IN SILICO SCREENING AND IDENTIFICATION OF LEAD MOLECULES WITH ANTI- HEPATITIS B ACTIVITY

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THESIS

Submitted in partial fulfilment of the

requirement for the degree of

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2018

DECLARATION

I hereby declare that this thesis entitled "***IN SILICO* SCREENING AND IDENTIFICATION OF LEAD MOLECULES WITH ANTI-HEPATITIS B ACTIVITY IN SELECTED SPICES**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
ΔG_{bind}	Free energy of binding
µg	Micro gram
µM	Micro molar
2 D	Two dimensional
3 D	Three dimensional
A°	Angstrom
BE	Binding energy
cccDNA	Covalently closed circular DNA
CHB	Chronic Hepatitis B
CHBV	Chronic Hepatitis B Virus
et al.	And others
etc.	So on and so forth
HBc	Hepatitis B core
HbeAg	Hepatitis B e Antigen
HbsAg	Hepatitis B s Antigen
HBx	Hepatitis B x
IgM	Immunoglobin M
Kcal/mol	Kilo calorie per mol
kDa	Kilo Dalton
kI	Inhibition constant
NA	Nucleos(t)ide Analogues
NCBI	National Center for Biotechnology Information
NIH	National Institute of Health
NMR	Nuclear Magnetic Resonance
ORF	Open Reading Frame
PDB	Protein Data Bank

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PEG-IFN α	Pegylated interferon α
Pol	Polymerase
RC	Relaxed circlar
RDD	Rational drug design
SBDD	Structure based drug design
vHTS	Virtual high throughput screening
Viz.	Namely
WHO	World Health Organization

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1. INTRODUCTION

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Liver is an essential organ that has many functions in the body. Many disease processes can occur in the liver, including infections such as hepatitis, cirrhosis, cancers and damage by medications or toxins. Hepatitis B is a serious worldwide problem and it is the most infectious viral disease, caused by the hepatitis B virus. According to World Health Organization ~ 240 million people have been chronically infected with hepatitis B and 6,86,000 of them die annually (WHO report, 2017). It is also reported that the chronically infected people are at a very high risk of developing hepatocellular carcinoma and liver cirrhosis (Loggi *et al.*, 2015). In India 40 million people have been reported as the carriers of hepatitis B and ~1.0 lakh of them are dying annually.

Hepatitis B virus is the prototype member of hepadnaviridae family and is partially double stranded, relaxed circular (RC) DNA of 3-2 kb size which replicates by reverse transcription. The genome of HBV encodes for overlapping Open Reading Frames(ORFs) that are translated into viral core protein, surface protein, polymerase/ reverse transcriptase and HBx (WHO report, 2017). The persistence of cccDNA in hepatocyte plays a key role in viral persistence, and reactivation of viral replication after cessation of antiviral therapy and resistance to therapy.

Therapeutic approach is mainly the suppression of antiviral replication. But the unique replication strategy employed by HBV enables its persistence within infected infected hepatocytes (Dandri and Petersen, 2016). Due to the high resistance rate of the virus and newly emerging viral mutants, currently approved antivirals have failed to prove their effectiveness. Vaccination (Recombivax HB, Engerix-B) is available for the prevention of hepatitis B and this has been effective for more than 20 years. There are now five oral nucleoside/ nucleotide analogues and two injectable versions of interferon that are approved for the treatment of CHBV (Ryan et al., 2015). But these antiviral strategies are either poorly effective or only effective for non-curative suppression of viral replication and its success is

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limited due to the formation of new drug resistance mutants. Hence an alternative therapy is the need of the hour.

Use of plants as a source of medicine has been an ancient practice and an important component of health care system in India. Plants being rich sources of secondary metabolites such as alkaloids, flavonoids, terpenoids, triterpenes, tannins, phenolic compounds, etc. have been used as treatment option including liver ailments (Subin et al., 2016). About 600,000 secondary compounds from plants have been already reported. Plants are also the best synthesizer of nanoparticles having pharmaceutical properties, which are used for site targeted drug delivery to treat disease like cancer (Sreekumar, 2016). The rich history of plant derived chemotherapeutic agents support the need to study natural products as the remedy for viral diseases. Many plants have been demonstrated to have phytochemicals against liver disease. *In silico* screening of phytochemicals coupled with *invitro* and *in vivo* screening may be the best option to validate the drug activity of herbal medicine as they induce less toxicity and side effects as compared to synthetic drugs

The unique genome structure and molecular biology of HBV pose a number of challenges and thus the development of bioinformatics tool has facilitated a more comprehensive and detailed analysis and understanding of origin, evolution, transmission and response to antiviral agents of HBV and its interaction with the host. Bioprospecting plants still remains a green area as it can provide us with fascinating structures which could be directly or indirectly used as therapeutic agents. It is high time to realize the potential of plant biodiversity and to exploit fully and judiciously.

In the present investigation, phytochemicals from *Elettaria cardamomum* (L.) Maton, *curcuma longa* and *Zingiber officinale* has been thoroughly studied to find out potential lead molecules which can act against hepatitis B viral activity and this is done through molecular docking.

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2. REVIEW OF LITERATURE

2.1 HEPATITIS B AND ITS IMPACTS

Hepatitis B is a life threatening liver infection of global importance caused by hepatitis B virus (HBV). According to World Health Organization (WHO) ~240 million people have been chronically infected with hepatitis B and 6,86,000 of them die annually all around the world (WHO report, 2017). Although it is a global serious health issue, its risk is higher in South America, Africa, Russia and Asia. India falls into the category of intermediate endemicity of HBV. It was estimated that India have over 40 million hepatitis B infected patients, which constitutes ~15% of the hepatitis B patients in the world, and around 1,15,000 people die per year.

Hepatitis B virus is highly infectious blood borne pathogen. HBV is an enveloped DNA virus that belongs to the family hepnaviridae. It is the smallest known DNA virus having spherical shape with diameter of about 42nm and genomic length of ~3.2 kb. It contains a small partially double stranded, relaxed circular DNA genome that replicates by reverse transcription of an RNA intermediate the pregenomic RNA (pgRNA) (Beck and Nassal, 2007). The genome encodes four overlapping open reading frames (ORFs) that are translated into viral core protein, surface proteins, polymerase/ reverse transcriptase (RT) and HBx (Wei *et al.*, 2010).

HBV infection is distributed worldwide in the form of eight different genotypes (A-H). There is atleast 8% nucleotide sequence dissimilarity among the 8 known HBV genotypes. HBV is transmitted via permucosal or percutaneous exposure to infected body fluids or blood products and it replicates through an RNA intermediate that can integrate itself into a host genome. The spectrum of HBV infection varies from acute to chronic depending on the duration of HBV surface antigen (HBsAg) in the serum.

Based on the period of infection hepatitis B infection is classified into two types, they are acute hepatitis B infection and chronic hepatitis B infection. It may

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last up to 6 months (with or without the symptoms) and infected person are able to pass the virus to others during this time. Most people are asymptomatic. A rare, life threatening condition called fulminant hepatitis can occur with a new acute infection and requires immediate, urgent medical attention since a person can go into sudden liver failure, which can lead to death (Pawlotsky *et al.*, 2015). Most people do not experience any symptoms during acute infection phase. However, some people have acute illness with symptoms that last for several weeks, including yellowing of skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain.

Acute HBV infection is characterized by the presence of HbsAg and immunoglobulin M (IgM) antibody to the core antigen, HbcAg. During the initial phase of infection, patients are also seropositive for hepatitis B e antigen (HBeAg). HbeAg is usually a marker of high level of replication of the virus. The presence of HbeAg indicates that the blood and body fluids of the infected individual are highly infectious (WHO, 2014). There is no specific treatment for acute infection B but may require treatment to relieve the symptoms.

Chronic hepatitis B (CHB) is seen in person if the virus or infection is persistent for more than 6 months i.e., the persistence of HbsAg for at least 6 months (with or without concurrent HBeAg). Persistence of HbsAg is the principle marker of risk for developing chronic liver disease and liver cancer (hepatocellular carcinoma) later in life. Most people with chronic hepatitis B are unaware of their infection, putting them at a serious risk of developing cirrhosis or liver cancer which are life threatening. In some people the chronic liver infection can later develop into hepatic decompensation, cirrohsis (scarring of the liver) or hepatocellular carcinoma. Long term/ chronic hepatitis B is often treated with medication to keep the virus under control.

A CHB cure can be defined at different levels. Basically, the most desirable end point is the elimination of both the viraemia (HBV-DNA) and the viral surface antigen (HBsAg), followed by sero-conversion to anti-HbsAg (anti-HBs)

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antibodies. This condition is largely satisfactory because it is associated with a substantial improvement of outcomes and a reduced risk of developing complications, at least in non-cirrhotic patients.

The likelihood that infection becomes chronic depends upon the age at which a person becomes infected. Children less than 6 years of age who become infected with hepatitis B virus are the most likely to develop chronic infection.

In infants and children

- 80-90% of infants infected during the first year of life develop chronic infections, and
- 30-50% of children infected before the age of 6 years develop chronic infections

In adults

- Less than 5% of otherwise healthy persons who are infected as adults will develop chronic infection and
- 20-30% of adults who are chronically infected will develop cirrhosis and/ or liver cancer (WHO factsheet, 2014).

2.1.1 Treatments

On a global basis, the hepatitis B virus (HBV) is the most important vaccine-preventable liver disease. As a consequence, the development of vaccine for the prevention of HBV represents one of the modern achievements of modern medicine. Universal immunization against HBV has been adopted in over 80 countries. Unfortunately, in many developing countries, HBV immunization coverage is still limited. The first commercially available vaccine was a plasma-derived product. Plasma derived vaccine currently comprise 80% of worldwide HBV vaccine production. The relatively inexpensive subunit vaccines are produced by the concentration, purification and chemical treatment of HBV surface antigen (HBsAg) particles isolated from the plasma of HBV carriers. Despite excellent immune-genecity and protective efficacy rates, physician acceptance of the first

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plasma-derived vaccine was impeded because of unfound concerns about the presence of bloodborne infectious agents in the vaccine. The first recombinant HBV vaccine was introduced in 1986 and a second was approved in 1989.

Recombivax HB is the first recombinant HBV vaccine which is formulated to contain a 10 μg adult dose of HbsAg protein. Engerix- B is formulated to contain a 20 μg dose of HbsAg protein. These vaccines are available for the prevention of hepatitis B and it has been effective for more than 20 years.

In case of chronic hepatitis B it is not curable but treatable this is mainly because of the peculiar feature of hepatitis B virus. The viral life cycle of HBV involves the formation of particularly stable episomal minichromosmes, covalently closed circular DNA (cccDNA) molecules, which serve as a template for transcription and reservoir for future replication cycles. Furthermore, the HBV genome is able to integrate into the host genome, thus reinforcing viral antigen production and favouring HBV oncogenesis. The inability to arrest this complex replicative machinery leads to the persistence of viral antigen production, which, in turn, progressively exacerbates the functional failure of the immune response; the immune response represent the most effective tool for viral control.

The goal of the therapy is to reduce the risk of complications including immature death. Treatment can help to prevent cirrhosis, liver failure and liver cancer by reducing Hepatitis B viral load and the loss of hepatitis HbeAg while improving liver enzyme.

A complete cure, however, would only be accomplished by elimination of cccDNA from infected hepatocytes, which represent definite viral eradication and ensure protection from the risk of reactivation in the case of immunosuppression. However, both of these end points still represent a challenge because they are adequately not met by current therapies. Therefore, clinicians must rely on a surrogate but more realistic end point, which is the induction of sustained virological remission.

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New strategies are designed for HBV elimination focuses on the following two main assumptions derived from the known mechanism underlying viral persistence: a) the need to target the virus directly and / or b) the need to restore an effective immune response.

2.1.2 Current HBV therapies

Two different therapeutic approaches are currently available for patients with chronic hepatitis B: (1) a finite antiviral and immunomodulatory treatment with interferon- α and (2) an indefinite treatment with nucleos(t)ide analogues (NAs), which can successfully achieve non-curative suppression of viral replication. Treatment with pegylated interferon-α (PEG-IFNα2a) can have a curative effect mediated by viral inhibition and an enhancement of the host immune response.

NAs inhibit the reverse transcriptase activity of the HBV polymerase i.e., NAs inhibit HBV DNA synthesis via a competitive interaction with the natural substrate of the HBV polymerase; however, they do not interfere with cccDNA formation. As a consequence, in most patients, HBV replication rebounds after antiviral therapy is discontinued.

The major advantage of these treatments is that in case of NAs, they show good tolerance and potent antiviral activity associated with high rates of on treatment response to therapy. PEG-IFN includes a finite course of treatment, the absence of drug resistance and an opportunity to obtain a durable post-treatment response to therapy.

Combination treatments capable of boosting anti-hbv reactivity while steadily suppressing viral replication are expected to accelerate the decline in HbsAg levels. Thus, the administration of PEG-IFNα2a, once complete suppression of HBV replication has been obtained with NAs, represents the newest therapeutic approach currently being evaluated.

There are now five oral nucleoside/ nucleotide analogues and two injectable versions of interferon that are approved for the treatment of CHBV (Ryan et al.,

2015). The currently available treatment for hepatitis B include Baraclude (entecavir), Epivir-HBV (lamivudine), Intron A (interferon alfa-2b), Hepsera (adefovir dipivoxil), Pegasys (peginterferon alfa-2a), Vemlidy (tenofovir alafenamide), Viread (tenofovir disoproxil fumarate).

The main limitations of current system of treatment are that these antiviral strategies are either poorly effective or only effective for non-curative suppression of viral replication and mainly the formation of new drug resistant mutants. The access to diagnosis and treatment of hepatitis B in many resource constrained settings are still narrow and many people are diagnosed only when they already have advanced liver disease (WHO factsheet, 2014). Liver cancer progress rapidly since treatment option are limited and this situation is mainly affected the middle class and poor people. Hence an alternate therapy which should be affordable to everybody is the need of the hour.

2.3 PLANTS AS A SOURCE OF DRUGS.

For millennia, nature has been a source of medicinal products, in which plants provide potential molecules to develop new drugs. The plant derived compounds have a long history of clinical use, better patient, tolerance and acceptance (Veeresham, 2012). To date, around 35000-70000 plant species have been screened for their medicinal use. In early drug discovery, plants especially those with ethnopharmacological uses have been the primary sources of medicine.

Plant provide the predominant ingredients of medicines in most medical tradition. The valuable medicinal properties of different plants are due to the presence of several constituents i.e., saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes, lactones, terpenoids and phorbol esters (Tiwar and Singh, 2004). Among them some act as synergistic and enhance the bioactivity of other compounds.

Plants have always been a common source of medicaments either in the form of traditional preparations or as pure active principles (Fransworth, 1985). Throughout the world medicinal plants are extensively utilized in two distinct areas

of health management, traditional system of medicine and modern system of medicine. At the household level many medicinal plants are used by women to take care of their families at the village level by men or tribal shamns, and by practitioners of classical traditional system of medicine such as Ayurveda Chinese medicine or the Japanese kamposystem.

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medicine and pharmaceutical research. Medicinal plants vary their effectiveness against any kind of cure. Plant based systems continue to play an essential role in health care and their use by different culture had been extensively documented. Among ancient civilization, India has been known to be rich repository of medicinal plants.

Indian system of medicines is among the well-known global traditional system of medicines. In India, Ayurveda, Unani, Siddha, and Folk (tribal) medicines are the major systems of indigenous medicine. The ancient health care practises are still relevant and followed by communities across the countries. India's contribution remains exemplary in the growth of traditional health care system. According to WHO, over 80% of the world population or 4.3 billion people rely upon such traditional plant-based system of medicine to provide them with primary healthcare. A large number of plant and plant products are using from antibiotics to anti-infective and from anti-cancer to anti-aging. Despite the current preoccupation with synthetic chemistry as a vehicle to discover and manufacture drugs, the contribution of plant to disease treatment and prevention is still enormous (Veeresham, 2012). Even at the dawn of 21st century, 11% of the 252 drugs considered are basic and essential by the WHO were exclusively of flowering plant origin.

The most striking feature of natural products in connection to their long-lasting importance in drug discovery is their structural diversity that is still largely untapped. Revitalization of the natural products is bringing newer challenges with respect to quality control and standardization along with cost effectiveness. Many

characterized human endogenous receptors, important in physiological function, are activated by plant derived chemicals, for example, the opioid and the more recently discovered cannabinoid receptors.

The advantage using herbal remedies is that it is not only cost effective but also safe and almost free from serious side effects. Treatment with medicinal plants is considered very safe as there is no or minimal side effects and also the use of herbal treatment is independent of any age groups and the sexes. The biggest advantage is that these remedies are in sync with nature.

Traditional knowledge of medicinal plants can provide leads for further scientific studies on species and genetic diversification with certain desirable traits that can be used or transferred into the modern biomedicine for prevention and cure of certain chronic disease. There has been widespread relief that green medicines are healthier and safer. Traditional knowledge is the totality of all knowledge and practises, whether explicit or implicit. This knowledge is established on past experience and observations (Mugabe, 1998).

Even at present time very limited knowledge about the ingredients in herbal medicines and their effect in humans, the lack of stringent quality control and the heterogeneous nature of herbal medicines all necessitate the continuous monitoring of the safety of these plant products (Chan, 1997). Although popularity of the synthetic products increased due to its production cost, time effectiveness, easy quality control, stringent regulation and quick effects, but their safety and efficacy has always remained questionable resulting in the dependence of natural products by more than 80% of the total population of the developing world, because of its time-tested safety and efficacy.

2.3.1 Bioprospecting

Bioprospecting, also known as biodiversity prospecting, is the exploration of biological material for commercially valuable genetic and biochemical properties (Rasoanaivo, 2011). In simple terms this means the investigation of living things to see how they can be commercially useful to humans. Small samples of natural

resources are collected for their potential value to industry, particularly in the pharmaceutical, biotechnology and agri-business fields. Local communities close to where the material originates may have specialised knowledge on how the resources are used, which can also be collected, and this is known as traditional or indigenous knowledge (IK).

Biological diversity (biodiversity) refers to all living things, including plants, animals, insects and marine life. New technologies are also now enabling microbes to be investigated. However, not all investigations on biodiversity are considered bioprospecting: academic or conservation research is excluded from the term. Nor does it include commercial use of natural resources e.g., medicinal plants as trade commodities.

The underlying aim of bioprospecting is to find new resources and products from nature that can be used by humans. Improving human health, through both medicine and better nutrition are key focal areas. Bioprospecting plays a dominant role in discovering leads for drug development, since existing/known compounds for developing drugs for human use are limited. Nature can provide original novelty and complexity that can be modified in the laboratory.

2.3.2 Constraints in bioprospecting

There is growing concern that a number of pharmaceutical firms and biotechnology companies are exploring the forests, fields and waters of developing world in search of biological riches and indigenous knowledge with sole aim of developing patented and profitable products. Although bioprospecting agreements are sanctioned by the multilateral convention on Biological diversity, in most cases commercial bioprospecting agreements cannot be officially monitored or enforced by source communities, countries or by the convention itself (Zakrzewski, 2002). Imbalance in ecosystem due to excessive exploitation of material resources is always a possibility. It is a fact that the tropical rainforest regions of the world, which constitute more than 50% of medicinal plants, are disappearing (Moran, 1992). This is mainly due to multitude of commercial interest including bioprospecting.

2.4 BIOINFORMATICS AND DRUG DISCOVERY

Bioinformatics is an interdisciplinary science spanning genomics, transcriptomics, proteomics, population genetics and molecular phylogenetics. Bioinformaticians in drug discovery use high throughput molecular data in comparisons between symptom-carriers (patients, animal disease models, cancer cell lines, etc.) and normal controls. The key objectives of such comparisons are to 1) connect disease symptoms to genetic mutations, epigenetic modifications and other environmental factors modulating gene expression, 2) identify drug targets that can either restore cellular function or eliminate malfunctioning cells, e.g., cancer cells, 3) predict or refine drug candidates that can act upon the drug target to achieve the designed therapeutic result and minimize side effects, and 4) assess the impact on environmental health and the potential of drug resistance

Bioinformatic analysis can not only accelerate drug target identification and drug candidate screening and refinement but also facilitate characterization of side effects and predict drug resistance (Xia, 2017). High-throughput data such as genomic, epigenetic, genome architecture, cistromic, transcriptomic, proteomic, and ribosome profiling data have all made significant contribution to mechanism-based drug discovery and drug repurposing. Accumulation of protein and RNA structures, as well as development of homology modeling and protein structure simulation, coupled with large structure databases of small molecules and metabolites, paved the way for more realistic protein-ligand docking experiments and more informative virtual screening.

Drug discovery and development is an intense, lengthy and an interdisciplinary endeavour. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to determine if such compounds satisfy a number of pre-set criteria for initiating clinical development. Traditionally, drugs were discovered by synthesizing compounds in a time-consuming multi-step processes against a battery of *in vivo* biological screens and

further investigating the promising candidates for their pharmacokinetic properties, metabolism and potential toxicity (Pugazhendhi and Umamaheswari, 2013). Such a development process has resulted in high attrition rates with failures attributed to poor pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse effects in humans (10%) and various commercial and miscellaneous factors. Today, the process of drug discovery has been revolutionized with the advent of genomics, proteomics, bioinformatics and efficient technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de novo design, *in vitro*, *in silico* ADMET screening and structure-based drug design (Kalyani *et al.*, 2013).

2.4.1 Different approaches for Drug-discovery

Bioinformatics plays a vital role in designing new drug compounds. Rational Drug Design (RDD) is a process used to discover and develop new drug compounds which uses a variety of computational methods. These methods can be categories based on the information available about drug targets and potential drug compounds. They are mainly used to identify novel compounds, design compounds for selectivity, efficacy and safety, and develop compounds into clinical trial candidates. The methods mainly include structure-based drug design, ligand based drug design, de novo drug design and homology modelling.

Structure-Based Drug Design (SBDD) - Structure-based drug design is one of several methods in the rational drug design toolbox. The key molecules often proteins and enzymes involved in a specific metabolic or cell signalling pathway that is known, or suggested, to be related to a particular disease state are taken as drug targets. The main purpose of drug compounds are to inhibit, restore or otherwise modify the structure and behaviour of disease-related proteins and enzymes. In SBDD, to assist in the development of new drug compounds the known 3D geometrical shape or structure of proteins are often used. The 3D structure of protein targets is most often derived from x-ray crystallography or nuclear magnetic resonance (NMR) techniques. X-ray and NMR methods can

resolve the structure of proteins to a resolution of a few angstroms (about 500,000 times smaller than the diameter of a human hair). This ability to work with both proteins and drug compounds at high resolution makes SBDD one of the most powerful methods in drug design.

Lead Optimization- The process by which the structure of the lead compound are systematically modified, docking each specific configuration of a drug compound in a proteins active site, and then testing how well each configuration binds to the site is known as lead optimization. SBDD techniques are especially effective in refining the 3D structures of lead to improve binding to protein active sites. Bioisosteric replacement is a common lead optimization method in which a specific functional groups in a ligand are substituted for other groups so as to improve the binding characteristics of the ligand.

Computer-Aided Drug Design (CADD) is a specialized discipline to simulate drug-receptor interactions which is highly dependent on bioinformatics tools, applications and databases. In drug discovery, to elicit interesting information and to identify vital genes and protein that speeds the process of drug discovery bioinformatics techniques are used.

