COMPUTATIONAL REPURPOSED LIGANDS OF LAMIACEAE FAMILY: POTENTIAL CURE FOR MALARIA

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CERTIFICATE

This is to certify that the dissertation titled, **COMPUTATIONAL REPURPOSED LIGANDS OF LAMIACEAE FAMILY : POTENTIAL CURE FOR MALARIA**" is an authentic record of work carried out by **VARSHA JAYASANKAR** under the supervision and guidance of **Dr. ASHA D.**, Assistant Professor, Department of Botany & Centre for Research, St. Teresas's College (Autonomous), Ernakulam in partial fulfilment of the requirement for the Master's Degree of Science in Botany.

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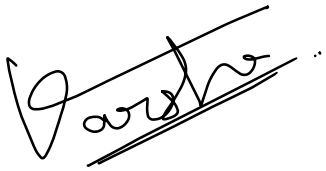

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1. INTRODUCTION

Although sciences and technology have progressed rapidly, de novo drug development has been a costly and time-consuming process over the past decades. In view of these circumstances, ‘drug repurposing’ (or ‘drug repositioning’) has appeared as an alternative tool to accelerate drug development process by seeking new indications for already approved drugs rather than discovering de novo drug compounds. In the meantime, the explosive and large-scale growth of molecular, genomic and phenotypic data of pharmacological compounds is enabling the development of new area of drug repurposing called computational drug repurposing.

Pathway-based drug-repurposing utilizes metabolic pathways, signaling pathways, and protein-interaction networks information to predict the similarity or connection between disease and drug. For example, using omics data processed from human patients or animals, disease-specific pathways are reconstructed to serve as new targets for repositioned drugs (Park, *et. al*.,2019).

Biomedical discovery has been reshaped upon the exploding digitization of data which can be retrieved from a number of sources, ranging from clinical pharmacology to cheminformatics-driven databases. Now, supercomputing platforms and publicly available resources such as biological, physicochemical, and clinical data, can all be integrated to construct a detailed map of signaling pathways and drug mechanisms of action in relation to drug candidates. Recent advancements in computer-aided data mining have facilitated analyses of ‘big data’ approaches and the discovery of new indications for pre-existing drugs has been accelerated (Karaman, & Sippl *et.al.*, 2019).

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus *Plasmodium*. The most serious and sometimes fatal type of malaria is caused by *Plasmodium falciparum*. The other human malaria species, *P. vivax, P. ovale, P. malariae,* and sometimes *P. knowlesi* can cause acute, severe illness but mortality rates are low. Malaria is the most important infectious disease in tropical and subtropical regions, and continues to be a major global health problem, with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries. It is estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of

whom 90% are children in sub- Saharan Africa. The number of malaria cases worldwide seems to be increasing, due to increasing transmission risk in areas where malaria control has declined, the increasing prevalence of drug resistant strains of parasites, and in a relatively few cases, massive increases in international travel and migration. The need for effective and practical diagnostics for global malaria control is increasing, since effective diagnosis reduces both complications and mortality from malaria. Differentiation of clinical diagnoses from other tropical infections, based on patients' signs and symptoms or physicians' findings, may be difficult. Therefore, confirmatory diagnoses using laboratory technologies are urgently needed (Tangpukdee *et.al.*,2009).

India contributes about 70% of malaria in the South East Asian Region of WHO. Although annually India reports about two million cases and 1000 deaths attributable to malaria, there is an increasing trend in the proportion of *Plasmodium falciparum* as the agent. There exists heterogeneity and variability in the risk of malaria transmission between and within the states of the country as many ecotypes/paradigms of malaria have been recognized. The pattern of clinical presentation of severe malaria has also changed and while multi-organ failure is more frequently observed in falciparum malaria, there are reports of vivax malaria presenting with severe manifestations (Tuteja, *et.al.*, 2007).

