MOLECULAR DOCKING OF ANTIVIRAL PROPERTIES OF *Glycosmis pentaphylla* (Retz) Correa

By

BRINDA O. P.
(2014-11-103)

THESIS

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CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680656
KERALA, INDIA

2017

DECLARATION

I hereby declare that the thesis entitled **"Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa"**is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
Date:30-3-2017

Brinda O. P.
(2014-11-103)

CERTIFICATE

Certified that the thesis entitled **"Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa"** is a record of research work done independently by **Ms. Brinda O. P. (2014-11-103)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship, fellowship to him.

30/03/2017

Place: Vellanikkara

Date : 30-03-2017

Dr. Valsala P. A.
(Chairpeerson, Advisory Committee)
Professor and Head (Retired)
CPBMB

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Brinda O. P. (2014-11-103),** a candidate for the degree of **Master of Science in Agriculture** with major field in **Plant Biotechnology,** agree that the thesis entitled **"Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa"**may be submitted by **Ms. Brinda O. P.** in partial fulfillment of the requirement for the degree.

30/03/2017

Dr. P. A. Valsala
(Chairperson, Advisory Committee)
Professor and Head (Retired)
Centre for Plant biotechnology and Molecular Biology
College of Horticulture, Vellanikkara

30/3/2017

Dr. Shylaja. M. R.
(Member, Advisory Committee)
Professor and Head
Centre for Plant biotechnology and
Molecular Biology
College of Horticulture, Vellanikkara

Dr. Anitha Cherian
(Member, Advisory Committee)
Professor
Department of Plant Pathology
College of Horticulture,
Vellanikkara

30/03/2017

Dr. Miniraj
(Member, Advisory Committee)
Professor
Department of Plantation Crops
College of Horticulture,
Vellanikkara

30/3/17

External Examiner
Dr. R. Jayaraj
Scientist B,
Forest ecology & Biodiversity Conservation
KFRI, Peechi

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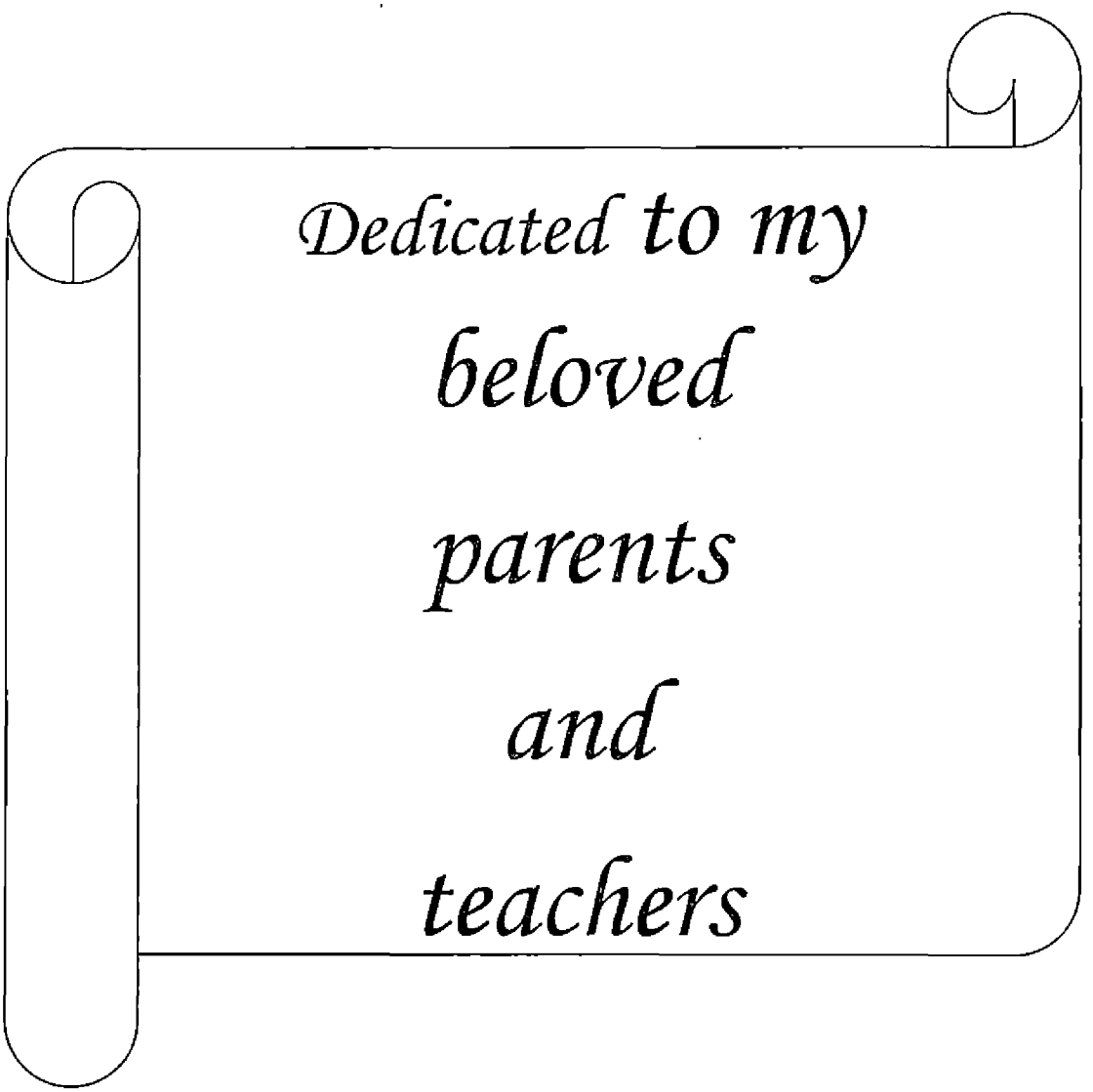
Dedicated to my
beloved
parents
and
teachers

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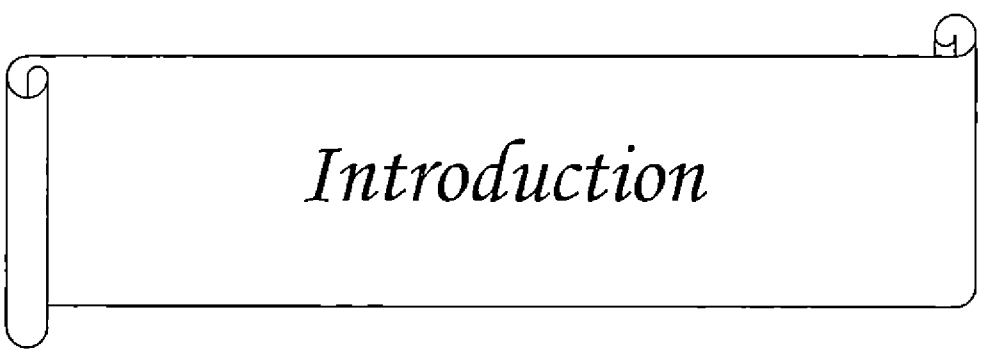
Abbreviations

%	Percentage
^0C	Degree Celsius
3D	3 dimensional
A	Acceptable
bp	Base pair
CCl_4	Carbon tetrachloride
CDC	Centre for Disease Control
CHIKV	Chikungunya virus
CPBMB	Centre for Plant Biotechnology and Molecular Biology
DENV	Dengue virus
DNA	Deoxyribose Nucleic Acid
Etc.	ex-cetra
g	Gram
G.pentaphylla	*Glycosmis pentaphylla*
GCMS	Gas Chromatography Mass Spectrometry
HA	Haemagglutinin
HBV	Hepatitis B virus
HBx	Hepatitis B virus X protein
HCV	Hepatitis C virus
HIV	Human deficiency syndrome virus
kcal	Kilocalorie
Kg	Kilogram
LCMS	Liquid Chromatography Mass Spectrometry
M	Molar
ml	Millilitre
mol	Mole
NSp	Non-structural protein
ORF	Open Reading frame
RdRp	RNA dependant RNA polymerase

TCMD	Traditional Chinese Medicine Database
WHO	World Health Organization



Introduction

1. Introduction

Right from the origin of stone age, human beings were depending on plants for the medicinal requisites.Initially the crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations were employed (Balick *et al.*,1997) and the traditional knowledgeon plant to be used against a specific disease and their method of application were passed orally, through generations. An important development in the history came when man focused on the isolation of active compounds present in medicinal plants marked by the isolation of morphine from opium (Kinghorn, 2001). Even in the present days, exploration for isolation and characterisation of pharmacologically active compounds from medicinal plants is done. The plant chemicals used for their medicinal purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants.

Glycosmis pentaphylla (Retz.) Correa, locally known as *'panal'* is one such plant used across the world. The medicinal properties of *G. pentaphylla* is available both in the written and non-written format as traditional knowledge since time immemorial. Indian traditional treatment system, Ayurveda has mentioned this plant for treating various ailments. Traditionally this plant is being used for cough, rheumatism, anaemia and jaundice (Sastri, 1956; Gopi, 2000). Although the various parts of this plant are used against different diseases ranging from ulcer to cancer (Ariful *et al.*, 2010), there is no strong evidence for proving its antiviral potential.

In recent past, there was an outbreak of new viral diseases like chikungunya and dengue. But so far no effective drugs are developed for these viral diseases. Nowadays, people are turning to natural products for medical uses. Leaf extracts from *Glycosmis pentaphylla* is used against viral disease jaundice in traditional system of medicine in India and other Asian countries (Oudhia, 2006). In modern medicine, drug development involves chromatographic technique coupled with bioinformatic tools like molecular docking.


Review

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Molecular docking will help us to screen the phyto-compounds present in *Glycosmis pentaphylla* that could be considered for its medicinal property against various diseases. This tool can be used for analyzing large number of phyto-compounds in lesser time and the drug suitability of the identified compounds with desirable properties can be further tested with wet lab studies.

Although various *in silico* docking studies have been reported against viral diseases, there is scarcity of specific antiviral drug against most of these viral diseases. The toxicity and side-effects produced by the adoption of synthetic drugs have changed the mind-set of people to look for safer medicines from natural origin. Thus, using a combination of modern computational techniques with the knowledge of traditional medicine will enable scientists to discover new phytocompounds and to convert them as effective medicines.

The present study entitled "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" was carried out with the objective to characterize the active ingredients in *Glycosmis pentaphylla* and to identify the compounds offering antiviral properties to this plant, through docking studies. Docking studies against protein targets for chikungunya, hepatitis, dengue and influenza were undertaken in this study.

2. Review of literature

The literature relevant to the investigation "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" is reviewed in this chapter.*Glycosmis pentaphylla* (Retz.) Correa, commonly known as orange berry and gin berry is a species of flowering plant belonging to Rutaceae family. It is an example for folklore medicinal plant used for treating various diseases all around the world (Sreejith *et al.*, 2012).

2.1 Vernacular Names

The plant has got various vernacular names. It is called *Ash shoura* in Bengali, *Obok* in Burma, *Komuong* in Chinese, *Ban nimbu* in Hindi, *Kirmira* in Marathi, *Panal* in Malayalam, *Annam, Konji* and *Kattukkonji* in Tamil, *Vanamimbuka* in Sanskrit and *Golugu* and *Gonjipandu* in Telugu.

2.2 Distribution

The plant is native to eastern, southern, and south-eastern Asia and north-eastern Australia. *Glycosmis pentaphylla* is having a wide distribution ranging from India, Malaysia and southern China to the Philippine islands where it occurs in tropical forests at low altitudes (Wang *et al.*, 2006). This plant is a small or medium sized evergreen shrub without thorns. It normally grows up to 4 m height and sometimes grown in gardens for its dark green glossy leaves and white or pinkish berries (Wealth of India, 2003).

2.3 Traditional Uses

The traditional Indian treatment system, Ayurveda, has been using this plant for treating various ailments. In India, roots pounded and mixed with sugar are given in low fever cases and the wood of this plant has also got its importance in order to treat snakebite or as an appetite enhancer for women after childbirth. Leaf paste in combination with ginger is used in eczema and skin affections (Chopra *et al.*, 1956). Leaf juice of this plant along with sugar is given in empty stomach in the morning to eradicate ascaris (Ambasta, 2000). Roots of this plant are used against facial inflammation, rheumatism, jaundice and anaemia (Oudhia, 2006).

In Ayurveda, plant is used against cough, rheumatism, anaemia and jaundice (Mohammed *et al.,* 2010).Stems and roots of plant are used for treatment of ulcer. Paste of leaves, with a bit of ginger, applied over the navel for worms and other bowel disorders (Sreejith *et al.,* 2012).But there is a need for proper scientific support for these traditional knowledge.

The various parts of this plant like root, stem and leaves are used in Kerala and Tamil Nadu for curing fever, rheumatism etc. Crushed root pieces mixed in water is taken in empty stomach in the morning against stomach pain. Juice of leaves is also used as a vermifuge (Balachandran *et al.,* 2008).

In many parts of Asia, *panal* is boiled and used for treating fever, liver complaints and for various intestinal parasites.This plant is ethnomedically exploited by the traditional healers in Gazipur district of Bangladesh against all forms of cancer (Ariful *et al.,* 2010). Medical practitioners in Bangladesh are using the stem and fruits of this plant against rheumatoid arthritis (Mohammed *et al.*, 2010). Apart from its internal uses, the plant is an aid for excessive skin dryness. *G. pentaphylla* along with other plants were used by folk medicinal practitioners in Bangladesh, against external wound bleeding and to treat bone fracture and its resultant pain (Farhana *et al.,* 2011).

Following the fibrous nature of stem, they are used as tooth brushes and has a slight bitter taste. The fruits are edible. Leaves have insect repellent activity and are reported to be used by natives of India, South Africa and Australia (Bonny *et al.,* 2005). *G. pentaphylla* is used by farmers in Kerala as an indigenous bio-pesticide (Namita *et al.,* 2011).

In a study conducted by Amran *et al.* (2011), methanolic extract of all parts of *G. pentaphylla* exhibited antibacterial activity against 12 test bacteria. Phytocompounds offering antifungal properties to this plant have been reported (Bandara *et al.,* 1990; Greger *et al.,* 1996). Studies also shows that *G. pentaphylla* has the capacity to display antioxidant (Amran *et al.,* 2011), antipyretic (Mandal *et al.,* 2011; Gupta *et al.,* 2011), antiproliferation and antitumour activity (Amran *et al.,* 2011).

Review of literature shows that in Indian Ayurvedic system of medicine and in traditional medicinal system of Bangladesh, this plant is used for the treatment of viral disease like jaundice.

2.4 Phytochemicals reported from *Glycosmis pentaphylla*

Various classes of compounds *viz.*, terpenoids (Amran *et al.*,2011), amides (Greger *et al.,* 1994), coumarins (Rahmani *et al.,* 1998) and flavonoids (Tian *et al.,* 1995) have been reported from this plant.

Some of the phytochemicals include arborinine, glycozolicine, 3-formyl carbazole, glycosinine, mupamine, varbazole, 3-methyl carbazole, glycolone, glycozolidol, glycozolinine, glycophymoline, glycophymine, glycomide, glycozoline, noracronycine, des-N-methylacronycine and des-N-methylnoracronycine (Govindhachari *et al.,* 1996; Chakraborty, 1969; Sarkar and Chakraborty, 1977; Sarkar and Chakraborty, 1979; Mukherjee *et al.,* 1983; Chowdhury and Bhattacharya, 1985; Bhattacharyya *et al.*,1985; Choudhury *et al.*,1987; Kamaruzzan and Chakraborty, 1989 and Jash and Biswas, 1992). Glycophymoline, glycophymine, glycomide, glycozoline, noracronycine, des N-methylacrocynine and des-N-methylnoracronycine have been reported from this plantand air dried leaves yielded two furoquinoline bases, kokusaginine and skimmianine (Sreejith *et al.,* 2012). Other alkaloids reported from the leaves include glycosine, arborine, glycosminine, arborinine (major), glycosamineglycorine, glycosmicine, γ-fagarinetriterpenes, arbinol and isoarbinol, arborinone, two isomeric terpene alcohols, myricyl alcohol, stigmasterol and ß-sitosterol (Sreejith *et al.,* 2012).

Roots contain the carbazole alkaloids, glycozolicine, 3-formylcarbazole, glycosinine, glycozoline, glycozolidine, skimmianine, gamma fagarine and dictamine (Sreejith *et al.,* 2012). Stems contain arborinine; other minor alkaloids. The alkaloids arborine, arbomine, skimmianine, glycorine, glycophymine, glycophymoline, glycosmicine and glycomide have been isolated from the flowers (Sreejith *et al.,* 2012). Glycoric acid has been isolated from the methanolic extract of the plant (Ghani, 2003). Six isoflavone glycosides like 3,7-dihydroxy-4',5,6

trimethoxyisoflavone 7-O-(5-O-trans-p-coumaroyl)-ß-d-apiofuranosyl(1>6)-ß-d-glucopyranoside, 2',7-dihydroxy - 4',5',5,6- tetramethoxyisoflavone 7-O-(5-O-trans-p-coumaroyl)-ß-d-apiofuranosyl-(1>6)-ß-d-glucopyranoside, 2',7-dihydroxy-4',5',5,6-tetramethoxyisoflavone 7-O-ß-dapiofuranosyl-(1>6)-ß-d-glucopyranoside,7-hydroxy-4',8dimethoxyisoflavone 7-O-ß-d-apiofuranosyl-(1>6)-ß-d-gluco-pyranoside, 7-hydroxy-4',6- dimethoxyisoflavone 7-O-ß-d-apiofuranosyl-(1>6)-ß-dglucopyranoside , and 4',5-dihydroxy-3',7-dimethoxyisoflavone 4'-O-ß-dapiofuranosyl-(1>6)-ß-d-gluco-pyranoside have been reported (Hinterberger *et al.,* 1986; Ito *etal.,*1999; Hofer and Greger, 2000; Wang *et al.,* 2005; Wang *et al.,*2006).Hydroquinone diglycoside acyl esters like, glypentosides and seguinoside, glypentosides as methoxyquinol 4-O-[(5-O-trans-p-coumaroyl)-ß-Dapiofuranosyl-(1 > 2)-ß-D-glucopyranoside] and 4-demethylantiarol 4-O[(3-methoxy-4-hydroxy-benzoyl)-ß-D-apiofuranosyl-(1 > 2)-ß-Dglucopyranoside were reported from the stem of *G. pentaphylla* (Junsong *et al.,* 2006).

2.5 Use of LCMS/MS in metabolomics

Liquid Chromatography-Mass spectroscopy (LCMS) is an analytical chemistry technique which combines physical separation ability of liquid chromatography and mass analysis ability of mass spectrometry (Aprino, 1992). Because of its high sensitivity, it has emerged as a powerful technique. It is having a wide range of applications oriented towards the separation, general detection and potential identification of chemicals of particular masses in the presence of other chemicals in complex mixtures, for example, natural products from natural-products extracts, and pure substances from mixtures of chemical intermediates.

Liquid chromatography in combination with mass spectrometry has become more efficient and convenient tool for chemical profiling of herbals. This technique has been more useful for the characterization and quantification of individual constituents in the plant extracts. Multi-component analysis (MCA) of herbal extracts can be done using LC/MS. Along with the sensitivity, selectivity

and fastness, separation and identification of different components of a mixture can be done simultaneously. LC performs the function of separation whereas MS performs the function of identification of components in a mixture on the basis of molecular mass and fragmentation pattern. This technique enable us to get a two dimensional information, the retention time gives the first dimensional information and mass detection in the form of molecular mass and fragmentation pattern provides the second dimensional information (Lee, 2002).

LC/MS has shown rapid expansion into the areas of structure elucidation and plays an important role in natural product research. Dan *et al.,* (2007) isolated three new sesquiterpenes from *Pencillium roqueforti* and established their structures. Several carotenoids were isolated and identified from the fruits of *Gardenia jasminoides.* Crocetin, new carotenoid was characterized by LC-MS (Manuel *et al.,* 2006). The active components and metabolites can be determined in pre-clinical studies using LC/MS. The characterization of two metabolites in rat urine was carried out based on the studies on the metabolites of piperine, an alkaloid constituent of *Piper nigrum* using LC/MS/MS and LC/NMR/MS/MS data (Bajad *et al.,* 2003).

In the past decade where a number of lead compounds and new natural products derived from medicinal herbs have been successfully isolated and identified, chemical analysis has played a central role in development and modernization of plant based medicine. But in recent past, mass spectroscopy coupled to LC is emerging as a technique of choice in identification of active ingredients, compositional analysis and chemical fingerprinting studies. Detecting a target compound in crude plant extract may be done using Tandem mass spectroscopy (MS/MS). Detection of camptothecin in an endophytic fungus *Entrophosphere infrquens,* which resides in the plant *Nothpodytes foetida* was done with the help of LC/MS/MS (Touseef*et al.,* 2006).

A liquid chromatography triple quadrupole tandem mass spectrometry (LC-MS/MS) based method has been reported by US Food and Drug Association to monitor the cyanuric acid (CYA) and melamine (MEL) in animal feed

(Turnipseed *et al.*, 2008). Similarly, a comparison of *Ocimum basilicum* and *Cassia fistula* (leaves and branch) aqueous extracts for their ability to detoxify aflatoxins AFB1 and AFB2 was also done (Iram*et al.*, 2016).The structural elucidation of degraded toxin products by LCMS/MS analysis had shown the formation of nine degraded products of AFB1 and AFB2.

2.6 Discovery of drugsfrom natural products

The secondary metabolites from natural sources are often perceived as the ones showing more "drug-likeness and biological friendliness than totally synthetic molecules". As they have been elaborated within living systems, they are regarded as good candidates for further drug development (Chin *et al.*, 2006). It is believed that chemical substances produced by living organisms (particularly the secondary metabolites) have evolved over time are more likely to have a specific biological activity than man-made synthetic chemicals.

In medicine, biotechnology and pharmacology, drug discovery is the process by which drugs are discovered and/or designed. In the past, most drugs have been discovered either by identifying the active ingredient from traditional remedies or by unexpected discovery. The process of drug discovery is so long, involving the identification of candidates, synthesis, characterization, screening, and assays for therapeutic efficacy. Once a compound has shown its value in these tests, the process of drug development will be initiated prior to clinical trials (Lahlou, 2012).

For the betterment and success of drug discovery using natural products, an integrative approach using a combination of the various discovery tools and the new disciplines of integrative biology is required (Lahlou, 2012). In a perspective for the search and development of new, safe and economical medicaments, natural products remain as an essential component. Therefore, pharmaceutical industry should change its mindset and resources are to be reoriented towards the drug discovery using natural compounds.

According to Lutz (2003), natural products not only complement synthetic molecules, but also exhibit drug-relevant features unsurpassable by any synthetic compound. There are many features for the natural products over the other. One such feature is their enormous structural and chemical diversity. Although the biosynthesis of these products involves their repeated interaction with different modulating enzymes, their actual biological function involves their binding with other proteins, hence their capability to interact with other molecules (Lutz, 2003).

The vast diversity of chemical structures and biological activities of natural products based on the forces of natural products chemistry, molecular and cellular biology, synthetic and analytical chemistry, biochemistry, and pharmacology paves the way for success of natural products. Searching structural chemical databases in connection with databases on target genes and proteins, will help in the creation of new chemical entities through computational molecular modelling for pharmacological evaluation (Nisbet *et al.*, 1997).

2.7 Molecular docking

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex (Lengauer and Rarey, 1996). The candidate structures produced by docking should be subjected for ranking by using scoring functions to identify the most compatible structure that may exist in nature.

The term docking had a restricted meaning when it took its birth in 1970s which meant refining a model of a complex structure by optimizing the separation between the interactors but keeping their relative orientations fixed. Later the concept of rigid docking evolved where the relative orientations of the interacting partners were allowed to vary, keeping the internal geometry of each partner fixed. Due to the advancements in computational power, scientists were able to model changes in internal geometry of interacting partners that may be formed during complex formation. This type of docking is called flexible docking.

2.7.1 *In-silico* drug discovery from traditional medicinal plants

Usages of natural products have been present in folk medicine for thousands of years. It is said that one-third of the adult population in industrially developed countries and more than 80 per cent of the population in developing countries adopts herbal medicinal products for the promotion of health and treatment of common diseases such ascold, inflammation, heart diseases, diabetes and central nervous system disorders (Lagunin *et al.*, 2014). More than 70 per cent of new chemical substances introduced into medical practice from 1981 to 2006 were derived from 25 natural products (Newman and Cragg, 2007). Thus the assertion by Dhawan (1995) that adopting a suitable study of plants based on their use in traditional medicinal system, is a viable and cost effective strategy for development of new drugs. Due to the presence of several thousands of pharmacological targets and due to the pleotropic effects exhibited by most of the natural compounds by their interaction with different targets, computational methods serve as a method of choice in natural product based drug discovery (Rollinger *et al.*, 2009).

An integrated approach by combining chemoinformatics and bioinformatics tools may facilitate the efforts for complementary lead and target identification using techniques like molecular docking (Yadav *et al.*, 2010) and pharmacophores (Rollinger *et al.*, 2009). Phytochemical constituents present in a plant are explored either based on a bioactivity guided fractionation or by employing random screening of plant extracts. To date, only the bioactive principles for traditional activities have been used as templates for new drug discovery for known bioactivities using molecular docking (Languin *et al.*, 2014). Therefore the large unexplored potential of these phytochemicals can be effectively investgated using multi-targeted *in silico* approaches. Thus, bioinformatics and systems biology approaches are gaining their importance for studying the therapeutic potential of medicinal plants (Barlow *et al.*, 2012). This may help us in selecting targets for docking and in identifying relationships between the revealed actions of phytochemicals on targets and the known therapeutic effects of medicinal plants.

Quantitative structure–activity relationship models (QSAR models) are regression or classification models in which the predictors include physico-chemical properties or theoretical molecular descriptors of chemicals. QSAR models help us in summarising an assumed relationship between chemical structures and their biological activity. It may also be helpful for predicting the activities of new chemical compounds. QSAR is used in drug discovery in order to identify the chemical structures that inhibit specific target along with low toxicity level. According to Lipinski's rule of five, "druglikeness" can be predicted using partition coefficient logP (Leo *et al.*, 1971). QSAR can also be used to study the interactions between the structural domains of proteins. QSAR models created on the homogenous data, also called local models are used for optimisation of hit or lead compounds.

2.7.2 Docking requirements

For docking, 3D structures of protein targets, which are inevitable, may be retrieved from PDB database (www.pdb.org) or can be made using molecular modelling methods.The use of data only for targets and the non-requirement of knowledge about the active compounds are advantages of docking. Some of the problems related to docking are the limitations in the availability of 3D structures of targets and in the estimation of results by selecting proper scoring function.

The study of ADME/T properties (absorption, distribution, metabolism, excretion and toxicity) for phytochemicals is essential for using the compound as a drug. Natural products are known to have more preferable ADME/T properties than synthetic drugs. The physical-chemical properties of the compounds along with their interaction with transporter proteins and blood proteins determine the adsorption, distribution and excretion in the body. Interactions with drug-metabolising enzymes (e.g., P450 cytochromes) decide the metabolism of phytochemicals (Languin *et al.*, 2014).

2.7.3 Docking in studies of phytochemicals

Molecular docking has been performed in various natural products inorder to use it as a drug. Khan *et al.* (2009) found that sieboldigenin which is a spirostane sterol found in *Smilax glabra* binds to the active site of soybean lipoxygenase (SLOX). In traditional chinese medicine, it was used as a 'heat clearing' herb, employed largely for arthritic joint pain and skin disease. Similarly, leucovorin was discovered as a potential anti-HIV agent by screening Chinese natural products using a molecular fingerprint based on the HIV protease inhibitor, saquinavir (Gao *et al.*, 2006).

As a result of combined molecular fingerprint studies and docking, Wang *et al.* (2007) discovered that aurantiamide acetate, from *Artemisia annua* (qinghao), inhibits severe acute respiratory syndrome coronavirus main proteinase (SARS-CoVMpro). Zeng *et al.* (2008) performed VS (virtual screening)studies of quorum sensing inhibitors of *Pseudomonas aeruginosa* in an experiment to search for different ways to prevent surface biofilm formation. Fifty-one traditional medicine compounds with known antibacterial activity were docked into the active site of the transcription activation factor TraR. *In vitro* screening of eight of the high scoring compounds subsequently showed that *Pseudomonas aeruginosa* growth was effectively inhibited by baicalein and that this compound acted synergistically with ampicillin.

By VS based on molecular descriptors, three Chinese herbal constituents viz., flavonoids myricetin, liquiritigenin and gossypetinwere selected by Paoletta *et al.*,(2008). These molecules were then docked into the active site of human aromatase and identified as potential aromatase inhibitors. Liquiritigenin was shown to have a lowest binding energy.

Epimedium spp. is commonly used as a medicine for 'yang invigoration' in which various medicines are used for strengthening the body. In an effort by Chen *et al.* (2009), to identify constituents of these herbs that might be able to mimic the phosphodiesterase 5 (PDE5) inhibitory effect of sildenafil, docking was used. Some of the potential inhibitors identified showed docking scores on par with that shown by another known PDE5 inhibitor taladafil (Chen *et al.*, 2009).

Three studies by Yu *et al.,* 2007; Deng *et al.*, 2008; Wang *et al.,* 2008 havebeen reported which involves *in silico* screening of herbs for active compounds against various kinases that can be used as targets for cancer treatment. In case of aurora-A kinase and polo like kinase as targets, Shen *et al.,* (2003) pharmacophores derived from known inhibitors which were used to screen the CNPD (Chinese Natural Product Database) and for KDR kinase as target, Qiao *et al.,* (2002), inhibitors were used to screen TCMD (Traditional *Chinese Medicine* - an online database). The hits were then filtered using the Lipinski rules, regression analysis predictions of IC50, and molecular docking to relevant protein targets. After the wet lab study, a pterocarpanglucoside which was identified as a hit from the TCMD was found to be good.