Virtual High-Throughput Screening (vHTS) - Virtual screening uses computer based method on the basis of biological structure to discover new ligands (Shoichet, 2014). In virtual screening, compounds are docked into a structurally defined biological target and the binding energy of the resulting complex is estimated, allowing compounds to be ranked. This technique has provided most successful where the target structure has been determined at high resolution. Virtual screening does not need any physical test samples, or even previously synthesized compounds.

Homology Modeling - Determining the 3-D structure of proteins is an another common challenge in CADD research. Most drug targets are proteins, so it's important to know their 3-D structure in detail. It's estimated that the human body has 500,000 to 1 million proteins. However, the 3-D structure is known for only a

small fraction of these. Homology modeling is one method used to predict 3-D structure. In homology modeling, the amino acid sequence of a specific protein (target) is known, and the 3-D structures of proteins related to the target (templates) are known. Soon after, to predict the 3-D structure of the target based on the known 3-D structures of the templates bioinformatic tools are used. MODELLER is a well-known tool in homology modeling, and the SWISS-MODEL Repository is a database of protein structures created with homology modeling. Similarity Searches: A common activity in biopharmaceutical companies is the search for drug analogues. With an outset of a favourable drug molecule, one can search for chemical compounds with similar structure or properties to a known compound. There are a variety of methods used in these searches, including sequence similarity, 2D and 3D shape similarity, substructure similarity, electrostatic similarity and others.

2.4.2 *In silico* screening and drug discovery from plant (standard approach)

In silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyse the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve binding characteristics.

The use of computers and computational methods permeates all aspects of drug discovery today and forms the core of structure-based drug design. High-performance computing, data management software and internet are facilitating the access of huge amount of data generated and transforming the massive complex biological data into workable knowledge in modern day drug discovery process (Pugazhendhi and Umamaheswari, 2013). The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets and elucidation of their functions to the discovery and development of lead compounds with desired properties. Computational tools offer the advantage of delivering new drug candidates more quickly and at a lower cost. Major roles of computation in drug

discovery are; (1) Virtual screening & de novo design, (2) *In silico* ADME/T prediction and (3) Advanced methods for determining protein-ligand binding (Jorgensen, 2004).

As structures of more and more protein targets become available through crystallography, NMR and bioinformatics methods, there is an increasing demand for computational tools that can identify and analyze active sites and suggest potential drug molecules that can bind to these sites specifically (Rao and Sreenivas, 2011). Also to combat life-threatening diseases such as AIDS, Tuberculosis, Malaria etc., a global push is essential. Time and cost required for designing a new drug are immense and at an unacceptable level.

2.5 TARGET IDENTIFICATION

Target identification and characterization begins with identifying the function of a possible therapeutic target (gene/protein) and its role in the disease. Identification of the target is followed by characterization of the molecular mechanisms addressed by the target. A good target should be efficacious, safe, meet clinical and commercial requirements and be 'druggable'.

Approaches:

- Data mining using bioinformatics - identifying, selecting and prioritizing potential disease targets
- Genetic association - genetic polymorphism and connection with the disease
- Expression profile - changes in mRNA/protein levels
- Pathway and phenotypic analysis - *In vitro* cell-based mechanistic studies
- Functional screening - knockdown, knockout or using target specific tools

Target validation shows that a molecular target is directly involved in a disease process, and that modulation of the target is likely to have a therapeutic effect. The most important criteria for target validation are to take multi-validation approach.

2.6 LIGAND PREPARATION

Ligand details can be retrieved from various databases. Some of the database which provide details about ligand are PubChem, ChemSpider, FooDB etc. The three dimensional structure of ligand can be retrieved from CORINA. PubChem has archived 35.6 million of unique chemicals. Among them, 25.3 million satisfy the rule of five, 1.85 million have been tested in at least one bioassay, and 0.8 million have been reported as active (Wang et al., 2013). Ligand structure can also be drawn with the help of software like ChemSketch.

2.7 MOLECULAR DOCKING

Molecular docking symbolizes an inevitable step in the current day drug discovery process. In the present study, docking was carried out using AutoDock 4.2, an automated molecular docking software package, following the standard procedure. Firstly water molecules from the target were removed and polar hydrogen atoms were added. Then root of the ligand molecule and torsion were selected. In the protein molecule torsions were checked for the selected residues. Precalculated grid maps required for running the program were calculated using the auto grid program. AutoDock 4.2 uses two algorithms namely Monto Carlo simulated Annealing and Lamarckian genetic algorithm for the generation of possible orientation of ligand at the binding site of the target. Lamarckian Genetic Algorithm (LGA) was selected to calculate the best conformers as it is the most efficient and reliable when compared to other algorithms. After the docking study the protein-ligand complexes were analysed considering the lowest binding energies and interaction forces. The molecules having free energy of binding less than or equal to $\leq$-5Kcal/mol were considered as the hit molecules. The interaction of the ligand in the target protein complexes were then analysed using ligplot.

2.8 APPLICATION OF BIOINFORMATICS AND ITS IMPACT ON DRUG DISCOVERY

The process of designing new drug using bioinformatics tools has opened a new area of research.

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In order to design a new drug one need to follow the following path.

1. Identifying target disease

2. Study interesting compounds

3. Detection of molecular bases for disease

4. Rational drug design techniques

5. Refinement of compounds

6. Quantitative structure activity relationships (QSAR)

7. Solubility of molecule

8. Drug testing

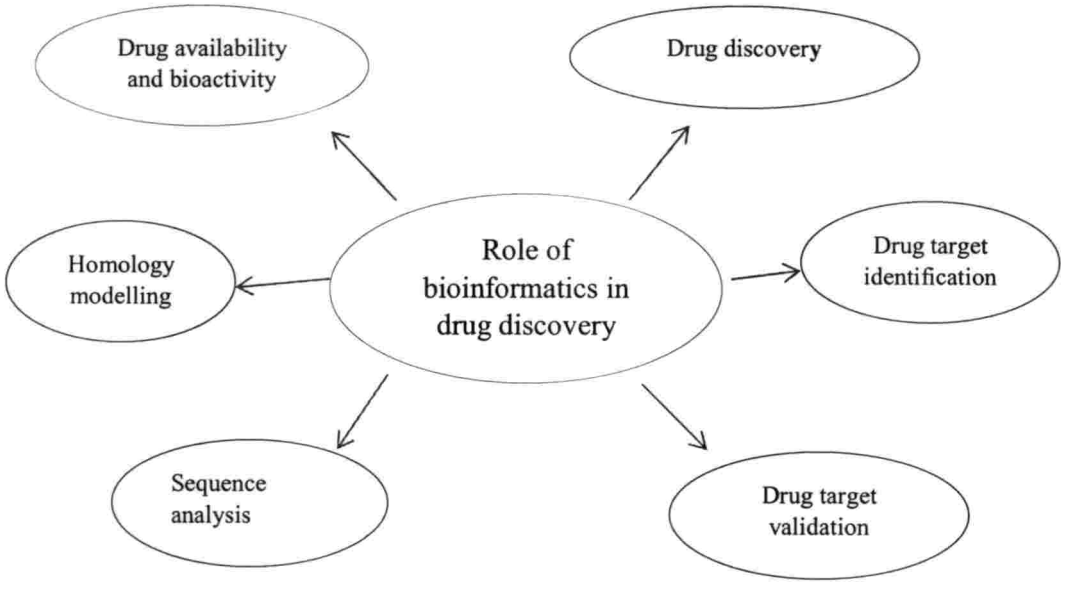

Figure 1. Role of bioinformatics in drug discovery

In drug discovery, the idea of using X-ray crystallography emerged more than 30 years ago, when the first 3 dimensional structure of protein was determined. Within a decade, a radical change in drug design has begun incarnating the

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knowledge of 3 dimensional structures of target protein into design process. Protein structure can influence drug discovery at every stage in design process. Classically, it is used in lead optimization a process that uses structure to guide the chemical modification of a lead molecule to give an optimized fit in term of shape, hydrogen bonds and other non-covalent interaction with the target.

2.9 SELECTED SPICE VARITIES FOR *IN SILICO* SCREENING

2.9.1 *Curcuma longa* L.

Curcuma longa is a member of the ginger family (Zingiberaceae). Its rhizomes (underground stems) are the source of a bright yellow spice and dye. Turmeric is sterile (does not produce seed, but it does grow vigorously from the rhizomes). It is thought to have arisen by selection and vegetative propagation of a hybrid between wild turmeric (*Curcuma aromatica*), native to India, Sri Lanka and the eastern Himalayas, and some other closely related species. It is only known as a domesticated plant and not found in the wild. India is the world's largest producer, consumer and exporter of turmeric. Turmeric is also cultivated extensively in Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and the Philippines.

2.9.2 *Zingiber officinale* Roscoe

Zingiber officinale is best known as the source of the pungent, aromatic spice called ginger. This spice is produced from the rhizome (underground stem) of the plant. Ginger has many medicinal uses. The fresh or dried rhizome is used in oral or topical preparations to treat a variety of ailments, while the essential oil is applied topically as an analgesic. Evidence suggests ginger is most effective against nausea and vomiting associated with surgery, vertigo, travel sickness and morning sickness. The topical use of ginger may cause allergic reactions. *Zingiber officinale* is possibly native to India. It is widely grown as a commercial crop in south and southeast Asia, tropical Africa (especially Sierra Leone and Nigeria), Latin America, the Caribbean (especially Jamaica) and Australia.

2.9.3 *Elettaria cardamomum* (L.) Maton

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Elettaria cardamomum is a member of the ginger family (Zingiberaceae). It is sometimes known as the 'queen of spices' alongside black pepper (Piper nigrum), which is known as the 'king of spices'. The dried ripe fruits of cardamom have been used as a spice and in medicines since the 4th century BC. *Elettaria cardamomum* is native to the Western Ghats of southern India. It has been introduced to other parts of tropical Asia and is widely grown for its aromatic seeds. It is grown as a crop in many countries, including Guatemala, Sri Lanka, Papua New Guinea and Tanzania.

3. MATERIALS AND METHODS

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To validate anti-hepatitis B activity and identification of lead compounds in indigenous spice of Kerala, most common and widely used spice belonging to the family Zingiberacea *viz*, *Elleteria 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 is a perennial herb belongs to the family Zingiberacea. The plants grow thick rooted rhizomes by which its reproduction takes place. Its rhizome is used either fresh or dried, which has unique medicinal properties. It acts as a carminative and also enhances the complexion and skin-tone. In addition, turmeric can be used to combat liver damage, respiratory disorders and ulcers. Turmeric's principal constituent is curcumin, which is a potent antioxidant. Nearly 226 phytochemicals were reported from *curcuma longa.*

3.1.2 *Zingiber officinale* Roscoe

Zingiber officinale, commonly known as ginger, is a spice consumed worldwide for culinary and medicinal purposes. It has a number of chemicals responsible for its medicinal properties, such as antiarthritis, antiinflammatory, antidiabetic, antibacterial, antifungal, anticancer, etc. Ginger has several active constituents, including aromatic ketones and terpenoids, and some of these may have anxiolytic and antidepressant properties. *Zingiber officinale* is possibly native to India. Nearly 309 phytochemicals were so far reported from *Z. officinale.*

3.1.3 *Elettaria cardamomum* (L.) Maton

Elettaria cardamomum belongs to the family Zingiberaceae. It is a rhizomatous herb having aromatic seeds. The fruits contain an essential oil. It possesses cineol, terpineol and terpinyl acetate. In Ayurvedic medicine, cardamom is used to treat disorders of the stomach and urinary system, asthma, bronchitis and

heart problems. The habitat of *E. cardamomum* is the evergreen forests of the Western Ghats. A total of 87 phytochemicals from *Elettaria cardamomum* was retrieved using open access database and by literature survey. Information on these phytochemicals was procured from databases like PubChem and ChemSpider databases.

3.2 *IN SILICO* SCREENING

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 and ethnobotanical databases'. The canonical SMILES of these phytochemicals were retrieved from chemical databases such as PubChem, ChemSpider and Dictionary of Natural products. The three dimensional structures of these phytochemicals were created using the online software CORINA. The structures of phytochemicals not available on databases were created using ChemSketch.

3.2.1 Dr. Duke's phytochemical and ethnobotanical databases

Dr. Duke's Phytochemical and Ethnobotanical Databases is an online database developed by James A. Duke at the USDA. The current Phytochemical and Ethnobotanical databases facilitate plant, chemical, bioactivity, and ethnobotany searches. A large number of plants and their chemical profiles are covered and data are structured to support browsing and searching in several user focused ways. For example, users can

- get a list of chemicals and activities for a specific plant of interest, using either its scientific or common name
- download a list of chemicals and their known activities in PDF or spreadsheet form
- find plants with chemicals known for a specific biological activity
- display a list of chemicals with their LD toxicity data
- find plants with potential cancer-preventing activity
- display a list of plants for a given ethnobotanical use

- find out which plants have the highest levels of a specific chemical 36

References to the supporting scientific publications are provided for each specific result. Also included are links to nutritional databases, plants and cancer treatments and other plant related databases. The database is available on the URL: www.ars-grin.gov/duke

3.2.2 PubChem

PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information (NCBI), a component of the National Library of Medicine, which is part of the United States National Institutes of Health (NIH). PubChem can be accessed for free through a web user interface. Millions of compounds' structures and descriptive datasets can be freely downloaded via FTP. PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. More than 80 database vendors contribute to the growing PubChem database. PubChem consists of three dynamically growing primary databases. As of 1 November 2017:

- Compounds, 93.9 million entries (up from 54 million entries in Sept 2014), contains pure and characterized chemical compounds.
- Substances, 236 million entries (up from 163 million entries in Sept 2014), contains also mixtures, extracts, complexes and uncharacterized substances.
- BioAssay, bioactivity results from 1.25 million (up from 6000 in Sept 2014) high throughput screening programs with several million values.

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

3.2.3 ChemSpider

ChemSpider is a free chemical structure database providing fast text and structure search access to over 63 million structures from hundreds of data sources. By integrating and linking compounds from more than 400 data sources, it enables researchers to discover the most comprehensive view of freely available chemical

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data from a single online search. 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.dnp.chemnetbase.com

3.2.4 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.

3.2.5 ChemSketch

ACD/ChemSketch freeware is a drawing package that allows you to draw chemical structures including organics, organometallics, polymers, and Markush structures. It also includes features such as calculation of molecular properties (e.g., molecular weight, density, molar refractivity etc.), 2D and 3D structure cleaning and viewing, functionality for naming structures (fewer than 50 atoms and 3 rings), and prediction of logP. It can be used to produce professionally looking structures and diagrams for reports and publications. ChemSketch is available for down on the URL: www.chemsketch.xtremedownload.com.

3.2.6 CORINA

CORINA is a fast and powerful 3D structure generator for small and medium sized, typically drug-like molecules. Its robustness, comprehensiveness, speeds and performance makes CORINA Classic a perfect application to convert large

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chemical datasets or databases from 2D to 3D structures. CORINA is available on the URL: www.molecularnetworks.com/onlinedemos/corina_demo

3.3 TARGET MOLECULE SELECTION

3.3.1 Target Molecule Preparation

In this investigation, three target proteins from HBV were selected namely HBx, pol and HBc. The 3D structure of HBc was retrived from Protein Data Bank (PDB ID: 1QGT) and the structure of HBx protein and pol was modelled using the software MODELLER. MODELLER is a computer program used for homology or comparative modelling of protein 3D structure.

3.3.2 MODELLER

MODELLER is used for homology or comparative modelling of protein three-dimensional structures. The user provides an alignment of a sequence to be modelled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modelling by satisfaction of spatial restraints and can perform many additional tasks, including *de novo* modelling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. MODELLER 9v.15 was used for the modelling of protein HBx and polymerase.

3.3.3 Sources of Target Molecule

The Protein Data Bank (PDB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organisations (PDBe, PDBj and RCSB).

The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB. The PDB is a key resource in areas of structural biology, such as structural genomics. Submission of structural data is a pre-requisite for publishing papers in major scientific journals and sanctioning fund by some funding agencies. Many other databases use protein structures deposited in the PDB. If the contents of the PDB are thought of primary data, then there are hundreds of derived databases that categorize the data differently. URL: www.rcsb.org.

3.3.4 PDBsum

The PDBsum is a pictorial database that provides at-a-glance overview of the contents of each 3D structure deposited in the Protein Data Bank (PDB). It shows the molecules that make up the structure (i.e., protein chains, DNA, ligands and metal ions) and schematic diagrams of their interactions. Extensive use is made of the freely available RasMol molecular graphics program to view the molecules and their interactions in 3D. 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/

3.3.5 CASTp

Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins. The measurement includes the area and volume of pocket or void by solvent accessible surface model (Richards' surface) and by molecular surface model (Connolly's surface), all calculated analytically. CASTp can be used to study surface features and functional regions of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures. CASTp is updated daily and can be accessed at http://cast.engr.uic.edu.

3.4 MOLECULAR DOCKING USING AUTODOCK

All selected phytochemicals were docked into the binding site of hepatitis B proteins HBx, HBc and polymerase using the open access software application tool, Autodock 4.2. 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 was positioned at the macromolecule with XYZ Co-ordinates set at 76.495, 178.463, 3.954 respectively and grid point spacing of 0.375 A° for HBx. Similarly for HBc protein XYZ Co-ordinates was set at 99.236, 93.525 and 44.402 and grid point spacing of 0.375A°. For polymerase XYZ was set at 5.64, -50.062, 31.768 and grid point spacing of 0.375A°. The docking calculations were done by setting all the docking parameters at default value.

For docking, all parameters were kept at default including population number. The ligand bound complexes were analysed for its binding affinity and possible orientation were ranked according to their lowest binding energy through cluster analysis. The top ranked molecules with free energy of binding ≤-Kcal/mol were considered as hit molecule.

3.4.1 Tools for Visualizing the Docked Results

The docked structures were visualized with the aid of following tools.

a) PyMol

PyMol produces high quality 3D structures of protein which is bound to the ligand molecule. The result file in the PDB format was uploaded to the PyMol software and the 3D structure was visualized. The software also provides the information about the number of hydrogen bond, the residue to which the bond is formed and type of bond. Various colours can be imparted to the protein as well as ligands to distinguish between them. The image of the particular protein can be saved in the .jpg format

b) LigPlot

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LigPlot software was used to visualize the 2D structure of the protein and ligand interaction. The PDB file was uploaded and the RUN button was clicked. The 2D structure of ligand bound to the protein residue was obtained as result. The Hydrogen bond formed between protein and ligand along with its bond length was also depicted. Hydrophobic interaction of protein residue with ligand was also obtained.

4. RESULTS

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4.1 *IN SILICO* SCREENING

4.1.1 Preparation of target molecule

Three hepatitis B virus proteins namely HBc, HBx and polymerase were selected as the target molecules. The 3D structure of HBc was retrieved from Protein Data Bank (PDB ID: 1QGT) and the structure of HBx protein and pol was modelled using the software MODELLER.

4.1.2 Structural visualization and active site detection

HBc is a small pliable protein of 21kDa, which is required for the formation of nucleocapsid. It consists of 142 amino acids. The active site of HBc was detected using the tool PDBsum. The active residues of HBc were PRO25, ASP29, LEU30, THR33, TRP102, SER106, PHE110 and VAL115. The structure of HBc was visualised using the tool, Pymol.

HBx is a small protein of 154 amino acids with a molecular mass of approximately 17.5kDa and plays a key role in viral infection *in vivo*. HBx has been suggested to transactivate a variety of viral and cellular promoters that facilitate viral replication. The active site was detected using the tool PDBsum and the active site residues include LEU53, PHE132 and ALA154. PyMOL was used to visualize the 3D structure of HBx protein.

Polymerase is an enzyme which exhibits both a DNA- dependent DNA polymerase and a RNA dependant DNA polymerase (reverse transcriptase) activity. It replicates the HBV genome from an encapsidated pregenomic RNA template. It contains 512 amino acids. There are several active residues in polymerase LYS1, ASN48, HIS175, PRO176, TYR218, ASN251, SER339, PRO340, TYR354, PRO355, ASN454, ASP458, PRO459, ARG468, and PRO512. The active sites were detected using the tool CASTp. The 3D structure of polymerase was visualized using the tool PyMol.

4.1.3 Preparation of ligand molecule

Out of the 571 phytochemicals screened from three selected spices such as *Elettaria cardamomum* (87), *Zingiber officinale* (273) and *curcuma longa* (211), 558 compounds were procured from open access databases and remaining 13 molecules were drawn using ChemSketch and the 3D structures of all phytomolecules were generated and saved in .pdb format using CORINA interface. The list of molecules downloaded from the open access databases along with their docked results were depicted in Appendix I. The structure of phytochemicals created using the tool ChemSketch were shown in figure 2.

4.2 DOCKING

4.2.1 Docked result of *Elettaria cardamomum*

Out of 87 phytochemicals from *Eletteria cardamomum* screened against HBx protein, 70 of them showed binding energy ≤- 5.0 kcal/mol and these molecules were considered as hit or active/inhibitory molecules against HBx protein. Among these, the top ranked five hit molecules having least binding energy were beta-sitostenone (-8.62 kcal/mol), beta sitosterol (-8.57 kcal/mol), stigmasterol (-8.22 kcal/mol), demosterol (-7.91kcal/mol) and cyanidin (-7.49kcal/mol) with inhibition constant 0.492μM, 0.519μM, 0.945μM, 1.6μM and 3.24μM respectively. The top hit molecules were thoroughly sorted out using the various parameters such as free energy of binding (kcal/ mol), inhibition constant (μM), number of hydrogen bonds, hydrophobic interactions, bond type and bond length (Å).