Mosquitoes of the genus *Anopheles* transmit malaria parasites. *Anopheles* mosquito species vary in their vector potential because of environmental conditions and factors affecting their abundance, blood-feeding behaviour, survival, and ability to support malaria parasite development. In the complex life cycle of the parasite in female mosquitoes, a process termed sporogony, mosquitoes acquire gametocyte-stage parasites from blood-feeding on an infected host. The parasites carry out fertilization in the midgut, transform to ookinetes, then oocysts, which produce sporozoites. Sporozoites invade the salivary glands and are transmitted when the mosquito feeds on another host. Most individual mosquitoes that ingest gametocytes do not support development to the sporozoite stage. Bottlenecks occur at every stage of the cycle in the mosquito. Powerful new techniques and approaches exist for evaluating malaria parasite development and for identifying mechanisms regulating malaria parasite–vector interactions (Beier, *et. al.*,1998).

Plasmodium falciparum is the etiological agent of malaria, the leading cause of death due to a vector-borne infectious disease, claiming 0.5 million lives every year. The single-cell eukaryote undergoes a complex life cycle and is an obligate intracellular parasite of

hepatocytes (clinically silent) and erythrocytes (disease causing). An infection can progress to a wide range of pathologies, including severe anaemia and cerebral malaria, which can lead to death. *P. falciparum* repeatedly replicates over the course of 48 h inside erythrocytes, resulting in exponential growth and rapid disease progression. As the single most important infectious disease afflicting children, no other pathogen has exerted a higher selection pressure on the human genome. Over 20 polymorphisms, including the sickle-cell trait, have been selected in human populations, despite severe fitness costs, since they offer protection against fatal *P. falciparum* infections. No effective vaccine exists, but several curative treatments are available (Joy, *et.al.*, 2003).

6YCZ, 6MPV, 3SRI are identified receptor proteins of *P. falciparum,* of which the protein structures are available in PDB (Protein Data Bank). The receptor proteins play a crucial role in the reproduction and life cycle of the plasmodium species. The denaturation of these proteins by any interaction, leads to the death and inability of the organism to multiply.

The medicinal use of plants as analgesic drugs in folk medicine is an ancient tradition, far older than the current sciences of medicine in developing countries. According to estimations, up to 70,000 plant species are used ethnomedicinally worldwide. Effects of herbal extracts have been studied by different pain tests including writhing test, light tail flick test, tail immersion test, hot-plate test, and formalin test.

The Lamiaceae family, one of the most important herbal families, incorporates a wide variety of plants with biological and medical applications. The most known members of this family are a variety of aromatic spices like thyme, mint, oregano, basil, sage, savory, rosemary, self-heal, hyssop, lemon balm, and some others with more limited use. Phytochemicals can be defined, in the strictest sense, as chemicals produced by plants. However, the term is generally used to describe chemicals from plants.

Plants in this family, are herbs or shrubs often with an aromatic smell. They are common in the Maltese Islands and other Mediterranean countries for the fact that some of them produce a high amount of essential oil that enables them to survive the hot summer season. Some examples from this family include mints, thyme, tulsi, spearmint and coleus. It is widely cultivated for medicinal, perfumery, culinary and ornamental purposes. Medicinal constituents include the strong aromatic essential oil, tannins, saponins and organic acids.

The plant has sedative, diuretic, tonic, antispasmodic and antiseptic properties (Raja, *et.al.,* 2012).

Lamiaceae is a family comprising 236 genera, and more than 7000 species. It is one of the most widely used and phytochemically studied families, because of their various compounds. Lamiaceae can be divided into two major categories, the first one includes all those species that mainly produce volatile terpenoids, found in the volatile compounds, such as Salvia sp., Mentha sp., Thymus sp. and Rosmarinus sp. While the second one comprises species that mainly biosynthesize constituents of the polar fraction, as Ajuga sp., Origanum sp., Teucrium sp., Melittis sp., and Stachys sp. Numerous researches on phytochemicals have led to the identification of many compounds, such as α- and β-pinene, menthol, thymol, eucalyptol, and limonene among the volatile constituents, and mono- and sesquiterpenes. From the other hand, Terpenes phenolic acids (rosmarinic, caffeic acids) and Alkaloids (apigenin, hesperidin), were detected (Skendi *et. al.,* 2019).

