed following DST and Zhang rule and selected the top ranked molecules from each plant *viz.* 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one from *C. longa,* Alpha-ylangene from *E. cardamomum* and Farnesal from *Z. officinale* as lead molecules. Mature seeds of *E. cardamomum* and mature rhizomes of *Z. officianale* and *C. longa* were air dried and extracted with 99% ethanol using a Soxhlet apparatus for 6-8 hours. The extracts were concentrated to dryness using a rotary evaporator and tested anti-mycobacterial activity by Luciferase reporter phage (LRP) assay against standard strain of *M. tuberculosis* H37RV at three different concentrations (25, 250 and 500 µg/ml). The results revealed that all the three plants have potential antituberculosis activity. In the order of merit *Z. officinale* rank first *E. cardamomum* rank second and *C. longa* rank third respectively. The results revealed the efficacy of anti-tuberculosis activity and the responsible phytomolecules in each plant. It also insights the discovery of novel drugs with desirable qualities from these plants that should be safe, effective and affordable to the poor people.