The top first ranked hit molecule, β-sitostenone (ΔGbind -8.62 kcal/mol) did not show any H-bond interaction with the target and there was no significant difference in binding energy when compared to the hit, β-sitosterol (-8.57kcal/mol). But latter showed 2 hydrogen bond interactions at the residue ARG72. The compound cyanidin showed 3 hydrogen bond interactions with the residue HIS94, LEU98 and THR97. Desmosterol and Stigmasterol showed 1 H bond interaction with the residues ARG77 and ALA76, respectively. Beta-sitosterol was selected as

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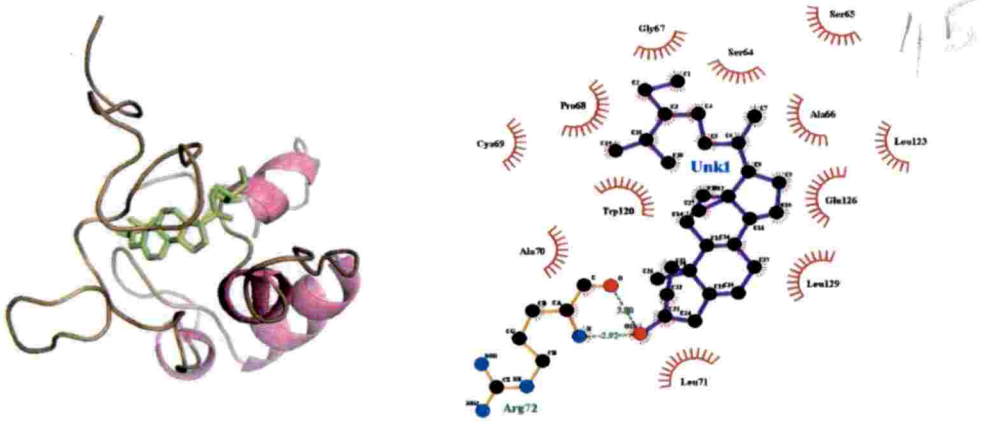
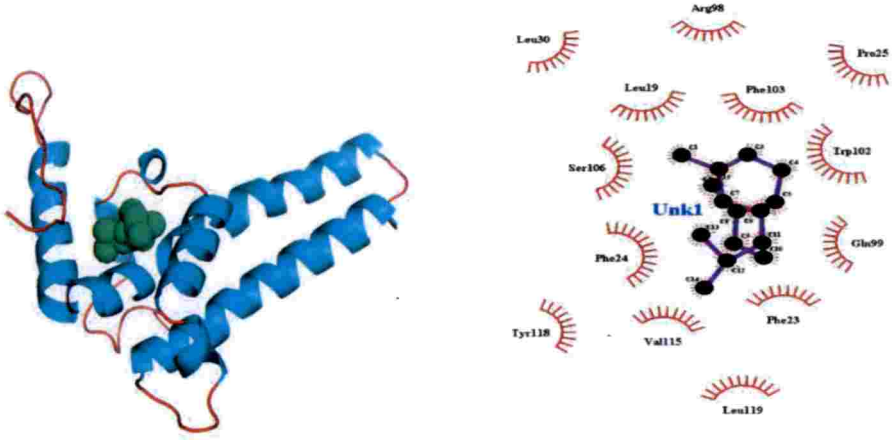
the best lead molecule as it showed least free energy of binding and lowest inhibition constant with 2 hydrogen bond interaction of bond length 3.00 and 2.92. It gave positive values in drug likeness properties.

The docked results between phytochemicals from *Eletteria cardamomum* and the third selected target, HBc revealed that out of 87 phytochemicals screened 49 of them have binding energy (ΔG_{bind}) $\leq$-5.0 kcal/mol and qualified as hit molecules. The detailed binding parameters analysis of the top five hit molecules such as α-ylangene (-8.04 kcal/mol), α-copaene (-7.82 kcal/mol), caryophyllene (-7.08 kcal/mol), humulene (-6.94 kcal/mol) and humulene oxide (-6.90kcal/mol) revealed that none of the phytochemicals with least binding energy exhibited H bond interaction with its target whereas all the phytochemicals have shown hydrophobic interaction with catalytic residue, TRP102. Among these, alpha ylangene was selected as the best lead molecule as it showed least free energy of binding, lowest inhibition constant and acceptable range of molecular weight.

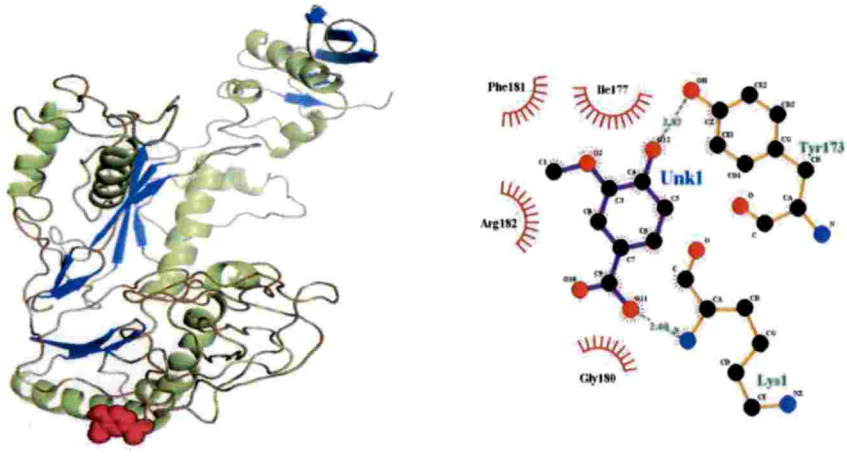
Docking between the target polymerase and the phytochemicals from *Eletteria cardamomum* (87) revealed that only three molecules have inhibitory effect on the target. They were vanilic acid, α-copaene and α-ylangene. Among the three compounds vanillic acid showed least free energy of binding (ΔGbind-5.50 kcal/mol), with inhibition constant (Ki) 92.28uM and established 2 H-bonds. The interacting residues include Lys1 and Tyr 173. Other two phytochemicals such as α-copaene (ΔG ≤-5.43 kcal/mol) and α-ylangene (ΔG ≤-5.21 kcal/mol) showed negligible difference in binding energy bud did not exhibit any H bond with the target protein. Therefore, vanillic acid was selected as the lead molecule. It also showed positive values in drug likeness properties.



A) β-sitosterol and HBx



B) α-ylangene and HBc



C) Vanillic acid and polymerase

Plate 1: Docked structures between the target proteins and leads from *Elettaria cardamomum* in pymol viewer and ligplot

Table 1. Docked results of selected hits from *Elettaria cardamomum*

Target	Lead molecule	BE kcal/mol)	KI(μM)	HBond	Bond type	Bond length
HBx	Beta-Sitostenone	-8.62	0.492	0		
	Beta-Sitosterol	-8.57	0.519	2	ARG72 OHO ARG72 NHO	3.00 2.92
	Stigmasterol	-8.22	0.945	1	ALA76 OHO	2.88
	Desmosterol	-7.91	1.6	1	ARG77 NHO	2.93
	Cyanidin	-7.49	3.24	3	LEU98 NHO HIS94 NHO THR97 OHO	2.81 2.53 2.52
	Alpha-Ylangene	-7.38	3.88	0		
	Alpha-Copaene	-7.28	4.65	0		
	Gamma- Tocopherol	-7.22	5.11	2	SER65 NHO VAL83 OHO	3.25 2.90
	Bisabolene	-6.94	8.19	0		
	Alpha- terpinylacetate	-6.46	18.51	2	LEU116 NHO CYS115 SHO	3.11 2.94
HBc	Alpha-Ylangene	-8.04	1.28	0		
	Alpha-Copaene	-7.82	1.86	0		
	Caryophyllene	-7.26	4.73	0		
	Humulene	-6.94	6.01	0		
	Humulene-Oxide	-6.90	8.76	0		
	Alpha-Terpinylacetate	-6.71	12.15	0		
	Eugenyl-Acetate	-6.64	0.44	0		
	Terpineol- Formiate	-6.45	18.75	0		
	Ascaridole	-6.41	20.15	1	SER106 OHO	2.75
	1,4-Cineole	-6.29	24.71	1	SER106 OHO	2.88
POL	anillic-Acid	-5.50	92.28	2	LYS1 NHO TYR173 OHO	2.60 2.82
	lpha-Copaene	-5.43	103.87	0		
	lpha-Ylangene	-5.21	151.61	0		

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4.2.2 Docked result of *Zingiber officinale*

A total of 273 phytochemicals derived from *Zingiber officinale* were docked with each of the target protein viz HBx, HBc and ploymerase. Out of the 273 phytochemicals, 203 compounds showed binding energy ≤-5kcal/mol with HBx. 156 compounds showed binding energy energy ≤-5kcal/mol with HBc and only 9 phytochemicals showed binding energy ≤-5 kcal/mol with polymerase. The details of the selected lead molecules and the best lead molecule against each target protein were as follows.

Of the 203 hit molecules (ΔGbind <-5.00 kcal/.mol) obtained against HBx the top ranked five hit molecules with least binding energy were β-carotene (-11.40 kcal/mol), 2-6-dimethyl-octa-3-7- diene-1-6-diol (-9.62 kcal/mol), β-sitosterol (-8.45 kcal/mol), stigmasterol (-8.22 kcal/mol) and myricetin (-8.30 kcal/mol) respectively. The inhibition constant of these molecules with the target protein was 4.44 uM, 0.088 uM, 0.639 uM, 0. 945 uM and 0.827 uM respectively. Further hydrogen bond interaction analysis of the docked structure of HBx showed that the compound β-carotene did not show any hydrogen bond interaction with HBx whereas the ligand 2-6-dimethyl-octa-3-7- diene-1-6-diol showed 5 hydrogen bond interactions at the residues ARG78, THR81 and ALA66. Myricetin also showed 5 hydrogen bond interactions with the residues SER153, PHE150, HIS94 and LEU98. β-sitosterol and stigmasterol showed 2 and 1 hydrogen bond interaction with ARG72 and ALA76 respectively. Although β-carotene did not show any H-bond interaction with the target, its binding energy level was significantly low due to the presence of more number of hydrophobic interactions. Therefore, β-carotene was selected as the best lead against HBx protein.

The docked result of phytochemicals with HBc revealed that out of 273 phytochemicals screened, 156 of them were qualified as hit molecules (free energy of binding less than -5 kcal/mol). The five molecules which showed least energy of binding were alpha-ylangene (-8.04 kcal/mol), β-himachalene (-7.91kcal/mol), gamma-muurolene (-7.91 kcal/mol), alpha-copaene (-7.82) and zingiberol (-

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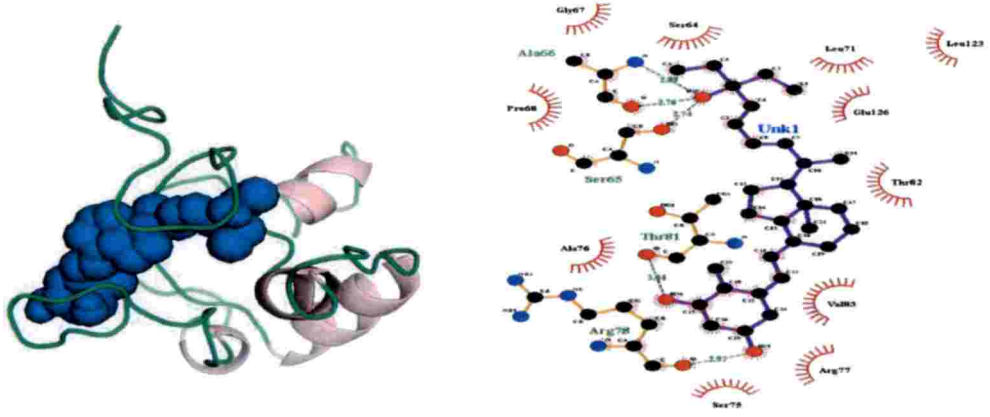
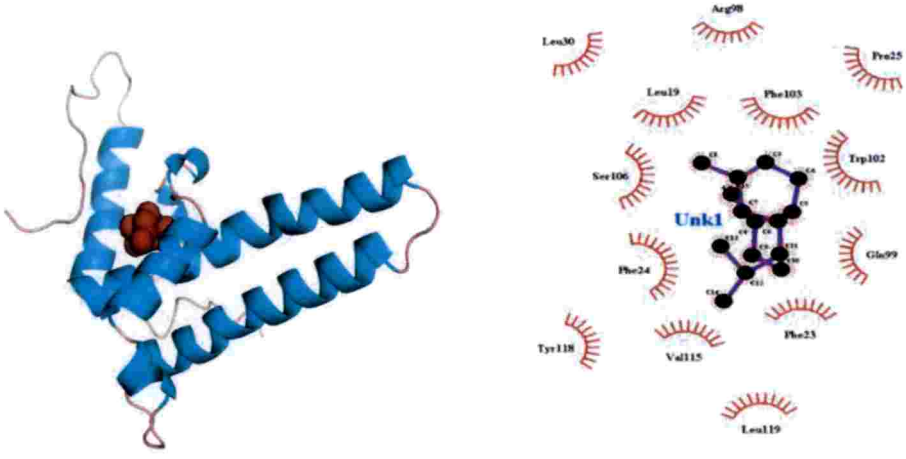
7.81kcal/mol) with inhibition constant 1.28μM, 1.58uμ, 1.58uM, 1.86μM and 1.87μM respectively. Except zingiberol the other ligands did not show any hydrogen bond interaction with the residues but all the phytochemicals have exhibited hydrophobic interaction with the active residue TRP 102. Zingiberol exhibited 1 hydrogen bond interaction with the active residue SER106 of bond length 3.12. Among the other hits, alpha-ylangene has the least free energy of binding (-8.04kcal/mol), least inhibition constant and the presence of more hydrophobic interaction. Hence alpha- ylangene was selected as the best lead molecule. It also follows the Lipinski's rule of five and has drug likeness properties.

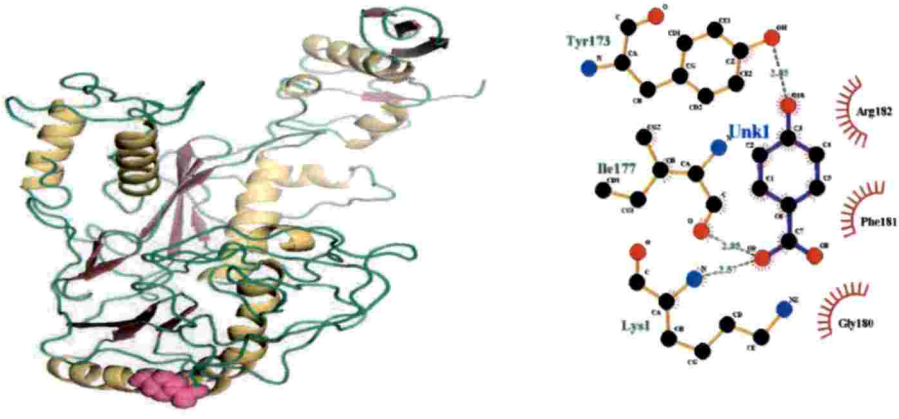
Against polymerase, out of 273 phytomolecules screened, only nine of them were qualified as hit molecules (ΔGbind <-5.00 kcal/.mol). The hit molecules based on least free energy of binding on the order of merit were p-hydroxy-benzoicacid (-5.67 kcal/mol), vanillic acid (-5.50 kcal/mol), alpha-copaene (-5.50 kcal/mol), cis-beta-sesquiphellandrol (-5.37 kcal/mol), alpha-ylangene (-5.21kcal/mol) beta-eudesmol (-5.14 kcal/mol), bisabolene (-5.06 kcal/mol), 4-gingerol (-5.02 kcal/mol), beta-santalol (-5.02 kcal/mol) and zingiberine (-5.02 kcal/mol). The inhibition constant of these molecules were as follows 69.54 uM, 92.28 uM, 99.14 uM, 115.91 uM, 151.73 uM, 172.17 uM, 194.70 uM, 209.55 uM, 208.24 uM, and 209.92 uM respectively. Out of the top 5 hit molecule p-hydroxy-benzoicacid, vanillic acid and cis-betasesquiphellandrol showed hydrogen bonding. P-hydroxy-benzoicacid exhibited 3 hydrogen bond interactions with critical residues LYS1, ILE177 and TYR173. Vanillic acid and cis-betasesquiphellandrol exhibits 2 hydrogen bond interaction with TYR173, LYS1, LEU 179 and ASP5 respectively. Considering the insignificant binding energy level difference and H-bond interactions both molecules p-hydroxy-benzoicacid and vanillic acids were equally competent to suggest as the best leads. However, p-hydroxy-benzoicacid was selected as the best lead molecule and it satisfied the Lipinski's rule of five.



A) 2-6dimethyl-octa-3-7-diene-1-6-diol and HBx



B) Alpha-ylangene and HBc



C) P-hydroxy benzoic acid and Polymerase

Plate 2: Docked poses of target and leads from *Zingiber officinale* in pymol viewer and ligplot

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Table 2. Docked results of selected hits of *Zingiber officinale*

Target	Lead molecule	BE (kcal/mol)	KI(μM)	HBond	Bond type	Bond length
HBx	Beta-Carotene	-11.40	4.44	0		
	2-6-Dimethyl-Octa-3-7-Diene-1-6-Diol	-9.62	0.088	5	RG78 OHO HR81 OHO ER65 OHO LA66 NHO LA66 OHO	2.93 3.04 2.74 2.89 2.76
	Beta-Sitosterol	-8.45	0.639	2	RG72 OHO RG72 NHO	3.00 2.92
	Stigmasterol	-8.22	0.945	1	LA76 OHO	2.88
	Myricetin	-8.30	0.827	5	IS94 NHO EU98 NHO HR97 OHO HE150 OHO ER153 OHO	2.57 2.87 2.54 2.71 2.91
	Kaempferol	-8.09	1.18	7	HR97 OHO IS 94 NHO IS94 NHO LA102 OHO LA 102 NHO ER153 OHO EU98 NHO	3.31 2.85 2.89 2.99 2.95 2.83 2.74
	Curcumin	-7.89	1.66	4	RG138 NHO YS130 NHO HR97 OHO EU98 NHO	2.51 3.03 2.62 2.85
HBc	Alpha-Ylangene	-8.04	1.28	0		
	Beta-Himachalene	-7.91	1.58	0		
	Gamma-Muurolene	-7.91	1.58	0		
	Alpha-Copaene	-7.82	1.86	0		
	Zingiberol	-7.81	1.87	0		
POL	P-Hydroxy-Benzoicacid	-5.67	69.54	3	YS1-NHO LE177 OHO YR173 OHO	2.57 2.85 2.85
	Vanillic Acid	-5.50	92.28	2	YS1 NHO YR173 OHO	2.60 2.82
	Alpha-Copaene	-5.46	99.14	0		
	Cis-Betasesquiphellandrol	-5.37	115.91	2	SP5 NHO EU179 OHO	3.11 2.86
	Alpha-Ylangene	-5.21	151.73	0		

4.2.3 Docked result of *Curcuma longa*

Out of 211 phytochemicals derived from *Curcuma longa* screened against the target HBx protein, 198 of them showed free energy of binding ≤-5 kcal/mol and identified as hit molecules. The top ranked hit molecules were beta-carotene (-11.40 kcal/mol), hopenone I (-10.38 kcal/mol), lupeol (-9.71 kcal/mol), hop-17(21)-en-3beta-ol (-9.30 kcal/mol) and gitoxigenin (-8.89 kcal/mol) respectively. The inhibition constant of the top five hit molecule was as follows 0.0044uM, 0.024uM, 0.076uM, 0.151uM and 0.303uM. Among the top ranked five hit molecules except β-carotene and hopenone I all others showed hydrogen bond interaction (bond type OHO and NHO). The compound hop-17(21)-en-3beta-ol and gitoxigenin have two hydrogen bond with the residues ARG78 and ALA85. Lupeol established one hydrogen bond with the residue ARG 72. The compound β-carotene and hopenone I have least free energy of binding but no hydrogen bond interactions. The number of hydrophobic interaction was significantly high between β-carotene with the target HBx. Hence, β-carotene was selected as the best lead.

The top ranked five hit molecules obtained against HBc were 2-hydroxy-methyl-anthraquinone (-8.00 kcal/mol), dicumyl peroxide (-7.87 kcal/mol), beta-turmerone (-7.76 kcal/mol), beta-sesqui phellandrene (-7.74 kcal/mol) and curlone (-7.71 kcal/mol) respectively. Among these, only 2-hydroxy-methyl-anthraquinone and dicumyl peroxide exhibits hydrogen bond interaction with the residue VAL115 and SER106 respectively. The compound 2-hydroxy-methyl-anthraquinone was selected as the best lead molecule since it has established hydrogen bond with HBC, least binding energy and inhibiton constant and presence of more hydrophobic interaction when compared with other hits. It also showed positive value in drug likeness properties.

Of the 211 phytochemicals screened against polymerase, only 12 were identified as hit molecules (ΔGbind <-5.00 kcal/.mol). Protocatechuic-acid (-5.75 kcal/mol), bisabola-3, 10-dien-2-one (-5.63 kcal/mol), vanillic-acid (-5.50

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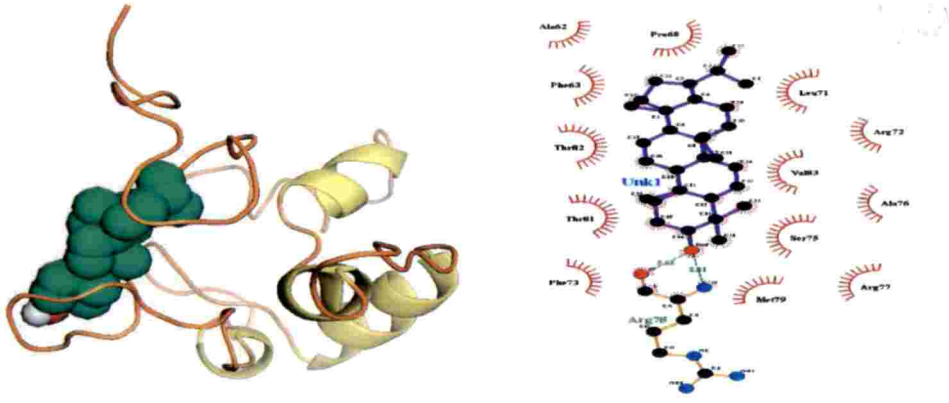
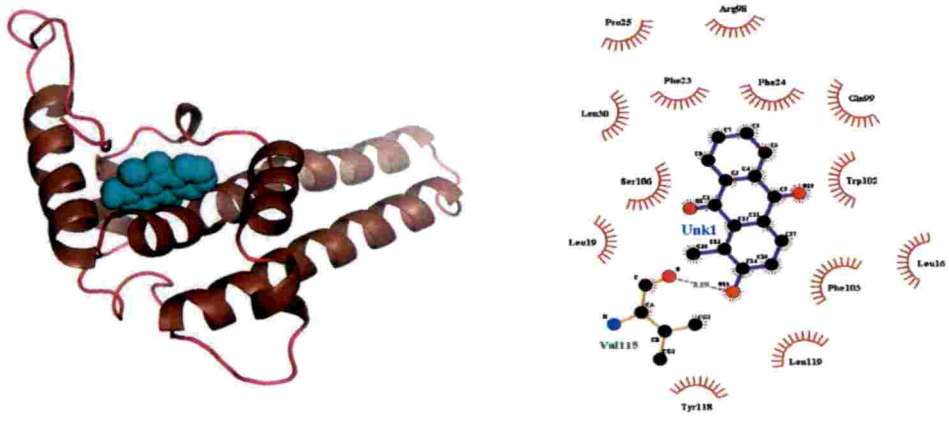
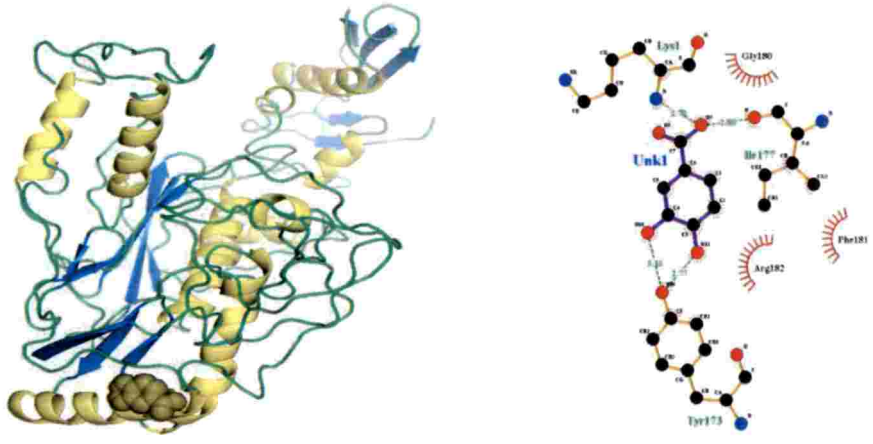
kcal/mol), dicinnamoylmethane (-5.44 kcal/mol) and bis-demethoxycurcumin (-5.42 kcal/mol) were the top ranked five hit molecules with inhibition constant 60.50 μM, 74.69μM, 92.28μM, 103.01μM and 106.08μM respectively. Here the top ranked five hit molecules showed negligible difference in binding energy. The compound protocatechuic-acid was selected as the lead molecule as it exhibited four hydrogen bond interactions with the residues TYR173, ILE177 and LYS1 and least binding energy with the target. It exhibits no violation from Lipinski's rule of five and has positive drug likeness properties.