Searches for multi-target ligands, where a single phytochemical with the potential to inhibit a variety of functionally/pathologically related targets were also carried out. Huang *et al.* (2007) have reported that several constituents of plants used in the Chinese formula Xuefuzhuyu tang are inhibitors of targets relevant to the treatment of cardiovascular disease. Candidate drug compounds with acceptable ADME profiles were first identified by Lipinski filtering, and these were then docked to a number of targets with known significance in cardiovascular disease, including renin, angiotensin-converting enzyme (ACE), vascular endothelial growth factor (VEGFR), 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) and P-glycoprotein (Pgp). Totally there were 283 compounds obtained as possible inhibitors of all the selected protein targets.A sum of 11 herbs studied among which the majority were identified as possible inhibitors of more than one target. Moreover, 10 of the herbs were shown to possess constituents with the potential to inhibit two or more targets, and 50 per cent of these showed the potential to inhibit five targets.

In 2010, Ehrman *et al.,* performed a protein-based screening study in which multiple PDB ligand–receptor complexes were employed to identify possible ligands for major anti-inflammatory targets – COX, p38 MAP kinase (p38 MAPK), cJun terminal NH2 kinase (JNK), and phosphodiesterase 4 (PDE4). A

conformational database of Chinese herbal constituents was first screened using Ligand Scoutpharmacophores. Resulting hits were subsequently docked into the relevant target proteins, and the docking scores compared against the median binding energy for the crystal ligands for that target. Cumulative score analysis for different phytochemical classes within each herb helped to identify 100 plants that are likely to mimic the known PDB ligands.

Barlow *et al.* (2012) reviewed the docking studies on chinese herbal medicines. There are also many papers where properties of Indian herbal medicines were studied.

The immuno-modulatory activity of derivatives of natural coumarino-lignoids isolated from the seeds of *Cleome viscosea* was explored using docking studies. Docking studies revealed the possible binding affinity of coumarino-lignoids to different immuno-modulatory receptors: TLR-4, iNOS, COX-2, CD14, IKK β, CD86 and COX1 (Yadav *et al.*,2010; Meena *et al.,* 2011). Similarly, the anticancer activity of glycyrrhetinic acid analogues against the human lung cancer cell line A-54994 and of the immuno-modulatory/anti-inflammatory activity of gallic acid derivatives were predicted (Yadav *et al.*, 2012). Glycyrrhetinic acid is a pentacyclictriterpenoid derivative of beta-amyrin obtained by hydrolysis of glycyrrhizic acid, found mainly in the root of *Glycyrrhiza glabra* (liquorice). The docking studies have shownhigh binding affinity of the predicted active compounds with the lung cancer target EGFR (Yadav *et al.*, 2013). A molecular docking of gallic acid derivatives showed that the compounds had high binding affinities for INFα-2, IL-6, and IL-4 35 receptors (Yadav *et al.*, 2012).

In 2012, Maurya *et al.* studiedimmuno-modulatory and antiinflammatory activity for the triterpenoids - ursolic acid and lupeol, isolated from *Eucalyptus tereticornis* and *Gentiana kurroo.*Docking results suggested that the studied triterpenoids showed immuno-modulatory and anti-inflammatory activity due to high binding affinity to human receptors and enzymes: NF-kappaB p52, tumour necrosis factor (TNF-alpha), nuclear factor NF-20 Kappa-B p50 and cyclooxygenase-2. Five novel polyhalogenated derivatives and an ester derivative

were synthesised from cleomiscosin - a methyl ether and studied by docking with Scigress Explorer (Sharma *et al.*, 2012). Docking results predicted that these compounds had high binding affinity to IL6, TNF-α and IL1β.

Withanolides from *W. somnifera* were studied for cytotoxic activity against a human breast cancer cell line (MCF7). In this study, AutoDock 4.2 was used for docking withanolides to aromatase (PDBID: 3EQM).This study by Prakash *et al.* (2013) showed that four selected compounds had promising binding affinity values with aromatase in comparison to the reference, the co-crystallised control compound androstenedione.

A study was done using natural inhibitors from *Glycosmis pentaphylla* against protein kinase C as a skin cancer target by Yasir *et al.* (2015). The study included docking works using Autodock 4.0 and Discovery studio 3.5. It was found that one of the secondary metabolite, glycosinine which is a carbazole derivative shows strong binding affinities with protein kinase C which can lead to the impairment of cellular physiological regulatory mechanism.

2.7.4 Docking studies using natural products against viral diseases

A study was undertaken by Mavuduru *et al.*, where, important phytoconstituents from 20 plants were used for docking studies using Maestro (Glide) and Lead IT (FlexX). Later, molecules which showed good docking scores were docked in Autodock 4.2. The docking study was conducted against influenza, dengue, HIV and chikungunya diseases. Quercetin and Kaemferol, which are flavonoids, gave good docking scores against most of the viral proteins. Quercetin interacted well with Neuramidase of Influenza (1L7F) in Maestro. Apart from flavonoids, gallic acid gave good docking score values against dengue, chikungunya and HIV proteins. Chemical constituents of *Euphorbia hirta* gave good docking scores on dengue, HIV and influenza proteins. Curcumin, an active ingredient in *Curcuma longa* gave good docking scores against HIV proteins like reverse transcriptase and protease. Mangiferin and gallic acid which are the main components of *Mangifera indica*, gave good docking studies on almost all the

viruses. Mangiferin is reported to antagonize cytopathic effects of HIV gave good docking studies against HIV protease.

Docking studies on anti-inflammatory compounds from medicinal plants against ulcerative colitis was conducted in 2013 by Hamsa *et al.* The target NF-kB p50/p65 was docked in two different ways; one with the glucocorticoid receptor protein using ZDOCK in Accelrys Discovery Studio 3.5 and the other was screening and docking of400 anti-inflammatory natural compounds. The results of the study showed that Ginkgetin, Bilobetin and Mesuaxanthone B displayed the best binding interactions among 400 anti-inflammatory compounds. The study also paves the way for further lab studies for the confirmation of the above results.

Mohan *et al.*, (2015) conducted docking studiesof phytochemicals from *Phyllanthus niruri* against hepatitis B DNA polymerase using the software Discovery studio 4.0. Docking studies revealed that a few phytochemicals from *Phyllanthus niruri* had good interactions with HBV DNA Polymerase.

Dengue infection which is turning into a serious disease due to the unavailability of proper treatment was studied using docking by Qamar *et al.*, 2015. NS1 glycoprotein of Dengue virus involved in its RNA replication was chosen as the target. Six flavonoids (Deoxycalyxin A; 3,5,7,3',4'-pentahydroxyflavonol-3-O-beta-D-galactopyranoside; Sanggenon O; (3R)-3',8-Dihydroxyvestitol; Epigallocatechingallate; Chamaejasmin) blocked the Asn-130 glycosylation site of NS1 and could be assumed to inhibit the viral replication.

2.7.5 Chikungunya – A viral disease

Belonging to the family *Togaviridae* and genus alphavirus, Chikungunya virus (CHIKV) is an emerging arthrogenic virus. The word ''Chikungunya'' translates to ''that which bends up'' relating to the stooped posture developed as a result of rheumatologic inflammation (Lumsden, 1955). CHIKV has been listed as a category C priority pathogen in 2008 by the US National Institute of Allergy and Infectious Diseases (NIAID): this category refers to the pathogens that could be engineered for mass dissemination in the future, or due to their high morbidity and

mortality rates and those with major health impacts (Powers and Logue, 2007 and Schwatz and Albert, 2010). An urban cycle, man to mosquito to man, or a sylvatic cycle, animal to mosquito to man can be seen in the cycle of CHIKV transmittance (Chhabra *et al.*, 2008).

Similar to the dengue virus, mosquitoes of the *Aedes* genus (*Aedes furcifer* in Africa and *Aedes aegypti* in Asia) act as vectors incase of human infection. A period lasting for 1–10 days normally occurs in case of acute infection and is characterized by a painful polyarthralgia, high fever, asthenia (weakness), headache, vomiting, rash, and myalgia (muscle pain). As only 19 percent of patients have been reported with rashes, it the least reliable symptom. Polyarthralgia is a major characteristic feature of the persistent chronic CHIKV attack (aches in the joints, joint pains) that can last from weeks to years. CHIKV attacks fibroblasts, explaining the involvement of muscles, joints, and skin connective tissues.

2.7.5.1 Virology of the CHIKV

The CHIKV genome is a positive sense, single stranded RNA genome. There are two open reading frames (ORFs), (Singh and Unni, 2011) the 5` end encoding the non-structural protein precursors:

(i) nsP_1: helps in viral mRNA capping via its guanine-7-methyltransferase and guanyltransferase enzymatic activities

(ii) nsP_2: acts as protease and helicase

(iii) nsP_3: part of the replicase unit and an accessory protein involved in RNA synthesis

(iv) nsP_4: RNA-dependent-RNA polymerase

For the synthesis of viral negative strand,the nsP_{123} precursor and nsP_4 function as a complex. The ORF at the 3' end encodes the structural proteins, the capsid (C), envelope glycoproteins E_1 and E_2 and two small cleavage products (E_3, 6K). The untranslated junction region (J) contains its internal promoter, a conserved sequence of 21 nucleotides.

2.7.5.2 Emerging novel chikv targets

As previously mentioned, the CHIKV genome is formed of 2 ORFs, one from the 5` end coding for nsP_1, nsP_2, nsP_3 and nsP_4. The 3' end ORF encodes the capsid (C), envelope proteins E_1, E_2, E_3 and 6k. These proteins, which acts during the essential steps in the lifecycle of the virus (Schwartz and Albert, 2010), could be the possible targets for drug design.

2.7.5.2.1 Non-Structural Protein

Non-structural protein 1- CHIKV nsP_1 is a palmitoylated 535 amino acid protein. Methyltransferase and guanylyltransferase are found in the 3' end are involved in capping and methylation for the formation of a new viral genomic and subgenomic RNAs (Solignat *et al.*, 2009).

Non-structural protein 2 - The non-structural protein 2 (nsP_2) of alphaviruses is a multifunctional protein (Hardy and Strauss, 1989). The proteolytic activity of nsP_2protein is crucial for the replication of virus and also plays a role in the cleavage of the non-structuralpolyprotein complex.

Non-structural protein 3 - Though the mutations affect different steps in replication of virus, the exact function of nsP_3 is not revealed (De *et al.*, 2003). It was found that the deletion of phosphorylated residues at the C-terminal region decreases the level of RNA synthesis (Vihinen *et al.*, 2001).

Non-structural protein 4 - The non-structural protein 4 acts as a RNAdependent-RNA polymerase (Shirako *et al.*, 2000). It was noticed that it has a role in suppression of the host cell unfold protein response (UPR), also named as the endoplasmic reticulum (ER) stress response (Rathore *et al.*, 2013) which is a mechanism that maintains the cellular protein homeostasis and prevents over-loading of unfolded protein in the lumen of the ER during normal and diseased cellular conditions.

2.7.5.2.2 Structural Proteins

There are mainly two glycoproteins, E_1 and E_2 that works in the invasion of susceptible cells and carry the basic antigenic determinants. They form the

icosahedral shell of the virion particle. They have major functions in receptor identification, fusion with cell membrane and elicitation of antigenic response in host cells (Dudha *et al.*, 2014). The viral entry into the host cells is being controlled by the E_1 and E_2: virus fusion into cell membranes under low pH condition is mediated by E1 (Kielian and Helenius, 1985), while interaction with a cellular receptor is done by E_2 (Dubuisson and Rice, 1993). E_3 is responsible for the proper localization of the structural polyprotein and its cleavage from E_2 is essential for spike maturation (Liljestrom and Garoff, 1991).

2.7.5.2.3 Heat shock 70 kDa protein

It belongs to a family of conserved ubiquitously expressed heat shock proteins which helps in the folding of protein and functions in cell protection under oxidative stress condition. HSP70 helps the chikungunya virus in mammalian cell entry by acting as a binding protein (Reddy *et al.*, 2014).

2.7.5.2.4 Interleukin-6 (IL-6)

This type of interleukin is being secreted by T cells and macrophages under inflammatory conditions. During chikungunya infection, the inflammatory response of IL-6 mediates the virus (Dhanwani *et al.*, 2014).

2.7.5.2.5 Tumour necrosis factor alpha

It is an adipokine that is found during the inflammation process and is expressed during the immune regulation. It helps in induction of apoptosis in cells. The activation of viral infection is associated with increased expression levels of TNF-alpha (Dhanwani *et al.*, 2014).

2.7.5.2.6 Interferon-beta

These proteins that are released by pathogens like viruses trigger immune response in host cells. Following the viral infection with enhanced expression, these interferons are expressed by fibroblasts (Rudd *et al.*, 2012).

2.7.5.2.7 Signal transducer and activator of transcription 2 (STAT2)

They function as transcription activators and are protein belonging to STAT family. Chikungunya virus utilises this housekeeping molecule to facilitate infection in mammals (Painqankar and Arankalle, 2014).

2.7.5.2.8 Human leukocyte antigen (HLA)

These genes encode antigen producing cells and other proteins. The alleles for HLA play an important role in susceptibility of infection by chikungunya virus (Thanapathi *et al.*, 2014).

2.7.5.2.9 Actin

Being a globular multi-functional protein, it is expressed during cell motility, cell functioning and muscle contraction. The action of actin molecule mediates the chikungunya viral infection (Painqanker and Arankalle, 2014).

2.7.6 Hepatitis B

The hepatitis B virus (HBV) is a small DNA virus with some similar features as that of retroviruses (Ganem and Schneider, 2001: Hollinger and Liang, 2001). It is a prototype virus of the *Hepadnaviridae* family. Based on sequence comparison, HBV is classified into eight genotypes, A to H. The infectious serums when viewed under electron microscope, visualization of three types of viral particles was done. Two of the viral particles are smaller spherical structures and filaments of variable length were also seen. The spheres and filaments are composed of hepatitis B surface antigen (HBsAg) and host-derived lipids without viral nucleic acids. Therefore they are non-infectious (Gavilanes *et al.*, 1982). The infectious HBV virion (Dane particle) has a spherical, double-shelled structure consisting of a lipid envelope containing HBsAg that surrounds an inner nucleocapsid composed of hepatitis B core antigen (HBcAg) complexed with virally encoded polymerase and the viral DNA genome.

The viral genome encodes four overlapping open reading frames (ORFs: S, C, P, and X). The S ORF encodes the viral surface envelope proteins, the HBsAg. Related but functionally distinct proteins are produced due to multiple in-frame translation initiation codons, which is a feature of the S and C genes. Depending

on the place of translation initiation, the C ORF encodes either the viral nucleocapsidHBcAg or hepatitis B e antigen (HBeAg). The core protein has the intrinsic property to self-assemble into a capsid-like structure (Hatton *et al.*, 1992). Although the function of HBeAg has been considered as an immune tolerogen, whose function is to promote persistent infection, its actual role remains largely undefined (Milich and Liang, 2003). The P ORF encodes a large protein that is functionally divided into three domains: the terminal protein domain, which has a role in encapsidation and initiation of minus-strand synthesis; the reverse transcriptase (RT) domain, which catalyzes genome synthesis; and the ribonuclease H domain, which is involved in the degradation of pregenomic RNA and facilitates replication. The HBV X ORF encodes a protein (HBxAg) with multiple functions, including signal transduction, transcriptional activation, DNA repair, and inhibition of protein degradation (Cross *et al.*, 1993; Bouchard and Schneider, 2004; Hu *et al.*, 2006; Zhang *et al.*, 2001). The mechanism of this activity and the biologic function of HBxAg in the viral life-cycle remain largely unknown. However, it is well establishedthat HBxAg is necessary for productive HBV infection in vivo and may contribute to the oncogenic potential of HBV.

2.7.6.1Hepatitis targets

2.7.6.1.1 Hepatitis B Virus X interacting Protein

The hepatitis B virus (HBV) X protein (HBx) is essential for virus infection and has been implicated in the development of liver cancer associated with chronic infection. HBx can interact with a number of cellular proteins. In cell culture, it exhibits pleiotropic activities, among which, one of its ability is to interfere with cell viability and stimulate HBV replication. HBx affects cell viability by a mechanism that requires its binding to DDB1, a highly conserved protein implicated in DNA repair and cell cycle regulation. HBx in association with DDB1 acts in the nucleus and stimulates HBV replication mainly by enhancing viral mRNA levels, regardless of whether the protein is expressed from the HBV genome itself or supplied in trans (Leupin *et al.*, 2005).

2.7.6.1.2 PDZ domain

Many of the human viruses with oncogenic capabilities, either in their natural host or in experimental systems (hepatitis B and C, human T cell leukaemia virus type 1, Kaposi sarcoma herpesvirus, human immunodeficiency virus, high-risk human papillomaviruses and adenovirus type 9), encode in their limited genome the ability to target cellular proteins containing PSD95/ DLG/ZO-1 (PDZ) interaction modules. Though it is not a must, it has been found in many cases that the viruses have evolved to bind the PDZ domains using the same short linear peptide motifs found in host protein-PDZ interactions, and in some cases regulate the interactions in a similar fashion by phosphorylation. But diverse viruses target a common subset of PDZ proteins that are intimately involved in controlling cell polarity and the structure and function of intercellular junctions, including tight junctions. Cell polarity is fundamental to the control of cell proliferation and cell survival and disruption of polarity and the signal transduction pathways involved is a key event in tumourigenesis (James and Roberts, 2015).

2.7.7 Hepatitis C - Target

The hepatitis C virus (HCV) is a small, enveloped, single-stranded, positive-sense RNA virus (Rosen, 2011). It is a member of the *Hepacivirus* genus in the family *Flaviviridae* (Ray *et al.*, 2009). HCV makes use of its viral genomic RNA as a template for both translation and generation of a complementary (-)-stranded RNA intermediate.A membrane-associated replicase enzyme complex consisting of virally encoded and host proteins is responsible for the replication of viral RNA. The catalytic subunit of the replicase complex is the HCV encoded non-structural 5B protein (NS5B), which contains all the sequence motifs highly conserved

2.7.8 Dengue Virus

Dengue virus (DENV) is a member of *Flaviviradae* family containing four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) (Weaver and Vasilakis,

2009). The DENV genome encodes a polyprotein. This polyprotein is cleaved into 10 viral proteins including three structural and seven non-structural proteins. The order of these proteins is capsid, premembrane, envelope protein, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Non-structural proteins are mainly involved in viral replication (Chambers *et al.,* 1990).

2.7.8.1Dengue – Targets

2.7.8.1.1 NS2BNS3 complex

According to recent studies, it has been found that NS3 has a serine protease domain at the N terminal region and its activity depends on its interaction with cofactor (NS2B). These two forms a complex called NS2BNS3pro complex (Qamar, 2014). Because of its ability to cleave viral proteins, this complex is very important.Any disruption in functional activities of this region results into the inhibition of viral replication. Hence, to screen and evaluate effects of different drug candidates, NS2BNS3 complex is considered an important target (Rothan *et al.,* 2012). Currently, there is no vaccine and effective drug available for the treatment of DENV infection (Idrees and Ashfaq, 2012)

2.7.8.1.2 Methyltransferase

The methyltransferase domain of dengue virus protein NS5 ensures efficient RNA synthesis initiation and elongation by the polymerase domain (Potisopon *et al.,* 2014). NS5 carries several essential enzymatic activities hosted in two domains: (i) the N-terminal methyltransferase domain (NS5-MTase) and (ii) the C-terminal RdRp domain (NS5-Pol). Both domains are connected by a flexible linker. The NS5-MTase domain catalyzes RNA cap methylation at both the N7 position of the cap guanosine and the 2-O position of the first nucleotide of the neo-synthesized positivestrand RNA (Dong, 2014). It might also harbor the DENV guanylyltransferase activity (Issur *et al.,* 2009). The NS5-Pol domain is responsible for the replication/transcription of the viral genome. NS5-Pol synthesizes RNA in three main phases: de novo initiation (i.e. primer synthesis), transition and elongation.

The NS5-MTase domain is involved RNA cap formation, whose precise timing remains unknown. Being part of NS5, the NS5-MTase domain may influence the different steps of RNA synthesis promoted by the NS5-Pol domain. Some evidence exists for the interaction between the two domains but little is known on a possible inter-regulation of their respective enzymatic activities.

2.7.9 Influenza

Influenza, commonly known as "the flu", is an infectious disease caused by an influenza virus (WHO, 2014.). Three types of influenza viruses affect people, called Type A, Type B, and Type C (Longo and Dan, 2012). The virion is pleomorphic; the envelope can occur in spherical and filamentous forms. In general, the virus's morphology is spherical. The major glycoprotein (HA) is interposed irregularly by clusters of neuraminidase (NA).

2.7.9.1 Influenza - Target

Viral neuraminidase is a type of neuraminidase found on the surface of influenza viruses that enables the virus to be released from the host cell. Neuraminidases are enzymes that cleave sialic acid groups from glycoproteins and are required for influenza virus replication.

When influenza virus replicates, it attaches to the interior cell surface using hemagglutinin, a molecule found on the surface of the virus that binds to sialic acid groups. Sialic acids are found on various glycoproteins at the host cell surface, and the virus exploits these groups to bind the host cell. In order for the virus to be released from the cell, neuraminidase must enzymatically cleave the sialic acid groups from host glycoproteins (CDC, 2014). Since the cleavage of the sialic groups is an integral part of influenza replication, blocking the function of neuraminidase with neuraminidase inhibitors is an effective way to treat influenza.

Materials and Methods

3. Materials and methods

The research work entitled "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" was carried at CPBMB, College of Horticulture during 2014-16. The materials used and methods followed are detailed in this chapter.Ligands for molecular docking were identified through wet lab studies and suitability of the same for drug development was ascertained through *in silico* studies.

3.1 Materials

3.1.1 Plant material

The traditional medicinal plant *Glycosmis pentaphylla* (Retz.) Correa belonging to the rutaceae family was selected for molecular docking to know the antiviral properties of various compounds present in stem, leaf and root of the plant. The plant known as "*panal*" in Kerala and used for medicinal and insecticidal properties was used for the study.

3. 1. 2 Other materials used for wet lab study

The other materials used in this study are methanol (90% and 75%), pure distilled water, glasswares like conical flask, funnel, glass rod, whatman filter paper (No. 1), tandem quadrupole UPLC for doing LCMS/MS study

3.2 Wet lab study

3.2.1 Plant material collection and processing

The plants were collected from Instructional Farm, College of Horticulture, Kerala Agricultural University, Vellanikkara (Plate 3.1). The above-ground portion of the plant was collected in September, 2015 while the roots were collected in July, 2016. The leaves, stemandroots were separately chopped into

Plate 1 *Glycosmis pentaphylla* (Retz.) Correa

Plate 2 Shade drying of *Glycosmis pentaphylla* leaf material

Plate 3 Shade drying of *Glycosmis pentaphylla* stem parts

Plate 4 Shade drying of *Glycosmis pentaphylla* root parts

small pieces and shade dried (Plates 3.2, 3.3 and 3.4). Subsequently, the materials were dried in hot air oven at 40 °C for 12 days. The material that has been dried to optimum moisture content was powdered to a coarse powder using a grinder. The powder was stored in a dry zip-lock cover and stored at room temperature under dry condition.

3. 2 Methods followed

3.2.1 Extraction of phytochemicals

For the extraction of the active molecules from the various parts of *Glycosmis*, hydro-alcoholic extraction was followed (Celeghini *et al.*, 2001) in whichboth volatile and non-volatile compounds are extracted simultaneously.

The quantity of leaf, stem and root powders obtained by powdering the dried samples in the grinder were 278, 286 and 18 g, respectively. For leaves, 100 g of powdered sample was first used in extraction using 90 per cent methanol. For getting hydroalcoholic extract of dry plant sample, 10 gram powderwas suspended in 100 ml 95 per cent methanol. Extraction was done in rotary shaker at 120 rpm and temperature 37 degree Celsius for 24 hours and then filtered using whatman No 1 filter paper (Plate 3.5). The crude extract was collected and the extraction for the residue was repeated 75 per cent methanol. The residue left after filtration was again extracted with distilled water for 24 hours in rotary shaker. The final extract was collected and added with the crude extract obtained in the previous steps, with 95 per cent and 75 per centmethanol (Kaneria *et al.*, 2012 and Sakeran *et al.*, 2014). The final extract collected was concentrated by evaporation in rotary evaporator till the final volume is reduced to one-third of the original composite extract and stored under cool condition. The same procedure was repeated for stem and root extract preparation also.

3.2.2 LCMS/MS Analysis

The LCMS/MS (Liquid Chromatography Mass Spectrometry) technique could be used to identify both non-volatile and volatile compounds present in a sample. The LCMS/MS analysis was carried out by outsourcing at the Analytical

Plate 5 Incubation of powdered plant samples in shaking incubator for hydro-alcoholic extraction

Plate 6 Extract under filteration

Plate 7 Hydro-alcoholic extract for LCMS/MS analysis

laboratory, Cashew Export Promotion Council, Kollam, Kerala. The instrument used for the analysis was Waters Acquity UPLC with triple quadrupole mass spectrometer. Crude extract (5 ppm) was infused using acetonitrile and water in the ratio 1:1 and formic acid. Electro spray ionization with a positive mode polarity (ES+), 3300 V of capillary voltage was used. Cone voltage of 35V and a gas flow of 8L/min. were applied. The source temperature was set at 150°C and desolution temperature was 500°C. The masses of various compounds present in the plantwere obtained after the analysis. Due to the absence of a library of compounds with mass and structure, a detailed literature survey on the reported molecules in *Glycosmis* was undertaken and the masses obtained were matched with that of the compounds already reported. A total of 23 compounds from the leaf sample and 14 compounds from the stem and root samples with the coinciding masses were taken as ligands for the molecular docking studies.

3.2.3 *In silico* study

3.2.3.1 Materials

Commercially licensed software Discovery Studio 4.0 developed and distributed by Accelrys, USA was used for the study at Distributed Information Centre (DIC), CPBMB. The supported computer system was of 4GB RAM and WINDOWS 7 operating system. PubChem database was used for downloading structures of structures of ligands. PDB was used for inputing protein structures.

3.2.3.2 Molecular docking

For molecular docking work a druggable target and ligand is necessary. In this study, druggable targets were proteins/ hormones associated with human viral diseases viz., chikungunya, hepatitis, dengue and influenza and ligands were the small biomolecules identified from root, stem and leafof the plant *Glycosmis pentaphylla* through LCMS/MS analysis.The software used for molecular docking was Discovery Studio 4.0.

For the target identification, Chikungunya Drug Target Database (CDTD – www.biocdtd.org) along with the viral proteins identified from Chikungunya with

a suitable resolution were selected and their structures were retrieved using the three dimensional structural data provided by the Protein Data Bank (PDB). The targets for other viral disease were selected from literature survey.

The major steps involved in molecular docking studies for drug development are preparation of target proteins, preparation of ligands, screening of ligands, docking and the ADMET analysis.

3.2.3.2.1 Preparation of proteins

The protein targets were retrieved from PDB and opened in Discovery Studio. By clicking in the view menu and then selecting hierarchy option, already present ligand groups and water molecules from the protein were deleted. Subsequently, the'dock ligands' option has been selected from 'receptor-ligand interaction' and further selected the 'prepare protein' option. A dialogue box hasappeared and 'run' option was chosen.

3.2.3.2.2 Preparation of ligands and filtration

After the preparation of protein, the active site of choice was selected and clicked the'current selection' in 'Define and Edit Binding Site'. Preparation of ligands was carried out using "prepare ligand" protocol of DS 4.0 option and then selectedthe structures of all ligands which have been retrived from PubChem Database. After submitting the ligands, the same were filtered using Lipinski's rule of five and Vebers protocol (Veber *et al.,* 2002; Lipinski, 2004) to identify the compounds having drug like properties coupled with bioavailability in human system. Lipinski's rule predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight (MWT) is greater than 500 and the calculated Log P (CLogP) is greater than 5 (Lipinski *et al.,* 1996).

3.2.3.2.3ADMET analysis

The level of pharmacokinetic interaction in human body is analysed using the ADMET screeningtool. ADMET refers to absorption, distribution, metabolism, excretion and toxicity of a ligand molecule in human body (Tian *et*

al., 2015).The 'ADMET descriptors' coming under the icon "small molecules" of DS 4.0 was usedto check these properties of a drug molecule. Parameters checked are human intestinal absorption, aqueous solubility, blood brain barrier (BBB), hepatotoxicity, CYP2D6 inhibition, plasma protein binding (PPB) and AlogP and the prescribed standards are given in table 2.

ADMET analysis was done for 21 phytocompounds from leaf samples and 13 compounds from stem and root sample. The ADMET descriptor provides mathematical values for each parameter.All the phytochemicals will be classified into three categories: Acceptable, Highly acceptable and Not acceptable based on the score values obtained. Highly acceptable category includes those compounds which strictly follows the acceptable limits for the different parameters. Acceptable compounds are those compounds which show a slight deviation from acceptable range. They may have up to 2 parameters not falling in the acceptable range. The non-acceptable compounds are those compounds which have 3 or more parameters not falling in the acceptable range.

In the ADMET descriptors absorption level was measured on a 0 (good) to 3 (very poor) scale. Only compounds showing values 0 and 1 were accepted. Solubility level predicts the solubility of compounds in water at 25°C. Its values ranged from 0 (extremely low) to 5 (too soluble) and suitable drug candidates show values between 2 and 4 (Kujawski *et al.,* 2012). For hepatotoxicity prediction, compounds were classified as toxic (true) or non-toxic (false).This parameter helps to predict liver toxicity that might be caused by the ligand molecule on human body. CYP2D6 classification helps to know whether the compound is an inhibitor of P450 2D6 enzyme (Usha *et al.,* 2014).Based on the values, the ligands may be classified as true (inhibitor) or false (non-inhibitor).BBB level was measured in values ranging from 0 (very high penetration) to 4 (very low) where a suitable value lies between 2 and 4 (Cecchelli *et al.,* 2007). PPB predicts the likeliness of a compound to bind to carrier proteins in blood. Based on the interaction, the compounds are classified as highly bound (true) or poorly bound (false) (Smith *et al.,* 2010).

Ligand molecules that pass the Lipinski's and Veber's rule and acceptable ADMET values were taken for docking studies with Discovery Studio 4.0. for drug development.