Various phytochemicals from different representatives of the family is as follows:

- Alpha-cadinol is a cadinane sesquiterpenoid that is cadin-4-ene carrying a hydroxy substituent at position 10. It has a role as a plant metabolite, a fungicide and a volatile oil component. It is a cadinane sesquiterpenoid, a carbobicyclic compound, a tertiary alcohol and a member of octahydronaphthalenes.

- Ursolic Acid is a pentacyclic triterpenoid found in various fruits, vegetables and medicinal herbs, with a variety of potential pharmacologic activities including anti-inflammatory, antioxidative, antiviral, serum lipid-lowering, and antineoplastic activities. Upon administration, ursolic acid may promote apoptosis and inhibit cancer cell proliferation through multiple mechanisms. This may include the regulation of mitochondrial function through various pathways including the ROCK/PTEN and p53 pathways, the suppression of the nuclear factor-kappa B (NF-kB) pathways, and the increase in caspase-3, caspase-8 and caspase-9 activities.

- Rosmarinic acid is a natural polyphenol antioxidant found in many Lamiaceae herbs used commonly as culinary herbs such as lemon balm, rosemary, oregano, sage, thyme, and peppermint. Chemically, rosmarinic acid is an ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid. Rosmarinic acid has powerful anti-inflammatory

properties, and research suggests that it may help treat inflammatory conditions like arthritis, asthma, and atopic dermatitis.

- Eugenol, also called clove oil, is an aromatic oil extracted from cloves that is used widely as a flavouring for foods and teas and as an herbal oil used topically to treat toothache and more rarely to be taken orally to treat gastrointestinal and respiratory complaints. Eugenol in therapeutic doses has not been implicated in causing serum enzyme elevations or clinically apparent liver injury, but ingestions of high doses, as with an overdose, can cause severe liver injury. Eugenol is a phenylpropanoid formally derived from guaiacol with an allyl chain substituted para to the hydroxy group. It is a major component of clove essential oil, and exhibits antibacterial, analgesic and antioxidant properties. It has been widely used in dentistry to treat toothache and pulpitis.

- Carvacrol is a phenol that is a natural monoterpene derivative of cymene. An inhibitor of bacterial growth, it is used as a food additive. Potent activator of the human ion channels transient receptor potential V3 (TRPV3) and A1 (TRPA1). It has a role as a volatile oil component, a flavouring agent, an antimicrobial agent, an agrochemical and a TRPA1 channel agonist. It is a member of phenols, a p-menthane monoterpenoid and a botanical anti-fungal agent. It derives from a hydride of a p-cymene. carvacrol is a phenolic monoterpene found in thyme, oregano, and several other species of the Lamiaceae. Long valued for their smell and taste, these substances also have antibacterial and anti-spasmolytic properties.

- Linalool is a monoterpenoid that is octa-1,6-diene substituted by methyl groups at positions 3 and 7 and a hydroxy group at position 3. It has been isolated from plants like *Ocimum canum*. It has a role as a plant metabolite, a volatile oil component, an antimicrobial agent and a fragrance. It is a tertiary alcohol and a monoterpenoid. Linalool naturally occurs in plants and spices as for example lavender, basil, or thyme. In contact with air linalool autoxidizes forming hydroperoxides.

- beta-caryophyllene is a beta-caryophyllene in which the stereocentre adjacent to the exocyclic double bond has S configuration while the remaining stereocentre has R

configuration. It is the most commonly occurring form of beta-caryophyllene, occurring in many essential oils, particularly oil of basil. It has a role as a non-steroidal anti-inflammatory drug, a fragrance, a metabolite and an insect attractant.