A) Hop-17(21)En-3β-ol and HBx



B) 2-hydroxy-methyl-anthraquinone and HBc



C) Protacatechuic acid and Polymerase

Plate 3: Docked structures between the target proteins and leads from *Curcuma longa* in pymol viewer and ligplot

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Table 3. Docked results of selected hits of *Curcuma longa*

Target	Lead Molecule	BE kcal/mol)	KI(μM)	HBond	Bond type	Bond length
HBx	Beta-Carotene	-11.40	0.004	0		
	Hopenone I	-10.38	0.024	0		
	Lupeol	-9.71	0.076	1	ARG72 OHO	2.70
	Hop-17(21)-En-3beta-Ol	-9.30	0.151	2	ARG78 OHO ARG78 NHO	2.65 2.81
	Gitoxigenin	-8.89	0.303	2	ARG72 NHO ALA85 NHO	2.89 2.80
	Campesterol	-8.88	0.308	1	ASP114 OHO	2.85
	Feruloyl-Pcoumaroyl-Methane	-8.80	0.357	3	LEU98 NHO THR97 OHO ARG138 NHO	3.12 2.45 3.01
	Beta-Sitosterol	-8.45	0.639	2	ARG72 OHO ARG72 NHO	3.00 2.92
HBc	2-Hydroxy-Methyl-Anthraquinone	-8.00	1.37	1	SER106 OHO	3.10
	Dicumyl Peroxide	-7.87	1.69	1	VAL115 OHO	3.19
	Beta-Turmerone	-7.76	2.06	0		
	Beta-Sesquiphellandrene	-7.74	2.14	0		
	Curlone	-7.71	2.23	0		
POL	Protocatechuic-Acid	-5.75	60.50	4	TYR173 OHO TYR173 OHO ILE177 OHO LYS1 NHO	3.16 2.77 2.80 2.70
	Bisabola-3,10-Dien-2- One	-5.63	74.69	2	TYR6 NHO ASP5 NHO	3.24 3.04
	Vanillic-Acid	-5.50	92.28	0		
	Dicinnamoylmethane	-5.44	103.01	0		
	Bis-Demethoxycurcumin	-5.42	106.08	3	TYR6 NHO ASP5 NHO ASP5 NHO	3.12 2.04 3.14
	1-(3-Cyclopentylpropyl)-2,4-Dimethylbenzene	-5.37	115.84	0		
	Beta-Turmerone	-5.22	148.56	0		

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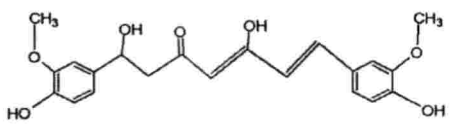
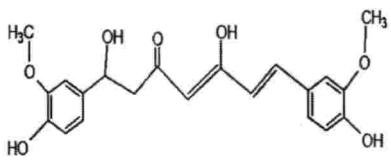
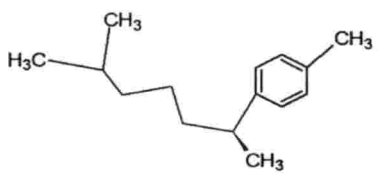
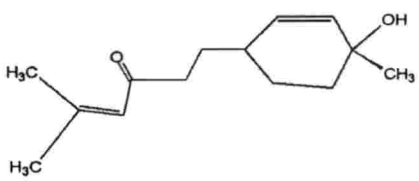
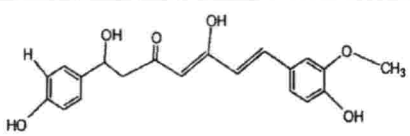
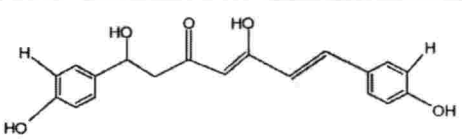
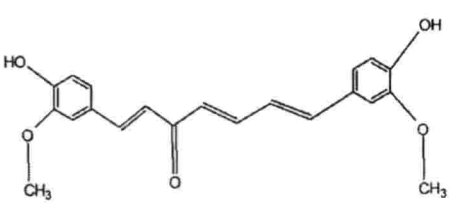
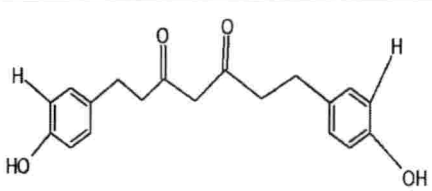
 1. 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	 2. 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl) -4,6-heptadiene-3-one
 3. Dehydrocurcumene	 4. 4-hydroxybisabola-2,10-diene-9-one
 5. 1,5-dihydroxy-1-7-4,6-heptadiene-3-one	 6. 1,5-dihydroxyl-1,7-bis (4-hydroxyphenyl)-4,6-heptadiene-3-one
 7. 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,4,66-heptadiene	 8. 1,7-bis(4-hydroxyphenyl)1-heptene-3,5-dione

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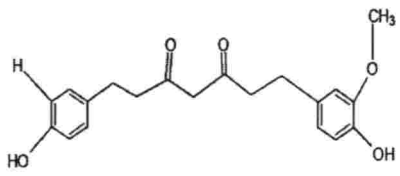
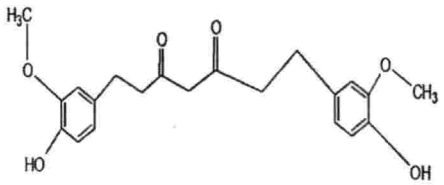
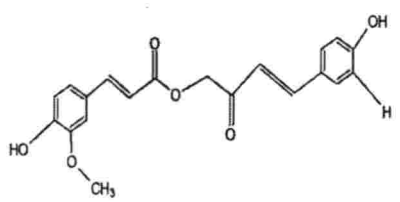


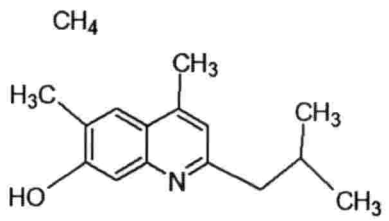
9. 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl) -4,6-heptadiene-3-one



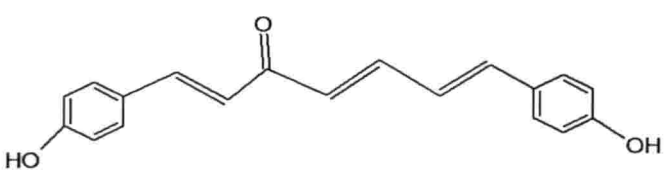
10. 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one



11. 4-(4'-hydroxyphenyl)-2-oxo-3-butenyl-3(4'-hydroxyphenyl-3'-methoxy)-propenoate



12. 2-(2'-methyl-1'-propenyl)-4,6-dimethyl-7-hydroxylquinoline



13. 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one

Figure 2. The structure of phytochemicals created using the tool ChemSketch.

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5. DISCUSSION

The inflammatory condition of liver is generally referred as hepatitis. The common cause of hepatitis is virus. Five different types of viruses such as type A,B,C,D and E are causing hepatitis. Among these, high rate of fatality is caused by hepatitis B virus (HBV). In addition to virus, other causes for hepatitis include toxic substances (e.g., alcohol, certain drugs) and autoimmune diseases. The HBV caused hepatitis induces liver cirrhosis and cellular carcinoma. The WHO (2017) reported that about 240 million people are chronically infected with HBV and in every year over 1,15,000 people die due its infection. HBV is highly endemic in many countries, especially in Asia and Africa, and is common in immigrant populations from endemic countries.

HBV is a DNA virus with partially double stranded circular DNA known as relaxed circular DNA or RC DNA. It codes for four proteins namely polymerase (P), core (C), surface (S) and X proteins. HBV replicates within the host through an intermediate, pregenomic RNA (pgRNA). For this, HBV –RC-DNA is end repaired to produce closed covalent circular DNA (cccDNA) by DNA repair enzyme. The HBV RNA is further transcribed from viral cccDNA using host DNA dependent RNA polymerase in cell nucleus and translated to individual protein in cell cytoplasm. HBV DNA polymerase or P protein has reverse transcriptase activity that helps in the production of viral genomic DNA from pgRNA intermediate. During the process of viral replication, HBV produces three DNA phases namely- RC DNA, cccDNA and linear DNA phase. RC DNA is required for mature virion formation, cccDNA get archived in host cell nucleus in episomal form and linear HBV DNA may integrate into host chromosome. Universal immunization against HBV has led to a dramatic reduction in the number of new cases, but a large number of HBV infected individuals suffer from chronic progressive liver disease leading to cirrhosis and its complications including portal hypertension, variceal hemorrhage and hepatocellular carcinoma. Despite the availability of safe and effective vaccines, a 100% effective antiviral treatment is not yet available for patients with chronic HBV. Current antiviral therapeutic

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agents can control the replication of HBV but not eliminate it because HBV can establish a stable covalently closed circular DNA (cccDNA). Interferon-α treatment can cure Hepatitis B by clearing HBV but it induces systemic side effects. in case of nucleos(t)ide analogs available therapy for HBV which mostly target HBV DNA polymerase by interfering with viral replication or formation of RC DNA from pgRNA. But they have no effect on viral RNA transcription from genomic cccDNA. These drugs control HBV only by reducing the viral load and are not helpful for eradication or cure. Therefore, there is a need for development of drug with novel mechanisms of action to achieve complete cure of HBV. Two possible proposed mechanisms are elucidated by Koniger *et al.* (2014) and Lucifora *et al.* (2014) for clearance of cccDNA. This include development of drug that can inhibit the RC DNA from forming into cccDNA and development of drug that can degrade cccDNA directly.

From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. This relationship has grown between plants and man, and many plants came to be used as drugs. The growth of knowledge to cure disease continues at an accelerating pace, and number of new plant-derived drugs increase likewise. Herbal medicine is currently experiencing a revival in Western society, along with other complementary therapies such as traditional Chinese Medicines, Osteopathy and Homeopathy (Shinwari and Gilani, 2003). Plant based systems continue to play an essential role in health care and their use by different culture had been extensively documented. Among ancient civilization, India has been known to be rich repository of medicinal plants. India has the unique distinction of having six recognised system of medicines. They are Ayurveda, Siddha, Unani and Yoga, Naturopathy and Homeopathy.

All these Indian systems of medicine have primarily claimed a curative potential for their medicinal preparations for all kinds of liver diseases. In spite of

the significant popularity of these medicinal systems, they are still to be recognized as being universally acceptable treatment modalities for chronic liver disease. The limiting factors that contribute to such an eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of randomized placebo controlled clinical trials; and (iii) lack of traditional toxicologic evaluations.

Plants being rich sources of secondary metabolites such as alkaloids, flavanoids, terpenoids, triterpenies, tannins, phenolic compounds, etc. have been used as treatment option including liver ailments (Subin *et al.*, 2016). But the efficacies of these herbal formulations are not scientifically validated due to several reasons such as lack of efficient screening method, high expense, slow and difficulties in executing the experimental works, lack of model organism for testing etc. Medicinal plants, the backbone of traditional medicine with excessive pharmacological studies are the potential source of lead compounds in drug development.

Kerala is blessed with globally accepted unique spices which have been used in the traditional system of medicine for curing many ailments. Of these the selected spices for present study *viz Elettaria cardamomum*, *Curcuma longa* and *Zingiber officinale* have been got popularity in all over the world (Siriruga, 1999; Bhowmik *et al.*, 2009; Kumar *et al.*, 2013).

Computer-aided drug discovery/design methods have played a major role in the development of therapeutically important small molecules for over three decades. These methods are broadly classified as either structure-based or ligand-based methods. Structure-based methods are in principle analogous to high-throughput screening in that both target and ligand structure information is imperative. Structure-based approaches include ligand docking, pharmacophore, and ligand design methods. Ligand-based methods use only ligand information for predicting activity depending on its similarity/dissimilarity to previously known active ligands. The significance of bioinformatics tools on drug discovery was well reviewed (Zagursky and Russell, 2001).

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Fortunately, a number of tools and methods have been developed to address the simple and yet very complex question of identifying a molecular starting point for a drug discovery program. Essentially, there are two general methods utilized in modern drug discovery programs, physical high throughput screening (HTS) methods and virtual high throughput screening methods. Biological screening is often repeated with the "hit" compounds in order to validate the HTS results. In virtual screening, compounds are docked into 3D model of structurally defined biological target and the binding energy of the resulting complex is estimated, allowing compounds to be ranked order. Structural information on biological targets may be available through X-ray crystallography, as a large number of protein crystal structures are available through the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). If a structure is not readily available, it may be possible to create a homology model of the biological target using crystal structure data of a closely related macromolecules. In either case, the individual compounds of the chemical libraries can then be "docked" in a hypothetical binding site in the target of interest to determine a relative rank order for the entire set of compounds. Automated data analysis tools are then employed that organize the predictions provided by the "docking" of the chemical libraries to the hypothetical binding sites of the biological targets. Among the *in silico* screening methods docking is widely applied one in practise. Several docking tools are available currently 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; Kichen *et al.*, 2004). Many authors had utilized the technique for the identification of lead molecules from Indian medicinal plants, which have been used against disease like tuberculosis (Nimmi *et al.*, 2016) hepatitis B (Subin *et al.*, 2016, Shefin *et al.*, 2016) and snakebites in Indian systems of medicine (Nisha *et al.*, 2014; Sreekumar *et al.*, 2014). Therefore, in the present investigation docking method was used for determining the efficacy of anti-hepatitis B plants and identification of lead molecules. Of these autodock is widely

used and was ilustrated by Mihasan (2012). Therefore, in the present study autodock tool Autodock 4.2 was used.

Success of the drug discovery process depends on the identification of the right target protein for *in silico* screening, the 3D structures of the target proteins are to be procured from available databases like PDB or to be created through a series of process such as sequence analysis, determination of closely related sequence, 3D structure modeling and its validation. In this investigation three target proteins from HBV were selected namely HBx, HBc and polymerase (P). The 3D structure of HBc was retrieved from Protein databank (PDB ID: 1QGT) and the structure of HBx and pol was modelled using the software MODELLER. MODELLER is a computer software using homology or comparative modelling of protein 3D structure.

Preparation of the ligand molecules is another important step in docking and in the present study phytochemicals derived from *Elettaria cardamomum*, *Curcuma longa* and *Zingiber officinale* all belongs to the family Zingiberaceae were selected for docking. The available structure of the selected plant-derived phytochemicals were retrieved from open access chemical databases and the remaining compound structures were created using ChemSketch which is an open access user friendly tool. The 2D structures of phytochemicals were converted into 3D structure using the tool CORINA, which is an open access widely used tool for 3D structure generation of small and medium sized chemical molecules especially drug molecules. Several docking tools based on different algorithms are available for docking. However, among the published papers in this line 70% authors used AutoDock tool (Mihasan, 2012). Structural investigation, detection of active site, right binding residues and pharmacophore nature of the target molecules are the key factors for the successful docking which leads to identification of the best lead molecules. It can be achieved by close observation of the target molecules using protein visualization and active site detection tools. For molecular visualization, the tools PyMol and LigPlot were used. All these tools are open access and widely

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used and analysis of the molecules using more than one tools generate more accuracy.

To avoid the unwanted docking exercise, 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). Generally, natural compounds are exception to Lipinski rule of five (Ganesan, 2008), however, due to the limitation in AutoDock tool, compounds with molecular weight more than 600 Da were excluded. Generally, lead molecules are selected based on the following criteria (1) Free energy of binding, which is a measure of the affinity of ligand-protein complex, or is the difference between the energy of complex and the sum of energies of each molecule separately. A docked molecule with the least free energy of binding was always considered as the best lead. (2) Inhibition constant, which is an indication of how potent an inhibitor is, it is the concentration required to produce half maximum inhibition. (3) H bond, which is the strongest type of intermolecular force or dipole interaction make the binding force more stable and (4) hydrophobic interactions. As a general principle the docked structures having ΔGbind less than -5 kcal/ mol were selected as hits or promising lead molecules. In order to find out the molecular interaction of the docked structure the tool LigPlot was used, in addition to H-bond interaction this tool clearly indicated other interactions such as hydrophobic, hydrophilic, van der Waals dispersion forces etc.

A total of 571 compounds from three plants viz *Elettaria cardamomum, Zingiber officinale* and *curcuma longa* were screened against the target proteins such as HBx, HBc and Polymerase. Maximum number of hit molecules was obtained against HBx protein followed by HBc and Polymerase respectively.

Out of 87 phytochemicals from *Elettaria cardamomum* docked with HBx protein, 70 of them showed inhibitory activity ($\Delta G_{bind} \leq$-5.0 kcal/mol). Of these, β-sitosterol was selected as the best lead molecule as it showed least free energy of binding and lowest inhibition constant with 2 hydrogen bond interaction of bond length 3.00 and 2.92. It gave positive values in drug likeness properties. Similarly,

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of the 273 phytochemicals screened from *Zingiber officinale* against HBX, 203 of them were qualified as hit molecules and out of 211 phytochemicals from *Curcuma longa* 198 of them were identified as hit molecules against HBx. In both plants the compound β-carotene was suggested as the best lead since it showed significantly least binding energy (-11.40 kcal/mol) due to the presence of more number of hydrophobic interactions. In general, more number of hydrophobic interactions is considered as a key factor for lead optimization, as this often enhancing ligand molecular weight, rotatable bonds and lipophilicity, these all affecting the ADMET properties of ligands (Chen *et al.*, 2016). It was also noted that the compound β-sitosterol was present in all the three tested plants and the compound was one of the top ranked hit molecules and it showed H-bond interaction with HBx.

Against HBc, the number of hit molecules obtained in the order of merit was 141 from *Curcuma longa*, 156 from *Zingiber officinale* and 49 from *Elettaria cardamomum*. The compound alpha ylangene was selected as the best lead molecule against HBC from the plants *Elettaria cardamomum* and *Zingiber officinale* since alpha-ylangene showed the least free energy of binding (-8.04kcal/mol), least inhibition constant and the presence of more hydrophobic interaction. While from *Curcuma longa* the compound 2-hydroxy-methyl-anthraquinone was selected as the best lead molecule since it showed hydrogen bond interaction with HBC, least binding energy, KI value, more number of hydrophobic interactions when compared to other hits and showed positive value in drug likeness properties.

Comparatively very less number of hit molecules was obtained against the target Polymerase. Maximum number of hit molecules from *Curcuma longa* (12) followed by *Zingiber officinale* (9) and *Elettaria cardamomum* (3). Vanillic acid was present in all the three selected plants and this molecule was found as the best lead against polymerase. However, in *Zingiber officinale* the compound p-hydroxy-benzoicacid and in *Curcuma longa* the compound protocatechuic-acid were equally competent as the best lead.

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It is interesting to note that certain phytochemicals, for example β-sitosterol, stigmasterol and vanillic acid have reported in all these plants and such molecules showed strong inhibitory activity against the target proteins. All the three species tested are spices and members of the family Zingiberaceae and that may be the reason to distribute certain compound as common and effective against a particular virus. The overall results support the traditional knowledge and practice. However false positive and false negative results may be encountered when selection made based on theoretical interpretation. Therefore, based on the theoretical insights *in vitro* and *in vivo* experimental demonstration of the activity is to be inevitable for further confirmation leading to the discovery of novel drug with more effective, faster and affordable drug against hepatitis B.

6. SUMMARY

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Hepatitis B is a viral infection that attacks the liver and is caused by hepatitis B virus of hepadnaviridae family. Based on the period of infection, it can be classified as acute and chronic infections. The virus is transmitted through contact with blood or other body fluids of infected person. It is a global disease and is found in higher risk in countries like South America, Russia, Africa and Asia. India falls into the region of intermediate endemicity and constitute about 15% of the entire pool of HBV. According to WHO, 40 million people are chronically infected with hepatitis and 1, 15, 000 of them die annually. Vaccination has been available for more than 20 years to prevent this disease. The current available therapy for hepatitis B is immunomodulatory treatment with interferon α and nucleo(s)tide analogue treatment. These treatments target the viral replication and inhibit the production of cccDNA, thus decreasing the viral load. The main limitations of current system of treatment are that the antiviral strategies are either poorly effective or only effective for non-curative suppression of viral replication and mainly the formation of new drug resistant mutants. Liver cancer progress rapidly and since treatment option are limited, the outcome is in general poor. Hence, an alternate therapy is needed.

Plants are a storehouse of many chemical molecules that have the potential to be a drug and only few of these compounds have been studied fruitfully. On basis of the traditional knowledge, spices have been used for treating many diseases including liver ailments. On this backdrop, the present investigation was aimed to validate anti-hepatitis B activity and identification of the best lead molecules in three common spices of Kerala belongs to a common family Zingiberaceae through *in silico* method. They were *Elettaria cardamomum, Curcuma longa* and *Zingiber officinale*.

For *in silico* screening, three target proteins such as HBx, HBc and polymerase from HBV were selected as the target molecule. The 3D structure of HBc was retrieved from PDB (PDB id: 1QGT) and the protein HBx and polymerase were modelled using the tool MODELLER 9v.15. For molecular visualization of

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the target the tool PyMol was used. The active site of HBx and HBc was detected using PDB Sum and Poly was detected using CASTp. A total of 571 phytochemicals (*Elettaria cardamomum* -87, *Curcuma* longa -211 and *Zingiber officinale* -273) were selected as ligand for docking. The information on the chemical molecules reported in selected spices was collected through literature survey and databases. The canonical SMILES were retrieved from chemical databases such as ChemSpider, Pubchem etc. Structures of 558 phytochemical were retrieved from databases and the remaining thirteen molecules structures were drawn using the tool ChemSketch. The 3D structures of all phytochemicals were generated as .pdb file format using CORINA. Docking was carried out using the tool AutoDock and detailed molecular visualisation was done using Pymol. All the selected phytochemicals were docked into the binding site of these 3 target protein and the docked structure having binding energy value ≤-5kcal/mol were selected as the hit molecule and from these hit molecule, best lead were selected based on the hydrogen bond, least binding energy and hydrophobic interaction with the active residues.