3.2.3.2.4 Docking with CDOCKER

Molecular docking was carried out by 'CDOCKER' protocol againstthe target selected for various viral diseases viz., chikungunya, hepatitis B, hepatitis C, dengue and influenza using the phytocompounds from *Glycosmis pentaphylla.* To go ahead with docking, the 'CDOCKER' was selected from 'Dock Ligands'. After docking, select the best pose by comparing CDOCKER energy and CDOCKER interaction energy. The best interaction is the one where there is minimum difference between the above mentioned energies. If any ligand displays CDOCKER and CDOCKER interaction energy to be same, then it is considered as the best interaction.In the present study, if the difference between the two exceeds more than 10, the interaction was considered to be unstable and rejected.Then go to scripts and select 'Show ligand binding site atom' to get the binding affinity of the ligand with target protein. The hydrogen bonds are displayed and note the number of hydrogen bonds and the amino acid molecules interacting in this reaction.The number of hydrogen bonds and their distance were noted.Active inhibitors of protein targets associated with the selected viral diseases were identified based on lowest binding energy. As the number of hydrogen bonds formed between the target and the ligand increases, the affinity of ligand with the target protein is better and the ligand is suitable for drug development. Shorter the hydrogen bond length, the distance between the target and ligand is lesser and is better.

A total of 19 protein targets; 12 for chikungunya viral disease, 2 for Hepatitis B, 2 for hepatatitis C, 2 for dengue and 1 for influenza as shown in table 1 were docked with ligands of phytocompounds from *Glycosmis pentaphylla* and results were documented and analysed.

3.2.3.3.2.5Steps in docking

(1) Open Discovery Studio 4.0, the software used for doing docking study.

(2) Go to file menu and open the protein in DS.

(3) Go to view menu and select hierarchy. Delete unwanted atoms like nativeligand group, undesired protein chain, water molecules and heteroatoms.

(4) Select the desired active site of the protein and select current selection option.A grid sphere appears at the site.

(5) Select 'Prepare protein' option from Receptor-ligand interactions.

(6) Go to Receptor-ligand interactions and select Prepare ligands option in orderto prepare the ligands to be docked.

(7) Go to Small molecules and filter the ligands using Lipinski's and Veber'srule.

(8) The filtered ligand molecules are subjected for docking using CDOCKERprotocol in receptor ligand interaction.

(9) After docking, the best pose (minimum difference between CDOCKERenergy and CDOCKER interaction energy) is selected. Go to Scripts andselect Show ligand binding site atom. Select the H bond and note the numberof H bonds and the amino acid interacting in this reaction.

(10) Go to small molecules and select ADMET descriptors. The ADMET valuesfor the ligand is displayed. The bioavailability of the ligand inside humanbody is decided using this protocol.

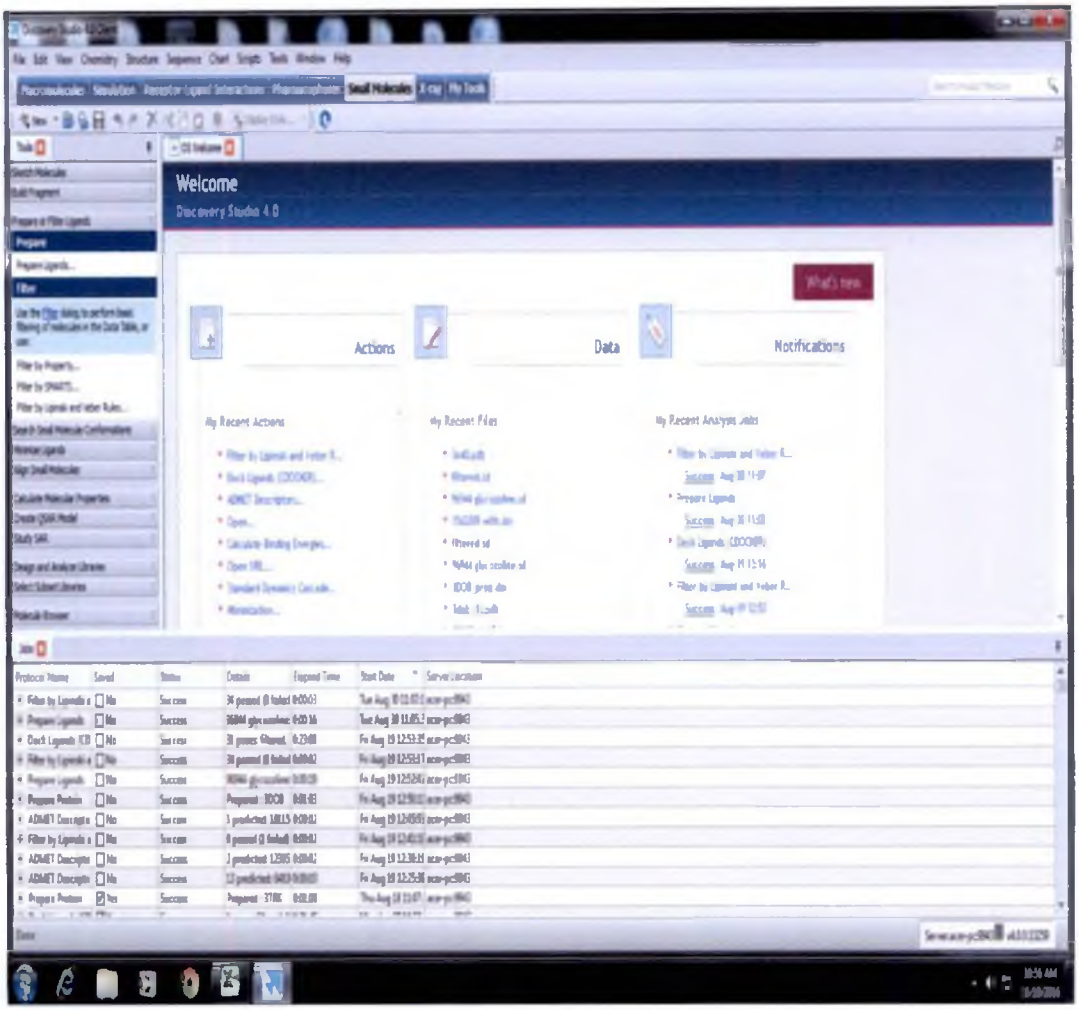


Plate 8 Home page of Discovery Studio

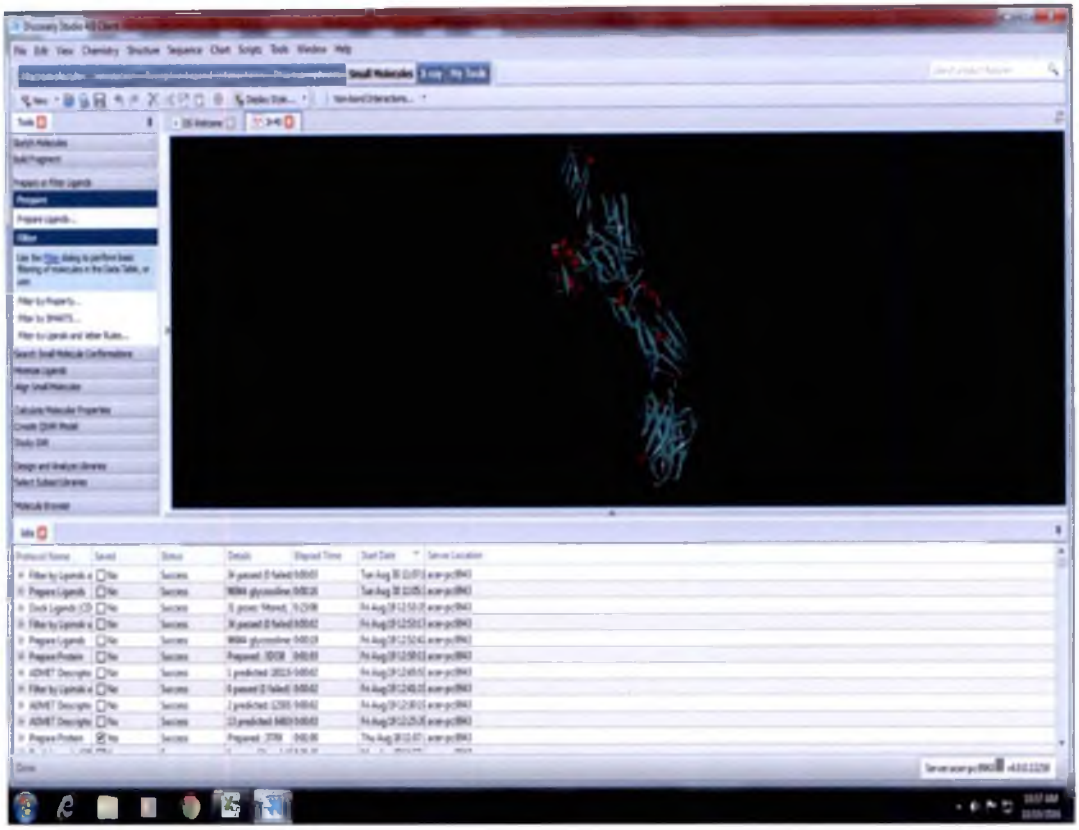


Plate 9 Opening Protein through DS

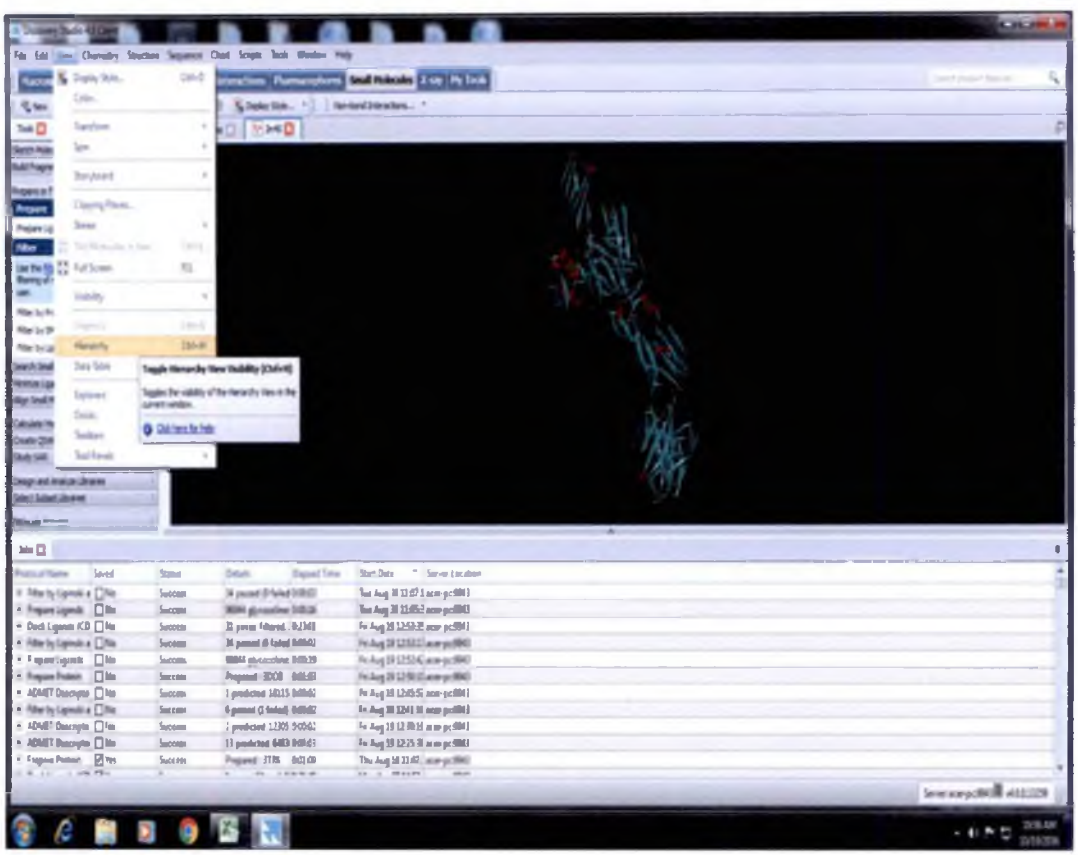


Plate 10 Modifying protein structures for docking

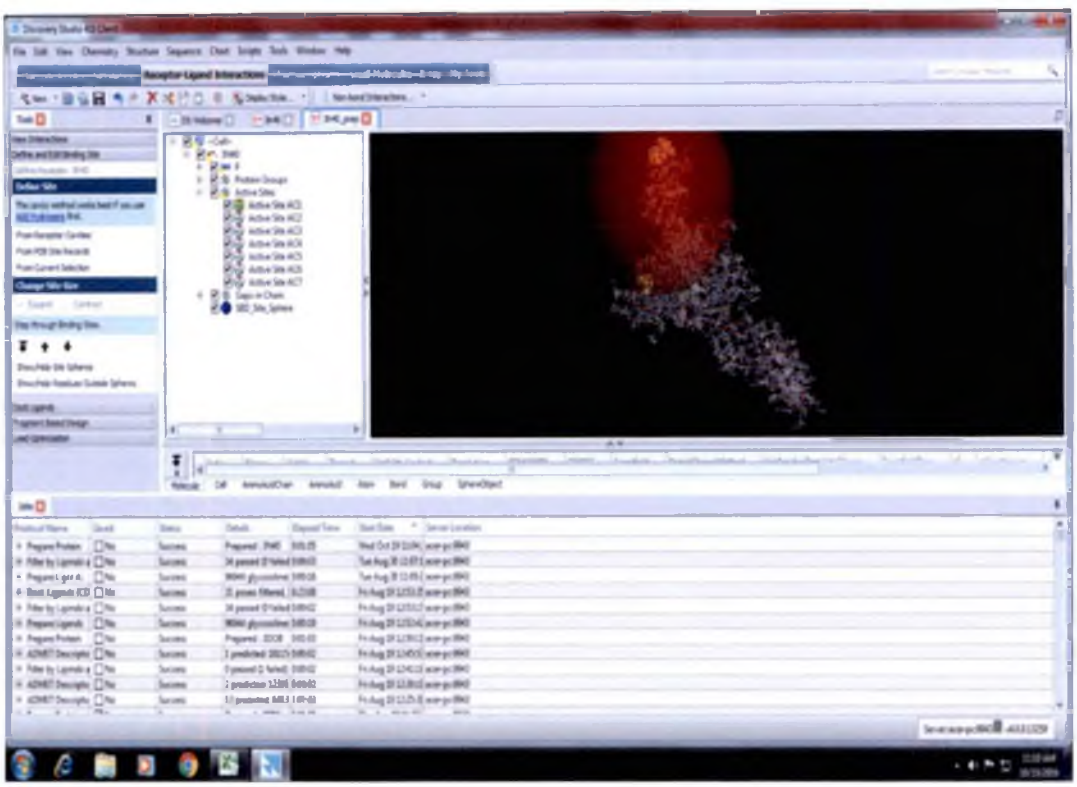


Plate 11 Selection of active site

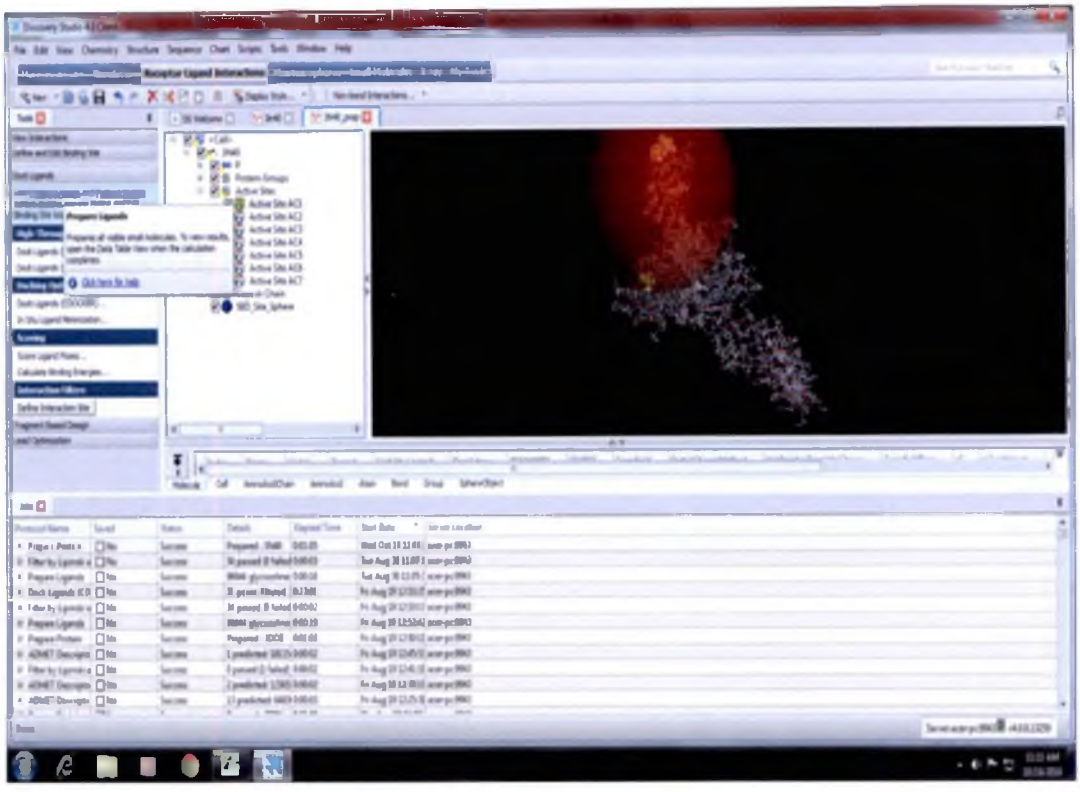


Plate 12 Preparation of Protein for docking

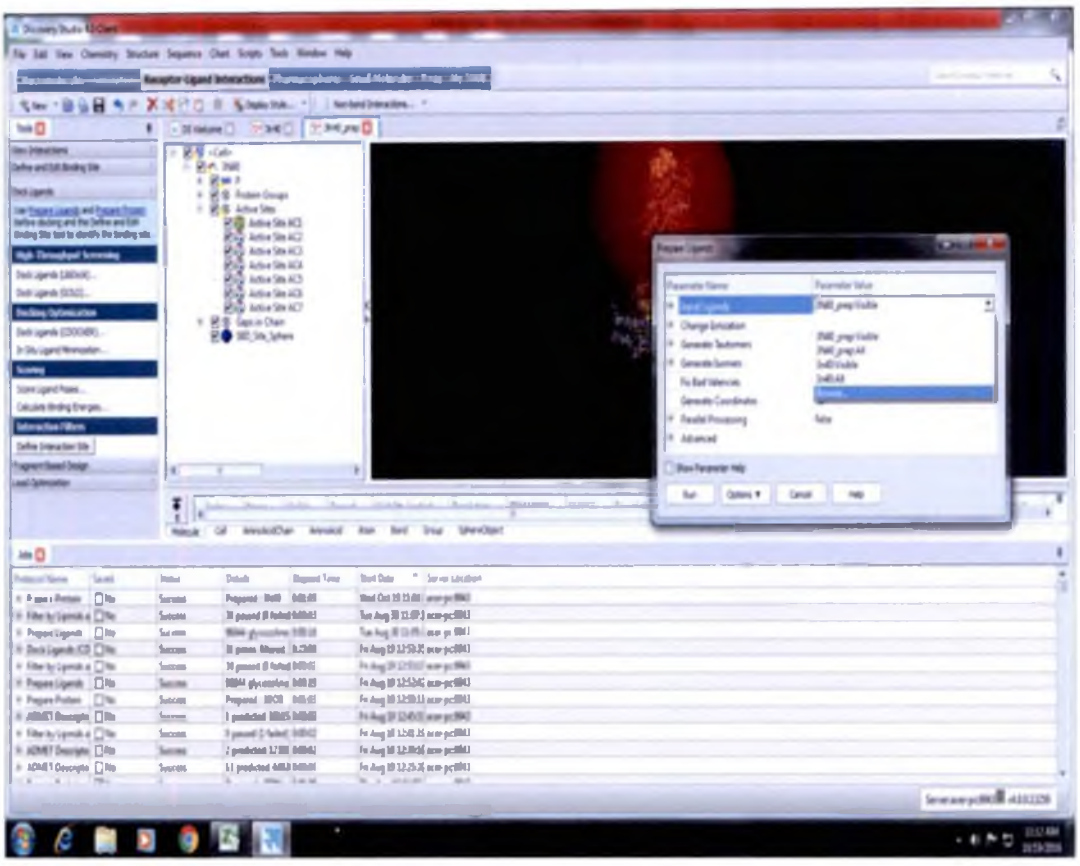


Plate 13 Preparation of ligands

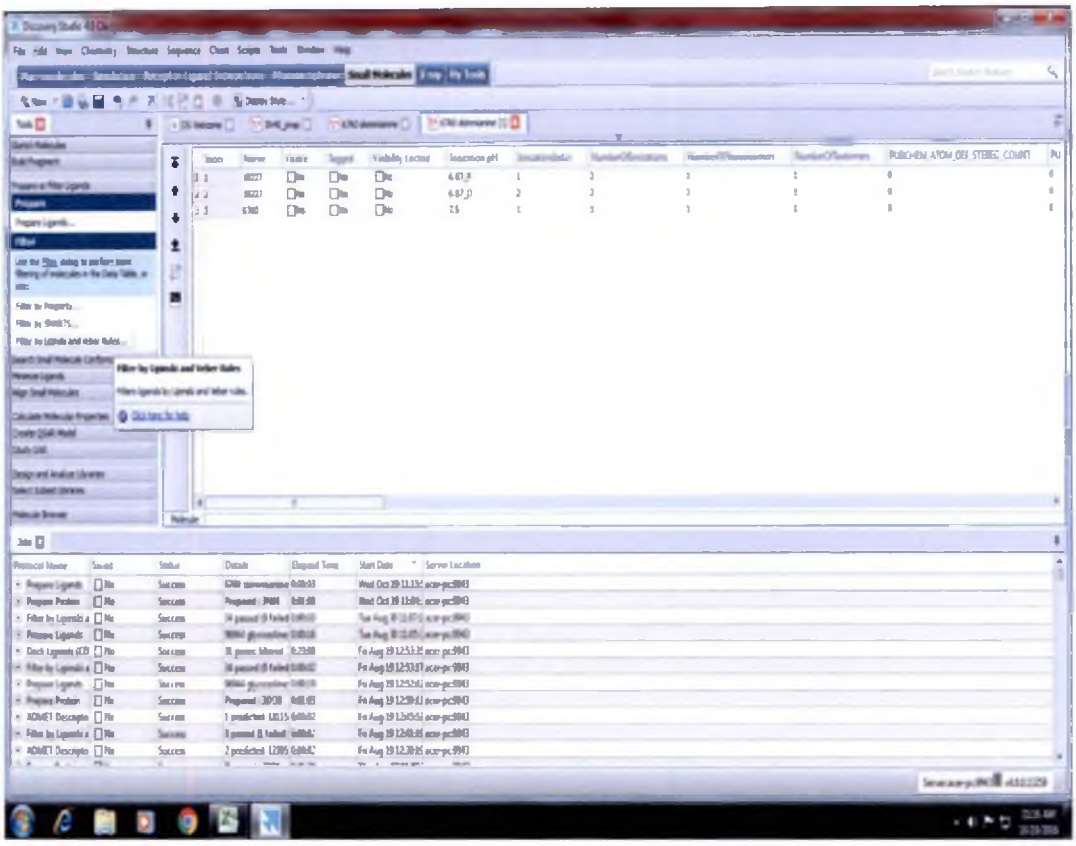


Plate 14 Filtration of ligands

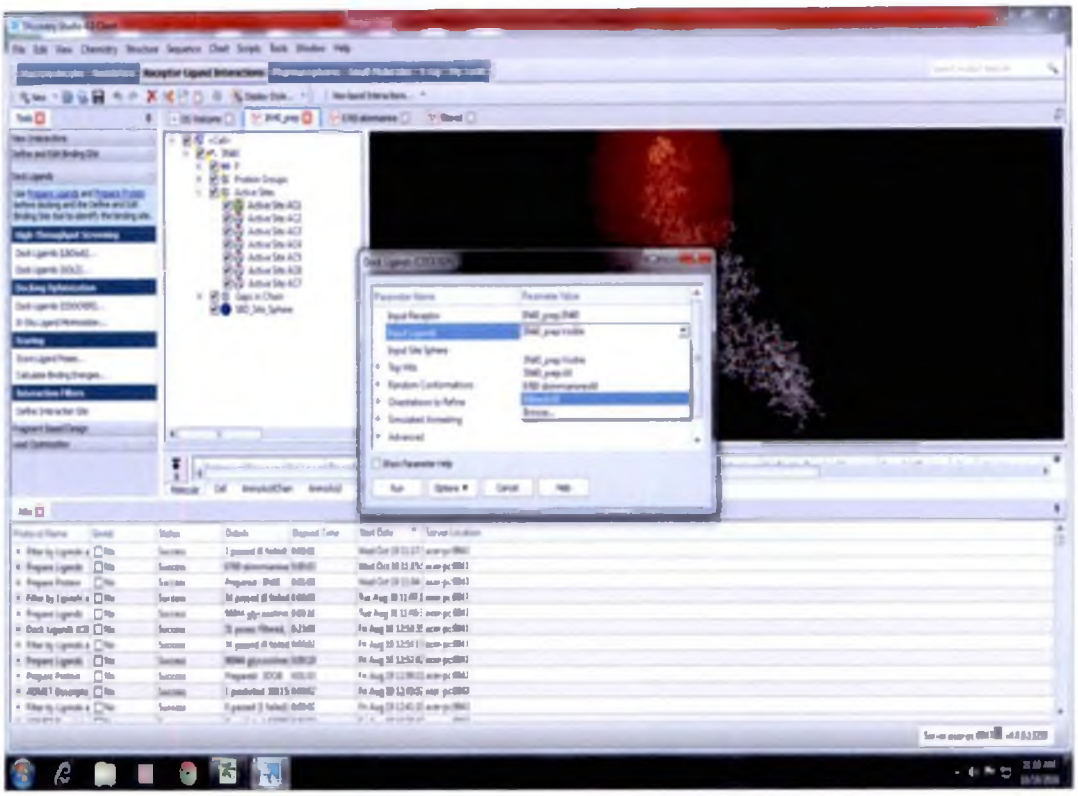


Plate 15 Docking

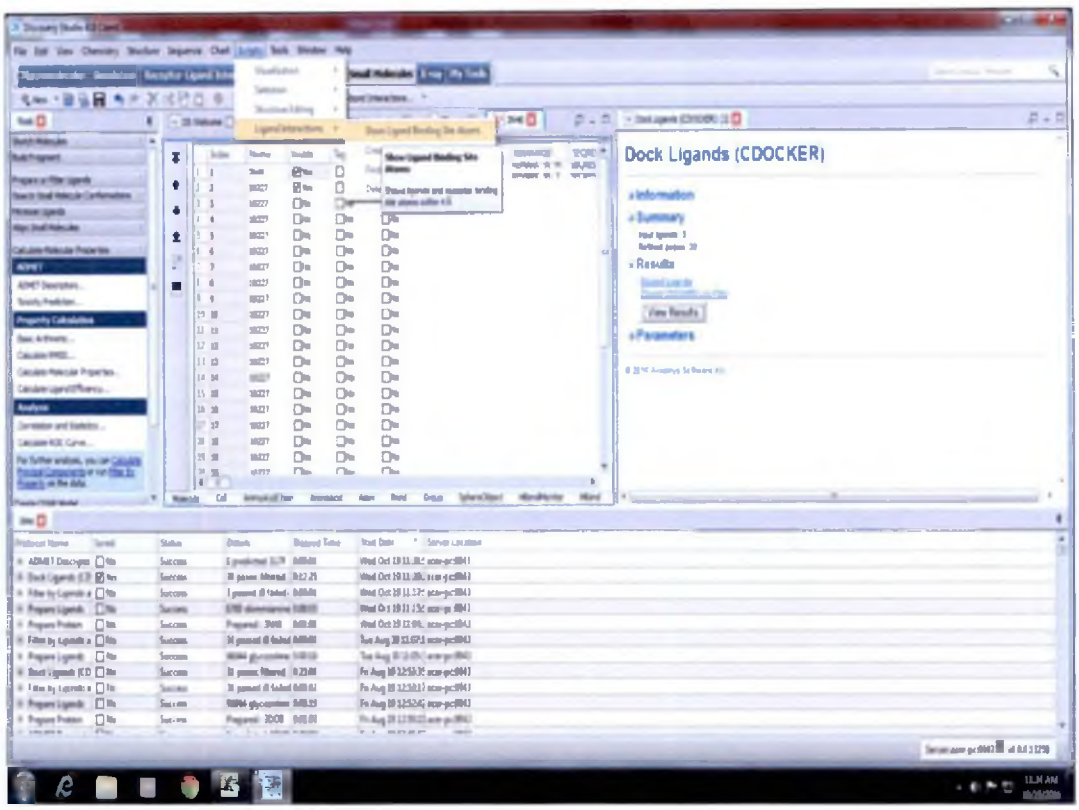


Plate 16 Checking the number of hydrogen bonds and aminoacid involved in the binding

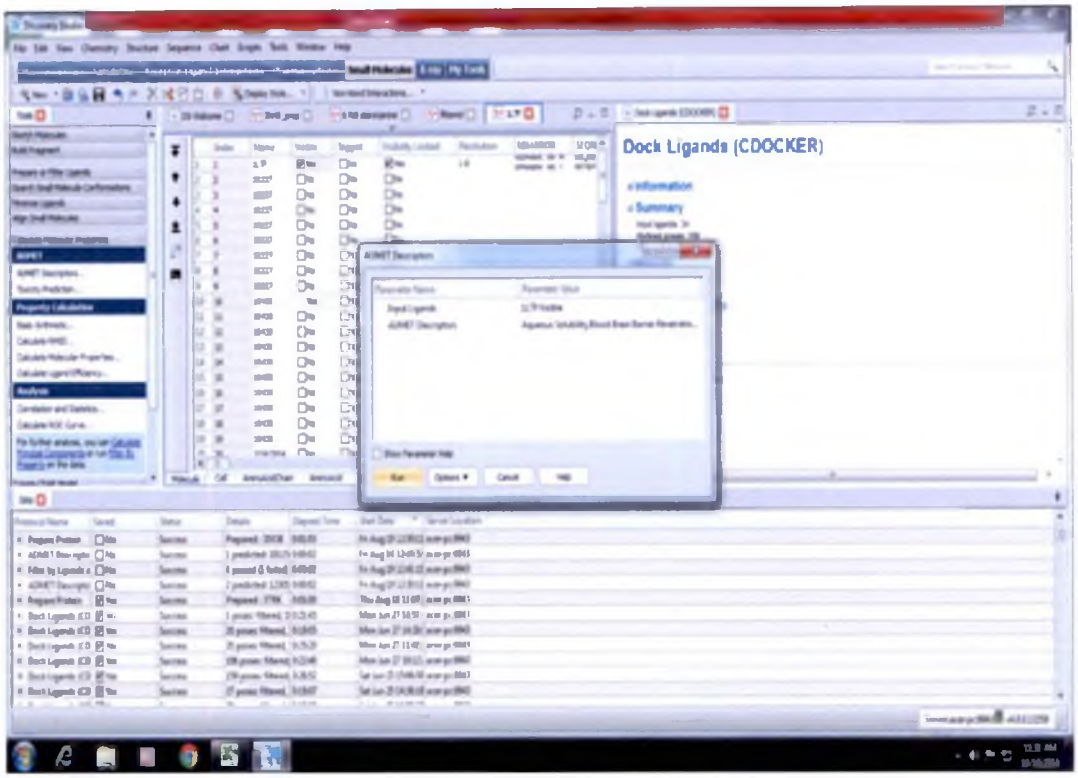


Plate 17 ADMET properties

Table 1. List of protein targets involved in various viral diseases selected for *in silico* molecular docking studies

Sl. No.	Target Name	PDB ID
A. Chikungunya		
1	Immature glycoprotein complex of chikungunya virus	3N40
2	Mature envelope glycoprotein complex of chikungunya virus	3N44
3	Macrodomain of chikungunya virus	3GPO
4	Chikungunya virus nsp3 macrodomain	4TU0
5	Chikungunya virus nsp2 protease	3TRK
6	Heat shock 70 kDa protein	3DOB
7	Interleukin 6	1ALU
8	Tumour necrosis factor alpha	3KMC
9	Interferon-beta	1AU1
10	Signal transducer and activator of transcription II	3ZMM
11	Human leukocyte antigen	2G9H
12	Actin	4M63

B. Hepatitis B		
13	Hepatitis B X-interacting protein	3MSH
14	PDZ domain-containing GIPC2	3GGE
C. Hepatitis C		
15	HCV NS5B RNA polymerase	2DXS
16	HCV NS5B polymerase inhibitors	4EO6
D. Dengue		
17	NS2B/NS3 protease	2FOM
18	Dengue methyl transferase	2P40
E. Influenza		
19	Neuraminidase of influenza virus	1L7F

Table 2. Range for ADMET descriptors

Sl No.	ADMET Descriptors	Range					
1	Solubility	0 (extremely low)	1(very low)	2 (low)	3 (good)	4 (Optimum)	5 (too soluble)
2	BBB level	0 (very high penetration)	1 (high)	2 (medium)	3 (low)	4 (very low)	
3	CYP2D6	False (non inhibitor)	True (inhibitor)				
4	Hepatotoxicity	False (non toxic - 0)	True (toxic – 1)				
5	Absorption	0 (good)	1 (moderate)	2 (poor)	3 (very poor)		
6	PPB	False (poorly bound)	True (highly bound)				
7	Alog P98 Value	< 4					

Results

4. Results

The results of the study entitled "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" are presented in two main headings in this chapter.