- Thymol is a phenol that is a natural monoterpene derivative of cymene. It has a role as a volatile oil component. It is a member of phenols and a monoterpenoid. It derives from a hydride of a p-cymene. It is a phenol obtained from thyme oil or other volatile oils. It is used as a stabilizer in pharmaceutic preparations. It has been used for its antiseptic, antibacterial, and antifungal actions, and was formerly used as a vermifuge.

- Nepetalactone is a name for multiple iridoid analog stereoisomers. Nepetalactones are produced by Nepeta cataria (catnip) and many other plants belonging to the genus Nepeta, in which they protect these plants from herbivorous insects by functioning as insect repellents.

- Camphene appears as a colourless to white crystalline solid with an insipid camphor-like odour. Dust and crystals are irritants to the eyes, nose and throat. Emits flammable vapors when heated. Emits acrid smoke and irritating fumes at high temperature. Used for the manufacture of synthetic camphor.

Cell membranes contain a host of proteins with diverse functions that support the life of a cell. Receptors are a special class of proteins that function by binding a specific ligand molecule. When a ligand binds to its receptor, the receptor can change conformation, transmitting a signal into the cell. In some cases, the receptors will remain on the surface of the cell and the ligand will eventually diffuse away. In other cases, ligand binding to a receptor triggers a series of events leading to internalization of the receptor: ligand complex in a process known as receptor-mediated endocytosis. Molecules have an innate affinity for one another due to electrostatic forces, such as Coulombic attractions, hydrogen bonds, and dispersion forces. The noncovalent interactions that result from this affinity are of particular importance in biological processes, including the catalysis of chemical reactions (by enzymes), neutralization of foreign toxins (by antibodies), and stimulation of cellular activities (by hormones). To initiate these processes, receptors and ligands exchange interactions with solvent and solute molecules for interactions with each other (Attie & Raines *et.al*., 1995).

Chimera docking is a novel method has been developed for the analysis of ligand–receptor interactions. The method utilizes binding data generated from the analysis of chimeric proteins with chimeric peptides. To each chimeric part of the peptide and receptor are assigned descriptors, thus creating a matrix of X descriptors. These descriptors are then correlated with the experimentally determined interaction binding affinities for each chimeric receptor/peptide pair by use of partial least-squares projection to latent structures (PLS) (Prusis *et.al*., 2001).

Chimera is used in docking the ligand and the receptor to produce a score which is analysed, to determine the level of denaturation of the protein. If the ligand interaction is suitable enough to denature the protein leading to an alteration in its function, the ligand (phytochemical) can be used to produce a potential drug against the disease.

2. AIMS AND OBJECTIVES

- To identify the ligand and receptor proteins
- Docking of the ligand with the receptor
- Computational analysis of the ligand receptor interaction
- Scoring and charting the interaction
- Producing the final list of ligands (phytochemicals) which can be used as an element in the potential drug production.

3. REVIEW OF LITERATURE

Cocan *et. al.* (2018) investigated the acivity of ethanolic extracts from the following medicinal plant species cultivated in western Romania: *Melissa officinalis* L., *Rosmarinus officinalis* L. (RO) and *Salvia officinalis* L. (SO). Antioxidant activity, total phenolics content and a profile of the main hydroxycinnamic acids (HCAs), including caffeic, ferulic, coumaric and rosmarinic acids, was determined for each plant extract. The *in vitro* antimicrobial activity against four bacterial strains (*Escherichia coli*, *Listeria-*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*), and the effect on cell viability in two melanoma cell lines (B164A5 murine melanoma and A375 human melanoma) was also assessed. The results indicated that total phenolics content was 73.76–274.73 mg GAE·g^{-1} and the antioxidant activity was 2.32–2.87 mM Fe^{2+}·100 g^{-1}. There was found a strong positive correlation (R=0.9691) between total phenolics content and the antioxidant activity in the investigated samples. Regarding the HCA profile obtained by high performance liquid chromatography, the results demonstrated that rosmarinic acid represents the main identified compound. The ethanolic extracts of RO and SO exhibited antibacterial activity against Gram positive and Gram-negative bacteria. RO was the most effective in terms of decreasing the cell viability of murine and human melanoma cell lines, while the HCAs did not exhibit any effect on cell viability. These findings suggested that plant extracts from the *Lamiaceae* family may be used in the clinic as natural antibacterial agents.