The result revealed that all three plants have inhibitory effect on the targets, HBx, HBc and Poly. The best lead molecules selected against HBx was β-carotein (ΔG_{bind} ≤-11.40 kcal/mol) derived from *Z. officinale* and *C. longa* followed by β-sitosterol (ΔG_{bind} ≤-8.62 kcal/mol) which was present in all the three plants. Similarly, the best lead obtained against HBc was α-ylangene (ΔG_{bind} ≤-8.04 kcal/mol) from *C. longa* and *Z. officinale* and the compound 2-hydroxy methyl anthraquinone (ΔG_{bind} ≤-8.00 kcal/mol) present in *C. longa*. Against polymerase vanillic acid (ΔG_{bind} ≤-5.50 kcal/mol) was found as the best lead and it was present in all three plants. The compound p-hydroxy-benzoicacid obtained from *Z. officinale* and protocatechuic-acid from *C. longa* were also considered as the best lead. The results support the traditional knowledge and practice. However, based on the foregoing results *in vitro* and *in vivo* experiments are to be essential for further confirmation.

7. REFERENCE

Arooj, M., Sattar, M., Safdar, M., Kalsoom, S., and Shahzad, S. 2012. *In silico* molecular docking and design of anti-hepatitis B drugs. *J. Pharma. Biol. Sci.* 3(6): 41-55.

Asadi-Samani, M., Kafash-Farkhad, N., Azimi, N., Fasihi, A., Alinia-Ahandani, E., and Rafieian-Kopaei, M. 2015. Medicinal plants with hepatoprotective activity in Iranian folk medicine. *Asian Pac. J. Trop. Biomed.* 5(2): 146-157.

Aspinal, E. J., Hawkins, G., Fraser, A., Hutchinson, S., and Goldberg, D. 2011. Hepatitis B prevention, diagnosis, treatment and care: A review. *Occup Med.* 61: 531-540.

Bell, T. G. and Kramis, A. 2016. Bioinformatics-updated features and application. Intech Publishers, South Africa, pp. 177-200.

Bosch, F. X., Ribes, J., Clerics, R., and Diaz, M. 2005. Epidemiology of hepatocellular carcinoma: *Clin Liver Dis*. 9(2):191-211.

Beasley, R. P. 1988. Hepatitis B virus, the major etiology of hepatocellular carcinoma. *Cancer J.* 61(10):1942-1956.

Beck, J. and Nassal, M. 2007. Hepatitis B virus replication. *World J. Gastroenterol.* 13(1): 48.

Bhowmik, D. C., Chandira, R. M., and Kumar, K. P. S. 2009. Recent trends of drug used treatment of tuberculosis. *JOCPR*. 3: 113-115

68

Chan, T.Y. 1997. Monitoring the safety of herbal medicines. *Drug Saf.* 17:209-215.

Dandri, M. and Petersen, J. 2016. Mechanism of hepatitis B virus persistence in hepatocytes and its carcinogenic potential. *Clin. Infect. Dis.* 62(4): S281-S288

Decorsiere, A., Mueller, H., Van Breugel, P. C., Abdul, F., Gerossier, L., Beran, R. K., Livingston, C. M., Niu, C., Fletcher, S., Hantz, O. and Strubin, M. 2016. Hepatitis B virus X protein identifies the Smc 5/6 complex as a host restriction factor. *Nature*, 531(7594): 386-389

Fabricant, D. S. and Farnsworth, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect.* 109(1): 69-75.

Farnsworth, N. R., Akerele, R. O., Bingel, A. S., Soejarto, D. D., Guo, Z. 1985. Medicinal plants in therapy. *Bull. World Health Organ.* 63: 965–981.

Ganesan, A. 2008. The impact of natural products upon modern drug discovery. *Curr. Opin. Chem. Biol.* 12(3): 306-317

Glebe, D. and Urban, S. 2007. Viral and cellular determinants involved in hepadnaviral entry. *World J. Gastroenterol.* 13(1): 22

Gallina, A., Bonelli, F., Zentilin, L., Rindi, G., Muttini, M., and Milanesi, G. 1989. A recombinant Hepatitis B Core Antigen Polypeptide with the Protamine-like Domain Deleted Self-Assembles into Capsid Particles But Fails to Bind Nucleic Acids. *J.Virol.* 63: 4645-4652.

69

Gudunguntla, S., Swarnalatha, D., Shivakala, T., and Mobeena, S. K. 2013. Hepatoprotective activity- A review. *Int J. Phytopharm.* 3(2): 37-49.

Horvart, R. T. 2011. Diagnostic and clinical relevance of HBV mutation. *Lab. Med.* 42(8): 488-496.

Jorgensen, W. L. 2004. The many roles of computation in drug discovery. *Science,* 303(5665): 1813-1818.

Jaya Sankar, R. V., Deval, R. G., and Mallikajuna, K. 2014. A review on hepatoprotective activity of some medicinal plants. *Int. J. Innov. Pharma. Sci.* 5(2): 395-404.

Kalyani, G., Sharma, D., Vaishnav, Y., and Deshmukh, V. S. 2013. A review on drug designing methods, its applications and prospects. *Int. J. Pharm. Res. Dev.* 5: 15-30

Kichen, D. B., Decornez, H., Furr, J. R., and Bajorath, J. 2004. Docking and scoring for drug discovery: methods and applications. *Nat. Rev. Drug Discov.* 3(11): 374-395.

Königer, C., Wingert, I., Marsmann, M., Rösler, C., Beck, J., and Nassal, M. 2014. Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses. *Proc. Natl. Acad. Sci. U S A.* 111:E4244–E4253.

Kumar , S., Sing, U. N., Kiran, S., and Ravi, S. 2013. Supplementation of ginger with anti- tuberculosis treatment (ATT): A better approach to treat anaemic pulmonary tuberculosis patients. *Int. J. Herb. Med.* 1: 17-20

Kshirsagar, A. D., Mohite, R., Aggrawal, A. S., and Suralkar, U., R. 2011. Hepatoprotective medicinal plants of Ayurveda – A review. *Asian J. Pharm. Clin. Res.* 4(3): 1-8.

Koff, R. S. 2001. Hepatitis vaccine. *Infect. Dis. Clin. North Am.* 15(1): 83-95

Lahlou, M. 2013. The success of natural products in drug discovery. *J. Pharm. Pharmacy.* 4:17-31.

Leroux-Roels, G., Cao, T., De Knibber, A., Meuleman, P., Roobrouck, A., Farhoudi, A., Vanlandschoot, P., and Desembere, I. 2001. Prevention of hepatitis B infection: Vaccination and its limitations. *Acta Clin. Belg.* 56(4): 209-219.

Lionta, E., Spyrou, G., Vassilatis, D. K., and Cournia, Z. 2014. Structure-based virtual screening for drug discovery: Principles, application and recent advances. *Curr. Top. Med. Chem.* 14: 1923-1938.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeny P. J. 1997. Experimental and computational approaches to solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* 23: 3-25

Loggi, E., Vitale, G., Conti, F., Bernadi, M., and Andreone, P. 2015. Digestive and liver disease. *Dig liver Dis.* 47: 836-841

Lounnas, V., Ritschel, T., Kelder, J., McGuire, R., Bywater, P. R., and Faloppe, N. 2013. Current progress in structure-based rational drug design marks a new mindset in drug discovery. *Comput. Struct. Biotechnol. J.* 5(6):1-14.

Loi, M.C., Maxia, L., Maxia, A., 2005. Ethno-botanical comparison between the villages of Escolca and Lotzorai (Saridinia, Italy). *J. Herbs Species Med. Plants*. 11(3):67-84

Lucifora, J., Xia, Y., and Reisinger F. 2014. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. *Science,* 343:1221–1228.

Meng, X. Y., Zhang, H. X., Mezei, M., and Cui, M. 2011. Molecular docking: a powerful approach for structure based drug discovery. *Curr. Comput. Aided Drug Discov.* 7(2): 146-157

McMahon, B. J. 2005. Epidermiology and natural history of hepatitis B. *Semin. Liver Dis*. 25(1): 3-8

Murakami, S. 1999. Hepatitis B Virus X protein: structure, function biology. *Intervirol.* 42: 82–99.

Murakami, S., 2001.Hepatitis B Virus X protein: a multifunctional regulatory protein. *J Gastroenterol.* 36: 651–60.

Murakami, S., J. Cheong, and Kaneko, S. 1994. Human hepatitis virus X gene encodes a regulatory domain that represses transactivation of X protein. *J. Biol. chem.* 269:15118-15123

Mazid, M., Khan, T. A. and Mohammad, F. 2012. Medicinal plants of rural India: a review of use by Indian folks. *Indo Global J. Pharm. Sci.* 2(3): 286-304.
Nisha, N. C., Sreekumar, S., Biju, C. K., and Krishnan, P. N. 2010. Snake anti-venom, virtual screening of plant derived molecules. *Biobytes,* 6: 14-22.

Mihasan, M. J. 2012. What in silico docking can do for the bench working biologist? *Biosci.* 37(6): 1089-1095.

Nimmi, H., Sreekumar, S., and Biju, C. K. 2016. *In silico* validation of anti-tuberculosis activity in Andrographis paniculata (Burm. F.) Nees. *Int. J. Pharma. Sci. Drug Res.* 9(4): 201-209

Nisha, N. C., Sreekumar, S., Biju, C. K., and Krishnan, P. N. 2014. Identification of lead compounds with cobra venom neutralizing activity in three Indian medicinal plants. *Int. J. Pharmacy Pharma. Sci.* 6(2): 536-541.

Nguyen, D. H., Ludgate, L. and Hu, J. 2008. Hepatitis B virus- cell interaction and pathogenesis. *J. Cell. Physiol.* 216(2): 289-294

Pandey, G. 2011. Medicinal plants against liver disease. *Int. Res. J. Pharm.* 2(5): 115-121.

Pawlotsky, J. M., Aghemo, A., Back, D., Dusheiko, G., Forns, X., Puoti, M. and Sarrazin, C. 2015. EASL recommendations on treatment of hepatitis C. *J. hepatol.* 63(1): 199-236

Pugazhendhi, D. and Umamaheshwari, T. S. 2013. Insilico methods in drug discovery-A review. *Int. J.* 3(5)

Pathak, R. K., Baunthiyal, M., Taj, G., and Kumar, A. 2014. Virtual screening of natural inhibitors to the predicted HBx protein structure of Hepatitis B Virus using molecular docking for identification of potential lead molecules for liver cancer. *Bioinformation* 10(7): 428-435

Perrillo, R. P. 2005. Current treatment of chronic hepatitis B: Benefits and limitations. *Semin. Liver Dis.* 25:20-28.

Rao, V. S. and Srinivas, K. 2011. Modern drug discovery process: an *insilico* approach. *J. Bioinform. Seq. Anal.* 3(5): 89-94

Ryan, J., Abdul- Basit, C. S. and Herrera, K. 2015. Consideration in first- line therapies for treatment- naïve chronic hepatitis B. *Med. Res. Arch.* (3)

Shefin, B., Sreekumar, S., and Biju, C., K. 2016. Identification of lead molecules with anti-hepatitis B activity in *Bacopa monnieri* (L.) Wettst. and *Cassia fistula* L. through *in silico* method. *J. Pharm. Biol. Sci.* 11(5): 16-21.

Sirirugsa, P. 1998. Thai Zingiberacea: Species diversity and their uses. *Pure Appl. Chem.* 70: 11

Schoichet, B. K. 2004. Virtual screening of chemical libraries. *Nature*, 432: 862-865.

Silwoski, G., Kothiwala, S. K., Meiler, J., and Edward, W. 2014. Computational methods in drug discovery. *Pharmacol. Rev.* 66(1): 334-395.

Shinwari, Z. K., and Gilani, S. S. 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Nothren Pakistan). *J. Ethnopharmacol.* 84: 289-298.

Sreekumar, S., Nisha, N. C., Biju, C. K., and Krishnan, P. N. 2014. Identification of potential lead compounds against cobra venom in Rauvolfia serpentine (L.) Benth. Ex Kurz. through molecular docking. 2014. *Int. J. Pharma. Res. Dev.* 6(8): 32-43.

Subin, M. S., Sreekumar, S., and Biju, C. K. 2016. Identification of lead compounds against human hepatitis B viral capsid protein in the three medicinal plants through *in silico* method. *J. Pharm. Biol. Sci.* 11(3): 1-6.

Tiwar, S. and Singh, A. 2004. Toxic and sub-lethal effects of oleadrin on biochemical parameters of freshwater air breathing murrel, Chant punctatus (Bloch.). *Indian J. Exp. Biolo.* 42:413-418.

Ved, D.K., Mudappa, A, and Shankar, D. 1998. Regulating export of endangered medicinal plant species-need for scientific vigour. *Curr. Sci.* 75: 341-344.

Veeresham, C. 2012. Natural products derived from plants as a source of drugs. *J. Adv. Pharm. Technol. Res.* 3(4): 200–201.

Wang, L., Ma, C., Wipf, P., Liu, H., Su, W. and Xie, X. Q. 2013. TargetHunter: an *in silico* target identification tool for predicting therapeutic potential of small organic molecules based on chemogenomic databases. *AAPS J.* 15(2): 395-406

75

WHO [World Health Organization]. 2016. Hepatitis B. Available: http://www.who. int/media centre/factsheet/fs2014/en/. [18 Oct. 2016].

WHO [World Health Organization]. 2014. Hepatitis B. Available: http://www. searo.who.int/thailand/factsheets/fs0031/en/. [23 June, 2014].

WHO [World Health Organization]. 2018. Hepatitis B. Available: http://www. who.int/news-room/fact-sheets/detail/hepatitis-b. [18 July, 2018].

Xia, X. 2017. Bioinformatics and drug discovery. *Curr. Top. Med. Chem.* 17(15): 1709-1726.

Zagursky, R. J., and Russel, D. 2001. Bioinformatics: use in bacterial vaccine discovery. *Biotechniques*. 31(3): 636-648.

Zoulim, F., Saputelli, J. and Seeger, C. 1994. Woodchuck hepatitis virus X protein is required for viral infection in vivo. *J Virol.* 68(3): 2026–2030

APPENDIX I

Phytochemicals selected from *E. cardamomum, C. longa* and *Z. officinale* along with docked results

Sl. no	Chemical compound	Docked results					
		HBx		HBc		Polymerase	
		ΔG	KI	ΔG	KI	ΔG	KI
	Elettaria cardamomum						
1.	(+)-Alpha-Terpineol $C_{10}H_{18}O$	-5.83	53.60	-6.25	26.63	-4.16	887.1
2.	(+)-Limonene $C_{10}H_{16}$	-5.52	89.85	-6.02	38.95	-4.78	326.6
3.	1,4-Cineole $C_{10}H_{18}O$	-5.51	92.05	-6.29	24.71	-4.18	862.3
4.	1,8-Cineole $C_{10}H_{18}O$	-5.55	85.60	-5.64	73.36	-3.76	1.77
5.	2-Undecane $C_{11}H_{24}$	-5.54	87.28	-5.18	159.1	-3.05	5.85
6.	3-Tridecane $C_{13}H_{28}$	-4.95	234.37	-5.24	143.7	-3.32	3.71
7.	Alpha-Copaene $C_{15}H_{24}$	-7.28	4.65	-7.82	1.85	-5.43	103.8
8.	Alpha-Phellandrene $C_{10}H_{16}$	-5.76	59.60	-5.97	42.22	-3.90	1.39
9.	Alpha-Pinene $C_{10}H_{16}$	-5.62	75.57	-5.67	69.80	-3.76	1.75
10.	Alpha-Terpinene $C_{10}H_{16}$	-5.63	75.23	-6.08	35.19	-4.44	556.9
11.	Alpha-Terpineol $C_{10}H_{18}O$	-5.95	43.85	-6.25	26.16	-3.74	1.81
12.	Alpha-Terpineol Acetate $C_{12}H_{20}O_2$	-6.52	16.55	-7.08	6.51	-4.02	1.13
13.	Alpha-Terpinylacetate $C_{12}H_{20}O_2$	-6.39	20.62	-6.71	12.15	-3.58	2.39
14.	Alpha-Thujene $C_{10}H_{16}$	-5.05	197.26	-5.56	84.18	-3.57	2.40
15.	Alpha-Tocopherol $C_{29}H_{50}O_2$	-6.28	24.87	+0.48		+73.87	
16.	Alpha-Ylangene $C_{15}H_{24}$	-7.38	3.88	-8.04	1.28	-5.21	151.6
17.	Arachidic-Acid $C_{20}H_{40}O_2$	-5.18	160.80	-0.95	200.3	-1.26	118.8
18.	Ascaridole $C_{10}H_{16}O_2$	-6.02	38.79	-6.41	20.15	-3.88	1.43
19.	Beta- Phellandrene $C_{10}H_{16}$	-5.68	68.90	-6.24	26.66	-4.52	488.7
20.	Beta-Pinene $C_{10}H_{16}$	-5.42	106.15	-5.76	59.68	-3.77	1.74
21.	Beta-Sitostenone $C_{29}H_{48}O$	-8.45	639.74	+14.78		+15.35	

22.	Beta-Sitosterol $C_{29}H_{50}O$	-8.60	492.74	-0.75	281.61	+42.59	
23.	Beta-Terpineol $C_{10}H_{18}O$	-5.62	75.52	-5.81	55.17	-3.64	2.14
24.	Bisabolene $C_{15}H_{24}$	-6.94	8.19	-5.09	185.6	-4.97	228.4
25.	Caffeic-Acid $C_9H_8O_4$	-6.07	35.76	-3.93	1.32	-4.56	458.15
26.	Camphene $C_{10}H_{16}$	-5.65	72.46	-5.30	130.1	-3.80	1.65
27.	Camphor $C_{10}H_{16}O$	-5.39	111.20	-5.09	185.6	-3.49	2.76
28.	Caprylic-Acid $C_8H_{16}O_2$	-5.22	148.64	-3.55	2.48	-3.94	1.29
29.	Carvone $C_{10}H_{14}O$	-5.86	50.83	-6.19	29.08	-4.79	307.17
30.	Caryophyllene $C_{15}H_{24}$	-6.23	27.03	-7.26	4.75	-4.24	785.85
31.	Citronellal $C_{10}H_{18}O$	-4.90	256.31	-5.40	109.4	-4.11	976.11
32.	Citronellic-Acid $C_{10}H_{18}O_2$	-6.55	31.07	-4.54	466.6	-3.42	3.10
33.	Citronellol $C_{10}H_{20}O$	-5.30	131.03	-5.27	137.1	-3.75	1.78
34.	Cyanidin $C_{15}H_{11}O_6$	-8.23	925.63	-4.79	308.37	-3.97	1.23
35.	D-Alpha-Terpinylacetate $C_{12}H_{20}O_2$	-6.46	18.51	-7.09	6.33	-3.97	1.23
36.	Decanoic-Acid $C_{10}H_{20}O_2$	-5.64	72.84	-3.83	1.55	-4.40	593.62
37.	Delta-Limonene $C_{10}H_{16}$	-5.47	97.34	-6.02	38.97	-4.58	400.25
38.	Delta-Terpineol $C_{10}H_{18}O$	-5.78	58.31	-6.26	25.84	-3.21	392.12
39.	Desmosterol $C_{27}H_{44}O$	-7.91	1.60	-2.03	32.67	+90.6	
40.	Eugenyl-Acetate $C_{12}H_{14}O_3$	-6.07	35.79	-6.63	13.82	-3.68	2.00
41.	Gamma-Terpinene $C_{10}H_{16}$	-5.59	79.25	-6.08	35.09	-4.09	1.01
42.	Gamma-Tocopherol $C_{28}H_{48}O_2$	-7.22	5.11	+5.34		60.96	
43.	Geranic-Acid $C_{10}H_{16}O_2$	-6.26	25.65	-4.80	304.85	-3.86	1.47
44.	Geraniol $C_{10}H_{18}O$	-5.81	55.09	-5.53	88.21	-3.94	1.30
45.	Geranyl-Acetate $C_{12}H_{20}O_2$	-5.57	83.09	-5.58	81.24	-4.77	316.72
46.	Heptanoic-Acid $C_7H_{14}O_2$	-4.94	241.11	-3.33	3.61	-4.08	407.64
47.	Hexanoic-Acid $C_6H_{12}O_2$	-4.41	585.35	-3.00	6.32	-3.87	1.46

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48.	Humulene $C_{15}H_{24}$	-6.41	20.02	-6.94	8.13	-3.77	1.72
49.	Humulene-Oxide $C_{15}H_{24}O$	-6.26	25.95	-6.90	8.76	-4.04	1.09
50.	Lauric-Acid $C_{12}H_{24}O_2$	-5.97	41.85	-4.51	496.18	-4.51	490.61
51.	Limonene $C_{10}H_{16}$	-5.50	92.34	-6.01	39.06	-4.61	416.47
52.	Linalool $C_{10}H_{18}O$	-4.71	353.93	-5.42	105.77	-3.26	4.09
53.	Linalool-Acetate $C_{12}H_{20}O_2$	-5.36	117.96	-5.86	50.58	-3.61	2.27
54.	Linoleic-Acid $C_{18}H_{32}O_2$	-5.68	68.12	-4.31	698.31	-3.63	2.20
55.	Linolenic-Acid $C_{18}H_{30}O_2$	-5.23	145.60	-5.30	130.72	-4.42	577.71
56.	Lysophosphatidylcholine $C_{10}H_{22}NO_7P$	-3.93	1.32	-3.01	6.25	-0.50	427.61
57.	Myrcene $C_{10}H_{16}$	-4.98	224.82	-5.51	90.76	-3.75	1.79
58.	Myristic-Acid $C_{14}H_{28}O_2$	-5.78	57.61	-4.19	847.38	-4.39	603.18
59.	Myrtenal $C_{10}H_{14}O$	-6.28	24.78	-5.49	94.13	-3.71	1.89
60.	N-Hentriacontene $C_{31}H_{64}$	-2.39	17.63	+28.49		+38.5	
61.	N-Heptacosane $C_{27}H_{56}$	-2.56	13.38	+7.35		-3.84	1.53
62.	N-Heptane C_7H_{16}	-4.71	353.17	-3.86	1.49	-3.84	1.54
63.	N-Nonacosane $C_{29}H_{60}$	-2.36	18.48	+18.49		+35.0	
64.	N-Pentacosane $C_{25}H_{52}$	-3.68	2.01	+3.77		+3.80	
65.	N-Tricosane $C_{23}H_{48}$	-3.35	3.50	-4.15	902.79	-0.55	397.09
66.	N-Tritriacontane $C_{33}H_{68}$	-2.42	16.94	+39.64		+34.6	
67.	Nerol $C_{10}H_{18}O$	-5.11	180.91	-5.43	104.31	-3.71	1.89
68.	Nerolidol $C_{15}H_{26}O$	-6.25	26.28	-5.95	43.70	-3.31	3.77
69.	Neryl-Acetate $C_{12}H_{20}O_2$	-5.37	115.30	-5.85	51.11	-4.63	404.66