4.1 Wet lab studies

4.1.1 Material collection and processing

Plant material from root, stem and leaves was collected and was dried under shade first.Then oven dried at 40^0C. The finely dried powder was used for the preparation of hydroalcoholic extracts of the plant parts using methanol and water. The extract was evaporated using rotary evaporator and then used for doing LCMS/MS study.

4.1.2 LCMS/MS analysis

Through LCMS/MS analysis, the mass:charge ratio of different phytochemicals present in the roots, stem and leaves of the plant were obtained.The molecular weight of various compounds already reported from the *Glycosmis* were taken through literature survey and compared with the masses of the compounds that were obtained from the LCMS/MS study. The compounds reported in the plant with molecular weights within a possible range with that of the molecular weights inferred from the analysis were matched and assumed to be present the sample which was analysed. Thus the phytochemicals in various parts of the plant were identified and were used as ligands for docking study.

The mass:charge ratio of different phytochemicals ranges from 104.14 to 497.00 in leaf extract (Figure 1) and 60.26 to 467.7 in stem and root extract (Figure 2). 25 compounds from leaves and 14 compounds from stem were identified as the possible ligands for the study. Table 3 indicates the list of phytochemicals identified from leaf extract based on the LCMS/MS masses. Table 4 lists out thephytochemicals identified from stem and root extracts using LCMS/MS masses.

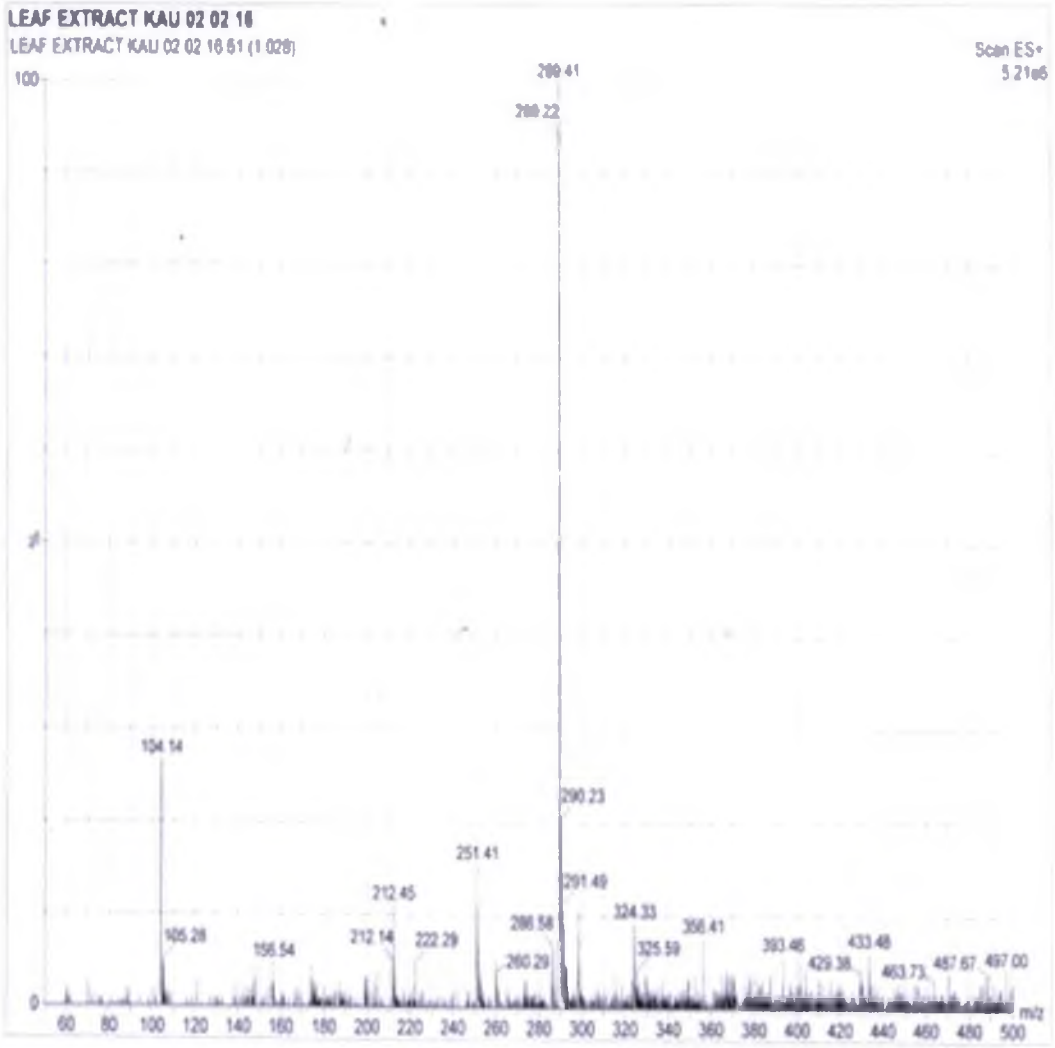


Figure 1 LCMS/MS chromatogram for leaf sample analysis

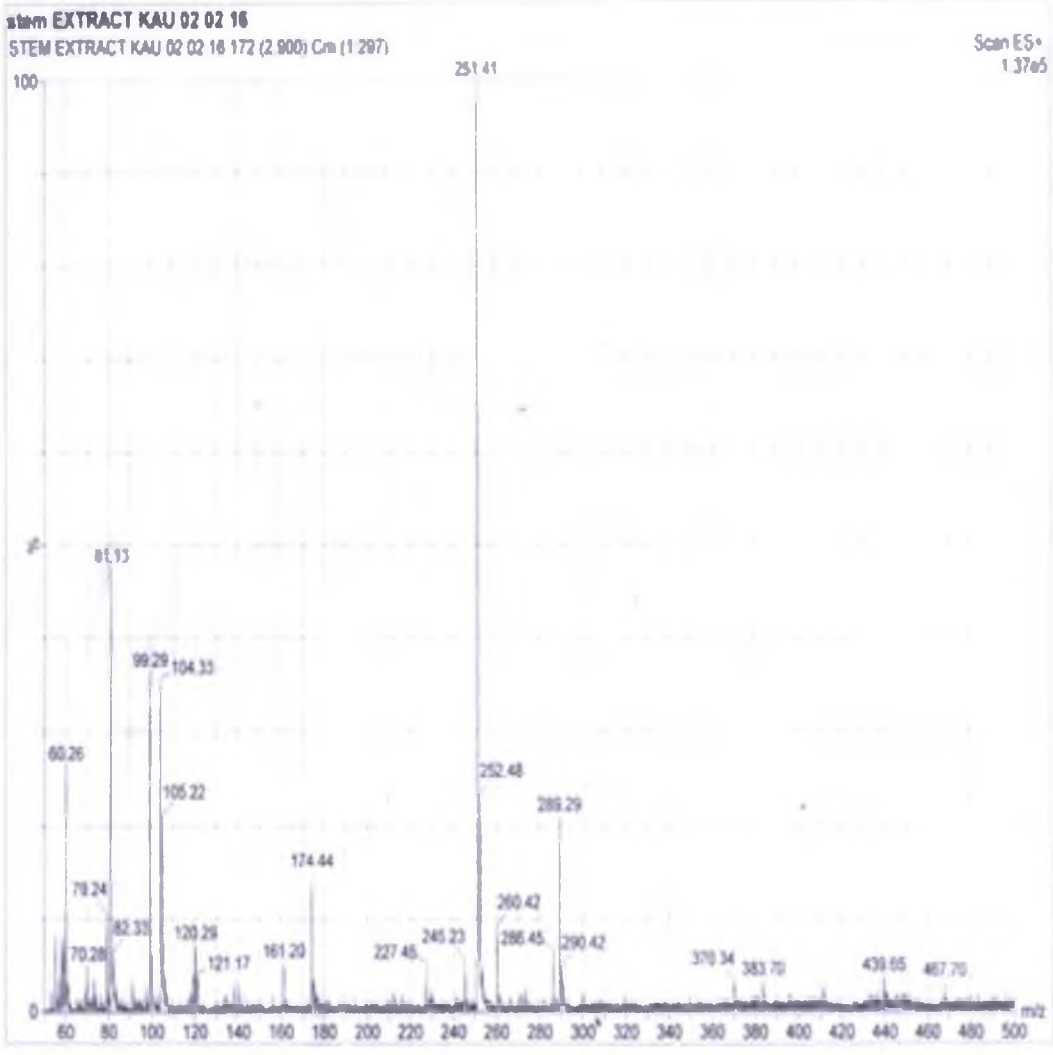


Figure 2 LCMS/MS analysis chromatogram for stem and root extracts

Table 3. Phytochemicals identified from leaf extract based on the LC-MS masses

LCMS/MS Value	Name of the compound	Molecular mass of the reported in literature	Reference
104.14	Senecioic acid	100.11	Greger, 1996
	Isovaleric acid	102.13	Greger, 1996
212.14	Glycozoline	211.25	Sreejith, 2012
222.29	(-)Guaiol	222.36	Sivakumar*et al.*, 2014
	Rosifoliol	222.36	Sivakumar*et al.*, 2014
	Glycosinine	225.24	Sreejith, 2012
	Glycozolidol	227.25	Sreejith, 2012
	Avicenol B	228.07	Wang *et al.*, 2006
	Gamma fagarine	229.23	Wang *et al.*, 2006
251.41	Arborine	250.29	Wang *et al.*, 2006
	Glycozolidal	255.27	Sivakumar*et al.*, 2014
	Avicequinone C	256.07	Wang *et al.*, 2006
	Kokusagine	259.25	Sivakumar*et al.*, 2014
	Skimmianine	259.25	Sivakumar*et al.*, 2014
260.29	Acrifoline	261.35	Wang *et al.*, 2006
286.58	Arborinine	285.29	Wang *et al.*, 2006
291.49	Mupamine	293.14	
356.41	Citracridone 1	353.36	Wang *et al.*, 2006
393.46	Glychalcone B	396.43	Wang *et al.*, 2006
429.38	Arborinone	424.70	Sreejith, 2012
	Beta Amyrin	426.71	Ahmed *et al.*, 2014
433.48	Myricylalcohol	438.82	Ahmed *et al.*, 2014
463.73	3-Epioleanolic acid	456.70	Ahmed *et al.*, 2014

Table 4. Phytochemicals identified from stem and root extracts using LC-MS masses

LCMS/MS value	Name of the compound	Molecular mass of the reported in literature	Reference
99.29	Senecioic acid	100.11	Greger, 1996
	Isovaleric acid	102.23	Greger, 1996
227.45	Glycosinine	225.24	Sreejith, 2012
	Glycozolidol	227.25	Sreejith, 2012
245.23	Marmesin	246.26	Sreejith, 2012
251.41	Glycozolidal	255.27	Sivakumar*et al.*, 2014
252.48	Arborine	250.29	Sivakumar*et al.*, 2014
260.42	Skimmianine	259.25	Sivakumar*et al.*, 2014
	Kokusaginine	259.25	Sivakumar*et al.*, 2014
	Acrifoline	261.35	Wang *et al.*, 2006
286.45	Arborinine	285.29	Wang *et al.*, 2006
290.42	e-N-methylnoracronycine	293.31	Wang *et al.*, 2006
370.34	Dehydroabietic acid	372	Sivakumar*et al.*, 2014
439.65	Myricylalcohol	438.82	Sivakumar*et al.*, 2014

4.2 Molecular docking studies

4.2.1 Retrieval of the possible phytocompounds from *Glycosmis*

Twenty three compounds form the leaf and fourteen compounds from the stem and roots were selected through the LCMS/MS analysis and matching withreported compounds in *Glycosmis pentaphylla* and is presented in table 3 and 4 respectively. Three diamensonal structures of these compounds were retrieved from the chemical structure database-Pubchem in .sdf format. Thus there were twenty six compounds as the possible ligands for the docking study.

4.2.2 Preparation of ligands and their filteration

Preparation of ligands was carried out using "prepare ligand" protocol of DS 4.0. Their absorption or permeability was tested using Lipinski's and Veber's rule. The results showed that a compound common in all three parts of the plant – Myricylalcohol (1-Triacontanol) failed to pass through Lipinski's and Vebers rule due to the presence of more number of rotatable bonds. All others, 22 from leaf and 13 from stem and root passed the test (Table 5 and 6 respectively).

4.2.3 ADMET results

ADMET analysis of those phytocompounds which has passed Lipinski's and Veber's rule was done using ADMET descriptors tool of DS 4.0. This tool make use of seven parameters for analysing the bioavailability and toxic effect of the candidate drug biomolecule in human body. These seven parameters are solubility level, absorption level, BBB level, PPB prediction, CYP2D6 prediction, hepatotoxicity prediction and AlogP and their standards for acceptance and non-acceptance for drug development is given in table 2 of section 3.5.3.

These parameters recorded by ADMET descriptor were analysed by scoring for certain parameter and compounds were classified into highly acceptable, acceptable and non-acceptable after giving weightage for all the 7 parameters tested as shown in 3.5.3.

Results of the ADMET tests for the 22 phytochemicals from leaf are given in the table 7. Out of the 22 leaf compounds, no compound fell into the highly acceptable category and 9 compounds fell into non-acceptable group.12 phytocompounds fell into acceptable group as they showed only slight deviation from the acceptable range for the parameters tested. They are kokusaginine, isovaleric acid, avicequinone C, Gamma fagarine, senecioic acid, glycozolidal, glycozolidol, citracridone I, acrifoline, glycosinine, skimmianine and epioleanolic acid (Table 7).

Results of the ADMET test for 13 phytochemicals from stem and root are given in table 8. Among them 3 compounds fell into the non-acceptable group and the rest 10 compounds are in the acceptable group.The compounds fell into the acceptable group arekokusaginine, isovaleric acid, senecioic acid,glycozolidal, glycozolidol, acrifoline, skimmianine,marmesin, arborinine, Des-N-methylnoracronycine (Table 8).

Table5. Screening of phytocompoundsfrom leaf using Lipinski's and Veber's rule

Sl. No.	Ligand	No. of H bond donors (<5)	No of H bond acceptors (<10)	AlogP (<5)	No. of rotatable bonds (<10)	Polar surface area (<140 A^{02})
1	Kokusaginine	1	5	2.56	3	54.97
2	Isovaleric acid	0	2	-0.304	2	40.12
3	Avicequinone C	1	4	2.473	1	67.51
4	Avicenol B	0	3	3.009	2	31.60
5	Gamma fagarine l	0	4	2.486	2	44.49
6	Senecioic acid	0	2	3.009	1	40.12
7	Epioleanolic acid	2	3	2.486	1	57.53
8	Arborinone	0	1	-0.133	1	17.07
9	Mupamine	2	3	4.973	1	35.07
10	Glychalcone B	1	6	3.009	6	74.22
11	Glycozolidal	2	4	3.707	3	52.14

Sl. No.	Ligand	No. of H bond donors (<5)	No of H bond acceptors (<10)	AlogP (<5)	No. of rotatable bonds (<10)	Polar surface area (<140 $A^{0\,2}$)
12	Glycozolidol	3	3	4.198	1	46.07
13	(-) Guaiol	1	1	2.177	1	20.23
14	Rosifoliol	1	1	2.678	1	20.23
15	Arborinine	1	5	3.905	2	59.00
16	Citracridone I	2	6	3.665	1	79.22
17	Acrifoline	2	3	2.885	0	41.73
18	Arborine	0	3	3.447	2	32.67
19	Glycosinine	2	3	-0.209	2	42.91
20	Skimmiamine	0	5	2.734	3	53.72
21	Beta amyrin	1	1	2.193	0	20.23
22	Glycozoline	2	2	2.469	1	25.02
Failed ligand						
23	Myricylalcohol	1	1	12.831	28	20.23

Table 6. Screening of phytocompounds from stem and roots using Lipinski's and Veber's rule

Sl. No.	Ligand	No. of H bond donors (<5)	No of H bond acceptors (<10)	AlogP (<5)	No. of rotatable bonds (<10)	Polar surface area (<140 A^{02})
1	Kokusaginine	1	5	2.56	3	54.97
2	Isovaleric acid	0	2	-0.304	2	40.12
3	Senecioic acid	0	2	3.009	1	40.12
4	Glycozolidal	2	4	3.707	3	52.14
5	Glycozolidol	3	3	4.198	1	46.07
6	Marmesin	1	4	2.029	1	55.76
7	Arborinine	1	5	3.905	2	59.00
8	Des-N-methylnoracronycine	2	4	3.656	0	62.58
9	Acrifoline	2	3	2.885	0	41.73
10	Dehydroabietic acid	0	2	2.734	4	26.3

Sl. No.	Ligand	No. of H bond donors (<5)	No of H bond acceptors (<10)	AlogP (<5)	No. of rotatable bonds (<10)	Polar surface area (<140 $A^{02)}$
11	Arborine	0	3	3.447	2	32.67
12	Glycosinine	2	3	-0.209	2	42.91
13	Skimmiamine	0	5	2.734	3	53.72
Failed ligands						
14	Myricylalcohol	1	1	12.831	28	20.23

Table 7. ADMET properties of phytocompounds from the leaf sample

Sl. No.	Ligand	Solubility level (2-4)	BBB level (2-4)	CYP2D6 prediction (false-non inhibitor)	Hepatotoxic prediction (false-non toxic)	Absorption level (0-1)	PPB prediction (false-poorly bound)	AlogP98 value (<4)	Remarks
1	Kokusaginine	2	2	FALSE	TRUE	0	TRUE	2.226	A
2	Isovaleric acid	3	2	FALSE	TRUE	0	TRUE	-0.304	A
3	Avicequinone C	3	2	FALSE	TRUE	0	TRUE	2.473	A
4	Avicenol B	2	1	FALSE	TRUE	0	TRUE	3.009	NA
5	Gamma fagarine	3	2	FALSE	TRUE	0	TRUE	2.346	A
6	Senecioic acid	4	3	FALSE	TRUE	1	FALSE	-0.133	A
7	Mupamine	2	1	FALSE	TRUE	0	TRUE	2.652	NA
8	Glychalcone B	2	2	FALSE	FALSE	0	TRUE	4.198	NA
9	Glycozolidal	3	2	FALSE	TRUE	0	TRUE	1.121	A
10	Glycozolidol	3	2	FALSE	TRUE	0	TRUE	1.622	A

11	(-)Guaiol	2	0	FALSE	TRUE	0	TRUE	3.905	NA
12	Rosifoliol	2	1	FALSE	TRUE	0	TRUE	3.665	NA
13	Arborinine	3	2	FALSE	TRUE	0	TRUE	2.885	NA
14	Citracridone I	2	2	FALSE	TRUE	0	TRUE	3.447	A
15	Acrifoline	3	2	FALSE	FALSE	0	TRUE	1.351	A
16	Arborine	2	1	FALSE	TRUE	0	TRUE	2.734	NA
17	Glycosinine	3	2	FALSE	TRUE	0	TRUE	1.138	A
18	Skimmianine	3	2	FALSE	TRUE	0	TRUE	2.33	A
19	Glycozoline	3	1	FALSE	TRUE	0	TRUE	1.864	NA
20	Beta amyrin	0	4	FALSE	FALSE	3	TRUE	7.303	NA
21	Epioleanolic acid	1	4	FALSE	FALSE	1	TRUE	6.447	A
22	Arborinone	0	4	FALSE	FALSE	3	TRUE	7.449	NA

A – Acceptable, HA – Highly acceptable, NA – Not acceptable

Table 8. ADMET properties of phytocompounds from stem and root sample

Sl. No.	Ligand	Solubility level (2-4)	BBB level (2-4)	CYP2D6 prediction (false-non inhibitor)	Hepatotoxic prediction (false-non toxic)	Absorption level (0-1)	PPB prediction (false-poorly bound)	AlogP98 value (<4)	Remarks
1	Kokusaginine	2	2	FALSE	TRUE	0	TRUE	2.33	A
2	Isovaleric acid	4	2	FALSE	FALSE	0	TRUE	1.17	A
3	Senecioic acid	4	2	FALSE	TRUE	0	FALSE	1.341	A
4	Glycozolidal	2	2	FALSE	TRUE	0	TRUE	3.049	A
5	Glycozolidol	2	1	FALSE	TRUE	0	TRUE	3.551	A
6	Marmesin	3	2	FALSE	TRUE	0	FALSE	2.029	A
7	Arborinine	3	2	FALSE	TRUE	0	TRUE	2.885	A
8	Des-N-methylnoracronycine	2	2	FALSE	TRUE	0	TRUE	3.499	A
9	Acrifoline	3	2	FALSE	FALSE	0	TRUE	1.351	A

Sl. No.	Ligand	Solubility level (2-4)	BBB level (2-4)	CYP2D6 prediction (false-non inhibitor)	Hepatotoxic prediction (false-non toxic)	Absorption level (0-1)	PPB prediction (false-poorly bound)	AlogP98 value (<4)	Remarks
10	Dehydroabietic acid	0	4	FALSE	FALSE	3	TRUE	7.338	NA
11	Arborine	2	1	FALSE	TRUE	0	TRUE	2.734	NA
12	Glycosinine	2	1	FALSE	TRUE	0	TRUE	3.066	NA
13	Skimmianine	3	2	FALSE	TRUE	0	TRUE	2.33	A

A – Acceptable, NA – Not acceptable

4.2.4 Target protein identification

Nineteen targets were selected against various viral diseases and is presented in table-1 along with their PDB IDs. Twelve target proteins were selected for chikungunya virus, two for hepatitis B, two for hepatitis C, two for dengue and one for influenza. Three diamensional crystal structures of these protein targets were retrieved from Protein Data Bank in .pdb format on the basis of X-ray diffraction and electron microscopy.

4.2.5 Preparation of protein molecules

The 'Prepare protein' protocol of Discovery Studio 4.0 makes use of CHARMM force-field for correcting all the selected protein structures by inserting missing atoms, modelling loop regions and side chains, adding hydrogen atoms, removing water molecules, natural ligands and hetero atoms inorder to attain optimum energy status for a stable conformation.

4.2.6 Active site identification

The active sites obtained from the DS 4.0 were selected and current selection option was used for specifying the targets at the time of docking. All the targets identified for various active sites were found to have more than one active site. Docking was carried out making use of all these active sites for checking their interaction with selected phytochemicals from *Glycosmis pentaphylla*.

4.2.7 Molecular docking with CDOCKER

Molecular docking was carried out by "CDOCKER" protocol against the targets selected for various diseases that is chikungunya, hepatitis B, hepatitis C, dengue and influenza using the phytocompounds from *Glycosmis* as ligands. Active inhibitors of protein targets associated with the selected viral diseases were identified based on lowest binding energy. The screening of ligands was done based on the difference between CDOCKER energy and CDOCKER interaction energy as shown in section 3.2.3.2.4 of Materials and methods chapter. The affinity of the ligand with the target protein was assessed based on

number and length of hydrogen bonds as shown in section 3.2.3.2.4. Results of the molecular docking involving the phytochemicals and specific target proteins for the various diseases selected are presented here under.

4.2.8 Docking results of target proteins involved in chikungunya

4.2.8.1 Immature glycoprotein complex of chikungunya

When table 9 depicts the dock scores for chikungunya target immature glycoprotein complex of chikungunya virus using the leaf sample phytochemicals, table 10 shows dock scores for chikungunya target immature glycoprotein complex of chikungunya virus using the stem and root sample phytochemicals. Two compounds showed a better interaction with the protein target using leaf compounds. A compound found common in leaf, stem and root – isovaleric acid, interacted forming a binding energy of -92.909 Kcal/mol. A hydrogen bond with a bond distance of 2.093A^0 is created. A compound found exclusively in the leaf extract - Avicequinone C, formed a single hydrogen bond with the protein where a critical aminoacid Lys 279 is involved. In total, 21 compounds from leaf and 13 compounds from stem and root showed a reaction with this target protein.

4.2.8.2 Mature envelope glycoprotein of chikungunya complex

Table 11 lists outdock scores using phytochemicals from leaf sample and table 12 lists dock scores using phytochemicals from stem and root sample for the mature envelope glycoprotein complex of chikungunya virus. With this protein, isovaleric acid and avicequinone C showed comparable interaction out of all the ligands docked from leaf, stem and root. Isovaleric acid formed a hydrogen bond involving an aminoacidAsn 231 with a bond distance of 2.072A^0. Avicequinone C is forming two hydrogen bonds involving aminoacids like His 232 and Lys 232. Isovaleric acid binds with energy of-144.711 Kcal/mol and avicequinone C has a binding energy of -44.211Kcal/mol. 17 compounds from leaf and 11 compounds from stem and root are showing interaction with the protein.

4.2.8.3 Macrodomain of chikungunya virus

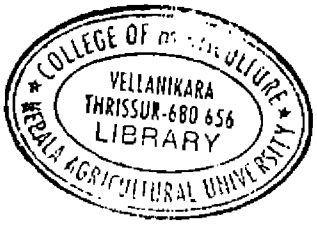
Dock scores using phytochemicals from leaf extract (table 13) and stem and root extract (table 14) for Macrodomain of chikungunya virus.Three hydrogen bonds are formed when isovaleric acid interacted with this protein, involving aminoacidsThr 111, Gly112 and Tyr 114. The binding energy obtained from this interaction is -169.707 Kcal/mol and the hydrogen bond distances are 2.377, 2.078 and 2.090A^0. AvicequinoneC formed two hydrogen bonds where the aminoacidsval33 and ser110 are involved. The bond distances are 2.146 A^0 and 2.299 A^0. 22 compounds from leaf and 13 compounds from stem root where found to react with the protein.

4.2.8.4 Chikungunya nsp_3 macrodomain

Table 15 depicts dock scores using phytochemicals from leaf extract and table 16 depicts dock scores using phytochemicals from stem and root extractfor Chikungunya virus nsp_3 macrodomain. 21 compounds from leaf and 13 compounds from stem and root reacted with the protein. Among these compounds, isovaleric acid formed two hydrogen bonds with bond distances of 2.329 A^0 and 2.043 A^0. Binding energy is -154.307 Kcal/mol. Avicequinone C formed three hydrogen bonds with binding energies of -76.983Kcal/mol. Hydrogen bond distances are 1.976, 2.344 and 2.278 A^0. Aminoacids involved are val35, ser112 and thr113.

4.2.8.5 Chikungunya nsp2 protease

Table 17 shows dock scores using phytochemicals from leaf extract and table 18 shows dock scores using phytochemicals from stem and root extract for chikungunya virus nsp_2 protease. Although 6 leaf compounds and 4 stem and root compounds reacted with the protein, no compound displayed a fair level of interaction.



4.2.8.6 Heat shock protein

Dock scores using phytochemicals from leaf extract is given in table 19 and for stem and root extract in table 20 for Heat shock 70 kDa protein. No good interaction was found among the three leaf compounds and two stem and root compounds.