In the study conducted by Dorman *et.al.,* (2003), De-odourised aqueous extracts of four commonly consumed herbs belonging to the Lamiaceae family, i.e., oregano (*Origanum vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), were investigated for their antioxidant properties. Various experimental models were used for the characterisation of the activity, including iron reduction capacity, DPPH radical dot, ABTS radical dot+ and radical dot OH radical-scavenging activities and the capacity of the extracts to inhibit copper-induced oxidation of human low-density lipoproteins (LDL) ex vivo. The extracts showed varying degrees of reductive and radical scavenging capacity, and were capable of a marked prolongation of the lag-time in the LDL oxidation assay. The hierarchy of the observed antioxidant activity of the extracts was dependent on the type of assay used. The observed antioxidant characteristics were not fully related to the total phenolic contents of the extracts in any of

the assays, but were presumably strongly dependent on rosmarinic acid, the major phenolic component present in this type of Lamiaceae extract.

Recent studies in West Africa and in Papua New Guinea by Greenwood (1989) have shown that the prevalence of malaria can vary widely between neighbouring villages and within different parts of the same village. Both genetic and environmental factors are likely to contribute to these variations. Clustering in households of genetically determined red cell abnormalities, and possibly of immune response genes, may contribute to differences in the prevalence of malaria within a village. Environmental factors probably play the major part in explaining differences between villages. The position of a village in relation to mosquito breeding sites, the design of houses and the level at which anti-mosquito measures are used will all influence the degree to which its inhabitants are exposed to infection. Attitudes to the treatment of a case of malaria may also contribute to local variations in the prevalence of malaria. Malaria parasitaemia and splenomegaly will be less frequent in a community where effective treatment is given immediately at home, or sought promptly from a primary health care worker, than in a neighbouring community where there is a much greater reliance on traditional medicines. Recognition of local variations in the prevalence of malaria is important because identification of the factors responsible for a low prevalence in one village but a high one in a neighbouring community may indicate a possible control measure. Local variations in the epidemiology of malaria were also taken into account when any kind of malaria intervention trial is planned.

According to the WHO, in 2008, there were 247 million reported cases of malaria and nearly one million deaths from the disease. Parasite resistance against first-line drugs, including artemisinin and mefloquine, is increasing. In this study the plant-derived compounds aglafolin, rocaglamid, kokusaginine, arborine, arborinine and tuberostemonine were investigated for their anti-plasmodial activity *in vitro* by Astelbauer *et.al.,* (2012). Fresh *Plasmodium falciparum* isolates were taken from patients in the area of Mae Sot, north-western Thailand in 2008 and the inhibition of schizont maturation was determined for the respective compounds. With inhibitory concentrations effecting 50%, 90% and 99% inhibition (IC_{50}, IC_{90} and IC_{99}) of 60.95 nM, 854.41 nM and 7351.49 nM, respectively, rocaglamid was the most active of the substances, closely followed by aglafoline with 53.49 nM, 864.55 nM and 8354.20 nM. The activity was

significantly below that of artemisinin, but moderately higher than that of quinine. Arborine, arborinine, tuberostemonine and kokusaginine showed only marginal activity against *P. falciparum* characterized by IC_{50} and IC_{99} values higher than 350 nM and 180 μM, respectively, and regressions with relatively shallow slopes $S > 14.38$.