70.	Nerylic-Acid $C_{26}H_{52}O_2$	-3.20	4.52	+10.94		+54.4	
71.	Niacin $C_6H_5NO_2$	-4.87	269.63	-3.72	1.88	-4.82	295.03
72.	Octan-1-ol $C_8H_{18}O$	-4.54	466.54	-4.13	935.31	-3.02	6.15
73.	Oleic-Acid $C_{18}H_{34}O_2$	-5.35	118.8	-4.25	770.90	-2.76	9.45
74.	P-Coumaric-Acid $C_9H_8O_3$	-6.23	26.95	-4.28	723.48	-4.26	750.11
75.	P-Cymene $C_{10}H_{14}$	-5.34	121.06	-5.72	64.37	-4.15	912.25
76.	P-Menth-Cis-2-En-1-Ol $C_{10}H_{18}O$	-6.22	55.73	-5.86	50.44	-3.69	1.96
77.	P-Menth-Trans-2-En-1ol $C_{10}H_{18}O$	-5.78	58.37	-5.94	43.96	-3.78	1.70
78.	P-Menthene $C_{10}H_{18}$	-5.59	79.55	-6.18	29.64	-4.67	378.18
79.	Perillic-Acid $C_{10}H_{14}O_2$	-6.22	79.55	-5.33	123.79	-4.73	338.39
80.	Sabinene $C_{10}H_{16}$	-5.46	99.43	-5.80	55.63	-4.54	466.30
81.	Sinapic-Acid $C_{11}H_{12}O_5$	-6.28	25.13	-4.02	1.12	-4.08	1.03
82.	Stearic-Acid $C_{18}H_{36}O_2$	-5.09	185.63	-3.16	4.79	-2.29	20.94
83.	Stigmasterol $C_{29}H_{48}O$	-8.22	945.74	+7.94		283.5	
84.	Terpinen-4-Ol $C_{10}H_{18}O$	-5.59	80.10	-5.80	56.24	-3.58	2.40
85.	Terpineol-Formiate $C_{11}H_{18}O_2$	-6.07	35.39	-6.45	18.75	-4.00	1.16
86.	Trans-Sabinenehydrate $C_{10}H_{18}O$	-5.32	126.41	-5.40	110.13	-3.24	4.23
87.	Vanillic-Acid $C_8H_8O_4$	-5.55	85.74	-4.17	881.95	-5.50	92.28

	Curcuma longa						
1.	(+)-(S)-Ar-Turmerone $C_{15}H_{20}O$	-6.47	18.18	-7.02	7.19	-3.21	2.54
2.	(+)-Alpha-Phellandrene $C_{10}H_{16}$	-5.76	60.15	-5.97	42.22	-4.23	351.34
3.	(1,2,3-Trimethyl-Cyclopent-2-Enyl)-Methanol $C_9H_{16}O$	-5.66	70.81	-5.14	170.80	-3.70	1.96
4.	(1E,4E)-1-(4-Hydroxy-3-Methoxyphenyl)-5-(4-Hydroxyphenyl)-1,4-Pentadien-3-One $C_{18}H_{16}O_4$	-6.84	9.69	-5.34	122.37	-4.69	363.86
5.	(1E,4E)-1,5-Bis(4-Hydroxyphenyl)-1,4-Pentadien-3-One $C_{17}H_{14}O_3$	-7.63	2.56	-5.58	80.77	-4.62	413.79
6.	(2-Methylpropenyl)Benzene $C_{10}H_{12}$	-5.21	150.80	-5.16	166.13	-4.09	996.17
7.	(4s,5s)-(+)-Germacrone 4,5-Epoxide $C_{15}H_{22}O_2$	-5.66	70.44	-5.65	71.79	-3.68	2.00
8.	(E)-Gamma-Bisabolene $C_{15}H_{24}$	-6.73	11.75	-7.38	3.88	-3.18	1.65
9.	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-Pentaene $C_{20}H_{32}$	-7.73	2.14	-6.52	16.71	-4.47	524.65
10.	(Z)-Cinerone $C_{10}H_{14}O$	-5.46	99.12	-6.01	39.60	-4.40	590.86
11.	1-(3-Cyclopentylpropyl)-2,4-Dimethylbenzene $C_{16}H_{24}$	-7.72	2.18	-7.57	2.81	-5.37	115.84
12.	1-(4-Hydroxy-3-Methoxyphenyl)-7-(3, 4-Dihydroxyphenyl)-1, 6-Heptadiene-3, 5-Dione $C_{20}H_{18}O_6$	-6.75	11.21	-5.34	122.37	-3.87	2.35
13.	1,10-Dehydro-10-Deoxy-9-Oxozedoarondiol $C_{16}H_{22}O_3$	-7.24	4.92	-3.94	1.29	-3.33	3.65
14.	1,5-Bis-(4-Hydroxy-3-Methoxyphenyl)-1,4-Pentadien-3-One $C_{19}H_{18}O_5$	-6.41	19.93	-4.66	385.92	-1.12	150.86
15.	1,5-Dihydroxy-1-(4-Hydroxy-3-Methoxyphenyl)-7-(4-Hydroxyphenyl)-4,6-Heptadiene-3-One $C_{21}H_{22}O_7$	-6.47	18.15	-0.75	281.00	+45.34	

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16.	1,5-Dihydroxy-1-(4-Hydroxyphenyl)-7-(4-Hydroxy-3-Methoxyphenyl)-4,6-Heptadiene-3-One $C_{10}H_{16}O_4$	-6.84	9.62	+1.90		+35.4	
17.	1,7-Bis-(4-Hydroxy-3-Methoxyphenyl)-1,4,6-Heptatrien-3-One $C_{21}H_{20}O_5$	-6.35	22.15	+0.40		-3.85	1.51
18.	1,7-Bis(4-Hydroxyphenyl)-1,4,6-Heptatrien-3-One $C_{19}H_{16}O_3$	-7.76	2.05	+11.83		-3.02	1.65
19.	1,7-Bis(4-Hydroxyphenyl)-1-Heptene-3,5-Dione $C_{19}H_{18}O_4$	-7.01	7.28	+9.15		+34.37	
20.	1,8-Cineole $C_{10}H_{18}O$	-5.55	86.15	+18.56		-2.01	1.29
21.	1-Methyl-2-Isopropylbenzene $C_{10}H_{14}$	-5.48	96.81	-5.56	84.58	-3.65	2.10
22.	1-Methyl-3-Isopropylbenzene $C_{10}H_{14}$	-5.49	95.30	-5.71	64.96	-4.81	297.72
23.	2-(2,5-Dihydroxy-4-Methylcyclohex-3-Enyl)Propanoic Acid $C_{10}H_{16}O_4$	-5.90	47.05	-4.77	320.4	-4.37	232.02
24.	2-(2'-Methyl-1'-Propenyl)-4, 6-Dimethyl-7-Hydroxyquinoline $C_{15}H_{19}O$	-7.37	3.95	-5.95	71.79	-4.87	254.78
25.	2,2,4-Trimethyl-3-(3,8,12,16-Tetramethyl-Heptadeca-3,7,11,15-Tetraenyl)-Cyclohexanol $C_{30}H_{52}O$	-6.45	18.58	-4.64	398.31	+195.3	
26.	2,2'-Oxybis[Octahydro-7,8,8-Trimethyl-4,7-Methanobenzofuran $C_{27}H_{40}O_3$	-7.67	2.37	-4.57	449.70	220.50	
27.	2,3,5-Trimethylfuran $C_7H_{10}O$	-5.32	125.78	-4.88	263.54	-4.15	909.59
28.	2,4-Dimethyl-3-Nitrobicyclo[3.3.1]Nonan-9-One $C_{11}H_{17}NO_3$	-6.99	7.52	-3.51	2690	-4.71	352.76
29.	2,4-Dimethyl-8-Oxabicyclo[3.2.1]Oct-6-En-3-One $C_9H_{12}O_2$	-4.68	371.28	-4.08	1030	-3.51	2.66
30.	2,6-Dimethyl-2,6-Octadiene-1,8-Diol	-6.76	11.12	-4.33	665.21	-4.01	1.15

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	$C_{10}H_{18}O_2$						
31.	2-Bornanol $C_{10}H_{18}O$	-5.26	139.51	-4.66	380.87	-3.38	3.34
32.	2-Carene $C_{10}H_{16}$	-5.28	135.77	-5.88	48.56	-3.83	1.57
33.	2-Hydroxy-Methyl-Anthraquinone $C_{15}H_{10}O_3$	-7.86	1.74	-8.00	1.37	-4.33	673.14
34.	2-Methoxy-5-Hydroxybisabola-3,10-Diene-9-One $C_{16}H_{26}O_3$	-6.98	12.66	-3.59	1.43	-3.84	2.5
35.	2-Methyl-6-(4-Formylphenyl)-2-Hepten-4-One $C_{16}H_{22}O_2$	-7.18	5.45	-4.60	373.9	-3.97	1.98
36.	3,3,5-Trimethyl-Cyclohexanol Acetate $C_{14}H_{18}O_2$	-6.24	26.70	-3.05	2.42	-3.68	2.00
37.	3,4,5,6-Tetramethyl-2,5-Octadiene $C_{12}H_{22}$	-5.34	121.12	-3.08	111.80	-3.82	1.59
38.	4"-(4"'-Hydroxyphenyl)-2"-Oxo-3"-Butenyl-3-(4'-Hydroxyphenyl-3'-Methoxy)-Propenoate $C_{20}H_{18}O_6$	-7.43	3.59	+28.9		-3.68	1.74
39.	4"-(4"'-Hydroxyphenyl-3-Methoxy)-2"-Oxo-3"-Butenyl-3-(4'-Hydroxyphenyl)-Propenoate $C_{20}H_{18}O_2$	-6.50	17.15	+33.2		-3.72	1.86
40.	4, 5-Dihydroxybisabola-2,10-Diene $C_{15}H_{26}O_6$	-7.12	6.05	-4.14	358.2	-2.94	1.64
41.	4,5-Dimethyl-2,6-Octadiene $C_{10}H_{18}$	-5.39	111.80	-4.28	488.2	-3.84	1.54
42.	4,8-Dimethyl-3,7-Nonadien-2-Ol $C_{11}H_{20}O$	-5.81	55.42	-4.02	589.4	-4.30	709.15
43.	4-Hydroxybisabola-2,10-Diene-9-One $C_{14}H_{22}O_2$	-7.09	6.33	-4.59	324.8	129.54	
44.	5-Hydroxyl-1-(4-Hydroxy-3-Methoxyphenyl)-7-(4-Hydroxyphenyl)-4,6-Heptadiene-3-One $C_{21}H_{24}O_6$	-5.64	73.66	-3.28	2.8	225.45	
45.	5-Hydroxyl-7-(4-Hydroxy-3-Methoxyphenyl)-1-(4-Hydroxyphenyl)-4,6-Heptadiene-3-One	-6.14	31.36	-3.89	1.42	247.21	

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	$C_{20}H_{22}O_5$						
46.	6-Cubebene $C_{15}H_{24}$	-6.87	9.13	-6.83	9.88	-4.72	347.34
47.	7-Epi-Sesquithujene $C_{15}H_{24}$	-6.39	20.65	-7.04	6.94	-4.62	410.40
48.	Acoradiene $C_{15}H_{24}$	-6.24	26.47	-7.13	5.90	-4.64	394.43
49.	Adoxal $C_{14}H_{26}O$	-5.78	58.42	-5.64	73.33	-3.81	1.62
50.	Alpha Selinene $C_{15}H_{24}$	-7.22	5.14	-7.33	4.24	-4.42	578.79
51.	Alpha-Atlantone $C_{15}H_{22}O$	-6.86	9.36	-7.10	6.26	-4.22	803.02
52.	Alpha-Bergamotene $C_{15}H_{24}$	-6.47	18.05	-7.07	6.57	-4.79	309.76
53.	Alpha-Curcumene $C_{15}H_{22}$	-6.67	12.93	-7.07	6.56	-4.84	281.83
54.	Alpha-Farnesene $C_{15}H_{24}$	-6.19	28.89	-6.21	27.87	-4.41	1.12
55.	Alpha-Pinene $C_{10}H_{16}$	-5.62	75.62	-5.68	69.18	-3.72	1.86
56.	Alpha-Santalene $C_{15}H_{24}$	-6.17	30.00	-7.20	5.27	-5.02	208.34
57.	Alpha-Santalol $C_{15}H_{24}O$	-6.19	29.22	-6.88	9.11	-5.04	201.36
58.	Alpha-Terpinene $C_{10}H_{16}$	-5.60	78.72	-6.08	35.19	-4.44	556.67
59.	Alpha-Terpineol $C_{10}H_{18}O$	-5.38	113.90	-6.25	26.16	-3.70	1.93
60.	Alpha-Thujene $C_{10}H_{16}$	-5.05	199.30	-5.56	84.18	-3.72	1.88
61.	Alpha-Tocopherol $C_{29}H_{50}O_2$	-6.61	14.29	+0.48		+52.35	
62.	Arabinose $C_5H_{10}O_5$	-2.99	6.48	-2.62	12.01	-1.24	124.10
63.	Aristolene $C_{15}H_{24}$	-7.35	4.10	-7.68	2.36	-4.50	502.66
64.	Ascaridole $C_{10}H_{16}O_2$	-6.25	26.29	-6.41	20.15	-4.62	411.19
65.	Azulene $C_{10}H_8$	-5.65	72.41	-5.79	57.23	-4.24	780.90
66.	Benzene, 1-Methyl-4-(1-Methylpropyl) $C_{11}H_{16}$	-5.47	98.37	-6.10	33.96	-4.01	1.14
67.	Beta-Bisabolene $C_{15}H_{24}$	-6.92	8.44	-7.15	5.73	-4.91	250.09
68.	Beta-Carotene $C_{40}H_{56}$	11.40	0.004	410800		343423	
69.	Beta-Caryophyllene $C_{15}H_{24}$	-6.23	26.91	-7.26	4.76	-4.24	785.74
70.	Beta-Curcumene $C_{15}H_{24}$	-6.48	17.94	-7.22	6.08	-4.87	268.46

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71.	Beta-Elemene $C_{15}H_{24}$	-6.33	22.97	-6.85	9.54	-4.12	959.51
72.	Beta-Myrcene $C_{10}H_{16}$	-5.38	113.61	-5.51	91.74	-3.71	1.92
73.	Beta-Phellandrene $C_{10}H_{16}$	-5.71	64.86	-6.26	26.66	-4.46	537.18
74.	Beta-Pinene $C_{10}H_{16}$	-5.28	135.92	-5.76	59.68	-3.77	1.73
75.	Beta-Sesquiphellandrene $C_{15}H_{24}$	-6.56	15.55	-7.74	2.14	-3.89	1.41
76.	Beta-Sitosterol $C_{29}H_{50}O$	-8.57	299.33	-0.75	281.61	374.73	
77.	Beta-Turmerone $C_{15}H_{22}O$	-7.04	6.87	-7.76	2.06	-5.22	148.56
78.	Bis-(4-Hydroxycinnamoyl)-Methane $C_{19}H_{16}O_4$	-8.07	1.21	-6.03	37.77	-4.44	559.68
79.	Bisabola-3,10-Dien-2- One $C_{15}H_{24}O$	-6.91	8.65	-7.48	3.29	-5.63	74.69
80.	Bisabolene $C_{15}H_{24}$	-6.68	12.70	-5.09	185.61	-5.06	194.70
81.	Bisacumol $C_{15}H_{22}O$	-6.97	7.79	-7.01	7.29	-3.85	1.51
82.	Bisacurone $C_{15}H_{24}O_3$	-6.87	9.19	-7.18	5.49	-4.03	1.11
83.	Bis-Demethoxycurcumin $C_{19}H_{16}O_4$	-8.32	791.10	-5.91	46.68	-5.42	106.08
84.	Borneol $C_{10}H_{18}O$	-5.38	113.93	-4.95	234.29	-4.72	348.97
85.	Bornyl Acetate $C_{12}H_{20}O_2$	-5.57	83.11	-5.83	53.21	-4.33	666.48
86.	Caffeic-Acid $C_9H_8O_4$	-6.25	26.03	-3.93	1.32	-4.40	596.82
87.	Calebin-A $C_{21}H_{20}O_7$	-7.18	5.42	+0.43		-1.73	54.39
88.	Campesterol $C_{28}H_{48}O$	-8.88	308.78	+22.86		-3.62	2.23
89.	Camphene $C_{10}H_{16}$	-5.65	72.45	-5.30	130.19	-3.80	1.65
90.	Camphor $C_{10}H_{16}O$	-5.39	111.32	-5.09	185.61	-3.49	2.76
91.	Caprylic-Acid $C_8H_{16}O_2$	-5.23	146.44	-3.55	2.48	-3.96	1.24
92.	Car-3-Ene $C_{10}H_{16}$	-5.36	117.68	-5.89	48.10	-3.70	1.93
93.	Carvacrol $C_{15}H_{22}$	-6.70	12.31	-7.05	6.85	-4.87	270.17
94.	Caryophyllene $C_{15}H_{24}$	-6.18	29.46	-7.26	4.75	-4.24	785.85
95.	Caryophyllene Oxide $C_{15}H_{24}O$	-6.55	15.68	-5.49	95.08	-3.60	2.30
96.	Chrysanthenyl Acetate $C_{12}H_{18}O_2$	-6.03	38.23	-6.13	32.06	-4.34	654.70

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97.	Cineol $C_{10}H_{18}O$	-5.53	87.86	+18.56		-3.75	1.79
98.	Cinnamic-Acid $C_9H_8O_2$	-5.84	52.23	-4.68	373.47	-4.78	316.09
99.	Citral $C_{10}H_{16}O$	-5.15	167.08	-5.57	82.70	-4.26	758.95
100.	Citronellal $C_{10}H_{18}O$	-5.66	70.73	-5.40	109.45	-4.11	976.11
101.	Citronellyl Valerate $C_{15}H_{28}O_2$	-5.40	110.71	-5.85	51.91	-3.31	3.77
102.	Corymbolone $C_{15}H_{24}O_2$	-6.07	35.80	-6.89	8.93	-3.86	1.47
103.	Cuminyl-Alcohol $C_{10}H_{14}O$	-5.65	72.71	-5.64	73.55	-4.02	1.13
104.	Curcumene $C_{15}H_{22}$	-6.69	12.48	-6.97	7.82	-4.91	250.43
105.	Curcumenol $C_{15}H_{22}O_2$	-7.49	3.24	-5.84	52.64	-4.51	490.60
106.	Curcumenone $C_{15}H_{22}O_2$	-7.43	3.60	-7.44	3.52	-4.79	3.8.79
107.	Curcumin $C_{21}H_{20}O_6$	-7.89	1.66	+1.28		-3.19	4.63
108.	Curcumol $C_{15}H_{24}O_2$	-6.80	10.38	-6.26	25.87	-4.73	342.98
109.	Curcuphenol $C_{15}H_{22}O$	-6.65	13.28	-7.28	4.59	-4.65	388.46
110.	Curdione $C_{15}H_{24}O_2$	-6.44	19.14	-7.57	2.83	-4.20	834.87
111.	Curlone $C_{15}H_{22}O$	-7.02	7.11	-7.71	2.23	-4.52	486.15
112.	Curzerenone $C_{15}H_{18}O_2$	-6.25	26.34	-6.50	17.23	-3.68	2.01
113.	Cyclocurcumin $C_{21}H_{20}O_6$	-7.45	3.49	-0.17	746.38	-4.11	971.12
114.	Cyclohexene $C_{10}H_{16}$	-5.81	55.38	-5.99	41.01	-4.63	405.34
115.	Cyclohexyl Formate $C_7H_{12}O_2$	-5.23	147.60	-4.66	383.95	-4.39	601.02
116.	D-Alpha-Phellandrene $C_{10}H_{16}$	-5.77	59.14	-5.97	42.19	-4.25	770.08
117.	D-Camphene $C_{10}H_{16}$	-5.65	72.60	-5.30	-5.30	-2.96	6.81
118.	D-Camphor $C_{10}H_{16}O$	-5.37	115.21	-5.07	191.14	-3.49	2.77
119.	D-Carvone $C_{10}H_{14}O$	-6.01	39.42	-6.19	29.07	-4.82	292.94
120.	Decaprenoic Acid $C_{10}H_{16}O_2$	-6.13	32.22	-4.71	355.14	-4.36	638.68
121.	Dehydrocurdione $C_{15}H_{22}O_2$	-6.43	19.26	-5.15	166.78	-3.94	1.29
122.	Dehydrozingerone $C_{11}H_{12}O_3$	-6.38	20.88	-5.58	80.71	-4.36	638.17
123.	Demethoxycurcumin $C_{20}H_{18}O_5$	-8.06	1.23	-6.16	30.65	-3.67	2.04

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124.	Desmethoxycurcumin $C_{20}H_{18}O_5$	-8.13	1.09	+1.38		-0.30	599.19
125.	Dicinnamoylmethane $C_{19}H_{16}O_2$	-8.12	1.11	-6.67	12.81	-5.44	103.01
126.	Dicumyl Peroxide $C_{18}H_{22}O_2$	-6.95	8.04	-7.87	1.69	-4.61	418.48
127.	Diferuloyl-Methane $C_{21}H_{20}O_6$	-6.71	12.10	-2.22	23.46	-4.54	471.43
128.	Dihydrocarvone $C_{10}H_{16}O$	-5.71	65.06	-6.11	32.96	-5.86	50.31
129.	Dihydrocurcumin $C_{21}H_{22}O_6$	-6.58	15.12	-3.62	2.22	-3.94	1.30
130.	D-Piperitone $C_{10}H_{16}O$	-5.90	47.12	-6.34	22.57	-4.81	298.41
131.	D-Sabinene $C_{10}H_{16}$	-5.19	158.19	-5.80	55.58	-3.81	1.62
132.	Eucalyptol $C_{10}H_{18}O$	-5.55	85.56	-5.39	111.74	-3.76	1.76
133.	Eudesma-3,7(11)-Diene $C_{15}H_{24}$	-7.14	5.79	-6.99	7.51	-4.77	321.04
134.	Eugenol $C_{10}H_{12}O_2$	-5.57	83.17	-5.64	73.34	-3.80	1.65
135.	Farnesol $C_{15}H_{26}O$	-6.16	30.34	-5.78	58.17	-4.53	479.54
136.	Ferulic Acid $C_{10}H_{10}O_4$	-6.12	32.73	-4.05	1.08	-4.13	935.49
137.	Feruloyl-Pcoumaroyl-Methane $C_{20}H_{18}O_5$	-8.80	357.20	-5.72	64.15	-4.34	662.73
138.	Gamma Elemene $C_{15}H_{24}$	-6.93	8.39	-6.44	19.03	-3.94	1.28
139.	Gamma-Atlantone $C_{15}H_{22}O$	-6.84	9.68	-7.55	2.92	-4.93	242.71
140.	Gamma-Curcumene $C_{15}H_{24}$	-6.41	20.10	-7.35	4.10	-5.20	153.46
141.	Gamma-Terpinene $C_{10}H_{16}$	-5.59	79.29	-6.08	35.09	-4.09	1.01
142.	Gamma-Terpineol $C_{10}H_{18}O$	-5.80	56.12	-6.04	37.41	-3.99	1.20
143.	Geraniol $C_{10}H_{18}O$	-5.57	83.07	-5.53	88.21	-3.94	1.30
144.	Geraniol Acetate $C_{12}H_{20}O_2$	-5.33	124.55	-5.72	63.86	-3.56	2.45
145.	Germacrene $C_{15}H_{24}$	-6.55	15.86	-7.25	4.88	-4.02	1.14
146.	Germacrene D $C_{15}H_{24}$	-7.45	3.44	-7.34	4.18	-4.54	469.46
147.	Germacron-(4s',5s)Epoxide $C_{15}H_{22}O_2$	-6.49	17.36	-4.93	244.64	-3.46	2.89
148.	Germacrone $C_{15}H_{22}O$	-7.06	6.69	-6.99	7.56	4.38	616.97
149.	Gitoxigenin $C_{23}H_{34}O_5$	-8.89	303.54	+14.79		+57.19	