4.2.8.7 Interleukin 6

Table 21 depicts dock scores using phytochemicals from leaf extract and table 22 shows the dock scores using phytochemicals from stem and root extract for Interleukin 6.18 leaf compounds and 11 compounds from stem and roots reacted with the protein. Isovaleric acid displayed the formation of three hydrogen bonds having bond distances of 2.491, 2.024 and 1.932 A^0. In all these bonds, arg179 is the aminoacid involved.Binding energy for this reaction is -141.095Kcal/mol. Avicequinone C interacted with the protein forming a single hydrogen bond. A bond distance of 1.754$A^0$was obtained.

4.2.8.8 Tumour necrosis factor alpha

Table 23 shows dock scores using phytochemicals from leaf extract and table 24 shows the dock scores using phytochemicals from stem and root extract for Tumour necrosis factor alpha. Although 19 leaf compounds and 12 compounds from the stem and root reacted, there is no hydrogen bond formation between the compounds showing better interaction and the protein. When isovaleric acid displayed a binding energy of -46.077Kcal/mol, avicequinone C showed a binding energy of -42.833Kcal/mol.

4.2.8.9 Interferon beta

Table 25 lists out the dock scores for all the ligands identified from leaf extract and table 26 lists out the dock scores for ligands identified from stem and root extracts. Only two compounds present common in all leaf, stem and root parts reacted with the protein. Isovaleric acid displayed better interaction and

formed a hydrogen bond with a bond distance of 2.27A^0. Ser12 is the aminoacid involved in this reaction. Binding energy displayed by this compound is -61.483Kcal/mol.

Table 9. Dock scores for Chikungunya target Immature glycoprotein complex of Chikungunya virus using theleaf sample phytochemicals

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	4.435	24.710				
2	Isovaleric acid	24.309	23.770	1	LYS279	2.093	-92.909
3	Avicequinone C	21.971	23.704	1	LYS279	1.873	-50.873
4	Avivenol B	-8.365	17.840				
5	Gamma fagarine I	-7.336	17.840				
6	Senecioic acid	6.193	22.507				
7	Epioleanolic acid	-74.331	18.379				
8	Arborinone	-75.800	21.192				
9	Mupamine	-5.088	21.192				
10	Glychalcone B	-3.636	26.956				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
11	Glycozolidal	1.384	25.993				
12	(-) Guaiol	4.409	22.859				
13	Rosifoliol	35.663	17.228				
14	Arborinine	-13.887	19.307				
15	Citracridone I	6.939	26.708				
16	Acrifoline	-3.068	24.543				
17	Arborine	-40.062	16.257				
18	Glycosinine	10.527	22.615				
19	Skimmiamine	3.893	22.370				
20	Beta amyrin	-75.487	16.133				
21	Glycozoline	3.860	21.538				

Table 10. Dock scores for Chikungunya targetImmature glycoprotein complex of Chikungunya virus using the stem sample phytochemicals

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-4.435	24.710				
2	Isovaleric acid	24.309	23.770	1	LYS279	2.093	-92.909
3	Senecioic acid	6.193	22.507				
4	Glycozolidal	1.384	25.993				
5	Glycozolidol	4.409	22.859				
6	Marmesin	3.786	27.647				
7	Arborinine	6.939	26.708				
8	Des-N-methylnoracronycine	-12.999	19.874				
9	Acrifoline	-40.062	16.257				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Mupamine	5.158	35.474				
10	Glychalcone B	23.349	61.180				
11	Glycozolidal	8.823	30.061				
12	Glycozolidol	8.210	28.942				
13	(-) Guaiol	-23.958	31.942				
14	Rosifoliol	-4.506	30.662				
15	Arborinine	17.481	37.950				
16	Citracridone I	7.932	35.917				
17	Acrifoline	-31.222	28.447				
18	Arborine	20.458	35.658				

Table 10. Dock scores for Chikungunya targetImmature glycoprotein complex of Chikungunya virus using the stem sample phytochemicals

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-4.435	24.710				
2	Isovaleric acid	24.309	23.770	1	LYS279	2.093	-92.909
3	Senecioic acid	6.193	22.507				
4	Glycozolidal	1.384	25.993				
5	Glycozolidol	4.409	22.859				
6	Marmesin	3.786	27.647				
7	Arborinine	6.939	26.708				
8	Des-N-methylnoracronycine	-12.999	19.874				
9	Acrifoline	-40.062	16.257				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Dehydroabietic acid	5.839	24.015				
11	Arborine	10.527	22.615				
12	Glycosinine	3.893	22.370				
13	Skimmiamine	-7.671	23.360				

Table 11. Dock scores using phytochemicals from leaf sample for theMature envelope glycoprotein complex of chikungunya virus

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-10.169	20.367				
2	Isovaleric acid	27.483	26.364	1	ASN231	2.072	-144.711
3	Avicequinone C	23.730	27.317	2	HIS232 LYS233	1.972 1.757	-44.211
4	Avicenol B	-3.574	21.062				
5	Gamma fagarine I	-3.345	22.486				
6	Senecioic acid	9.371	26.136				
7	Mupamine	-2.890	22.275				
8	Glychalcone B	6.261	40.214				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Glycozolidal	-0.136	22.630				
10	(-) Guaiol	-0.722	17.670				
11	Rosifoliol	-37.666	16.078				
12	Arborinine	-22.467	9.887				
13	Citracridone I	-7.124	24.045				
14	Arborine	11.753	23.889				
15	Glycosinine	-0.244	18.960				
16	Skimmiamine	-8.721	22.049				
17	Glycozoline	-1.171	16.803				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^{0})	Binding energy (Kcal/mol)
9	Acrifoline	11.485	22.783				
10	Dehydroabietic acid	1.065	20.254				
11	Skimmiamine	-8.186	23.234				

Table 13. Dock scores using phytochemicals from leaf extract forMacrodomain of chikungunya virus

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	0.923	32.358				
2	Isovaleric acid	33.005	32.157	3	THR111 GLY112 TYR114	2.377 2.078 2.090	-169.707
3	Avicequinone C	34.083	37.629	2	VAL33 SER110	2.146 2.299	-33.266
4	Avicenol B	0.414	24.981				
5	Gamma fagarine l	9.099	34.316				
6	Senecioic acid	11.521	27.917				
7	Epioleanolic acid	-101.102	16.424				
8	Arborinone	-73.749	27.377				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Mupamine	5.158	35.474				
10	Glychalcone B	23.349	61.180				
11	Glycozolidal	8.823	30.061				
12	Glycozolidol	8.210	28.942				
13	(-) Guaiol	-23.958	31.942				
14	Rosifoliol	-4.506	30.662				
15	Arborinine	17.481	37.950				
16	Citracridone I	7.932	35.917				
17	Acrifoline	-31.222	28.447				
18	Arborine	20.458	35.658				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
19	Glycosinine	6.383	24.869				
20	Skimmiamine	2.572	34.573				
21	Beta amyrin	-149.313	13.749				
22	Glycozoline	7.146	25.224				

Table 14. Dock scores using phytochemicals from stem and root extract for Macrodomain of chikungunya virus

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	0.923	32.358				
2	Isovaleric acid	33.005	32.157	3	THR111 GLY112 TYR114	2.377 2.078 2.090	-169.707
3	Senecioic acid	11.521	27.917				
4	Glycozolidal	8.823	30.061				
5	Glycozolidol	8.210	28.492				
6	Marmesin	13.316	35.571				
7	Arborinine	17.481	37.950				
8	Des-N-methylnoracronycine	5.641	40.398				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Acrifoline	-31.222	28.447				
10	Dehydroabietic acid	16.908	37.992				
11	Arborine	20.458	35.658				
12	Glycosinine	6.383	24.869				
13	Skimmiamine	2.572	34.573				

Table 15. Dock scores using phytochemicals from leaf extract for Chikungunya virus nsp_3 macrodomain

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	0.666	31.733				
2	Isovaleric acid	29.279	28.410	2	SER112 GLY114	2.329 2.043	-154.307
3	Avicequinone C	36.955	39.294	3	VAL35 SER112 THR113	1.976 2.344 2.278	-76.983
4	Avicenol B	1.128	26.429				
5	Gamma fagarine	5.308	30.065				
6	Senecioic acid	10.669	27.154				
7	Epioleanolic acid	-222.917	-23.406				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance ($A^{0)}$	Binding energy (Kcal/mol)
8	Mupamine	6.330	36.795				
9	Glychalcone B	11.891	45.966				
10	Glycozolidal	9.304	30.540				
11	Glycozolidol	8.674	27.636				
12	(-) Guaiol	-21.594	31.398				
13	Rosifoliol	-3.381	30.638				
14	Arborinine	19.715	39.958				
15	Citracridone I	8.859	36.057				
16	Acrifoline	-44.333	13.299				
17	Arborine	24.968	36.803				
18	Glycosinine	7.368	27.193				

Sl. No	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^{0})	Binding energy (Kcal/mol)
19	Skimmiamine	2.993	34.478				
20	Beta amyrin	-209.19	-15.126				
21	Glycozoline	8.075	25.715				

Table 16. Dock scores using phytochemicals from stem and root extract for Chikungunya virus nsp3 macrodomain

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	0.666	31.733				
2	Isovaleric acid	29.279	28.410	2	SER112 GLY114	2.329 2.043	-154.307
3	Senecioic acid	10.669	27.154				
4	Glycozolidal	9.304	30.540				
5	Glycozolidol	8.674	27.636				
6	Marmesin	13.546	34.412				
7	Arborinine	19.715	39.958				
8	Des-N-methylnoracronycine	2.885	36.144				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Acrifoline	-44.333	13.299				
10	Dehydroabietic acid	9.106	35.693				
11	Arborine	24.968	36.803				
12	Glycosinine	7.368	27.193				
13	Skimmiamine	2.993	34.478				

Table 17. Dock scores using phytochemicals from leaf extract for Chikungunya virus nsp_2 protease

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	-19.960	-6.218				
2	Gamma fagarine	-5.595	21.159				
3	Senecioic acid	-26.859	-1.099				
4	Glycozolidol	-21.507	12.799				
5	Glycosinine	-507.138	-134.178				
6	Glycozoline	-25.153	1.369				

Table 18. Dock scores using phytochemicals from stem and root extract for Chikungunya virus nsp2 protease

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	-19.960	-6.218				
2	Senecioic acid	-26.859	-1.099				
3	Glycozolidol	-21.507	12.799				
4	Glycosinine	-507.138	-134.178				

Table 19. Dock scores using phytochemicals from leaf extract for Heat shock 70 kDaprotein

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Senecioic acid	14.673	13.686				
2	Glycozolidol	4.339	20.909				
3	Glychalcone B	-0.175	30.213				

Table 20. Dock scores using phytochemicals from stem and root extract for Heat shock 70 kDa protein

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Senecioic acid	14.673	13.686				
2	Glycozolidol	4.339	20.909				

Table 21. Dock scores using phytochemicals from leaf extract for Interleukin 6

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-6.073	23.508				
2	Isovaleric acid	22.947	21.856	3	ARG179	2.491 2.029 1.932	-141.095
3	Avicequinone C	20.906	23.541	1	LYS171	1.754	-46.131
4	Avicenol B	-6.033	18.785				
5	Gamma fagarine	-6.391	18.726				
6	Senecioic acid	2.793	19.211				
7	Epioleanolic acid	-7.945	17.856				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
8	Mupamine	3.596	38.923				
9	Glychalcone B	1.452	24.805				
10	Glycozolidal	3.401	22.131				
11	Glycozolidol	-31.305	22.437				
12	(-) Guaiol	-14.579	18.579				
13	Rosifoliol	6.913	25.023				
14	Arborinine	-4.173	23.798				
15	Citracridone I	9.327	21.218				
16	Arborine	2.433	20.736				
17	Glycosinine	-7.427	24.007				
18	Skimmiamine	2.367	20.247				

Table 22. Dock scores using phytochemicals from stem and root extract for Interleukin 6

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-6.073	23.508				
2	Isovaleric acid	22.947	21.856	3	ARG179	2.491 2.029 1.932	-141.095
3	Senecioic acid	2.793	19.211				
4	Glycozolidal	1.452	24.805				
5	Glycozolidol	3.401	22.131				
6	Marmesin	2.284	22.896				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
7	Arborinine	6.913	25.023				
8	Des-N-methylnoracronycine	-11.101	21.869				
9	Acrifoline	9.327	21.218				
10	Dehydroabietic acid	2.433	20.736				
11	Arborine	-7.427	24.007				

Table 23. Dock scores using phytochemicals from leaf extract for Tumour necrosis factor alpha

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	6.312	35.744				
2	Isovaleric acid	22.212	20.995	nil			-46.077
3	Avicequinone C	27.992	31.378	nil			-42.833
4	Avicenol B	0.984	25.948				
5	Gamma fagarine	1.653	26.731				
6	Senecioic acid	2.596	19.598				
7	Epioleanolic acid	12.906	39.461				
8	Mupamine	10.030	43.411				
9	Glychalcone B	15.040	36.750				
10	Glycozolidal	15.970	34.650				
11	Glycozolidol	-24.440	30.163				
12	(-) Guaiol	-3.114	30.209				
13	Rosifoliol	13.467	32.101				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
14	Arborinine	4.660	32.738				
15	Citracridone I	-27.581	31.526				
16	Acrifoline	22.852	36.383				
17	Arborine	14.667	34.812				
18	Glycosinine	-1.437	29.526				
19	Skimmiamine	15.730	34.282				

Table 25. Dock scores using phytochemicals from leaf extract for Interferon-beta

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	23.381	22.456	1	SER12	2.279	-61.483
2	Senecioic acid	4.318	20.792				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
14	Arborinine	4.660	32.738				
15	Citracridone I	-27.581	31.526				
16	Acrifoline	22.852	36.383				
17	Arborine	14.667	34.812				
18	Glycosinine	-1.437	29.526				
19	Skimmiamine	15.730	34.282				

Table 24. Dock scores using phytochemicals from stem and root extract for Tumour necrosis factor alpha

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	6.312	35.744				
2	Isovaleric acid	22.212	20.995	nil			-46.077
3	Senecioic acid	2.596	19.598				
4	Glycozolidal	15.040	36.750				
5	Glycozolidol	15.970	34.650				
6	Marmesin	8.560	29.536				
7	Arborinine	13.467	32.101				
8	Des-N-methylnoracronycine	-0.543	34.701				
9	Acrifoline	-27.581	31.526				
10	Arborine	22.852	36.383				
11	Glycosinine	14.667	34.812				
12	Skimmiamine	-1.437	29.282				

Table 25. Dock scores using phytochemicals from leaf extract for Interferon-beta

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	23.381	22.456	1	SER12	2.279	-61.483
2	Senecioic acid	4.318	20.792				

Table 26. Dock scores using phytochemicals from stem and root extract for Interferon-beta

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	23.381	22.456	1	SER12	2.279	-61.483
2	Senecioic acid	4.318	20.792				

Table 27. Dock scores using phytochemicals from leaf extract for Signal transducer and activator of transcription II

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	5.414	37.141				
2	Isovaleric acid	17.074	16.557				
3	Avicequinone C	27.641	29.209	1	ARG980	1.889	-37.835
4	Avicenol B	3.795	28.350				
5	Gamma fagarine	2.758	27.591				
6	Senecioic acid	1.700	17.995				
7	Epioleanolic acid	7.185	32.679				
8	Glychalcone B	15.085	37.086				
9	Glycozolidal	16.044	34.522				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Glycozolidol	-25.999	27.866				
11	(-) Guaiol	-2.825	29.343				
12	Rosifoliol	15.507	33.543				
13	Arborinine	10.170	37.386				
14	Citracridone I	-28.91	29.485				
15	Acrifoline	18.38	31.599				
16	Arborine	13.739	32.261				
17	Glycosinine	-0.697	30.794				
18	Skimmiamine	16.597	34.898				

Table 28. Dock scores using phytochemicals from stem and root extract for Signal transducer and Activator of transcription II

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^{0})	Binding energy (Kcal/mol)
1	Kokusaginine	5.414	37.141				
2	Isovaleric acid	17.074	16.557				
3	Senecioic acid	1.700	17.995				
4	Glycozolidal	15.085	37.086				
5	Glycozolidol	16.044	34.522				
6	Marmesin	5.817	27.256				
7	Arborinine	15.507	33.543				
8	Des-N-methylnoracronycine	0.956	33.543				
9	Acrifoline	-28.91	29.485				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance ($A^{0)}$	Binding energy (Kcal/mol)
10	Arborine	18.38	31.599				
11	Glycosinine	13.739	32.261				
12	Skimmiamine	-0.697	30.794				

Table 29. Dock scores using phytochemicals from leaf extract for Human leukocyte antigen

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	2.99	34.959				
2	Isovaleric acid	15.157	14.698				
3	Avicequinone C	24.045	26.706	1	HIS167	2.393	-48.604
4	Avicenol B	-3.384	22.535				
5	Gamma fagarine	-0.230	24.764				
6	Senecioic acid	-3.206	13.057				
7	Epioleanolic acid	-0.581	24.656				
8	Mupamine	-3.852	28.544				
9	Glychalcone B	10.790	32.390				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Glycozolidal	14.527	32.949				
11	Glycozolidol	-23.736	29.711				
12	(-) Guaiol	-8.672	23.967				
13	Rosifoliol	9.262	27.276				
14	Arborinine	-5.544	20.877				
15	Citracridone I	-32.321	24.190				
16	Acrifoline	13.980	25.893				
17	Arborine	14.198	32.639				
18	Glycosinine	-4.539	26.254				
19	Skimmiamine	14.324	32.189				

Table 30. Dock scores using phytochemicals from stem and root extract for Human leukocyte antigen

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	2.99	34.959				
2	Isovaleric acid	15.157	14.698				
3	Senecioic acid	-3.206	13.057				
4	Glycozolidal	10.790	32.390				
5	Glycozolidol	14.527	32.949				
6	Marmesin	1.136	21.799				
7	Arborinine	9.262	27.276				
8	Des-N-methylnoracronycine	-7.166	25.578				
9	Acrifoline	-32.321	24.190				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Arborine	13.980	25.893				
11	Glycosinine	14.198	32.639				
12	Skimmiamine	-4.539	26.254				

Table 31. Dock scores using phytochemicals from leaf extract for Actin

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-3.704	28.721				
2	Isovaleric acid	33.132	32.114	2	LYS18	2.147 1.912	-170.727
3	Avicequinone C	34.931	42.015	2	TYR306 LYS336	2.167 1.724	-63.378
4	Avicenol B	2.998	30.451				
5	Gamma fagarine	4.032	30.204				
6	Senecioic acid	13.991	30.288				
7	Epioleanolic acid	-171.383	4.279				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
8	Mupamine	1.233	27.772				
9	Glychalcone B	16.462	56.500				
10	Glycozolidal	11.314	32.695				
11	Glycozolidol	17.751	36.497				
12	(-) Guaiol	-29.105	35.648				
13	Rosifoliol	-15.037	21.339				
14	Arborinine	18.818	39.015				
15	Citracridone I	7.950	47.854				
16	Acrifoline	-40.177	25.688				
17	Arborine	29.274	41.451				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
18	Glycosinine	14.270	36.013				
19	Beta amyrin	-213.698	-2.787				
20	Skimmiamine	7.541	39.887				
21	Glycozoline	3.782	23.554				

Table 32. Dock scores using phytochemicals from stem and root extract for Actin

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-3.704	28.721				
2	Isovaleric acid	33.132	32.114	2	LYS18	2.147 1.912	-170.727
3	Senecioic acid	13.991	30.288				
4	Glycozolidal	11.314	32.695				
5	Glycozolidol	17.751	36.497				
6	Marmesin	9.608	34.300				
7	Arborinine	18.818	39.015				
8	Des-N-methylnoracronycine	2.688	38.697				

4.2.8.10 Signal transducer and activator

Table 27 shows dock scores using phytochemicals from leaf extract and table 28 shows dock scores using phytochemicals from stem and root extract for Signal transducer and activator of transcription II. 18 compounds from leaf and 12 compounds from stem and root showed some interaction with the protein. Only a single compound from stem showed good interaction. Avicequinone C formed a hydrogen bond where arg980 is involved. Bond distance formed is $1.889A^0$.

4.2.8.11 Human leukocyte antigen

Table 29 shows dock scores using phytochemicals from leaf extract and table 30 depicts the dock scores using phytochemicals from stem and root extract for Human leukocyte antigen. Among the 19 leaf compounds and 12 compounds from stem and root, only a single compound, avicequinone C displayed better interaction. An aminoacid his167 is involved in the hydrogen bond formation. A bond distance of 2.393 A^0 is created. A binding energy of -48.604Kcal/mol is obtained from the interaction.

4.2.8.12 Actin

Table 31 shows dock scores using phytochemicals from leaf extract and table 32 depicts dock scores using ligands from for actin. 21 leaf compounds and 13 compounds from stem and root displayed interaction with the protein. Isovaleric acid formed two hydrogen bonds. Lys18 is the aminoacid involved in this reaction. Bond distances of 2.147 A^0 and 1.912 A^0. Binding energy obtained in this reaction is -170.727 Kcal/mol. Avicequinone C showed the formation of two hydrogen bonds where the bond lengths are $2.167A^0$ and $1.912A^0$. Aminoacids involved are tyr306 and lys336.

4.2.9 Docking results of target proteins involved in hepatitis

Table 32. Dock scores using phytochemicals from stem and root extract for Actin

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-3.704	28.721				
2	Isovaleric acid	33.132	32.114	2	LYS18	2.147 1.912	-170.727
3	Senecioic acid	13.991	30.288				
4	Glycozolidal	11.314	32.695				
5	Glycozolidol	17.751	36.497				
6	Marmesin	9.608	34.300				
7	Arborinine	18.818	39.015				
8	Des-N-methylnoracronycine	2.688	38.697				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Acrifoline	-40.177	25.688				
10	Dehydroabietic acid	29.274	41.451				
11	Arborine	29.274	41.451				
12	Glycosinine	14.270	36.013				
13	Skimmiamine	7.541	39.887				

4.2.8.10 Signal transducer and activator

Table 27 shows dock scores using phytochemicals from leaf extract and table 28 shows dock scores using phytochemicals from stem and root extract for Signal transducer and activator of transcription II. 18 compounds from leaf and 12 compounds from stem and root showed some interaction with the protein. Only a single compound from stem showed good interaction. Avicequinone C formed a hydrogen bond where arg980 is involved. Bond distance formed is $1.889A^0$.

4.2.8.11 Human leukocyte antigen

Table 29 shows dock scores using phytochemicals from leaf extract and table 30 depicts the dock scores using phytochemicals from stem and root extract for Human leukocyte antigen. Among the 19 leaf compounds and 12 compounds from stem and root, only a single compound, avicequinone C displayed better interaction. An aminoacid his167 is involved in the hydrogen bond formation. A bond distance of 2.393 A^0 is created. A binding energy of -48.604Kcal/mol is obtained from the interaction.

4.2.8.12 Actin

Table 31 shows dock scores using phytochemicals from leaf extract and table 32 depicts dock scores using ligands from for actin. 21 leaf compounds and 13 compounds from stem and root displayed interaction with the protein. Isovaleric acid formed two hydrogen bonds. Lys18 is the aminoacid involved in this reaction. Bond distances of 2.147 A^0 and 1.912 A^0. Binding energy obtained in this reaction is -170.727 Kcal/mol. Avicequinone C showed the formation of two hydrogen bonds where the bond lengths are $2.167A^0$ and $1.912A^0$. Aminoacids involved are tyr306 and lys336.

4.2.9 Docking results of target proteins involved in hepatitis

4.2.9.1 Hepatitis B X-interacting protein

Table 33 shows the dock scores using phytochemicals from leaf extract and table 34 depicts the dock scores using ligands for Hepatitis B X-interacting protein. 17 leaf compounds and 11 compounds from stem and root interacted with theprotein. Only one compound-avicequinone C displayed good interaction. Avicequinone C formed a single hydrogen bond with a bond distance of 2.270A^0. The aminoacid involved in the hydrogen bond is asp80. Binding energy of -26.55 Kcal/mol is obtained from the reaction.

4.2.9.2 PDZ domain

Table 35 indicates dock scores using phytochemicals from leaf extract and table 36 indicates dock scores using ligands from stem and root extract for PDZ domain-containing GIPC2. Isovaleric acid and avicequinone C showed better interaction out of the 18 leaf compounds and 11 compounds from stem and root. Isovaleric acid displays binding energy of -119.283 Kcal/mol. Aminoacid found to interact in the hydrogen bond reaction is gly113. Hydrogen bond distance is 1.890 A^0. Avicequinone C shows the formation of three hydrogen bonds with bond distances of 2.465, 2.116 and 2.433 A^0. The aminoacids found to interact in these hydrogen bond formations are gly113 and lys119.

4.2.9.3 HCV ns5B RNA polymerase

Table 37 Dock scores using phytochemicals from leaf extract and table 38 shows ligands from stem and root extract for HCV NS5B RNA polymerase. From the total 17 leaf compounds and 12 compounds from the root and stem reacted with the protein. Binding energy displayed by the isovaleric acid is -64.97 Kcal/mol. There is no hydrogen bond formed when this compound reacts with the protein. Avicequinone C shows a hydrogen bond formation with a distance of 1.883A^0. Binding energy displayed is -41.888 Kcal/mol. Aminoacid found to react with the protein is arg503.

4.2.9.4 HCV ns5B polymerase inhibitors

Table 39 shows dock scores using phytochemicals from leaf extract and table 40 shows dock scores using ligands from stem and root extract for HCV NS5B RNA polymerase inhibitors. 17 leaf compounds and 12 compounds from stem and root interacted with the protein. Isovaleric acid formed three hydrogen bondsinvolving the aminoacid arg501 and lys533. Binding energy formed is -257.107 Kcal/mol. Hydrogen bond distances are 2.255, 2.027 and 1.881A^0. Avicequinone C displayed a hydrogen bond of bond length 1.959A^0. Arg501 is involved in this reaction. Binding energy obtained from this reaction is -36.251 Kcal/mol.

Table 33. Dock scores using phytochemicals from leaf extract for Hepatitis B X-interacting protein

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-3.016	27.679				
2	Isovaleric acid	15.095	14.047				
3	Avicequinone C	19.87	22.061	1	ASP80	2.270	-26.556
4	Avicenol B	-3.820	21.010				
5	Gamma fagarine	-5.076	19.632				
6	Senecioic acid	-3.987	12.658				
7	Mupamine	0.249	25.423				
8	Glychalcone B	0.601	32.668				
9	Glycozolidal	7.416	29.507				
10	Glycozolidol	6.890	25.630				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
11	(-) Guaiol	-32.821	20.523				
12	Arborinine	2.693	20.631				
13	Citracridone I	-7.117	29.561				
14	Arborine	11.533	23.913				
15	Glycosinine	6.243	25.140				
16	Skimmiamine	-9.066	22.435				
17	Glycozoline	8.029	25.686				

Table 34. Dock scores using phytochemicals from stem and root extract for Hepatitis B X-interacting protein

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-3.016	27.679				
2	Isovaleric acid	15.095	14.047				
3	Senecioic acid	-3.987	12.658				
4	Glycozolidal	7.416	29.507				
5	Glycozolidol	6.890	25.630				
6	Marmesin	3.914	24.64				
7	Arborinine	2.693	20.631				
8	Des-N-methylnoracronycine	-8.601	24.658				
9	Arborine	11.533	23.913				
10	Glycosinine	6.243	25.140				
11	Skimmiamine	-9.066	25.686				

Table 35. Dock scores using phytochemicals from leaf extract for PDZ domain-containing GIPC2

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-9.022	19.805				
2	Isovaleric acid	26.209	26.710	1	GLY113	1.890	-119.283
3	Avicequinone C	18.340	20.229	3	GLY113 LYS119	2.465 2.116 2.433	-38.535
4	Avivenol B	-7.620	17.206				
5	Gamma fagarine	-6.970	18.088				
6	Senecioic acid	9.395	25.731				
7	Mupamine	-1.303	24.672				
8	Glychalcone B	-1.823	28.036				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Glycozolidal	-1.199	20.272				
10	Glycozolidol	1.671	20.137				
11	(-) Guaiol	-38.825	14.285				
12	Rosifoliol	-14.391	17.547				
13	Arborinine	4.372	22.591				
14	Citracridone I	-16.36	10.606				
15	Arborine	9.340	22.762				
16	Glycosinine	0.111	21.611				
17	Skimmiamine	-10.495	20.446				
18	Glycozoline	-0.192	17.890				

Table 36. Dock scores using phytochemicals from stem and root extract for PDZ domain-containing GIPC2

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-9.022	19.805				
2	Isovaleric acid	26.209	26.710	1	GLY113	1.890	-119.283
3	Senecioic acid	9.395	25.731				
4	Glycozolidal	-1.199	20.272				
5	Glycozolidol	1.671	20.137				
6	Marmesin	1.148	22.255				
7	Arborinine	4.372	22.591				
8	Des-N-methylnoracronycine	-12.456	21.110				
9	Arborine	9.340	22.762				
10	Glycosinine	0.111	21.611				
11	Skimmiamine	-10.495	20.446				

Table 37. Dock scores using phytochemicals from leaf extract for HCV NS5B RNA polymerase

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-1.962	28.632				
2	Isovaleric acid	21.837	20.652	No H bonds			-64.97
3	Avicequinone C	26.184	27.815	1	ARG503	1.88309	-41.888
4	Avicenol B	-0.028	24.586				
5	Gamma fagarine	0.027	25.420				
6	Senecioic acid	4.611	20.960				
7	Mupamine	-3.001	24.024/				
8	Glycozolidal	7.484	28.76				
9	Glycozolidol	7.964	27.673				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	(-) Guaiol	-25.449	27.417				
11	Rosifoliol	-7.567	25.112				
12	Arborinine	8.918	27.161				
13	Acrifoline	-32.517	24.693				
14	Arborine	16.671	28.277				
15	Glycosinine	7.151	25.700				
16	Skimmiamine	-4.353	28.519				
17	Glycozoline	6.335	24.103				

Table 38. Dock scores using phytochemicals from stem and root extract for HCV NS5B RNA polymerase

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-1.962	28.632				
2	Isovaleric acid	21.837	20.652	No H bonds			-64.97
3	Senecioic acid	4.611	20.960				
4	Glycozolidal	7.484	28.76				
5	Glycozolidol	7.964	27.673				
6	Marmesin	12.425	32.869				
7	Arborinine	8.918	27.161				
8	Des-N-methylnoracronycine	-6.899	26.115				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Acrifoline	-32.517	24.693				
10	Arborine	16.671	28.277				
11	Glycosinine	7.151	25.700				
12	Skimmiamine	-4.353	28.519				

Table 39. Dock scores using phytochemicals from leaf extract for HCV NS5B polymerase inhibitors

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-7.514	21.718				
2	Isovaleric acid	31.725	30.812	3	ARG501 LYS533	2.255 2.027 1.881	-257.107
3	Avicequinone C	23.015	26.172	1	ARG501	1.959	-36.251
4	Avicenol B	0.144	25.419				
5	Gamma fagarine	0.937	26.081				
6	Senecioic acid	14.400	30.705				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
7	Mupamine	-4.342	22.011				
8	Glycozolidal	-1.344	21.489				
9	Glycozolidol	-0.026	19.134				
10	Rosifoliol	-10.997	21.391				
11	Arborinine	11.402	36.976				
12	Citracridone I	-1.975	25.925				
13	Acrifoline	-37.999	19.140				
14	Arborine	15.737	27.260				
15	Glycosinine	-0.141	18.346				
16	Skimmiamine	-4.764	28.146				
17	Glycozoline	0.487	18.473				

Table 40. Dock scores using phytochemicals from stem and root extract for HCV NS5B polymerase inhibitors

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-7.514	21.718				
2	Isovaleric acid	31.725	30.812	3	ARG501 LYS533	2.255 2.027 1.881	-257.107
3	Senecioic acid	14.400	30.705				
4	Glycozolidal	-1.344	21.489				
5	Glycozolidol	-0.026	19.134				
6	Marmesin	5.693	26.404				
7	Arborinine	11.402	30.976				
8	Des-N-methylnoracronycine	-6.000	27.040				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Acrifoline	-37.999	19.140				
10	Arborine	15.737	27.260				
11	Glycosinine	-0.141	18.346				
12	Skimmiamine	-4.764	28.146				

4.2.10Docking targets against dengue target

4.2.10.1 Dengue ns2B/ns3 protease

Table 41 depicts dock scores using phytochemicals from leaf extract and table 42 depicts dock scores using ligands from stem and root extract for dengue NS2B/NS3 protease. Two compounds (isovaleric acid and senecioic acid) common in all the extracts – leaf, stem and root reacted with the protein. Out of these two compounds, isovaleric acid displayed good interaction with three hydrogen bonds of bond distances 2.414, 1.851 and 2.162 A^0. Binding energy involved in this reaction is -47.985 Kcal/mol.

4.2.10.2 Methyltransferase

Table 43 indicates dock scores using phytochemicals from leaf extract and table 44 indicates dock scores using ligands from stem and root extract for dengue methyl transferase. Twenty two leaf compounds and 13 compounds from stem and root interacted with the protein. Isovaleric acid made two hydrogen bonds of bond distances 1.841 and 2.132 A^0. Aminoacid interacting in this bond formation is lys29. Binding energy for this reaction is -201.268 Kcal/mol. Avicequinone C formed a hydrogen bond of distance 1.725A^0. Binding energy for this reaction is -29.516 Kcal/mol and lys14 is the aminoacid involved in this hydrogen bond formation.

4.2.11 Targets for influenza

4.2.11.1 Neuraminidase

Table 45 indicates dock scores using phytochemicals from leaf extract and table 46 indicates dock scores using ligands from stem and root extract for neuraminidase of influenza virus. 17 leaf compounds and 10 compounds from root and stem are involved in the reaction. Isovaleric acid displays five hydrogen bonds involving aminoacids arg118, arg292, arg371. Bond distances are 2.064, 2.244, 2.117, 1.871 and 1.971A^0. Avicequinone C showed three hydrogen bonds with bond distances of 1.897, 2.057 and 2.33A^0. Aminoacids involved in these reactions are arg118, glu227 and arg371.

Table 41. Dock scores using phytochemicals from leaf extract for dengue NS2B/NS3 protease

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	22.674	21.697	3	LYS145	2.414 1.851 2.162	-47.985
2	Senecioic acid	5.374	21.736				

Table 42. Dock scores using phytochemicals from stem and root extract for dengue NS2B/NS3 protease

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Isovaleric acid	22.674	21.697	3	LYS145	2.414 1.851 2.162	-47.985
2	Senecioic acid	5.374	21.736				

Table 43. Dock scores using phytochemicals from leaf extract for dengue methyl transferase

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-0.364	30.295				
2	Isovaleric acid	29.403	28.194	2	LYS29	1.842 2.132	-201.268
3	Avicequinone C	27.021	29.126	1	LYS14	1.725	-29.516
4	Avicenol B	-1.844	23.341				
5	Gamma fagarine	-0.722	24.290				
6	Senecioic acid	11.151	27.510				
7	Epioleanolic acid	-63.569	31.184				
8	Arborinone	-64.261	33.902				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
9	Mupamine	2.212	29.417				
10	Glychalcone B	10.745	43.00				
11	Glycozolidal	11.604	34.526				
12	Glycozolidol	12.001	32.399				
13	(-) Guaiol	-22.941	30.439				
14	Rosifoliol	-3.560	29.555				
15	Arborinine	16.086	35.302				
16	Citracridone I	6.576	37.85				
17	Acrifoline	-37.829	20.451				
18	Arborine	16.257	27.962				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
19	Glycosinine	11.393	31.654				
20	Skimmiamine	-0.651	30.158				
21	Beta amyrin	-60.852	31.074				
22	Glycozoline	9.414	27.171				

Table 44. Dock scores using phytochemicals from stem and root extract for dengue methyl transferase

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	-0.364	30.295				
2	Isovaleric acid	29.403	28.194	2	LYS29	1.842 2.132	-201.268
3	Senecioic acid	11.151	27.510				
4	Glycozolidal	11.604	34.526				
5	Glycozolidol	12.001	32.399				
6	Marmesin	6.819	28.634				
7	Arborinine	16.086	35.302				
8	Des-N-methylnoracronycine	-2.488	30.950				
9	Acrifoline	-37.829	20.451				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Dehydroabietic acid	15.117	33.556				
11	Arborine	16.257	27.962				
12	Glycosinine	11.393	31.654				
13	Skimmianine	-0.651	30.158				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
5	Gamma fagarine	3.724	30.268				
6	Senecioic acid	14.577	31.034				
7	Mupamine	14.231	39.395				
8	Glycozolidal	19.207	43.926				
9	Glycozolidol	21.718	43.238				
10	(-) Guaiol	-21.965	31.877				
11	Rosifoliol	-1.214	30.758				
12	Arborinine	20.372	39.253				
13	Acrifoline	-28.180	35.469				
14	Arborine	19.734	32.310				
15	Glycosinine	21.299	45.387				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
10	Dehydroabietic acid	15.117	33.556				
11	Arborine	16.257	27.962				
12	Glycosinine	11.393	31.654				
13	Skimmianine	-0.651	30.158				

Table 45. Dock scores using phytochemicals from leaf extract for Neuraminidase of influenza virus

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	13.408	42.692				
2	Isovaleric acid	32.639	32.048	5	ARG118 ARG292 ARG371	2.064 2.244 2.117 1.871 1.976	-94.446
3	Avicequinone C	31.874	33.950	3	ARG118 GLU227 ARG371	1.897 2.057 2.330	-68.082
4	Avicenol B	5.065	29.843				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
5	Gamma fagarine	3.724	30.268				
6	Senecioic acid	14.577	31.034				
7	Mupamine	14.231	39.395				
8	Glycozolidal	19.207	43.926				
9	Glycozolidol	21.718	43.238				
10	(-) Guaiol	-21.965	31.877				
11	Rosifoliol	-1.214	30.758				
12	Arborinine	20.372	39.253				
13	Acrifoline	-28.180	35.469				
14	Arborine	19.734	32.310				
15	Glycosinine	21.299	45.387				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
16	Skimmiamine	5.374	36.396				
17	Glycozoline	20.557	38.379				

Table 46. Dock scores using phytochemicals from stem and root extract for Neuraminidase of influenza virus

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
1	Kokusaginine	13.408	42.692				
2	Isovaleric acid	32.639	32.048	5	ARG118 ARG292 ARG371	2.064 2.244 2.117 1.871 1.976	-94.446
3	Senecioic acid	14.577	31.034				
4	Glycozolidal	19.207	43.926				
5	Glycozolidol	21.718	43.238				
6	Arborinine	20.372	39.253				

Sl. No.	Ligand	(-)CDOCKER energy (Kcal/mol)	(-) CDOCKER Interaction energy (Kcal/mol)	No. of H bonds	H bond	Distance (A^0)	Binding energy (Kcal/mol)
7	Acrifoline	-28.180	35.469				
8	Arborine	19.734	32.310				
9	Glycosinine	21.299	45.387				
10	Skimmianine	5.374	36.396				

Discussion

5. Discussion

Glycosmis pentaphylla (Retz.) Correa, also called as *panal* in Malayalam is a traditional medicinal plant of Asia. In Ayurveda, the traditional Indian system of medicine it is used for treating cough, rheumatism, anaemia, jaundice, worms and facial inflammation (Ambasta, 2000; Balachandran *et al.,* 2008; Mohammed *et al.,* 2010 and Oudhia, 2006). In Bangladesh also traditional medicinal practitioners use this plant for treating fever, liver complaints, various intestinal parasites, rheumatoid arthritis, cancer, wound bleeding, bone fracture and skin infections (Chopra *et al.,* 1956; Farhana *et al.,* 2011and Mohammed *et al.,* 2010). But detailed studies focusing on the phytochemical composition and its exploitation to tackle the ailments mentioned above and new viral diseases like chikungunya is a necessity of the day. Development of science and technology, allowing the use of sophisticated techniques, replacing the traditional tests (tests for various types of compounds present in a plant like tests for flavonoids, test for coumarins etc., where mere presence of the type of phytochemical is confirmed but not the chemical or biological properties of each of these phytochemicals coming under each class) has significantly increased the efficiency of such studies. Modern day analysing methods like GCMS, LCMS, LCMS/MS has significantly improved the efficiency of such studies for the phytochemical detection along with their chemical properties.

Superiority of herbal system for drug development is an important factor for future medicine as it has less side-effects, natural abundance, and lower cost for its production.

In silico tools like molecular docking helps us to analyse the potential of the phytocompounds present in traditional medicinal plants to be used as drug to fight against various diseases. It also helps to screen an array of compounds present in a plant and to select suitable compounds alone for wet lab studies, thus reducing labour and time involved in screening whole compounds present in the plant through wet lab studies. The present study "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" was carried out to look into

the antiviral property of the plant mainly against chikungunya, and also other diseases like hepatitis, dengue and influenza, so as to detect phytochemicals with capability to be developed into drug. Twenty eight phytocompounds from this plant identified through LCMS/MS from various parts of this plant were taken as the ligands against various protein targets of selected viral diseases for this study.

5.1 Wetlab studies

Phytocompounds present in stem, root and leaf samples of *G. pentaphylla* were identified by LCMS/MS by outsourcing.

5.1.1 Collection and preparation of sample

Phytocompounds produced in *G. Pentaphylla* differ in quantity and quality in various plant parts like leaf stem and root (Junsong *et al.*, 2006).So samples were collected from root, stem and leaf. Powdered samples were prepared from each part and hydroalcoholic extractswere prepared using two different concentrations of methanol (90 and 75 per cent) and water, hydroalcoholic extracts have been prepared from each of the powdered samples and were evaporated with the aid of rotary evaporator at 60°C. The extracts from each of these plant parts were then sent for LCMS/MS analysis to investigate the phytochemical compounds present in *panal.*

5.1.2 LCMS/MS study

A total of 23 compounds from leaf and 14 compounds from stem and roots were identified. Identical compounds were present in stem and root.These compounds were subjected to molecular docking by taking them as the candidate ligands/ drug molecules against viral disease causing proteins and viral proteins

LC along with the tandem mass spectrometry enables better sensitivity and thus increases the efficiency of the detection of various chemicals present in the crude extract. Due to the absence of library for detecting the phytochemicals, only the mass to charge ratio of the compounds were received from the LCMS analysis. With the help of literature review on the chemical components reported from *Glycosmis pentaphylla,* the chemical compounds were identified based on

their molecular weight (tables 3 and 4). The compounds identified in leaf are senecioic acid, isovaleric acid, glycozoline, (-) guaiol, rosifoliol, glycosinine, glycozolidol, avicenol B, gamma fagarine, arborine, glycozolidal, avicequinone C, kokusaginine, skimmianine, acrifoline, arborinine, mupamine, citracridone I, glychalcone B, arborinone, beta amyrin, myricyl alcohol and 3-epioleanolic acid and in stem and root are senecioic acid, isovaleric acid, glycosinine, glycozolidol, marmesin, arborine, kokusaginine, skimmianine, acrifoline, arborinine, e-N-methylnoracronycine, dehydroabietic acid and myricyl alcohol. The compounds marmesin, acrifoline, e-N-methylnoracronycine and dehydroabietic acid were found exclusively in stem and root.

5.2 *In silico* analysis

Molecular docking of phytocompounds present in *Glycosmis pentaphylla* was done for finding out antiviral properties of them so as to develop drugs from the biomolecules against chikungunya, hepatitis, dengue and influenza viral diseases.

The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein, which in turn results in a therapeutic benefit to the patient. Drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and therefore will bind to it. Nowadays, drug design frequently but not necessarily relies on computer modeling techniques are referred to as computer-aided drug design. Drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design (Reynolds, 2010).

A biomolecular target (most commonly a protein or nucleic acid) is a key molecule involved in a particular metabolic or signalling pathway that is associated with a specific disease condition. Potential drug targets are not necessarily disease causing but must by definition be disease modifying (Dixon and Stockwell, 2009). In some cases, small molecules will be designed to enhance or inhibit the target function in the specific disease modifying pathway. Small

molecules such asreceptor agonists, antagonists, inverse agonists, modulators, enzyme activators or inhibitors or ion channel openers or blockers will be designed that are complementary to the binding site of target (Anderson, 2003; Imming *et al.*, 2006).

In the present study, molecular docking was done using Discovery Studio 4.0 (Accelerys, USA).The steps involved in *in-silico* analysis include preparation of protein targets and ligands, ADMET analysis and molecular docking.

5.2.1 Preparation of protein targets and ligands

The three dimensional structures of the 23 compounds from the leaf and 14 compounds from stem and root were retrieved from reliable database Pubchem and prepared following the protocol prescribed in Discovery Studio version 4.0 as described in section 3.2.3.2.2. The details regarding name and mass of the phytocompounds are mentioned in the tables 3 and 4. These compounds were further filtered using Lipinski's Rule of Five and Veber's protocol, a criterion for screening compounds for drug likeliness based on number of H-bond donors, number of H-bond acceptors, molecular weight and calculated Log P. All the compounds other than Myricyl alcohol passed the test. A common compound present in leaf, stem and root - Myricylalcohol failed to pass the Lipinski's and Veber's protocol due to more number of rotatable bonds. Myricyl alcohol is a natural plant growth regulator found in epicuticular waxes (Naeem *et al.*, 2012). Since this compound got failed to pass the Lipinski's and Veber's rule, it is evident that this molecule is not having enough chemical properties to be used as a drug. Using this screening protocol, themolecules which were capable of fitting into the active site and thus deactivating the target proteins were identified. A total of 26 phytocompounds excluding Myricylalcohol were selected for ADMET analysis and docking study.

5.2.2 ADMET analysis

Before starting with molecular docking, the identified phytochemicals were subjected to ADMET analysis. Parameters such as solubility level, absorption

level, BBB level, PPB prediction, CYP2D6 prediction, hepatotoxicity prediction and AlogP were checked using mathematical values such that the compounds were screened for knowing bioavailability inside human body (Baby *et al.*, 2016). Out of 22 leaf compounds, no compounds came under the highly acceptable group, showing that no compound agrees with the limits prescribed for each character in a strict fashion. Ten compounds came under non-acceptable group. They were either found to have more than 2 characters lying away from the strictly acceptable range. Rest 10 of the compounds fell in the acceptable category where they dissatisfy any 2 parameters out of the seven parameters employed for screening in ADMET analysis. The compounds that belong to the acceptable group are Kokusaginine, isovaleric acid, avicequinone C, gamma fagarine, senecioic acid, glycozolidol, glycozolidal, citracridone I, acrifoline, glycosinine, skimmiamine and epioleanolic acid.

Among the compounds from stem and root, 3 compounds came under the non-acceptable category. These compounds are dehydroabietic acid, arborine, glycosinine. All other compounds came under the acceptable group.Two of these alkaloids, kokusaginine and skimmianine, were found to inhibit the proliferation of cancer cells and to induce a cell cycle arrest in a concentration-dependent manner in HeLa cells, as evidenced by flow cytometry (Judit *et al.*, 2013). Isovaleric acid showed excellent wound healing properties in a clinical study on 72 patients with ulcus cruris (Papageorgiou, 1978). The alkaloids like Kokusaginine and the sterol, β-sitosterol were found to show antibacterial activity (Onyancha *et al.*, 2014). The compounds showed antibacterial activity ranging from mild to moderate activity (Onyancha *et al.*, 2014). The quinoline alkaloid gamma-fagarine exhibited antiplasmodial activity (Randrianarivelojosia*et al.*, 2003). Furoquinoline alkaloids skimmianine and γ-fagarine exhibited antileishmanial activitiy (Ostan *et al.*, 2007). It was demonstrated that Glycozolidal exhibits moderate antibacterial activity against the periodontopathic bacteria, Porphyromonas gingivalis (Rodanant *et al.*, 2015). Glycosinine was found to suppress skin cancer target protein kinase C (Yasir *et al.*, 2015).

5.2.3 Molecular docking

Molecular docking was performed using the "CDOCKER" protocol of the software Discovery Studio Version 4.0. Dock scores obtained after the docking were used to analyse the targets and phytocompounds. This study was oriented to identify the phytocompounds in *Glycosmis pentaphylla* (Retz.) Correa which helps to tackle viral diseases mainly chikungunya where there is lack of medicines to suppress it effectively. Lowest binding energy was also usedas a criterion for identifying the potent inhibitors against each target protein (Ramakanth *et al.*, 2012). According to Ahmed *et al.* (2014), the highly negative binding energy of a protein-ligand complex indicates that it has released more free energy and moved to a lower, thermodynamically stable energy state. The difference between CDOCKER energy and CDOCKER interaction energy were also used for screening ligands where lesser difference between the two, better is the interaction. The lock and key model is adopted in this method of docking where protein is the lock and ligand is the key. A combination of energy produced by adding the internal ligand strain energy and receptor-ligand interaction energy makes up the CDOCKER energy. CDOCKER interaction energy is the interaction energy between the protein and ligand. The values of these two energies indicate the strength of interaction between the proteins and ligands. The interactions of phytocompounds from the *panal* with identified targets for different viral diseases are discussed hereunder.

5.2.3.1 Interaction of phytocompounds with chikungunya drug targets

The interaction of 22 leaf compounds and 13 compounds from stem and root,which have satisfied the Lipinski's and Veber's rule, were subjected for docking with 12 targets related to chikungunya. The protein targets were immature glycoprotein complex of chikungunya virus, mature envelope glycoprotein complex of chikungunya virus, macrodomain of chikungunya virus, chikungunya virus nsp3 macrodomain, chikungunya virus nsp2 protease, heat shock 70 kDa protein, interleukin 6, tumour necrosis factor alpha, interferon-beta, signal transducer and activator of transcription II, human leukocyte antigen

and actin. Out of all the phytocompounds subjected for docking, only 2 compounds viz., isovaleric acid and avicequinone C showed good interaction with low binding energy.

Good interaction of the above mentioned two phytocompounds viz., isovaleric acid and avicequinone C for inhibition was observed for targets such as immature glycoprotein complex, mature glycoprotein complex, macrodomain, nsp3 macrodomain, interleukin 6, interferon beta, signal transducer and activator, human leukocyte antigen, and actin. The compound isovaleric acid interacted with 8 targets with a good dock score where a binding energy of -170 Kcal/mol was obtained with an interaction with the actin protein. Another compound avicequinone C from leaf interacting with all these 8 targets showed a highest negative binding energy of 76.983 Kcal/mol. Thus, it could be assumed that these two compounds may be able to give an antiviral property to the plant against chikungunya.

The glycoprotein complexes of chikungunya virus are a complex of structural proteins E1, E2 and E3. The E1 and pE2 (precursor to the E3 and E2 proteins prior to furin cleavage) glycoproteins are assembled as heterodimers in the endoplasmic reticulum (ER). E3 is cleaved from pE2 by furin in the Golgi, and the resultant E1–E2 heterodimers are then transported to the plasma membrane. These heterodimers self-assemble into 80 trimeric spikes on the virus surface (Von Bondorff and Harrison 1975; 1978). E1 is responsible for fusion of the viral membrane with the endosomal membrane, and E2 is involved in receptor binding and the subsequent receptor-mediated endocytosis. The E3 mediates proper folding of pE2 and controls the spike functions by interacting with the fusion protein E1. Thus, E3 is required for efficient particle assembly, mediating both spike folding and spike activation for viral entry. The high viral load during the acute phase of chikungunya was characterised by the production of pro-inflammatory cytokine, interleukin (IL)–6. Persistent arthralgia, seen during chronic chikungunya infection was also associated with higher levels of IL-6 (Chow *et al.*, 2011).

The mortality resulting from chikungunya is associated with undetectable levels of alpha/beta interferon (IFN-α/β) in serum. A 10 fold increase in the levels of tumor necrosis factor (TNF) was also reported (Rudd *et al.,* 2012). Studies suggest that inadequate IFN-α/β responses following virus infection can be sufficient to induce hemorrhagic fever and shock (Rudd *et al.,* 2012).After the secretion of IFN-β from the infected cell, it binds to the IFN-α/β receptor IFNAR in an autocrine or paracrine manner to amplify the signal or to prime uninfected cells to establish an antiviral state, respectively. Subsequently, the Janus kinases JAK1 and TYK2 are phosphorylated and, in turn, phosphorylate signal transducers and activators of transcription 1 and 2 (STAT1 and STAT2) (Randall and Goodbourn, 2008).

Genes coding for human leukocyte antigen (HLA) class II molecules are polymorphic and have been shown to influence susceptibility to chikungunya (Thanapati *et al.,* 2014). HSP70 and actin were identified as virus binding proteins in mammalian cells (Paingankar and Arankalle, 2014). So it can be concluded that by suppressing these target proteins, chikungunya can be controlled.

Chikungunya is a disease of recent days and has been reported to have no specific antiviral drug or medicine for its cure or treatment. To date, patients affected with chikungunya have been advised to take rest, increase the intake of fluid food and medicines like paracetamol to relieve pain. Through this study, two compounds were identified with antiviral property and the same can be developed as a novel drug after wet lab and clinical studies.

With the improvement in technology, there is an important role for the computational tools for identifying the proteins and other metabolites present within a cell of an organism to cause a certain disease and to screen the compounds capable of inhibiting these disease causing target and thus suppressing it. Using *in silico* techniques of virtual screening and docking, Rashad and Keller (2013) identified the novel binding sites and inhibitors for the chikungunya virus envelope proteins. Bassetto *et al.* (2013) have developed a homology-based model of CHIKV virus using the crystal structure of nsP2 of the alphavirus VEE as a

template. A recent study had shown that the nsP4 protein of CHIKV is involved in the mechanism of action of T-705 (favipiravir) that was seen to inhibit CHIKV replication in vitro and in vivo (Jochmans *et al.*, 2013). Thus, it is vital to target other viral proteins also for structure-based drug design.

When we conducted docking study against various chikungunya protein targets, two phytocompounds – isovaleric acid and avicequinone C interacted with 7 target proteins. Against two targets – chikungunya virus nsp2 protease and heat shock protein, none of the phytocompounds has shown good interaction. With other targets, only one phytocompound exhibited good interaction. The highest binding energy of -170.727 Kcal/mol was formed when isovaleric acid interacted with the protein target-actin. As actin protein plays a significant role during muscle contraction and chronic chikungunya disease is characterised by severe body pain, suppression of this target protein will lead a significant development for pharmaceutical industry. From the overall results of docking study against chikungunya, it can be concluded that two phytocompounds from *G. pentaphylla* which displayed a fair interaction with the targets can be subjected for further wet lab studies for a novel anti-chikungunya drug development programme.

Praveena *et al.* (2014) conducted a docking study by homology modelling using structural polyprotein as the drug target. This study indicated that Eupatorin isolated from *Eupatorium prostratum* has higher affinity to the protein binding site. Further, Nguyen *et al.* (2014) conducted a docking study using nsP3 macrodomain as the target and identified some compounds from database called NCI Diversity Set II that can be assumed to inhibit the target protein for chikungunya.

The results of molecular docking of phytochemicals from *Glycosmis pentaphylla* revealed the possibility of making use of the two compounds-isovaleric acid and avicequinone C which have shown better dock scores for drug development. Although they had better interaction with the targets identified for chikungunya, the binding energy is not high (<100). Thus it can be assumed that these phytocompounds can be used as an ingredient for the development of

antiviral medicines for chikungunya. In other words, a combination of these compounds can be used as a drug along with other potential molecules.

5.2.3.2 Interaction of phytocompounds with Hepatitis targets

The target proteins for hepatitis B were Hepatitis B X-interacting protein and PDZ domain-containing GIPC2. Out of the 26 ligands tested, only two compounds-isovaleric acid and avicequinone C gave good interaction.Avicequinone C was present only in the leaves and the other compound, isovaleric acid in all the plant parts. Isovaleric acid gave a good binding energy value with one of the target. The variation between CDOCKER energy and CDOCKER interaction energy was also satisfactory.

Hepatitis B virus X-interacting protein is a human protein encoded by the *HBXIP* gene (Melagari *et al.*, 1998). This complexes with the C-terminus of hepatitis B virus X protein (HBx). The function of this protein is to negatively regulate HBx activity and thus to alter the replication life cycle of the virus (*Entrez* gene). PDZ domain of this protein is also an important target for drug design (Arooj *et al.*, 2012).

The targets for hepatitis C are HCV NS5B RNA polymerase and HCV NS5B polymerase inhibitors. Though isovaleric acid showed a negative binding energy of 64.97Kcal/mol, the ligands failed to establish hydrogen bonds. But isovaleric acid interacted with another protein target of the same disease with good binding energy. This interaction also had shown the formation of 3 hydrogen bonds, which indicated that the interaction is fairly good. Avicequinone C interacted with both the target proteins identified for this disease and had shown only a single hydrogen bond interaction between the targets and the ligand. The binding energy was satisfactory and the difference between CDOCKER energy and CDOCKER interaction energy was less.

Glycosmis pentaphylla is well known for its hepatoprotective activity in traditional medicine. Several studies were conducted to establish a strong foundation for the usage of this plant for the same. In 2010, Nayak and co-

workers have experimented this plant for its hepatoprotective effect against paracetamol-induced toxicity. The methanol extract (400 mg/kg) of *G. pentaphylla* was able to alter the toxic condition of the hepatocytes and protected the membrane integrity against paracetamol-induced leakage of marker enzymes. A similar study was conducted by Raju and Rao (2010) for evaluating *panal*'s hepatoprotective activity against CCl4-induced acute liver injury in rats. Administration of methanolic extracts of *Glycosmis pentaphylla* showed recovery against the toxic effects of CCl4.

A study was conducted by Reddy *et al.* (2012) where a potent inhibitor for Hepatitis-B virus X-associated protein was designed through molecular docking. Another*in-silico*study found thatthe compounds from *G.glabra* have good docking scores for both Hepatitis B and C target proteins (Vani and Rajarajan, 2015).

A study on homology modelling and molecular docking on phytocompounds from the traditional antidote *Phyllanthus niruri* and other nucleoside analogues against HBV DNA Polymerase, was done using the software Discovery Studio 4.0 (Mohan *et al.*, 2015). The study has proven the treatment potential of this plant. Pathak *et al.* (2014)have screened known natural compounds against the HBX protein using molecular docking. The structure of HBX was predicted and used for docking against plant derived natural compounds (curcumin, oleanolic acid, resveratrol, bilobetin, luteoline, ellagic acid, betulinic acid and rutin), by Molegro Virtual Docker. The screening identified rutin with the binding energy of -161.65 Kcal/mol.

A study to investigate the inhibitory activities of 24 different compounds from 11 plants against the NS3 helicase protein of HCV was carried out using computational techniques (Arumugam *et al.*, 2013). The study has revealed the potent compounds.

5.2.3.3 Interaction of phytocompounds with dengue targets

The target dengue proteins identified for docking the phytocompounds of *Glycosmis* were Dengue ns2B/ns3 protease and Methyltransferase. When isovaleric acid interacted with the protein NS2B/NS3 protease, the ligand had shown a low binding energy (<100) but 3 hydrogen bonds were formed. The same ligand has also displayed good binding with other dengue targets. The interaction between the isovaleric acid and the dengue methyltransferase protein also has shown the formation of 2 hydrogen bonds which indicated a better interaction. Avicequinone C showed an interaction where the binding energy is low and it is marked by the presence of only one hydrogen bond. The difference between the CDOCKER energy and CDOCKER interaction energy was less, assuming a good interaction between these two phytocompounds and their interaction with the respective targets.

Dengue virus has three structural proteins and seven nonstructural proteins. The structural proteins are capsid C, envelope E and premembrane (prM). The nonstructural proteins are NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Idress and Usman, 2012). The replication of dengue virus depends upon correct cleavage of polypeptide which requires both host cell proteases and the virus-encoded two component protease NS2B/NS3. Thus NS2B/NS3 protease plays a central role in replication of dengue virus (Stevens *et al.*, 2009). Thus we selected this protein as one of our protein targets against dengue. Isovaleric acid displayed a strong interaction with a binding energy of -47.985 Kcal/mol along with the formation of three hydrogen bonds.