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150.	Guaiacol $C_7H_8O_2$	-5.33	124.37	-4.49	510.94	-4.28	727.40
151.	Guaiane $C_{15}H_{28}$	-7.11	6.18	-7.65	2.47	-4.55	464.61
152.	Himachalene $C_{15}H_{24}$	-6.82	10.05	-7.36	4.02	-4.77	317.32
153.	hop-17(21)-en-3beta-ol $C_{30}H_{50}O$	-9.30	151.88	156.79		608.02	
154.	Hopenone I $C_{30}H_{48}O$	10.38	24.77	-5.92	38.24	785.65	
155.	Humulene $C_{15}H_{24}$	-6.61	14.38	-6.94	8.13	-3.77	1.72
156.	Iso-Artemisia Ketone $C_{10}H_{16}O$	-5.51	91.26	-5.88	48.73	-3.35	3.53
157.	Isoborneol $C_{10}H_{18}O$	-5.20	155.00	-4.67	377.51	-3.38	3.31
158.	Isoprocurcumenol $C_{15}H_{22}O_2$	-6.43	19.30	-5.90	47.54	-3.91	1.36
159.	L-Alpha-Curcumene $C_{15}H_{22}$	-6.62	14.11	-7.08	6.47	-4.98	225.29
160.	Limonene $C_{10}H_{16}$	-5.57	83.26	-6.01	39.06	-4.61	416.47
161.	Linalool $C_{10}H_{18}O$	-4.78	313.71	-5.42	105.77	-3.26	4.09
162.	Linoleic Acid $C_{18}H_{32}O_2$	-4.35	643.15	-4.31	698.31	-3.06	5.74
163.	Lupeol $C_{30}H_{50}O$	-9.71	76.36	169.18		+931.9	
164.	Menthofuran $C_{10}H_{14}O$	-5.93	44.81	-6.47	18.16	-5.04	203.39
165.	Menthol $C_{10}H_{20}O$	-6.02	38.81	-6.66	13.07	+27.01	
166.	Methyleugenol $C_{11}H_{14}O_2$	-5.17	163.29	-5.49	94.32	-3.35	3.50
167.	Monodemethoxycurcumin $C_{20}H_{18}O_5$	-7.32	4.32	-6.13	32.08	-3.89	1.41
168.	Nerolidyl Propionate $C_{18}H_{30}O_2$	-5.77	59.19	-6.32	23.42	-3.54	2.53
169.	Nerylacetone $C_{13}H_{22}O$	-6.12	32.90	-6.11	33.18	-4.41	584.26
170.	Niacin $C_6H_5NO_2$	-5.05	197.54	-3.72	1.88	-4.82	295.03
171.	O-Coumaric-Acid $C_9H_8O_3$	-6.20	28.74	-4.29	717.67	-4.82	293.26
172.	Palmitic Acid $C_{16}H_{32}O_2$	-5.15	168.87	-4.71	873.52	-3.06	5.69
173.	P-Coumaric-Acid $C_9H_8O_3$	-6.11	32.96	-4.28	770.90	-4.44	558.03
174.	P-Coumaroylferuloyl-Methane $C_{20}H_{18}O_5$	-7.65	2.48	-3.29	3.87	-3.79	1.66
175.	P-Cymen-8-Ol $C_{10}H_{14}O$	-5.49	95.27	-5.78	58.23	-4.29	717.28

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176.	P-Cymene $C_{10}H_{14}$	-5.34	121.09	-5.72	64.37	-4.15	912.25
177.	Phellandral $C_{10}H_{16}O$	-6.14	31.58	-6.20	28.45	-3.90	1.39
178.	Phytol $C_{20}H_{40}O$	-5.13	173.62	-4.89	262.32	-3.40	3.24
179.	Piperitone-Epoxide $C_{10}H_{16}O_2$	-5.64	73.60	-6.24	26.48	-3.90	1.38
180.	P-Mentha-1,4(8)-Diene $C_{10}H_{16}$	-5.51	91.12	-6.19	28.84	-4.53	475.17
181.	P-Methoxy-Cinnamicacid $C_{10}H_{10}O_3$	-6.06	36.27	-4.49	509.98	-4.54	466.97
182.	P-Methylacetophenone $C_9H_{10}O$	-5.43	105.06	-5.52	90.30	-4.34	663.34
183.	Procurcumadiol $C_{15}H_{22}O_3$	-7.44	3.52	-5.99	40.54	-3.57	2.40
184.	Procurcumenol $C_{15}H_{22}O_2$	-7.05	6.74	-5.96	42.64	-4.48	517.00
185.	Protocatechuic-Acid $C_7H_6O_4$	-5.30	129.31	-3.97	1.24	-5.75	60.50
186.	P-Tolylmethylcarbinol $C_9H_{12}O$	-5.42	107.26	-5.28	134.01	-4.48	520.22
187.	Pyrazolo[1,5-A]Pyridine, 3,3a,4,7-Tetrahydro-3,3-Dimethyl-, (3as)-, 3,3-Dimethyl-4,7-Dihydro-3ah-Pyrazolo[1,5-A]Pyridine $C_9H_{14}N_2$	-5.53	88.22	-5.96	42.64	-4.23	798.02
188.	Quercetin $C_{15}H_{10}O_7$	-6.73	11.75	-4.17	870.70	-3.71	4.76
189.	R-Citronellene $C_{10}H_{18}$	-5.31	125.77	-5.53	88.37	-3.47	2.84
190.	Riboflavin $C_{17}H_{20}N_4O_6$	-5.47	97.46	-2.87	7.82	+2.45	
191.	Stearic Acid $C_{18}H_{36}O_2$	-5.83	53.18	-3.16	4.79	-2.55	13.49
192.	Stigmasterol $C_{29}H_{48}O$	-8.86	321.55	+7.94		+283.52	
193.	Sylvestrene $C_{10}H_{16}$	-5.80	55.63	-5.98	41.04	-4.62	411.14
194.	Syringic Acid $C_9H_{10}O_5$	-4.93	242.17	-4.26	754.31	-2.75	9.65
195.	Teresantalol $C_{10}H_{16}O$	-5.27	136.69	-4.88	264.46	-3.38	3.36
196.	Terpinenol-4 $C_{10}H_{18}O$	-5.59	79.63	-5.80	56.24	-3.58	2.40
197.	Terpinolene $C_{10}H_{16}$	-5.43	105.10	-6.19	28.83	-4.53	474.38
198.	Tetrahydrocurcumin $C_{21}H_{24}O_6$	-6.23	27.05	-3.76	1.76	-0.42	491.34
199.	Thymol $C_{10}H_{14}O$	-5.61	76.70	-6.14	31.80	-4.73	340.52

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200.	Turmerone $C_{15}H_{20}O$	-6.59	14.84	-7.16	5.65	-4.39	608.94
201.	Turmeronol-A $C_{15}H_{20}O_2$	-7.13	5.98	-6.68	12.61	-4.33	672.95
202.	Turmeronol-B $C_{15}H_{20}O_2$	-5.48	96.45	-5.15	167.06	-2.95	6.89
203.	Vanillic-Acid $C_8H_8O_4$	-5.57	83.07	-4.17	881.95	-5.50	92.28
204.	Vanillin $C_8H_8O_3$	-5.53	88.25	-5.55	85.12	-4.36	636.85
205.	Xanthorrhizol $C_{15}H_{22}O$	-6.88	8.98	-6.78	10.76	-4.90	256.59
206.	Z-Alpha-Bergamotene $C_{15}H_{24}$	-6.65	15.83	-7.37	3.95	-4.84	283.02
207.	Z-Cinerone $C_{10}H_{14}O$	-5.38	113.74	-6.00	40.16	-4.40	590.86
208.	Zedoarondiol $C_{15}H_{24}O_3$	-6.67	12.95	-4.58	438.19	-3.58	2.38
209.	Z-Ferulicacid $C_{10}H_{10}O_4$	-6.09	34.40	-4.35	646.18	-4.58	441.25
210.	Zingerone $C_{11}H_{14}O_3$	-6.10	33.88	-5.55	85.12	-3.54	2.54
211.	Zingiberene $C_{15}H_{24}$	-6.26	25.61	-7.52	3.07	-4.71	355.21
	Zingiber officinale						
1.	(+)-6-Gingerol $C_{17}H_{26}O_4$	-5.41	107.82	-4.91	250.75	-3.78	1.71
2.	(+)-Alpha-Curcumene $C_{15}H_{22}$	-6.69	12.44	-7.05	6.79	-4.69	364.27
3.	(+)-Angelicoidenol $C_{10}H_{18}O_2$	-5.23	147.01	-4.35	652.17	+1.14	
4.	(+)-Aromadendrene $C_{15}H_{24}$	-7.17	5.54	-4.57	449.19	-4.95	237.03
5.	(+)-Beta-Phellandrene $C_{10}H_{16}$	-5.71	65.08	-6.24	26.60	-4.42	573.36
6.	(+)-Borneol $C_{10}H_{18}O$	-5.25	142.09	-4.95	234.29	-3.38	3.34
7.	1-(4-Hydroxy-3-Methoxyphenyl)-3,5-Diacetoxyoctane $C_{19}H_{28}O_6$	-4.91	249.87	-3.58	2.37	-1.75	52.21
8.	1-(4-O-Beta-Dglucopyranosyl-3-Methoxyphenyl)-3,5-Dihydroxydecane $C_{25}H_{42}O_7$	-4.93	244.87	+ 1.20		+94.0	
9.	1,8-Cineole $C_{10}H_{18}O$	-5.55	85.67	-5.64	73.05	-3.76	1.75
10.	10-Dihydrogingerdione $C_{58}H_{76}O_{14}$	-5.57	171.87	-2.74	9.78	-1.50	78.86

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11.	10-Epizonarene $C_{15}H_{24}$	-6.59	14.71	-7.79	1.94	-4.54	473.36
12.	10-Gingediol $C_{21}H_{36}O_4$	-4.24	776.56	-3.02	6.15	+7.57	
13.	10-Gingerdione $C_{21}H_{32}O_4$	-3.76	1.75	-4.54	467.71	-1.74	50.64
14.	10-Gingerol $C_{21}H_{34}O_4$	-5.74	61.97	-0.50	429.63	-1.75	51.75
15.	10-Shogaol $C_{21}H_{32}O_3$	-6.79	10.46	-4.36	640.85	-1.86	43.64
16.	2-(2'-3'-Epoxy-3-Methylbutyl)-3-Methyl-Furan $C_{10}H_{14}O_2$	-5.58	81.70	-5.85	51.46	-4.14	922.01
17.	2,2,4-Trimethylheptane $C_{10}H_{22}$	-4.65	388.06	-5.45	101.59	-3.40	3.23
18.	2-6-Dimethyl-Hept-5-En-1-Al $C_9H_{16}O$	-4.96	231.76	-5.21	152.76	-3.89	1.41
19.	2-6-Dimethyl-Octa-2-6-Diene-1-8-Diol $C_{10}H_{18}O_2$	-4.99	417.53	-5.04	200.58	-3.56	2.44
20.	2-6-Dimethyl-Octa-3-7-Diene-1-6-Diol (Seocalcitrol) $C_{30}H_{46}O_3$	-9.17	189.68	+57.05		145.50	
21.	2-Nonanone $C_9H_{18}O$	-4.76	325.92	-4.92	246.98	-3.65	1.65
22.	2-Undecanone $C_{11}H_{22}O$	-5.51	92.20	-5.19	155.75	-3.73	1.85
23.	3-Phenylbenzaldehyde $C_{13}H_{10}O$	-6.61	14.30	-6.79	10.56	-4.74	332.02
24.	4-Gingerol $C_{15}H_{22}O_4$	-5.33	123.85	-5.24	143.28	-5.02	209.55
25.	4-Phenylbenzaldehyde $C_{13}H_{10}O$	-6.51	16.95	-7.04	6.96	-4.52	485.26
26.	4-Terpineol $C_{10}H_{18}O$	-5.04	200.93	-5.80	55.84	-3.45	2.98
27.	6-Dehydrogingerdione $C_{17}H_{22}O_4$	-4.21	817.77	-5.05	197.08	-4.88	266.19
28.	6-Gingerdiol $C_{17}H_{28}O_4$	-4.20	829.83	-5.53	88.44	-3.66	2.09
29.	6-Gingerdione $C_{17}H_{24}O_4$	-5.30	131.37	-5.08	190.06	-3.57	1.54
30.	6-Gingerol $C_{17}H_{26}O_4$	-5.73	62.94	-4.91	250.75	-3.78	1.71
31.	6-Gingesulfonic-Acid $C_{17}H_{26}O_6S$	-5.51	91.24	-4.18	866.39	-2.92	7.24

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32.	6-Methylgingediol $C_{18}H_{30}O_4$	-4.89	260.88	-4.76	322.28	-2.34	11.70
33.	6-Methyl-Hept-5-En-2-One $C_{15}H_{21}N_4O_4S^+$	-6.09	34.14	-4.96	229.60	-3.56	1.54
34.	6-Methyl-Hept-5-En-2-Ol $C_8H_{16}O$	-5.01	214.39	-4.97	226.9	-3.74	1.82
35.	6-Paradol $C_{17}H_{26}O_3$	-5.59	79.69	-5.49	94.48	-4.33	671.5
36.	6-Shogaol $C_{17}H_{24}O_3$	-6.21	27.90	-6.09	34.59	-4.09	1.01
37.	7-(3-4-Dihydroxyphenyl)-1-(4-Hydroxy-3- Methoxy-Phenyl)-Hept- 4-En-3-One $C_{20}H_{22}O_5$			-4.55	464.8	-1.02	177.7
38.	7-Gingerol $C_{18}H_{28}O_4$	-5.64	44.24	-4.96	229.6	-2.80	8.87
39.	8-Gingediol $C_{19}H_{32}O_4$	-4.50	499.04	-5.08	188.7	-1.07	163.4
40.	8-Gingerol $C_{19}H_{30}O_4$	-4.53	481.04	-4.48	523.1	-1.31	109.2
41.	8-Shogaol $C_{19}H_{28}O_3$	-5.58	81.22	+21.79		+1.59	
42.	9-Gingerol $C_{20}H_{32}O_4$	-4.91	250.00	-4.07	1.04	-1.48	81.77
43.	9-Oxo-Nerolidol $C_{15}H_{24}O_2$	-5.63	74.97	-6.16	30.65	-2.74	9.84
44.	Acetaldehyde CH_3CHO	-2.64	11.59	-2.03	32.53	-2.39	17.64
45.	Acetone C_3H_6O	-3.13	5.08	-2.64	11.62	-2.97	6.60
46.	Aframodial $C_{20}H_{30}O_3$	-6.52	16.54	-6.29	24.69	-4.24	785.5
47.	Allo-Aromadendrene $C_{15}H_{24}$	-7.17	5.54	-7.60	2.71	-4.03	1.12
48.	Allo-Aromadendrine $C_{14}H_{22}O$	-7.06	6.73	-7.59	2.71	-4.54	2.68
49.	Alpha-Cadinene $C_{15}H_{24}$	-6.87	9.15	-7.76	2.04	-4.87	267.5
50.	Alpha-Cadinol $C_{15}H_{26}O$	-7.02	7.17	-6.79	10.57	-4.01	1.14
51.	Alpha-Cedrol $C_{15}H_{26}O$	-7.27	4.71	-7.06	6.74	-4.32	679.8
52.	Alpha-Copaene $C_{15}H_{24}$	-7.23	5.02	-7.82	1.86	-5.46	99.14
53.	Alpha-Cubebene $C_{15}H_{24}$	-6.83	9.89	-7.52	3.08	-4.66	381.4

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54.	Alpha-Curcumene $C_{15}H_{22}$	-6.44	19.18	-6.99	7.52	-4.39	607.0
55.	Alpha-Farnesene $C_{15}H_{24}$	-6.29	24.58	-6.45	18.60	-4.03	1.12
56.	Alpha-Linolenic-Acid $C_{18}H_{30}O_2$	-5.23	146.36	-4.91	250.6	-3.19	4.59
57.	Alpha-Muurolene $C_{15}H_{24}$	-6.87	9.17	-7.75	2.09	-4.62	411.9
58.	Alpha-Phellandrene $C_{10}H_{16}$	-5.76	60.08	-5.97	42.23	-4.29	719.0
59.	Alpha-Pinene $C_{10}H_{16}$	-5.62	75.84	-5.52	89.38	-3.72	1.86
60.	Alpha-Selinene $C_{15}H_{24}$	-7.23	4.99	-6.08	35.07	-4.05	1.08
61.	Alpha-Terpinene $C_{10}H_{16}$	-5.63	74.83	-6.08	35.19	-4.44	556.6
62.	Alpha-Terpineol $C_{10}H_{18}O$	-5.93	44.75	-6.19	28.80	-3.70	1.93
63.	Alpha-Ylangene $C_{15}H_{24}$	-7.23	5.03	-8.04	1.28	-5.21	151.7
64.	Alpha-Zingiberene $C_{15}H_{24}$	-6.10	33.91	-7.08	6.46	-4.64	397.3
65.	Angelicoidenol-2-Obeta-Dglucopyranoside $C_{16}H_{28}O_7$	-5.85	51.20	-2.41	17.13	-1.59	68.41
66.	Anti-Methyl-10shogaol $C_{16}H_{13}N_3O_3$	8.01	1.34			-3.08	5.57
67.	Ar-Curcumene $C_{15}H_{22}$	-6.57	15.16	-6.73	11.67	-4.90	255.6
68.	Aromadendrene $C_{15}H_{24}$	-7.17	5.55	-6.81	10.26	-4.14	926.8
69.	Aromadendrine $C_{15}H_{12}O_6$	-7.06	6.70	-4.57	449.1	-4.27	737.5
70.	Benzaldehyde C_7H_6O	-5.09	185.45	-4.31	692.7	-3.88	1.43
71.	Beta-Bisabolene $C_{15}H_{24}$	-6.95	8.01	-7.15	5.73	-4.91	250.0
72.	Beta-Bisabolol $C_{15}H_{26}O$	-7.13	5.90	-7.11	6.13	-4.32	684.8
73.	Beta-Carotene $C_{40}H_{56}$	11.40	0.004	+41080		+34342	
74.	Beta-Caryophyllene $C_{15}H_{24}$	-6.63	13.74	-7.26	4.76	-4.24	785.7
75.	Beta-Elemene $C_{15}H_{24}$	-6.29	24.57	-6.45	18.86	-4.12	959.5
76.	Beta-Eudesmol $C_{15}H_{26}O$	-7.72	2.21	-6.82	10.00	-5.14	172.17

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77.	Beta-Farnesene $C_{15}H_{24}$	-5.65	72.06	-6.44	18.88	-4.11	965.47
78.	Beta-Himachalene $C_{15}H_{24}$	-6.82	10.04	-7.91	1.58	-4.77	316.61
79.	Beta-Ionone $C_{13}H_{20}O$	-7.20	5.31	-7.04	6.91	-4.80	304.1
80.	Beta-Myrcene $C_{10}H_{16}$	-4.98	224.67	-5.51	91.74	-3.71	1.92
81.	Beta-Phellandrene $C_{10}H_{16}$	-5.71	65.48	-6.26	26.66	-4.46	537.1
82.	Beta-Pinene $C_{10}H_{16}$	-5.42	106.30	-5.76	59.68	-3.77	1.73
83.	Beta-Santalol $C_{15}H_{24}O$	-6.66	13.19	-7.33	4.27	-4.67	784.9
84.	Beta-Selinene $C_{15}H_{24}$	-6.82	10.08	-7.41	3.73	-4.55	460.4
85.	Betasesquiphellandrene $C_{15}H_{24}$	-6.96	7.88	-7.74	2.14	-3.89	1.41
86.	Betasesquiphellandrol $C_{15}H_{24}O$	-6.82	10.05	-7.67	2.37	-4.68	95.80
87.	Beta-Sitosterol $C_{29}H_{50}O$	-8.44	651.47	+26.45		+374.7	
88.	Beta-Thujone $C_{10}H_{16}O$	-5.62	75.46	-5.90	47.01	-4.22	811.6
89.	Beta-Zingiberene $C_{15}H_{24}$	-7.23	5.02	-7.49	3.21	-4.82	291.3
90.	Bisabolene $C_{15}H_{24}$	-6.89	8.95	-5.09	185.6	-5.06	194.7
91.	Borneol-Acetate $C_{12}H_{20}O_2$	-5.99	40.78	-5.80	56.28	-4.65	305.4
92.	Bornyl-Acetate $C_{12}H_{20}O_2$	-6.03	37.87	-5.83	53.21	-4.33	666.4
93.	Caffeic-Acid $C_9H_8O_4$	-6.00	40.04	-3.93	1.32	-4.40	596.8
94.	Calamenen $C_{15}H_{22}O$	-6.80	10.43	-7.50	3.20	-4.42	579.3
95.	Campesterol $C_{28}H_{48}O$	-4.95	9.25	+22.86		-3.62	2.23
96.	Camphene $C_{10}H_{16}$	-5.65	72.43	-5.30	130.1	-3.80	1.65
97.	Camphene-Hydrate $C_{10}H_{18}O$	-5.11	178.77	-5.26	139.4	-3.40	3.21
98.	Camphor $C_{10}H_{16}O$	-5.36	118.43	-5.09	185.6	-3.49	2.76
99.	Capric-Acid $C_{10}H_{20}O_2$	-6.12	32.42	-4.20	827.6	-4.43	565.3

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100.	Caprylic-Acid $C_8H_{16}O_2$	-5.11	178.12	-3.55	2.48	-4.25	647.2
101.	Capsaicin $C_{18}H_{27}NO_3$	-5.94	44.15	-6.05	36.62	-3.29	3.91
102.	Car-3-Ene $C_{10}H_{16}$	-5.35	120.70	-5.89	48.10	-3.70	1.93
103.	Caryophyllene $C_{15}H_{24}$	-6.63	13.71	-7.26	4.75	-4.24	785.8
104.	Cedorol $C_{15}H_{26}O$	-7.27	4.71	-6.91	8.67	-4.05	1.07
105.	Chavicol $C_9H_{10}O$	-5.28	134.90	-5.12	176.7	-3.85	1.50
106.	Chlorogenic-Acid $C_{16}H_{18}O_9$	-6.55	15.83	-3.50	2.72	-2.96	6.71
107.	Chrysanthemin $C_{21}H_{21}O_{11}^+$	-5.55	86.06	+3.88		+265.5	
108.	Cineole $C_{10}H_{18}O$	-5.55	85.52	+18.56		-3.75	1.79
109.	Cis-10-Shogaol $C_{21}H_{32}O_3$	-5.62	76.56	-4.52	489.2	-0.98	190.3
110.	Cis-12-Shogaol $C_{23}H_{36}O_3$	-5.64	73.63	-2.15	26.61	-0.62	349.7
111.	Cis-6-Shogoal $C_{17}H_{24}O_3$	-5.79	56.55	-5.81	55.06	-3.82	1.59
112.	Cis-Betasesquiphellandrol $C_{15}H_{24}O$	-6.99	7.48	-7.06	6.72	-5.37	115.9
113.	Cis-Geranic-Acid $C_{10}H_{16}O_2$	-6.00	39.73	-4.66	385.8	-4.13	945.9
114.	Cis-Hexan-3-Ol $C_{10}H_{18}O$	-3.26	12.18	-3.33	3654	-3.78	1.69
115.	Cis-Nerolidol $C_{15}H_{26}O$	-4.27	55.66	-6.39	20.78	-4.42	575.26
116.	Cis-Rose-Oxide $C_{10}H_{18}O$	-3.21	32.01	-6.53	16.34	-4.08	1.02
117.	Cis-Selinen-4-Ol $C_{15}H_{26}O$	-4.98	89.12	-7.13	5.89	-4.24	773.6
118.	Cis-Sesquiabinenehydrate $C_{15}H_{26}O$	-4.28	224.58	-5.98	41.62	-4.63	403.1
119.	Citral $C_{10}H_{16}O$	-5.80	56.22	-5.57	82.70	-4.26	758.9
120.	Citronellal $C_{10}H_{18}O$	-5.62	76.18	-5.40	109.4	-4.11	976.1
121.	Citronellol-Acetate $C_{12}H_{22}O_2$	-4.91	253.35	-5.52	89.86	-4.73	341.6
122.	Cumene C_9H_{12}	-4.96	229.93	-5.30	129.6	-4.64	395.6