The nonstructural proteins (five) contain methyltransferase (MTase) and RNA-dependent RNA polymerase (RdRp) activity. The function of MTase is methylation and catalyzing the capping of viral RNA, which is an essential step for viral replication (Khromykh *et al.,* 1999). MTase catalyzes the transfer of methyl group from substrate methyl donor S-adenosyl-L-methionine (SAM). So MTase inhibition can prevent the methylation step of viral RNA (Singh *et al.,* 2016). Two phytocompounds viz., isovaleric acid and avicequinone C displayed a favourable interaction. The interaction of Isovaleric acid with this target protein

resulted in the two hydrogen bond formation with the binding energy of -201.268 Kcal/mol. Avicequinone C displayed a single hydrogen bond formation. Thus we can say that these two compounds have an antiviral potential against dengue.

Andrographis paniculata is a potent drug used in Ayurveda, Siddha and Homoeopathy in many formulations and is effective for the treatment of various diseases such as malaria, diabetes, viral hepatitis, cirrhosis, liver cancer. An*in silico* docking study has revealed the efficiency of phytocompounds andrographolide and 14-deoxy-11-oxoandrographolide from this plant against the dengue viral protein (Nithya *et al.*, 2014).

Evaluation of inhibitory potential of triterpenoids from *Azadirachta indica* against therapeutic target of dengue virus NS2B/NS3 protease was carried out *in silico* (Dwivedi *et al.*, 2016). Nimbin, desacetylnimbin and desacetylsalannin had good binding affinity with DENV NS2B/NS3 protease, but azadirachtin and salannin did not show any interaction with the target protein.Another study had shown that ellagic acid is a potential ligand with antiviral properties against the dengue viral glycoproteins (Bupesh *et al.*, 2014). Manikandan *et al.* (2014) revealed the possibility for using small molecules as ligands to exploit their medicinal properties and to find a potent phytocompound to suppress viral diseases like dengue. Naringenin, Quercetin and Fisetin molecules showed excellent docking results with very minimal toxicity, suggesting that these compounds obtained from plants can be exploited against viral diseases. These studies indicate that there is a good scope for drug development against viral diseases from natural sources.

This study revealed that isovaleric acid and avicequinone C have the potential to be developed as a drug against dengue.

5.2.3.4 Interaction of phytocompounds against influenza

Neuraminidase of influenza virus was used as the target for searching the potential phytocompounds capable of suppressing the disease. Among all the ligands subjected for docking against influenza target, isovaleric acid displayed

good results. The difference between CDOCKER energy and CDOCKER interaction energy was marginal (0.591 Kcal/mol). Although the negative binding energy obtained from the interaction between the above ligand and the protein target selected was not high, it was found capable of establishing 5 hydrogen bonds. Thus, from the above results we can conclude that the ligand-isovaleric acid can be considered for its antiviral activity against this disease and further studies may be conducted for better understanding of its drug properties.

Neuraminidase is important at various stages of viral infection. During the first stage, it helps the virus to approach the target cells by cleavage of sialic acids from respiratory tract mucins (Matrosovich *et al.,* 2004). Secondly, it may take part in the fusion of viral and cell membranes. It has also got a role in facilitating the budding of new virions by preventing their aggregation, caused by the interaction of the haemagglutinin (HA) of the first virus with the sialylated glycans of the second one (Wagner *et al.,* 2002). NA amplifies HA haemagglutinating activity by cleavage of the terminal neuraminic acid residues of the oligosaccharides surrounding the receptor-binding site of HA (Ohuchi *et al.,* 1996).Thus we selected neuraminidase as an effective target against influenza. Suppression of this novel target can lead to an effective antiviral drug development. The Neu5Ac binding site is located above the first strands of the third and the fourth motifs in a big loop on the NA surface. The enzyme active site consists of functional amino acid residues Arg118, Asp151, Arg152, Arg224, Glu276, Arg292, Arg371, and Tyr406, and structural amino acid residues Glu119, Arg156, Trp178, Ser179, Asp (or Asn in N7 and N9) 198, Ile222, Glu227, Glu277, Asp293, and Glu425 (Shtyrya *et al.*, 2009). In this study, isovaleric acid was found to have hydrogen bonding with 3 active site residues- Arg118 and Arg292 and Arg 371 in case of isovaleric acid. Arg118, Arg371 and Glu 227 in case of avicequinone C.

Thus the results show that these two phytochemicals are able to form a strong interaction with the active site residues of neuraminidase which indicates its strong antiviral potential against influenza.

In a parallel study, H1N1 proteins (neuraminidase and hemagglutinin) were screened with phytocompounds isolated from *tulsi* plant (*Ocimum sanctum L.*) using molecular docking tools. From this study, it was identified that Apigenin can serve as an alternative to Oseltamivir and Zanamivir with improved predicted binding properties (Alhazmi, 2015). In another *in silico* study, small molecules from alternate medical systems, andrographolide from *Siddha,* gelsemine from Homeopathy, eugenol from Ayurveda, and two natural products namely vitamin C and vitamin E were selected as potential ligands. The best docking simulation was reported by vitamin C interacting through six hydrogen bonds into proteins hemagglutinin and neuraminidase (Raja *et al.*, 2014). The suppressive effects of Curcurin, a ribosome-inactivating protein, from *Jatropha curcas* was investigated against Neuraminidase and Hemagglutinin proteins of Pandemic Influenza H1N1/2009 (Chavan *et al.*, 2015).

Although all the phytocompounds identified from *Glycosmis pentaphylla*through LCMS/MS analysis were subjected for analysis, good interaction was shownonly by isovaleric acid and avicequinone C. Isovaleric acid derivatives were reported from *Glycosmis* spp. by Greger *et al.*(1996). Isovaleric acid belongs to the sulfonyl group of amides. This group of amides is known for their diverse biological functions. Sulfonamides are widely used as antimicrobial (Ozbek 2007; Genc *et al.*, 2008), anticancer (El-Sayed *et al.*, 2011; Mun *et al.*, 2012), anti-inflammatory (Borne *et al.*, 1974) and antiviral agents as well as HIV protease inhibitors (De Clercq, 2001). Avicequinone C is reported to have significant antimicrobial activity (Han *et al.*, 2007). Thus, it can be assumed that these two compounds-isovaleric acid and avicequinone C have a potential to be considered for drug development.

5.2.4 Conclusion

Through LCMS/MS study, 26 phytochemicals were identified in *G. pentaphylla* and were subjected for docking studies against targets for chikungunya, hepatitis, dengue and influenza. Results revealed that isovaleric acid present in all plant parts and avicequinone C, present only in leaf can be considered for drug

development against all viral diseases said above. This study emphasises that there is abundant scope for development of medicines from natural plant sorces for viral diseases where there is no specific antiviral drug.This study paves some scientific footing for the use of this plant against viral disease-jaundice in traditional system of medicine like Ayurveda.

Summary

6. SUMMARY

The study entitled "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" was carried out to identify metabolites present in this plant that can be used for the treatment of viral diseases viz., chiungunya, hepatitis, dengue and influenza. The various secondary metabolites present in stem, leaf and roots of the plant were identified through LCMS/MS by outsourcing. The identified compounds as ligands were subjected for *in silico* molecular docking study with proteins assosciated with viral diseases as targets so as to develop drug molecules for viral disease treatment. Salient findings of the study are summarised below.

i) The plant material was collected from the Instructional farm,Vellanikkara. Separate dry samples were prepared from root, stem and leaves and were powdered. The finely ground powder was converted into a hydroalcoholic extract and was concentrated using rotary evaporator. The concentrated extract was subjected to LCMS/MS analysis through outsourcing.

ii) The mass to charge ratio obtained through LCMS/MS analysis was compared with the masses of various compounds identified from this plant though literature review. The compounds thus identified were assumed to be present in the plant samples.Twenty three compounds from leaf are isovaleric acid, senecioic acid, glycozoline, (-)guaiol, rosifoliol, glycosinine, glycozolidol, avicenol B, gamma fagarine, arborine, glycozolidal, avicequinone C, kokusaginine, skimmianine, acrifoline, arbornine, mupamine, citracridone I, glychalcone B, arborinone, beta amyrin, myricyl alcohol, 3-epioleanolic acid and fourteen compounds from stem and roots, viz., are isovaleric acid, senecioic acid, glycosinine, glycozolidol, marmesin, glycozolidal, arborine, skimmianine, kokusaginine, acrifoline, arborine,kokusaginine, skimmianine, acrifoline, arbornine, e-N-

methylnoracronycine, dehydroabietic acid and myricyl alcohol were identified.

iii) *In silico* docking works were carried out using these phytochemicals as ligands for drug developmentagainst selected viral diseases.Nineteen proteins responsible for causing chikungunya, hepatitis, dengueand influenza viral disease were used as targets for molecular docking studies.In this study, more emphasise was given on chikungunya target proteins. Molecular docking studies were conducted using Discovery Studio 4.0 developed and distributed byAccelerys, USA.

iv) Three dimensional structure of proteins and phytocompounds were retrieved from biological databases ie., PDB and Pubchem. The target proteins were prepared for docking using 'prepare protein' protocol of Discovery Studio 4.0.

v) Phytocompounds were prepared as ligands using 'prepare ligands' protocol and filtered through Lipinski's rule of 5 and Veber's protocol. All 22 ligands from leaf and 13 from stem and roots passed the test indicating that they have drug likeliness properties. Only one compound Myricylalcohol present in all the parts of the plant failed this test.

vi) ADMET screening was done to check the bioavailability of all the phytocompounds when used as drug. Out of the total 26, ligands screened 12 compounds from leaf and 10 from stem and root were identified to be acceptable as drugs with respect to their bioavailability.

vii) Molecular docking was carried out by 'CDOCKER' protocol of Discovery Studio against the target selected for various viral diseases viz., chikungunya, hepatitis, dengue and influenza.

viii) The results of molecular docking for chikungunya revealed that only two compounds – isovaleric acid and avicequinone C interacted with 10 targets out of 12 targets tested.The targets that has shown good

interaction are immature glycoprotein complex of chikungunya, mature envelope glycoprotein of chikungunya complex, macrodomain of chikungunya virus, chikungunya nsp3 macrodomain, interleukin 6, tumour necrosis factor alpha, interferon beta, signal transducer and activator, human leukocyte antigen and actin.

ix) Out of the two targets identified for hepatitis B, better interaction was displayed when isovaleric acid interacted with the PDZ domain where there is a single hydrogen bond formation. With the same target avicequinone C formed three hydrogen bonds.

x) Between the two targets identified for hepatitis C, good interaction was given when isovaleric acid interacted with ns_5B polymerase inhibitors with a binding energy of -257.107 Kcal/mol. Avicequinone C displayed a single hydrogen bond with the same target.

xi) Isovaleric acid formed two hydrogen bonds with dengue target methyltransferase with a good binding energy (-201.268 Kcal/mol). With another target (ns2B/ns3 protease), the same compound formed three hydrogen bonds.

xii) Avicequinone C formed a single hydrogen bond with dengue methyltransferase protein.

xiii) A very good interaction resulting in the formation of five hydrogen bonds was formed when isovaleric acid interacted with influenza target neuraminidase. With the same target, avicequinone C was able to form three hydrogen bonds with active site residues viz., arg118, glu 227 and arg 371.

xiv) With the influenza target neuraminidase, these compounds give good interaction and can be used as the possible candidates for drug development.

Thus, it can be concluded that out of total 26 phytocompounds used as ligands against target proteins of chikungunya, hepatitis, dengue and influenza, only two compounds-isovaleric acid and avicequinone C displayed good interaction. These

two compounds can be considered for drug development against these viral diseases.

References

References

Ahmed, D., Kumar, V., Sharma, M., and Verma, A. 2014. Target guided isolation, *in-vitro* antidiabetic, antioxidant activity and molecular docking studies of some flavonoids from *Albizzia lebbeck Benth.* bark. *BMC Complement. Altern. Med.* **14**: 2-12.

Alhazmi, M. 2015. Molecular docking of selected phytocompounds with H1N1 proteins. *Bioinformation* **11**(4): 196-202.

Ambasta, S. P. 2000. *The Useful Plants of India* (4th Ed.). Published by National Institute of Science and Communication. New Delhi, pp. 380-381.

Amran, H., Farhana, R., Shapna, S., Mohammad, R. R., Shams-Ud-Doha, K. M., Rumana, M. and Apurba, S. A. 2011. Antimicrobial, antioxidant and cytotoxic effects of methanolic extracts of leaves and stems of *Glycosmis pentaphylla* (Retz.) Correa. *J. App. Pharm. Sci.* **8**: 137-140.

Anderson, A. C. 2003. The process of structure- based drug designs. *Chem. Biol.* **10**(9): 787-797.

Anonymous, 2003. *The Wealth of India -A Dictionary of Indian Raw Materials and Industrial Products*, Products publication and information directorate, Council of Scientific and Industrial Research (CSIR), New Delhi,**4**: 225.

Aprino, P. 1992. Combined liquid chromatography mass spectrometry part III Applications of thermospray. *Mass Spectrometry Rev.* **11**: 3.

Ariful, H. M., Romeo, M., Rohimul, F., Kishore, K. T., Azmal, I. H., and Bulbul, A. 2010. *Abstract A101*: Ethnomedicinal uses of some medicinal plants for prevention against all forms of cancer by the traditional healers in Gazipur district of Bangladesh. AACR International conference on frontiers in cancer prevention research. *Cancer Prev Res.* **3** (1 supplement): A101.

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. Bio. Sci.* 3(6): 41-55.

Arumugam, M., Lulu, S., Kumari, S., and Kumari, D. V. 2013. Computational screening and evaluation of bioactive compounds against ns$_3$helicase of HCV. *Int. J. Pharma. Pharma. Sci.* 4(4): 370-376.

Baby, B., Antony, P., Halabi, W. A., Al Homedi, Z and Vijayan, R. Structural insights into the polypharmacological activity of quercetin on serine threonine kinases. *Drug Des. Dev. Ther.*10: 3109-3123.

Bajad, S., Coumar, M., Khajuria, R.K., Bedi, K.L., and Suri, O.P., 2003. Characterization of a new metabolite 3,4-methylenedioxy phenyl-2E,4E-pentadienoic acid-N-(3yl-propionoic acid) amide of piperine, an ominipresent food component by LC/NMR/MS. *Eur. J. Pharma. Sci.* 19(5): 413-421.

Balachandran, I., Udayan, P. S., Harinarayanan, M. K., and Tushar, K. V. 2008. Some common plants used by kurichiar tribes of tirunelli forest, Wayanad district, Kerala, in medicine and other traditional uses. *Indian J. Tradit. Knowl.* 7: 250-255.

Balick, M. J. and Cox, P. A. 1997. *Plants, People and Culture: the Science of Ethnobotany, Scientific American Library,* New York, NY. **60.**

Bandara, B. M. R., Hewage, C. M., Jayamanne, D. H. L. W., Karunaratne, V., Adikaram, N. K. B., Bandara, K. A. N. P., Pinto, M. R. M., and Wijesundara, D. S. A. 1990. Biological activity of some steam distillates from leaves of ten Rutaceous plants. *J. Nat. Sci. Coun. Sri Lanka.* **18**: 71-77.

Barlow, D. J., Buriani, A., Ehrman, T., E. Bosisio, E., Eberini, I., and Hylands, P. J. 2012. *In silico* studies in Chinese herbal medicines research: evaluation of *in silico* methodologies and phytochemical data sources and a review of research to date. *J. Ethnopharmacol.* **140**(3): 526-534.

Bassetto, M., De Burghgraeve, T., Delang, L., Massarotti, A., Coluccia, A, Zonta, N., Gatti, V., Colombano, G., Sorba, G., Silvestri, R., Tron, G. C., Neyts,

J., Leyssen, P., and Brancale, A. 2013. Computer-aided identification, design and synthesis of a novel series of compounds with selective antiviral activity against chikungunya virus. *Antiviral Res.* **98**(1):12-18.

Bhattacharyya, P., Chakrabartty, P. K., and Chowdhury, B. K. 1985. Glycozolidol, an antibacterial carbazole alkaloid from *Glycosmis pentaphylla. Phytochemistry* **24**: 882-883.

Borne, R. F., Peden, R. L., Waters, I. W., Weiner, M., Jordan, R., and Coats, E. A. 1974. Anti-inflammatory activity of para-substituted N-benzenesulfonyl derivatives of anthranilic acid. *J. Pharm. Sci.* **63**(4):615-617.

Bouchard, M. J., and Schneider, R. J. 2004. The enigmatic X gene of hepatitis B virus. *J. Virol.* **78**: 12725–12734.

Bupesh, G., Raja, R. S., Saranamurali, K., Kumar, V. S., Saran, N., Kumar, M., Vennila, S., Sheriff, K., Kaveri, K., and Gunasekaran, P. 2014. Antiviral activity of ellagic acid against envelope proteins from dengue virus through *in silico* docking. *Int. J. Drug Dev. Res.* **6**(2): 205-210.

CDC (Centres for Disease Control and Prevention), 1999. Neuraminidase inhibitors for treatment of influenza A and infections. *Morbidity and Mortality Weekly Report*, 48 (RR14): 1-9.

Cecchelli, R., Berezowski, V., Lundquist, S., Culot, M., Renfttel, M., Dehouck, M. P., and Fenart, L. 2007. Modelling of the blood-brain barrier in drug discovery and development. *Nat. Rev. Drug Discov.* **6**: 650-661.

Celeghini, R., Vilegas, J. H., and Lancas, F. M. 2001. Extraction and quantitative HPLC analysis of coumarin in hydroalcoholic extracts of *Mikania glomerata* Spreng. (guaco) leaves. *J. Brazilian Chem. Soc.* **12**: 706-709.

Chakraborty, D. P. 1969. Glycozoline, a carbazole derivative, from*Glycosmis pentaphylla. Phytochemistry* **8**: 769-772.

Chambers, T. J., Hahn, C. S., Galler, R., and Rice, C. M. 1990. Flavivirus genome organization, expression, and replication. *Annu. Rev. Microbiol.* **44**: 649-688.

Chavan, R., Samant, L., and Bapat, S. 2015. Protein modelling and docking of curcurin against neuraminidase, hemagglutinin proteins of pandemic influenza H1N1/2009. *J. Pharm. Sci. Res.* **7**(2): 70-75.

Chen, C.Y., Chang, Y.H., Bau, D.T., Huang, H.J., Tsai, F.J., Tsai, C.H., and Chen, C.Y.C. 2009. Discovery of potent inhibitors for phosphodiesterase 5 by virtual screening and pharmacophore analysis. *Acta Pharmacol. Sin.* **30:** 1186–1194.

Chhabra, M., Mittal, V., Bhattacharya, D., Rana, U., and Lal, S. 2008. Chikungunya fever: a reemerging viral infection. *Indian J. Med. Microbiol.* **26**: 5-12.

Chin, Y. W., Balunas, M. J., Chai, H. B., and Kinghorn, A. D. 2006. Drug discovery from natural sources. *Am. Assoc. Pharma. Sci. J.* 8(2):239-242.

Chopra, R. N., Nayar, S. I., and Chopra, I. C. 1956. *Glossary of Indian medicinal plants*,Publication and Information Directorate, CSIR, New Delhi, **204:**330.

Chow, A., Her, Z., Ong, E. K. S., Chen, J., Dinatatac, F., Kwek, D. J. C., Barkham, T., Yang, H., Renia, L., Leo, Y., and Ng, L. F. P. 2011. Persistent arthragalia induced by chikungunya virus infection is associated with IL-6 and granulocyte macrophage colony stimulating factor. *J. Infect. Dis.* **203**(2): 149-157.

Chowdhury B. K. and Bhattacharyya P. 1985. A further quinazoline alkaloid from*Adhatoda vasica. Phytochemistry* **24**: 3080-3082.

Chowdhury, A., Mustapha, M., Garba, and Bhattacharyya, P. 1987. Carbazole and 3-methylcarbazole from*Glycosmis pentaphylla. Phytochemistry* **26**: 2138–2139.

Cross, J. C., Wen, P., and Rutter, W. 1993. Transactivation by hepatitis B virus X protein is promiscuous and dependent on mitogen-activated cellular serine/threonine kinases. *Proc. Natl. Acad. Sci. USA* **90**:8078–8082.

Dan, S., Annie, R., Laird, A. T., Barbara, A. B., Mark, W. S., and David, J.M. 2007. Isolation and structure elucidation by LC-MS-SPE/NMR: PR Toxin- and Cuspidatol-related eremophilane sesquiterpenes from *Penicillium roqueforti*. *J. Nat. Prod.* **70** (1): 121–123.

De Clercq, E. 2001. New developments in anti-HIV chemotherapy. *Curr. Med. Chem.* **8**(13): 1543-1572.

De, I., Fata-Hartley, C., Sawicki, S. G., and Sawicki, D. L. 2003. Functional analysis of nsP3 phosphoprotein mutants of Sindbis virus. *J. Virol.* **77**:13106-13116.

Deng, X.Q., Wang, H.Y., Zhao, Y.L., Xiang, M.L., Jiang, P.D., Cao, Z.X., Zheng, Y.Z., Luo, S.D., Yu, L.T., Wei, Y.Q., and Yang, S.Y. 2008. Pharmacophore modelling and virtual screening for identification of new Aurora-A kinase inhibitors. *Chem. Biol. Drug Des.* **71**: 533–539.

Dhanwani, R., Khan, M., Lomash, V., Rao, P. V., Ly, H., and Parida, M. 2014. Characterization of chikungunya virus induced host response in a mouse model of viral myositis. *PLoS One* **9**(3): e92813.

Dhawan, B.N., 1995. *Centrally acting agents from Indian plants* In: Koslow, S. H., Murthy, R. S, and Coelho, G. V., (eds.)*Decade of the Brain: India/USA Research in Mental Health and Neurosciences Rockville*, National Institute of Mental Health and Neurosciences, Bangalore, pp. 197-202.

Dixon, S. J. and Stockwell, B. R. 2009. Identifying druggable disease-modifying gene products, *Curr. Opn. Chem. Biol.* **13**(6): 549-555.

Dong, H., Fink, K., Zust, R., Lim, S. P., Qin, C. F. and Shi, P. Y. 2014. Flavivirus RNA methylation. *J. Gen. Virol.* **95**: 763–778.

Dubuisson, J., and Rice, C. M. 1993. Sindbis virus attachment: isolation and characterization of mutants with impaired binding to vertebrate cells. *J. Virol.* **67**(6):3363-3374.

Dudha, N., Rana, J., Gabrani, R., Gupta, A., Chaudhary, V. K., and Gupta, S., 2014. Small scale expression, solubilisation and characterisation of chikungunya virus structural proteins. *Asian J. Pharma. Clin. Res.* 7(5): 268-271.

Dwivedi, V. D., Tripathi, I. P., and Mishra, S. K. 2016. *In silico* evaluation of inhibitory potential of triterpenoids from *Azadirachta indica* against therapeutic target of dengue virus, NS2B-NS3 protease. *J. Vector Borne Dis.* **53**: 156–161.

Ehrman, T.M., Barlow, D.J., and Hylands, P.J. 2010. *In-silico* search for multi-target anti-inflammatories in Chinese herbs and formulas. *Bioorg. Med. Chem.* **18**: 2204–2218.

El-Sayed, N. S., El-Bendary, E. R., El-Ashry, S. M., and El-Kerdawy, M. M. 2011. Synthesis and antitumor activity of new sulfonamide derivatives of thiadiazolo[3,2-α] pyrimidines. *Eur. J. Med. Chem.* **46**(9):3714-3720.

Farhana, I., Farhana, I. J., Syeda, S., Ishita, M., Nazmus, S., Shaiful, A. B., Auditi, S., Sherejad, S., and Mohammed, R. 2011. Variations in disease and medicinal plant selection among folk medicinal practitioners: a case study in Jessore district, Bangladesh. *Am. Eurasian J. Sustain. Agric.* **5**: 282-291.

Ganem, D. and Schneider, R. J. 2001. Hepadnaviridae and their replication, In: Knipe, D. M., Howley, P. M., Griffin, D. E., Martin, M. A., Lamb, R. A., Roizman, B., (Eds.), *Fields Virology* 4 ed., Philadelphia, PA, Lippincott-Raven Publishers.

Gao, W. N., Li, Y., Zhang, R., Gao, H., Xu, W.R., Li, A.X., Du, Q.S., Zhang, X., and Wei, D.Q. 2006. Screening of new HIV inhibitors based on the

database of traditional Chinese medicine. *Yaoxue Xuebao* (*Acta Pharm. Sin.*) **41**: 241–246.

Gavilanes, F., Gonzales-Ros, A., and Peterson, D. 1982. Structure of hepatitis B surface antigen: characterization of the lipid components and their association with the viral proteins. *J. Biol. Chem.* **257**:7770– 7777.

Gene, Y., Ozkanca, R., and Bekdemir, Y. 2008. Antimicrobial activity of some sulphonamide derivatives on clinical isolates of *Staphylococcus aureus*. *Anm. Clin. Microb.* **7**: 17-22.

Ghani, A.2003. *Medicinal plants of Bangladesh,* The Asiatic Soc. Bangladesh, **430**: 502-504.

Gopi, K. S. 2000. *Encyclopaedia of Medicinal Plants Used in Homoeopathy.* AIY Publications Pvt. Ltd: Vol I.

Govindachari, T. R., Pai, B. R. and Subramaniam, P. S. 1996. Alkaloids of *Glycosmis pentaphylla* (Retz.) Correa. *Tetrahedron* **22***:* 3245-3252.

Greger, H., Zechner, G., Hofer, O., and Vajrodaya, S. 1996. Bioactive amides from *Glycosmis* species. *J. Natl. Prods.* **59**:1163-1168.

Greger, H., Hofer, O., Zechner, G., Hadacek, F. and Wurz, G. 1994. Sulphones derived from methylthiopropenoic acid amides from *Glycosmis angustifolia. Phytochemistry* **37***:* 1305-1310.

Gupta, N. A., Subhramanyam, E. V. S., Jha, S., Bhatia, V., and Narang, E. 2011. A comparative antipyretic activity of the crude extracts of the plant *Leucas aspera* and *Glycosmis pentaphylla. J. Chem. Pharm. Res.* **03**: 320-323.

Hamsa, N. S., Nair, V. P., Chandramohan, V., and Patel, S. 2013. Pharmacore elucidation and docking studies on anti-inflammatory compounds of medicinal plants for ulcerative colitis. *Asian J. Pharma. Clin. Res.* **6**(3): 56-61.

Han, L., Huang, X., Dahse, H. M., Moellmann, U., Fu, H. Z., and Grabley, S. 2007. Unusual naphthoquinone derivatives from the twigs of *Avicennia marina*. *J. Nat. Prod.* **70**(6): 923-927.

Hardy, W. R. and Strauss, J. H. 1989. Processing the non-structural polyproteins of Sindbis virus non- structural proteinase is in the C terminal half of nsp_2 and functions both in cis and trans. *J. Virol.* **63**: 4653-4664.

Hatton, T., and Zhou, S. 1992. Standring DN. RNA- and DNA-binding activities in hepatitis B virus capsid protein: a model for their roles in viral replication. *J. Virol.* **66**:5232–5241.

Hinterberger, S., Hofer, O., and Greger, D. H. 1986. Synthesis and corrected structures of sulphur containing amides from *Glycosmis* species: sinharines, penimides, and illukumbins. *Tetrahedron* **50**: 6279-6286.

Hofer, O., and Greger, H. 2000. Sulphur containing amides from *Glycosmis* species (Rutaceae). *Progress in the Chemistry of Organic Natural Products*, Springer Vienna, pp. 187-223.

Hollinger, F. B., and Liang, T. J. 2001. Hepatitis B virus. In: Knipe, D. M., Howley, P. M., Griffin, D. E., Lamb, R. A., Martin, M. A., Roizman, B., Eds.,*Fields Virology* Lippincott-Raven Publishers, Philadelphia, PA, 2971-3036.

Hu, Z., Zhang, Z., Kim, J. W., Huang, Y., and Liang, T. J. 2006. Altered proteolysis and global gene expression in hepatitis B virus X transgenic mouse liver. *J. Virol.* **80**:1405–1413.

Huang, Q., Qiao, X.B., and Xu, X. J. 2007. Potential synergism and inhibitors to multiple target enzymes of Xuefu Zhuyu decocotion in cardiac disease therapeutics: a computational approach. *Bioorg. Med. Chem. Lett.* **17**: 1779–1783.

Idress, S., and Ashfaq, U. A. 2012. A brief review on dengue molecular virology, diagnosis, treatment and prevalence in Pakistan. *Genet Vaccines Ther.* **10**(1): 6.

Idrees, S. and Usman, A. A. 2012. A brief review on dengue molecular virology, diagnosis, treatment and prevalence in Pakistan. *Genet. Vaccines Ther.* **10**:6.

Imming, P., Sinning, C., and Meyer, A. 2006. Drugs, their targets and the nature and number of drug targets. *Drug Discov.* **5**(10): 821-834.

Iram, M., Rani, S. R. H., and Pais, N. 2016. Therapeutic implications of metoprolol chronotherapy in hypertension. *World J. Pharma. Res.* **5**(9): 1223-1233.

Issur, M., Geiss, B. J., Bougie, I., Picard-Jean, F., Despins, S., Mayette, J., Hobdey, S. E. and Bisaillon, M. 2009. The flavivirus NS5 protein is a true RNA guanylyltransferase that catalyzes a two-step reaction to form the RNA cap structure. *RNA* **15**: 2340 - 2350.

Ito, C., Kondo, Y., Ruangrungsi, N., and Furukawa, H. 1999. New quinazoline alkaloids from *Glycosmis cochinchinensis. Chem. Pharm. Bull.* **47**: 1491-1493.

James, C. D., and Roberts, S. 2015. Viral interactions with PDZ domain-containing proteins An oncogenic trait? *Pathogens* **5**(8): 1-22.

Jash, S. S., and Biswas, G. K. 1992. Carbazole alkaloids from *Glycosmis pentaphylla. Phytochemistry* **31**: 2503-2505.

Jochmans, D., Guerrero, N. S., and Delang, L. 2013. Inhibition of chikungunya virus replication by T-705 (favipiravir) and identification of resistance associated mutations in the RNA-dependent RNA polymerase. In Abstracts: *26th International Conference on Antiviral Research,* Vol. 169: 1.

Judit, M., Imre, O., Laszlo, P., Tannaz, G., Judit, H., and Istvan, Z. 2013. Investgation of antiproliferative action of the quinolone alkaloids Kokusaginine and skimmiamine on human cell lines. *Curr. Signal Transduction Ther.* **8**(2): 148-155.