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123.	Curcumin $IC_{21}H_{20}O_6$	-7.23	5.04	+1.28		-2.94	7.01
124.	Cyanin $C_{27}H_{31}O_{16}^+$	-4.31	697.88	+91.87		+531.7	
125.	D-Borneol $C_{10}H_{18}O$	-5.24	143.45	-4.66	384.5	-3.38	3.34
126.	Decan-1-Al $C_{10}H_{17}N$	-5.88	49.37	-5.10	182.2	-3.43	3.05
127.	Decanal $C_{10}H_{20}O$	-4.56	453.69	-5.06	196.3	-3.19	4.61
128.	Dec-Trans-2-En-1-Al $C_{10}H_{18}O$	-5.31	129.01	-5.21	152.8	-3.67	2.03
129.	Delphinidin $C_{15}H_{11}O_7$	-6.87	9.23	-4.32	685.1	-3.98	1.20
130.	Delta-Cadinene $C_{15}H_{24}$	-7.29	4.55	-7.66	2.44	-4.85	279.7
131.	Delta-Car-3-Ene $C_{10}H_{16}$	-5.36	117.75	-5.89	48.13	-3.70	1.93
132.	Diethylsulfide $C_4H_{10}S$	-3.69	1.97	-2.91	7.42	-2.84	8.29
133.	Dihydrogingerol $C_{11}H_{12}O_3$	-6.11	53.55	-5.93	44.76	-3.28	2.09
134.	Dodecanoic-Acid $C_{12}H_{24}O_2$	-5.24	143.26	-4.34	654.9	-4.57	446.2
135.	Elemol $C_{15}H_{26}O$	-6.26	25.90	-6.71	12.12	-3.98	1.20
136.	Ethyl-Isopropylsulfide $C_5H_{12}S$	-4.28	730.66	-3.55	2.51	-3.44	2.99
137.	Ethyl-Myristate $C_{16}H_{32}O_2$	-4.83	288.65	-5.45	100.6	-4.84	283.1
138.	Eugenol $C_{10}H_{12}O_2$	-5.45	100.35	-5.64	73.34	-3.80	1.65
139.	Farnesal $C_{15}H_{24}O$	-6.27	25.54	-6.04	37.22	-4.19	848.3
140.	Farnesene $C_{15}H_{24}$	-6.13	31.87	-6.21	28.11	-4.19	851.0
141.	Farnesol $C_{15}H_{26}O$	-6.18	29.76	-5.78	58.17	-4.53	479.5
142.	Ferulic-Acid $C_{10}H_{10}O_4$	-6.30	24.17	-4.05	1.08	-4.13	935.4
143.	Furanogermenone $C_{15}H_{20}O_2$	-7.14	5.80	-7.30	4.43	-3.72	1.89
144.	Furfural $C_5H_4O_2$	-4.60	426.58	-3.50	2.74	-4.84	281.3
145.	Gadoleic-Acid $C_{20}H_{38}O_2$	-4.93	244.15	-3.46	2.92	+0.06	

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146.	Galanolactone $C_{20}H_{30}O_3$	-7.43	3.58	-5.06	195.8	-4.02	1.12
147.	Gamma-Aminobutyricacid $C_4H_9NO_2$	-3.37	3.38	-2.42	16.97	-3.27	4.03
148.	Gamma-Bisabolene $C_{15}H_{24}$	-6.43	19.52	-7.45	3.45	-5.08	187.5
149.	Gamma-Eudesmol $C_{15}H_{26}O$	-7.68	10.63	-6.44	18.89	-3.99	1.20
150.	Gamma-Muurolene $C_{15}H_{24}$	-7.06	6.67	-7.91	1.58	-4.65	390.4
151.	Gamma-Selinene $C_{15}H_{24}$	-7.28	4.59	-7.40	3.77	-4.88	265.6
152.	Gamma-Terpinene $C_{10}H_{16}$	-5.62	75.74	-6.08	35.13	-4.09	1.01
153.	Geranial $C_{10}H_{16}O$	-5.04	201.94	-5.65	72.30	-4.34	653.3
154.	Geraniol $C_{10}H_{18}O$	-5.33	124.46	-5.43	103.9	-3.94	1.30
155.	Geraniol-Acetate $C_{12}H_{20}O_2$	-6.08	35.14	-5.72	64.10	-3.56	2.45
156.	Gingediacetate $C_{21}H_{32}O_6$	-4.98	224.73	-2.92	6.74	+9.15	
157.	Gingerenone-A $C_{21}H_{24}O_5$	-6.48	17.71	-5.13	174.7	-2.31	20.14
158.	Gingerenone-B $C_{22}H_{26}O_6$	-5.61	77.37	-4.00	1.17	-2.19	24.92
159.	Gingerenone-C $C_{20}H_{22}O_4$	-7.60	2.71	-6.13	31.87	-3.17	4.74
160.	Gingerglycolipid-A $C_{33}H_{56}O_{14}$	-0.86	233.31	+341.1		+280.6	
161.	Gingerglycolipid-B $C_{33}H_{58}O_{14}$	-3.52	2.65	+621.2		+1056	
162.	Gingerone $C_{11}H_{14}O_3$	-5.71	64.76	-5.77	58.82	-3.93	1.31
163.	Glycol Monopalmitate $C_{18}H_{36}O_3$	-4.10	990.19	-3.74	1.82	-0.36	547.2
164.	Glyoxal $C_2H_2O_2$	-3.02	6.08	-2.06	30.98	-2.71	10.28
165.	Guaiol $C_{15}H_{26}O$	-6.80	10.29	-6.91	8.54	-4.64	399.4
166.	Heptadecanoic-Acid $C_{17}H_{34}O_2$	-6.01	39.48	-4.08	1.02	-4.30	701.9
167.	Heptan-2-Ol $C_7H_{16}O$	-4.15	902.07	-4.19	843.2	-3.15	4.93
168.	Heptan-2-One $C_7H_{14}O$	-4.76	325.65	-4.36	639.0	-3.54	2.55

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169.	Hexacosanoic Acid 2,3-Dihydroxypropyl Ester $C_{29}H_{58}O_4$	-2.21	23.98	+38.26		+81.44	
170.	Hexahydrocurcumin $C_{21}H_{26}O_6$	-5.74	61.89	-5.07	193.57	+6.37	
171.	Hexan-1-Al $C_6H_{12}O$	-4.24	780.28	-3.61	139.6	-3.54	2.54
172.	Hexan-1-Ol $C_6H_{14}O$	-4.33	665.06	-3.50	138.4	-2.81	8.75
173.	Humulene $C_{15}H_{24}$	-6.91	8.59	-7.12	6.01	-3.77	1.72
174.	Humulene-Epoxide-2 C15H24O	-6.56	15.64	-6.55	15.84	-4.01	1.15
175.	Isoborneol $C_{10}H_{18}O$	-5.20	154.87	-4.67	378.1	-3.38	3.31
176.	Isoeugenol $C_{10}H_{12}O_2$	-5.88	49.28	-5.52	90.16	-3.91	1.36
177.	Isogingerenone-B C22H26O6	-6.39	20.72	-3.68	2.02	+6.37	
178.	Isovaleraldehyde $C_5H_{10}O$	-4.08	1.02	-3.37	3.37	-3.06	5.76
179.	Isovanillin C8H8O3	-5.29	132.68	-4.98	222.9	-4.21	817.2
180.	Juniper-Camphor C15H26O	-7.52	3.06	-7.69	2.31	-3.71	1.92
181.	Kaempferol $C_{15}H_{10}O_6$	-8.09	1.18	-4.86	292.4	-3.88	1.43
182.	Labda-Trans-8(17)-12diene-15-16-Dial C20H30O2	-3.32	72.16	-7.24	4.97	-2.83	8.37
183.	Lauric-Acid C12H24O2	-5.53	88.14	-4.16	897.0	-4.51	490.6
184.	Limonene $C_{10}H_{16}$	-5.50	92.84	-6.01	39.06	-4.61	416.4
185.	Linalool $C_{10}H_{18}O$	-4.80	300.77	-5.42	105.7	-3.26	4.09
186.	Linalool-Oxide C10H18O2	-5.11	179.95	-5.97	42.35	-3.64	2.15
187.	Linalool-Propionate C13H22O2	-5.47	98.38	-6.05	36.69	-3.37	3.40
188.	Maleimide-5-Oxime $C_4H_4N_2O_2$	-5.14	171.36	-3.94	1.29	-4.16	893.5
189.	Menthol-Acetate C12H22O2	-6.44	19.00	-6.67	12.98	-4.13	935.4
190.	Methyl-12-Gingediol C24H42O4	-4.52	284.62	+5.02		+5.37	

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191.	Methyl-6-Gingerol $C_{18}H_{28}O_4$	-4.65	323.2	-5.64	72.83	-2.78	9.19
192.	Methyl-8-Gingerol $C_{20}H_{32}O_4$	-4.83	288.83	-3.16	4.80	-1.46	84.91
193.	Methyl-Allyl-Sulfide C_4H_8S	-3.63	2.17	-2.86	7.96	-2.88	7.73
194.	Methyl-Caprylate $C_9H_{18}O_2$	-4.61	419.03	-4.59	434.0	-4.15	900.99
195.	Methyl-Gingerol $C_{18}H_{28}O_4$	-4.70	359.80	-4.70	357.7	-3.28	3.91
196.	Methyl-Glyoxal $C_3H_4O_2$	-3.81	1.62	-2.66	11.30	-3.26	4.11
197.	Methyl-Heptenone $C_8H_{14}O$	-5.38	113.85	-4.95	234.4	-3.96	1.25
198.	Methyl-Isobutylketone $C_6H_{12}O$	-4.58	441.04	-4.07	1.04	-3.63	2.20
199.	Myrcene $C_{10}H_{16}$	-5.18	158.58	-5.52	90.62	-3.75	1.79
200.	Myricetin $C_{15}H_{10}O_8$	-8.30	827.90	-3.95	1.27	-0.94	203.5
201.	Myristic-Acid $C_{14}H_{28}O_2$	-5.27	137.11	-4.14	918.9	-4.39	603.18
202.	Myrtenal $C_{10}H_{14}O$	-5.64	73.76	-5.65	71.95	-3.31	3.75
203.	N-Butyraldehyde C_4H_8O	-3.40	3.22	-3.88	1.43	-2.83	8.41
204.	N-Decanal $C_{10}H_{20}O$	-5.51	670.08	-3.43	3.04		
205.	Neoisopulegole $C_{10}H_{18}O$	-5.81	55.17	-6.00	39.73	-4.73	342.3
206.	Neral $C_{10}H_{16}O$	-5.43	104.49	-5.65	71.72	-3.71	1.92
207.	Nerol C10H18O	-5.58	303.51	-5.38	113.3	-3.71	3.77
208.	Nerolidol $C_{15}H_{26}O$	-5.39	111.73	-5.84	52.02	-3.31	3.77
209.	Nerol-Oxide C10H16O	-5.67	70.09	-6.16	30.54	-4.00	1.16
210.	N-Heptane C_7H_{16}	-4.77	320.73	-3.88	1.44	-3.84	1.54
211.	N-Nonane C_9H_{20}	-5.69	67.93	-4.69	366.3	-3.25	4.15
212.	N-Nonanol $C_9H_{20}O$	-4.96	231.63	-4.56	451.9	-2.78	9.20
213.	N-Octane C_8H_{18}	-5.05	198.69	-4.30	704.4	-3.64	2.14

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214.	N-Octanol $C_8H_{18}O$	-4.87	269.07	-4.21	817.6	-2.89	7.66
215.	Nonan-1-Al $C_9H_{18}O$	-4.53	478.04	-4.66	385.7		
216.	Nonan-2-Ol C9H19O	-4.96	231.11	-5.02	209.1	-3.32	3.69
217.	Nonan-2-One C18H36O2	-3.31	3.74	-3.06	5.71	-3.61	2.27
218.	Nonyl-Aldehyde C9H18O	-5.18	159.01	-4.69	366.5	-3.33	3.64
219.	N-Propanol C_3H_8O	-3.10	5.31	-2.44	16.39	-2.68	10.94
220.	Octan-1-Al $C_8H_{16}O$	-4.16	896.06	-4.28	729.9	-3.28	3.93
221.	Octan-1-Ol-Acetate $C_{10}H_{21}O_3^-$	-2.12	27.84	-0.12	817.9	-2.23	23.31
222.	Octan-2-Ol $C_8H_{18}O$	-5.29	132.22	-4.60	423.9	-3.39	3.25
223.	Oct-Trans-2-En-1-Al C8H14O	-5.29	133.48	-4.57	448.2	-3.48	2.82
224.	Pantothenic-Acid C9H17NO5	-3.43	3.05	-2.32	19.91	-2.31	20.18
225.	Paradol $C_{17}H_{26}O_3$	-5.31	128.74	-5.00	216.0	-4.33	666.9
226.	Patchouli-Alcohol $C_{15}H_{26}O$	-6.62	13.93	-5.58	81.55	-3.91	1.37
227.	P-Coumaric-Acid $C_9H_8O_3$	-6.25	26.23	-4.28	770.9	-4.44	558.0
228.	P-Cymen-8-Ol $C_{10}H_{14}O$	-5.45	133.94	-5.78	58.15	-4.29	717.2
229.	P-Cymene $C_{10}H_{14}$	-5.34	121.22	-5.72	64.45	-4.15	912.2
230.	Pentadecanoic-Acid C15H30O2	-5.61	76.75	-4.17	883.6	-3.84	1.53
231.	Pentan-2-Ol $C_5H_{12}O$	-4.00	1.17	-3.45	2.96	-3.30	3.82
232.	Perillaldehyde $C_{10}H_{14}O$	-6.12	32.72	-6.04	37.51	-3.97	1.24
233.	Perillene $C_{10}H_{14}O$	-5.90	47.47	-5.56	84.40	-4.34	663.0
234.	P-Hydroxy-Benzoicacid $C_7H_6O_3$	-5.06	194.27	-3.52	2640	-5.67	69.54
235.	Pin-2-En-5-Ol $C_{10}H_{16}O$	-5.39	111.88	-5.48	96.13	-3.38	3.33
236.	Pipecolic-Acid $C_6H_{11}NO_2$	-4.98	223.40	-4.02	1.12	-3.94	1.30

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237.	P-Mentha-1,5-Dien-7-Ol $C_{10}H_{16}O$	-6.44	19.14	-5.91	46.84	-3.75	1.79
238.	P-Mentha-1-8-Dien-7-Ol $C_{10}H_{16}O$	-6.16	30.77	-5.85	51.17	-4.04	1.09
239.	P-Mentha-2,8-Dien-1-Ol $C_{10}H_{16}O$	-5.73	63.52	-5.67	70.28	-3.71	1.91
240.	Propionaldehyde C_3H_6O	-3.07	5.65	-2.43	16.56	-2.56	13.40
241.	Quercetin $C_{15}H_{10}O_7$	-6.94	8.24	-4.17	870.7	-3.71	4.76
242.	Raffinose $C_{18}H_{32}O_{16}$	-0.03	954.83	+31.08		+212.3	
243.	Rosefuran $C_{10}H_{14}O$	-5.48	96.35	-5.84	51.98	-4.17	876.4
244.	Sabinene $C_{10}H_{16}$	-5.50	93.13	-3.15	4.91	-4.54	466.3
245.	Selina-3,7(11)-Diene $C_{15}H_{24}$	-7.41	5.79	-6.99	7.50	-4.77	321.0
246.	Sesquiphellandrene $C_{15}H_{24}$	-7.29	4.53	-7.53	3.02	-4.72	345.0
247.	Sesquithujene $C_{15}H_{24}$	-6.75	11.32	-7.07	6.55	-4.06	1.05
248.	Shikimic-Acid $C_7H_{10}O_5$	-5.26	139.83	-3.72	1.86	-4.53	477.6
249.	Stigmasterol $C_{29}H_{48}O$	-8.40	690.92	+7.94		+283.5	
250.	Terpinen-4-Ol $C_{10}H_{18}O$	-5.52	82.61	-5.80	56.24	-3.58	2.40
251.	Terpinolene $C_{10}H_{16}$	-5.43	105.16	-6.19	28.83	-4.53	474.3
252.	Tert-Butanol $C_4H_{10}O$	-3.60	2.30	-3.15	4.92	-2.74	9.87
253.	Trans-10-Shogaol $C_{21}H_{32}O_3$	-5.82	54.27	-4.41	583.87	-3.07	2.47
254.	Trans-12-Shogaol $C_{23}H_{36}O_3$	-6.04	37.58	-4.76	326.55	-2.83	8.45
255.	Trans-Beta-Farnesene $C_{15}H_{24}$	-5.73	62.57	-6.07	35.57	-4.02	1.12
256.	Trans-Betasesquiphellandrol $C_{15}H_{24}O$	-7.06	5.11	-7.13	5.98	-4.78	315.55
257.	Trans-Geranic-Acid $C_{10}H_{16}O_2$	-6.28	24.99	-4.66	385.58	-4.99	220.33
258.	Trans-Linalool-Oxide $C_{10}H_{18}O_2$	-5.19	158.14	-5.97	41.80	-3.67	2.04
259.	Trans-Nerolidol $C_{15}H_{26}O$	-6.14	31.34	-5.92	45.47	-2.61	12.30

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260.	Trans-Octen-2-Al $C_8H_{14}O$	-5.53	88.21	-4.59	435.4	-3.36	2.31
261.	Trans-Rose-Oxide $C_{10}H_{18}O$	-5.85	51.42	-6.53	16.32	-4.07	1.04
262.	Tricyclene $C_{10}H_{16}$	-5.16	165.37	-5.27	137.1	-4.28	731.6
263.	Undecan-2-Ol $C_{11}H_{24}O$	-5.00	215.20	-5.41	107.7	-3.27	4.04
264.	Undecan-2-One $C_{11}H_{22}O$	-5.05	197.57	-5.20	155.2	-3.61	2.24
265.	Vanillic Acid $C_8H_8O_4$	-5.64	73.21	-4.17	881.9	-5.50	92.28
266.	Vanillin $C_8H_8O_3$	-5.21	152.14	-5.55	85.12	-4.36	636.8
267.	Xanthorrhizol $C_{15}H_{22}O$	-6.81	10.20	-6.78	10.76	-4.90	256.5
268.	Zerumbodienone $C_{15}H_{24}O$	-6.72	11.89	-6.89	8.88	-4.40	592.5
269.	Zingiberenol $C_{15}H_{26}O$	-7.05	6.83	-7.19	5.37	-4.34	326.8
270.	Zingiberine $C_{15}H_{24}$	-6.73	11.66	-7.30	4.47	-5.02	209.9
271.	Zingiberol $C_{16}H_{28}O$	-7.04	6.86	-7.81	1.87	-3.79	1.67
272.	Zingiberone $C_{11}H_{14}O_3$	-5.45	101.21	-5.81	55.19	-3.65	2.10
273.	Zonarene $C_{15}H_{24}$	-6.87	9.21	-7.78	1.98	-4.46	542.1

IN SILICO SCREENING AND IDENTIFICATION OF LEAD MOLECULES WITH ANTI- HEPATITIS B ACTIVITY

Submitted by

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Abstract of Thesis

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COLLEGE OF AGRICULTURE

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ABSTRACT

Hepatitis B is an infectious liver disease which causes high morbidity and mortality worldwide. The present treatment of chronic hepatitis B (CHB) infection concentrates on clearing the HBV DNA and to prevent the development of complications. Currently seven drugs are available for the treatment of CHB: 5nucleo(s)tide analoque and 2 interferon based therapies. In order to find out safe and better drug, in the present investigation a total of 571 phytochemicals from three plants viz *Elettaria cardamomum* (L.) Maton, *Curcuma longa* and *Zingiber officinale* were screened against three Hepatitis B Virus proteins such as HBx, HBc and polymerase (Poly) through docking using the tool AutoDock 4.2. For docking out of 571 phytochemicals derived from *E. cardamomum* (87), *Z. officinale* (273) and *C. longa* (211), the structure of 558 compounds were downloaded from chemical databases and remaining 13 molecules structures were drawn using ChemSketch. The 3D structures of all phytochemicals structures were generated using the tool CORINA in .pdb format. The 3D structure of the target protein HBc was retrieved from Protein Data Bank (PDB ID: 1QGT) and the structure of HBx and Poly were modelled using the software MODELLER. The active site and residues of the target proteins HBx and HBc was detected using PDB Sum and Poly was detected using CASTp. Docking was performed using the tool AutoDock and the docked structures having binding energy $\leq$ -5.0 kcal/mol were considered as the active/hit molecules. Top ranked five hit molecules with least binding energy obtained from each plant were further analysed based on other criteria such as hydrogen bond, other molecular interactive forces like hydrophobic interactions and drug likeness properties and selected the best lead molecules. The result showed that all three spices have inhibitory effect on the targets, HBx, HBc and Poly. The best lead molecules selected against HBx was β-carotein ($\Delta G_{bind} \leq$-11.40 kcal/mol) derived from *Z. officinale* and *C. longa* followed by β-sitosterol ($\Delta G_{bind} \leq$-8.62 kcal/mol) which was present in all the three plants. Similarly, the best lead obtained against HBc was α-ylangene ($\Delta G_{bind} \leq$-8.04 kcal/mol) from C. *longa* and *Z. officinale* and the compound 2-hydroxy methyl anthraquinone ($\Delta G_{bind} \leq$-8.00

kcal/mol) present in *E. cardamomum* was also equally competent as the best lead. Against polymerase vanilic acid ($\Delta G_{bind} \leq$-5.50 kcal/mol) was found as the best lead and it was present in all three plants. The compound p-hydroxy-benzoicacid obtained from *Z. officinale* and protocatechuic-acid from *C. longa* were also equally competent as the best lead. The results support the traditional knowledge and practice. However, based on the foregoing results *in vitro* and *in vivo* experiments are to be essential for further confirmation.

Key words: Auto Dock, *E. cardamomum, Z. officinale, C. longa,* Phytochemicals, Hepatitis B