Junsong, W., Yingtong, D., Xianwen, Y., Shunlin, L., Yuehu, W., and Xiaojiang, H. 2006. Hydroquinone diglycoside acyl esters from the stems of*Glycosmis pentaphylla. Phytochemistry* **67**: 486–491.

Kamaruzzman, S. R., and Chakraborty, D. P. 1989. Mupamine from *Glycosmis pentaphylla. Phytochemistry* **28**: 677-678.

Kaneria, M., Kanani, B., and Chanda, S. 2012. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian plants. *Asia Pacific J. Trop. Biomed.* **2**: 195-202.

Khan, I., Nisar, M., Ebad, F., Nadeem, S., Saeed, M., Khan, H., Ullah, S., Khuda, F., Karim, N., and Ahmad, Z. 2009. Anti-inflammatory activities of sieboldigenin from *Smilax china* Linn.: experimental and computational studies. *J. Ethnopharmacol.* **121**: 175-177.

Khromykh, A. A., Sedlak, P. L., and Westway, E. G. 1999. Trans-complementation analysis of the flavivirus kunjin ns5 gene reveals an essential role for translation of its N terminal half in RNA replication. *J. Virol.* **73**: 9247-9255.

Kielian M, and Helenius A. 1985. pH-induced alterations in the fusogenic spike protein of Semliki Forest virus. *J. Cell Biol.* **101**(6):2284-2291.

Kinghorn, A. D. 2001. Pharmacognosy in the 21stcentury. *J. Pharmacy Pharmacol.* **53**: 135-148.

Kujawski, J., Popielarska, H., Myka, A., Beata, D., and Marek, B. K. 2012. The logP parameter as a molecular descriptor in the computer-aided drug design - an overview. *Comput. Methods Sci. Technol.* **18**(2): 81-88.

Lagunin, A. A., Goel, R. K., Gawande, D. Y., Pahwa, P., Gloriozoma, T. A., Dmitriev, A. V., Ivanov, S. M., Rudik, A. V., Konova, V. I., Pogodin, P. V., Druzhilovsky, D. S., and Poroikov, V. V. 2014. Chemo and bioinformatics resources for *in silico* drug discovery from medicinal plants beyond their traditional use: a critical review. *Nat. Prod. Rep.* **31**: 1585-1611.

Lahlou, M. 2012. *De la Plante au Médicament: Une Passerelle entre Tradition et Science*. Édition LAWNE, Maroc. ISBN 978-9954-31-103-5, p. 272.

Lee, Y., Hoaglund-Hyzera, C., Barnes, S. C., Hilderbrand, A., Valentine, S., and Clemmer, D. 2002. Development of high-throughput liquid chromatography injected ion mobility quadrupole time-of-flight techniques for analysis of complex peptide mixtures. *J. Chromatogr. B. Anal. Technol. Biomed. Life Sci.* **782**: 343–351.

Lengauer, T., and Rarey, M. 1996. Computational methods forbio molecular docking. *Curr. Opin. Struct. Biol.* **6** (3): 402-406.

Leo, A., Hansel, C., and Elkins, D. 1971. Partition coefficients and their uses. *Chem. Rev.* 71(6):525-616.

Leupin, O., Bontron, S., Schaeffer, C., and Strubin, M. 2005. Hepatitis B virus X protein stimulates viral genome replication via a DDBI-dependant pathway distinct from that leading to cell death. *J. Virol.* **77**:6274–6283.

Liljeström, P., and Garoff, H. 1991. Internally located cleavable signal sequences direct the formation of Semliki forest virus membrane proteins from a polyprotein precursor. *J. Virol.* **65**(1):147-154.

Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. 1996. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **46**: 3-26.

Lipinski, C.and Hopkins, A. 2004. Navigating chemical space for biology and medicine. *Nature* **432**(7019): 855-861.

Longo, and Dan, L. 2012. Influenza. Harrison's Principles of Internal Medicine, 18th ed., Mc. Graw-Hill, New York.

Lumsden, W. H. 1955. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. II. General description and epidemiology. *Trans. R. Soc. Trop. Med. Hyg.* **49**: 33-57.

Lutz, M. K. 2003. Putting nature back into drug discovery. *Nat. Biotechnol.* **21**(3): 602-604.

Mandal, S., Upadhyay, N., Sharma, I., Rohit, S., Mandloi, A. 2011. A comparative antipyretic activity of the crude extracts of the ariel parts of *Glycosmis pentaphylla* and *Bauhinia variegate*. *Rec. Res. Sci. Tech.* **03**: 16-18.

Manikandan, P., Muthuselvam, A., and Manibalan, S. 2014. Small molecules screening against type-2 dengue virus envelope protein (DenVe) from phyto antiviral ligands-an *in silico* analysis. *Asian. J. Med. Pharma. Sci.* **2**(2): 143-148.

Manuel, C., Amaya, Z., Ana, M., Jose, L.N., and Gonzalo, L.A. 2006. Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and *Gardenia jasminoides* fruits: Tentative identification of seven new compounds by LC-ESI-MS. *J. Agric. Food Chem.* **54** (3): 973-979.

Matrosovich, M. N., Matrosovich, T. Y., Gray, T., Roberts, N. A., and Klenk, H. D. 2004. Neuraminidase is important for the initiation of influenza virus infection in human airway epithelium. *J. Virol.* **78**(22): 12665-12667.

Maurya, A., Khan, F., Bawankule, D. U., Yadav, D. K., and Srivastava, S. K. 2012. QSAR, docking and in vivo studies for immunomodulatory activity of isolated triterpenoids from *Eucalyptus tereticornis* and *Gentiana kurroo*. *Eur. J. Pharma. Sci.* **47** (1): 152-161.

Mavuduru, G., Awasthi, P., Timiri, A. K., and Ghosh, M. Computer aided perspective of selection of plants against viruses. Conference paper, Birla institute of technology, Mesra, Ranchi, Jharkhand, India, available at: https://www.researchgate.net/profile/Ajay_Timiri/publications?pubType=inProceedings, retrieved 21.11.2016.

Meena, A., Yadav, D. K., Srivastava, A., Khan, F., Chanda, D., and Chattopadhyay, S. K. 2011. *In silico* exploration of anti-inflammatory

activity of natural coumarinolignoids. *Chem. Boil. Drug Des.* **78** (4): 567-579.

Melagari, M., Scaglioni, P. P., and Wands, J. R. 1998. Cloning and characterization of a novel Hepatitis B virus X binding protein that inhibits viral replication. *J. Virol.* **72**(3): 1737-1743.

Milich, D., and Liang, T. J. 2003. Exploring the biological basis of hepatitis B antigen in hepatitis B virus infection. *Hepatology* **38**: 1075–1086.

Mohammed, R., Rownak, J., Azad, A. K., Syeda, S., Mahbubur, R., Anita, R. C., Rahima, B., Dilruba, N., Zubaida, K., Mohammad, S. H., Afsana, K., and Emdadullah, M. 2010. A randomized survey of medicinal plants used by folk medicinal practitioners in six districts of Bangladesh to treat rheumatoid arthritis. *Adv. Nat. Appl. Sci.* **4**: 124-127.

Mohan, M., James, P., Valsalan, R., and Nazeem, P. A., 2015. Molecular docking studies of phytochemicals from *Phyllanthus nirui* against hepatitis B DNA polymerase. *Bioinformation* **11**(9): 426-431.

Mukherjee, M., Mukherjee, S., Shaw, A. K. and Ganguly, S. N. 1983. Mukonicine, a carbazole alkaloid from leaves of *Murraya koenigii. Phytochemistry* **22**: 2328-2329.

Mun, J., Jabbar, A. A., Devi, N. S., Liu, Y., Van Meir, E. G.,and Goodman MM. 2012. Structure–activity relationship of 2,2-dimethyl-2H-chromene based arylsulfonamide analogs of 3,4-dimethoxy-N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]-N-phenylbenzenesulfonamide, a novel small molecule hypoxia inducible factor-1 (HIF-1) pathway inhibitor and anti-cancer agent. *Bioorg Med Chem.* **20**(4): 4590–4597.

Naeem, S., Duffy, J. E., and Zavaleta, E. 2012. The functions of biological diversity in an age of extinction. *Science* **336**(6087): 1401-1406.

Namita, A., Ranawat, M. S., Pankaj, A., and Zaman, K. 2011. Pharmacognostic and preliminary phytochemical studies on the leaves of *Glycosmis pentaphylla* (Retz.) Corr. *J. pharm. Res.* **4**: 1800-1801.

Nayak, S. S., Jain, R., and Sahoo, A. 2010. Hepatoprotective activity of *Glycosmis pentaphylla* against paracetamol-induced hepatotoxicity in swiss albino mice. *Pharma. Biol.* **49**(2): 111-117.

Newman, D. J. and Cragg, G. M. 2007. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **70**(3): 461-477.

Nguyen, P., Yu, H., and Keller, P. 2014. Discovery of *in silico* hits targeting the nsp_3 macrodomain of chikungunya virus. *J. Mol. Modeling* **20**(5): 2212-2216.

Nisbet, L. J., Moore, M., and Soejarto, D. D. 1997. Will natural products remain an important source of drug research for the future? *Curr Opin. Biotechnol.* **8**(6): 708-712.

Nithya, P., Jeyram, C., Sundaram, K. M., Chandrasekhar, A., and Ramaswamy, M. S. 2014. Anti-dengue viral compounds from *Andrographis paniculata* by *in silico* approach. *World J. Altern. Med.* **1**(2): 10-16.

Onyancha, E. M., Tarus, P. K., Machochlo, A. K., and Chhabra, S. C. 2014. Phytochemical and antimicrobial studies of *Teclea nobilis Del.* used in traditional medicine in Kenya. *J. Kenya Chem. Soc.* **8**(1): 89-97.

Ostan, I., Saglam, H., Limoncu, M. E., Ertabaklar, H., Toz, S. O., Ozbel, Y., and Ozbiligin, A. 2007. *In vitro* and *in vivo* activities of *Haplophyllum myrtifolium* against *Leishmania tropica.New Microbiologica* **30**: 439-445.

Ouchi, A., Nerome, K., Kanegae, Y., Ishida, M., Nerome, R., Hayashi, K., Hashimoto, T., Kaji, M., Kaji, Y., and Inaba, Y. 1996. Large outbreak of swine influenza in southern Japan caused by reassortant (H1N2) influenza viruses: its epizootic background and characterization of the causative viruses. *J. Gen. Virol.* 77: 1751–1759.

Oudhia, P. 2006. Traditional medicinal knowledge about *Aleuro marginatus* sp. feeding on Ban-Nimbu *(Glycosmis pentaphylla)* in Chhattisgarh, India. Ethno botanical surveys, available at: http://www.Ecoport.org.

Ozbek, N., Katircioqlu, H., Karacan, N., and Baykal, T. 2007. Synthesis, characterization and antimicrobial activity of new aliphatic sulphonamide. *Bioorg. Med. Chem.* **15**(15): 5105-5109.

Painqankar, M. S., and Arankalle, V. A. 2014. Identification of chikungunya virus interacting proteins in mammalian cells. *J. Biosci.* **39**(3): 389-399.

Paoletta, S., Steventon, G.B., Wildeboer, D., Ehrman, T.M., Hylands, P.J., and Barlow, D.J. 2008. Screening of herbal constituents for aromatase inhibitory activity. *Bioorg. Med. Chem.* **16**: 8466–8470.

Papageorgiou, V. P. 1978. Wound healing properties of naphthaquinone pigments from *Alkanna tinctoria. Experientia* **34**: 499.

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.

Potisopon, S., Pret, S., Collet, A., Decroyly, E., Canard, B., and Selisko, B., 2014. The methyltransferase domain of dengue virus protein NS5 ensures efficient RNA synthesis initiation and elongation by the polymerase domain. *Nucleic Acids Res.* **42**(18):11642–11656.

Powers, A. M. and Logue, C. H. 2007. Changing patterns of chikungunya virus: re-emergence of a zoonotic arbovirus. *J. Gen. Virol.* **88**: 2363-2377.

Prakash, O., Khan, F., Sangwan, R. S., and Misra, L. 2013. ANN-QSAR model for virtual screening of androstenedione C-skeleton containing phytomolecules and analogues for cytotoxic activity against human breast cancer cell line MCF-7. *Comb. Chem. High Throughput Scrn.* **16**(1):57.

Praveena, P., Sudarsanam, D., and Saravanan, K. 2014. GC, GC/MS characterization of the *Enicostema littorale. World J. Pharma. Res.* **3**(5): 844-852.

Qamar, M. T., Mumtaz, A., Ashfaq, U. A., Adeel, M. M., and Fatima, T. 2015. Potential of plant alkaloids as dengue ns3 protease inhibitors: Molecular docking and simulation approach. *Bioinformation* 9:262-267.

Qamar, M. T., Mumtaz, M. A., Naseem, R., Ali, A., Fatima, T., Jabbar, T., Ahmad, Z. and Ashfaq, U. A. 2014. Molecular docking based screening of plant flavonoids as dengue NS1 inhibitors. *Bioinformation* 10(7):460-465.

Qiao, X., Hou, T.J., Zhang, W., Guo, S.L., Xu, X.J., 2002. A 3D structure database of components from Chinese traditional medicinal herbs. *J. Chem. Inf. Comput. Sci.* **42**: 481–489.

Rahmani, M., Ling, C. Y., Sukari, M. A., Ismail, H. B. M., Meon, S. and Aimi, N. 1998. 7-methoxyglycomaurin: A new carbazole alkaloid from *Glycosmis rupestris. Planta Med.* **64**: 780-780.

Raja, K., Prabahar, A., Selvakumar, S., and Raja, T. K. 2014. *In silico* analysis to compare the effectiveness of associated drugs prescribed for swine flu in diverse medicine systems. *Indian J. Pharm. Sci.* **76**(1): 10-18.

Raju, N. J., and Rao, B. G. 2010. Evaluation of hepatoprotective activity of *Glycosmis pentaphylla* roots against CCl_4-induced acute liver injury in rats. *Int. J. Pharma. Sci. Rev. Res.* 4(2): 81-86.

Randall, R. E., and Goodbourn, S. 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus counter measures. *J. Gen. Virol.* **89**: 1-47.

Randrianarivelojosia, M., Rasidimanana, V. T., Rabarison, H., Cheplogia, P. K., Ratsimbason, M., Mulholland, D. A., and Mauclere, P. 2003. Plants traditionally prescribed to treat tazo (malaria) in eastern region of Madagascar. *Malaria J.* 2(25): 1-9.

Rashad, A. A., and Keller, P. P. 2013. Structure based design towards the identification of novel binding sites and inhibitors for the chikungunya virus envelope proteins. *J. Mol. Graphics Modelling* 44: 241-252.

Rathore, A. P., Ng, M. L., and Vasudevan, S. G. 2013. Differential unfolded protein response during Chikungunya and Sindbis virus infection: CHIKV nsP4 suppresses eIF2 alpha phosphorylation. *Virol. J.* **10**: 36.

Ray, S. C., and Thomas, D. L. 2009. Hepatitis C. In: Mandell, G. L., Bennet, J. E.,and Dolin, R.,(Eds.) *Mandell Douglas and Bennet's Principles and Practice of Infectious Diseases,* 7th ed., Churchill Livingstone, Philadelphia, PA, 7: 154.

Reddy, A. J. M., Barik, M. R., Muli, G. L. and Parthasarathy, T. 2012. Computational approach for designing and development of potent inhibitor for hepatitis B virus X assosciated protein through molecular docking studies. *J. Chem. Pharma. Res.* 4(1): 265-271.

Reddy, V., Mani, R. S., Desai, A., and Ravi, V. 2014. Correlation of plasma viral loads and presence of chikungunya IgM antibodies with cytokine/ chemokine levels during acute chikungunya virus infection. *J. Med. Virol.* **86**(6): 1393-1401.

Reynolds, C. H. 2010. Computer Aided Drug Design: A practical guide to protein structure based modelling. In: *Drug Design: Structure and Ligand Based Approaches* 1Ed.,Merz Jr, K. M., Ringe, D. and Reynolds, C. H. (Eds.), Cambridge University Press,Cambridge, UK, pp. 181-196.

Rodanant, P., Surarit, R., Laphookhieo, S., and Kuvatanasuchati, J. 2015. *In vitro* evaluation of antibacterial and anti-inflammation activities of *Clausena lansium* (Lour.) Skeels. *Songklanakirim J. Sci. Technol.* **37**(1): 43-48.

Rollinger, J. M., Schuster, D., Danzl, B., Schwaiger, S., Markt, P., Schmidtke, M., Gertsch, J., Raduner, S., Markt, P., Schmidtke, M., Gertsch, J., Raduner, S., Wolber, G., Langer, T. and Stuppner, H. 2009. *In silico* target fishing for rationalized ligand discovery exemplified on constituents of *Ruta graveolens*. *Planta Med.* 75: 195-204.

Rosen, H. R. 2011. Clinical practice. Chronic hepatitis C infection. *New England J. Med.* **364**(25): 2429-2438.

Rothan, H. A., Han, H. C., Ramasamy, T. S., Othman, S., Rahman, N. A., and Yusof, R. 2012. Inhibition of dengue NS2B-NS3 protease and viral replication in Vero cells by recombinant retrocyclin-1. *BMC Infect. Dis.* **12**: 314.

Rudd, P. A., Wilson, J., Gardner, J., Larcher, T., Babarit, C., Le, T. T., Anaraku, I., Kumagai, Y., Loo, Y. M., GaleJr, M., Akira, S., Khormykh, A. A., and Suhebier, A. 2012. Interferon response factors 3 and 7 protect against chikungunya virus hemorrhagic fever and shock. *J. Virol.* **86**(8): 9888-9898.

Sakeran, M. I., Zidan, N., Rehman, H., Aziz, A. T., and Saggu, S. 2014. Abrogation by *Trifolium alexandrium* root extract on hepatotoxicity induced by acetaminophen in rats. *Redox. Rep.* **19** (1): 26-33.

Sarkar, M. and Chakraborty, D. P. 1979. Glycophymoline, a new minor quinazoline alkaloid from *Glycosmis pentaphylla. Phytochemistry* **18**: 694-695.

Sarkar, M. and Chakraborty, D. P. 1977. Some minor constituents from *Glycosmis pentaphylla. Phytochemistry* **16**:2007-2008.

Sastri, B. N. 1956. *The Wealth of India: Raw Materials.* CSIR, New Delhi. **4**: 150.

Schwartz, O. and Albert, M. L. 2010. Biology and pathogenesis of chikungunya virus. *Nat. Rev. Microbiol.* **8**: 491-500.

Sharma, S., Chattopadhyay, S. K., Yadav, D. K., Khan, F., Mohanty, S., and Maurya, A. 2012. QSAR, docking and in vitro studies for anti-inflammatory activity of cleomiscosin A methyl ether derivatives. *Eur. J. Pharma. Sci.* **47**(5): 952-964.

Shen, J. H., Xu, X. Y., Cheng, F., Liu, H., Luo, X. M., Shen, J. K., Chen, K. X., Zhao, W. M., Shen, X., and Jiang, H. L. 2003. Virtual screening on natural products for discovering active compounds and target information. *Curr. Med. Chem.* **10**: 1241–1253.

Shirako, Y., Strauss, E. G., and Strauss, J. H. 2000. Suppressor mutations that allow Sindbis virus RNA polymerase to function with nonaromatic amino acids at the N-terminus: Evidence for interaction between nsP1 and nsP4 in minus-strand RNA synthesis. *Virology* **276**: 148-160.

Singh, J., Kumar, M., Mansuri, R., Sahoo, G. C., and Deep, A. 2016. Inhibitor designing, virtual screening, and docking studies for methyltransferase: A potential target against dengue virus. *J. Pharm. Bioallied. Sci.* **8**(3): 188-194.

Singh, S. K., and Unni, S. K. 2011. Chikungunya virus: host pathogen interaction. *Rev. Med. Virol.* **21**: 78-88.

Sivakumar, M., Chamundeeswari, D., and Susithra, E. 2014. Comparative in-vitro antiarthritic studies on the various extracts of *Glycosmis pentaphylla* DC roots. *J. Pharmacy Res.* 8(7): 986-989.

Smith, D. A., Di, L., and Kerns, E. H. 2010. The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nat. Rev. Drug Discov.* **9**: 929-939.

Solignat, M., Gay, B., Higgs, S., Briant, L., and Devaux, C. 2009. Replication cycle of chikungunya: a re-emerging arbovirus. *Virology* **393**: 183-197.

Sreejith, P. S., Praseeja, R. J. and Asha, V. V. 2012. A review on the pharmacology and phytochemistry of traditional medicinal plant, *Glycosmis pentaphylla* (Retz.) Correa. *J. Pharmasy Res.* **5**(5): 2723-2728.

Stevens, A. J., Gahan, M. E., Mahalingam, S., and Keller, P. A. 2009. The medicinal chemistry of dengue fever. *J. Med. Chem.* **52**: 7911-7926.

Thanapati, S., Hande, A., Das, R., Gurav, Y., and Tripathy, A. S. 2014. Association of human leukocyte antigen class II allele and haplotype in chikungunya infection in a western Indian population. *Trans. R. Soc. Trop. Med. Hyg.* **108**(5): 277-282.

Tian, S., Wang, J., Li, Y., Li, D., Xu, L., and Hou, T. 2015. The application of *in silico* drug-likeness predictions in pharmaceutical research. *Adv. Drug Delivery Rev.* **86**: 2-10.

Touseef, A., Khajuria R.K., Puri, S.C., Verma, V., and Qazi, G.N. 2006. Determination and quantification of camptothecin in an endophytic fungus by LC/MS/MS method. *Curr. Sci.* **91**(2): 208-212.

Turpinseed, A. A., Burns, S. P., Moore, D. J. P., Hu, J., Guenther, A. B., and Monson, R. K. 2009. Controls over ozone deposition to a high elevation subalpine forest. *Agr. For. Meteorol.* **149**: 1447-1459.

Usha, T., Goyal, A. K., and Lubna, S. 2014. Identification of anti-cancer targets of eco-friendly waste Punica granatum peel by dual reverse virtual screening and binding analysis. *Asian Pac. J. Cancer Prev.* **15**: 10345-10350.

Vani, G., and Rajarajan, S. 2015. A study on *in silico* analysis of phytochemicals targeting the proteins of hepatitis B and C virus. *Int. J. Curr. Microbiol. App. Sci.* 4(12): 638-691.

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. and Kopple, K. D. 2002. Molecular properties that influence the oral bioavailabilty of drug candidates. *J. Med. Chem.* **45**: 2615-2623.

Vihinen, H., Ahola, T., Tuittila, M., Merits, A., and Kaariainen, L. 2001. Elimination of phosphorylation sites of Semliki Forest virus replicase protein nsP3. *J. Biol. Chem.* **276**: 5745-5752.

Wagner, R., Matrosovich, M., and Klenk, H. D. 2002. Functional balance between haemagglutinin and neuraminidase in influenza virus infections. *Med. Virol.* **12**(3): 159-166.

Wang, J. S., Zheng, Y. T., Efferth, T., Wang, R. R, Shen, Y. M., and Hao, X. J. 2005. Indole and carbazole alkaloids from *Glycosmis species. Phytochemistry* **66**: 697-701.

Wang, J., Di, Y., Yang, X., Li, S., Wang, Y., Hao, X. 2006. Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. *Phytochemistry* **67**: 486-491.

Wang, S.Q., Du, Q.S., Zhao, K., Li, A.X., Wei, D.Q., and Chou, K.C. 2007. Virtual screening for finding natural inhibitor against cathepsin-L for SARS therapy. *Amino Acids* **33**: 129–137.

Wang, L., Li, D., Bao, C., You, J., Wang, Z., Shi, Y., and Zhang, H. 2008. Ultrasonic extraction and separation of anthraquinones from *Rheum palmatum* L. *Uttrason Sonochem.***15**: 738-746.

Weaver, S. C., and Vasilakis, N. 2009. Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infection Genetics and Evolution* **9**: 523-540.

WHO (World Health Organization), 2014. Influenza (seasonal) Fact Sheet No. 211. www.who.int/mediacentre/factsheet/fs211/en.

Yadav, D. K., Meena, A., Srivastava, A., Chanda, D., Khan, F., and Chattopadhyay, S. K. 2010. Development of QSAR model for immunomodulatory activity of natural coumarinolignoids, *Drug Des. Devel. Ther.***4**: 173-186.

Yadav, D. K., Khan, F., and Negi, A. S. 2012. Pharmacore modelling, molecular docking, QSAR and *in silico* ADMET studies of gallic acid derivatives for immunomodulatory activity. *J. Mol. Model.* **18**(6): 2513-2525.

Yasir, M., Tripathi, M. K., Singh, P., Prakash, V., Sarkar, R., Srivastava, R. 2015. Efficacy of natural inhibitors from Glycosmis pentaphylla against protein kinase C: an *in silico* approach to combat skin cancer. *Oxidation Communications* **38**(1): 114-121.

Yu, H., Wang, Z. L., Zhang, L. R., Zhang, J. F., and Huang, Q. 2007. Pharmacophore modelling and *in silico* screening for new KDR kinase inhibitors. *Bioorg. Med. Chem. Lett.* **17**: 2126–2133.

Zeng, Z. R., Qjan, L., Cao, L. X., Tan, H. M., Huang, Y. L., Xue, X. L., Shen, Y., and Zhou, S. N., 2008. Virtual screening for screening for novel quorum sensing inhibitors to irradicate biofilm formation of *Pseudomonas aeruginosa, Appl. Microbial. Biotechnol.* **79**: 119-126.

Zhang, Z., Torii, N., Hu, Z., Jacob, J., and Liang, T. J. 2001. X-deficient woodchuck hepatitis virus mutants behave like attenuated viruses and induce protective immunity *in vivo. J. Clin. Invest.* **108**:1523–1531.

Annexures

ANNEXURE I

List of laboratory equipments used for the study

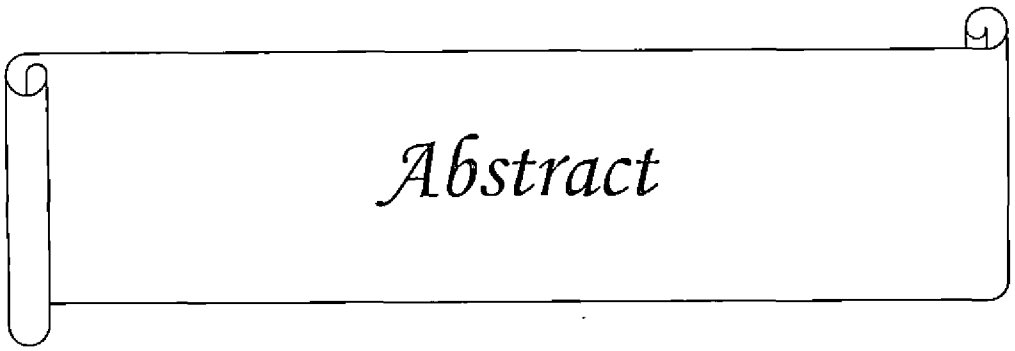
Sl No.	Equipment Name
1	Rotary evaporator
2	Shaker cum incubator
3	LCMS/MS unit
4	Computer with 4 GB RAM and Windows 7 operating system

ANNEXURE II

Reagents required:

Carbinol (99.9%)

Pure water


Abstract

MOLECULAR DOCKING OF ANTIVIRAL PROPERTIES OF *Glycosmis pentaphylla* (Retz.) Correa

By

BRINDA O. P.
(2014-11-103)

ABSTRACT OF THE THESIS

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Master of Science in Agriculture

(Plant Biotechnology)

Faculty of Agriculture
Kerala Agricultural University, Thrissur

CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA
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Abstract

Glycosmis pentaphylla (Retz.) Correa, a tropical shrub, locally known as '*panal*', is widely used in Ayurveda, for cough, rheumatism, anaemia and jaundice. Although, various parts of this plant are used against different diseases ranging from ulcer to cancer and also as an antiviral agent, there is no scientific evidence for proving its antiviral potential. Molecular docking facilitates the screening of large number of phytocompounds for their capability to interact with and deactivate the disease effecting proteins, within a short period of time. Hence the present study entitled "Molecular docking of antiviral properties of *Glycosmis pentaphylla* (Retz.) Correa" was undertaken with the objective to characterize the active ingredients in *Glycosmis pentaphylla* and to identify the compounds offering antiviral properties to this plant, through docking studies.

Separate dry samples were prepared from root, stem and leaf and were powdered. The finely ground powder was converted into a hydroalcoholic extract and was concentrated using rotary evaporator. The concentrated extract was subjected to LCMS/MS analysis through outsourcing. The mass to charge ratio obtained through LCMS/MS analysis was compared with the masses of various compounds identified from this plant though literature review.

Identified 23 phytochemicals from leaves and 14 from stem and root were subjected for molecular docking against viral protein targets of chikungunya, hepatitis, dengue and influenza using Discovery Studio 4.0. The protein targets were identified on the basis of information provided in the Chikungunya Drug Target Database. Protein targets for other viral diseases were selected based on the literature. Twelve proteins were selected for chikungunya. Two target proteins each were selected for Hepatitis B, hepatitis C and dengue and one for influenza. Suitability of phytocompounds to be developed as a drug was identified by screening with Lipinski's and Veber's rule and ADMET analysis. The ligands with good interaction with the protein target were identified based on the minimum difference between CDOCKER energy and CDOCKER interaction energy.

Two phytocompounds, isovaleric acid and avicequinone C, have shown acceptable interaction with the protein targets of all selected viral diseases. These two phytocompounds were found to have acceptable ADMET values (out of the seven parameters five were falling in the acceptable range). Isovaleric acid and avicequinone C were able to establish good interaction with majority of protein targets for the selected viral diseases with minimum difference between CDOCKER energy and CDOCKER interaction energy. They were capable to form hydrogen bonds with the involvement of aminoacid residues along with the establishment of good binding energy.

The isovaleric acid and avicequinone C molecules with strong capability to interact and deactivate the disease causing proteins are the basis for the antiviral properties of *Glycosmis pentaphylla*. Futher isolation and purification of these molecules and wet lab analyses on animal models have to be done to develop antiviral drugs from this plant.