Lambros & Vanderberg *et.al.*, (1979) studied the synchronous development of the erythrocytic stages of a human malaria parasite, Plasmodium falciparum, in culture was accomplished by suspending cultured parasites in 5% D-sorbitol and subsequent reintroduction into culture. Immediately after sorbitol treatment, cultures consisted mainly of single and multiple ring-form infections. At the same time, varying degrees of lysis of erythrocytes infected with the more mature stages of the parasite was evident. Approximately 95% of the parasites were in the ring stage of development at 48 and 96 hr after sorbitol treatment; likewise, a high percentage of trophozoite and schizont stages was observed at 24, 72, and 120 hr. D-Mannitol produced similar, selective, lytic effects.

The constant emergence of resistant strains of *Plasmodium falciparum* has necessitated the continuous screening of traditional plants such that novel and effective antimalarial drugs will be developed. Enenebeaku *et.al*., (2021) investigated the antiplasmodial activity of the methanol root extract of *Dictyandra arborescens* against Plasmodium and was determined in vivo and the active compounds responsible for the observed activity identified in silico. Column chromatography was used to determine the solvent fraction containing the active compounds. All fractions reduced percentage parasitaemia in the treated mice, and hexane fraction showed significant antimalarial activity. The hexane fraction gave two eluates coded EA and EB whose bioactive components were determined using Gas Chromatography-Mass Spectrometry (GC-MS). Eluate EA gave 11 compounds (propane 1,2-dichloro propane, hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9,17 octadecadienal, (Z)-, cis-13-octadecenoic acid, methyl ester, 6-octadecenoic acid, methyl ester, (Z)-, heptadecanoic acid, 16-methyl, methyl ester and bis (2-ethylhexyl) phtalate). In comparison, eluate EB gave 16 compounds (carbonic acid, prop-1-en-2-yl tetradecyl ester, 5-octadecene, (E)-, isobutyl tetradecyl carbonate, hexadecanoic acid, methyl ester, n-hexadecanoic acid butyl octadecyl ether, 10-octadecenoic acid, methyl ester, cyclopropaneoctanoic acid, 2-hexyl-2,3-divinyloxirane and carbonic acid, dodecyl 2,2,2-trichloroethyl ester). These compounds were subjected to molecular docking against Lactate dehydrogenase and Plasmepsin II enzymes from P.

berghei. Bis(2-ethylhexyl) phthalate and bis(3-methylbutan-2-yl) phthalate gave binding affinity values close to artesunate for the two protein targets. The antimalarial potential of D. *arborescens* root as a novel source of an antimalarial drug is thus validated.

Every year, malaria caused by *Plasmodium falciparum* leads to 1 million deaths. Disease condition is alarming due to acquired resistance in parasite against antimalarial drugs in circulation. It brings the necessity to design novel inhibitors against newly identified drug targets. RIO-2 kinase regulates ribosome biogenesis and represents a promising drug target. North eastern region of India is a biodiversity hub with a rich source of medicinal plants. Medicinal plants represent a source of phytochemical library to be screened to develop an inhibitor against the PfRIO-2 kinase. In current report, we selected plants with known antimalarial activity and performed in silico screening with phytochemicals against PfRIO-2 as a target. The majority of antimalarial phytochemicals docked very well into the ATP binding pocket of the PfRIO-2 kinase. A competition assay with substrate ATP indicates that a total of 5 phytochemicals, rutin, bebeerines, isochondrodendrine, nimbin and punicalagin, share similar interactions with protein residues within the ATP binding pocket and have potential to inhibit ATP binding. A significant relationship was found between docking energy and experimentally determined antimalarial values of rutin, bebeerines, isochondrodendrine, nimbin and punicalagin ($R^2 = 0.91$, $p < 0.001$). Docking and virtual screening has identified lead phytochemicals, namely rutin, bebeerines, isochondrodendrine, nimbin and punicalagin, as a potent PfRIO-2 inhibitor, but cannot replace experimental verification (Prasad & Trivedi *et.al.*, 2012).

Vladimir *et.al.,* (2014) conducted a study to evaluate acetylcholinesterase (AChE) inhibitory and antioxidant activities of Lamiaceae medicinal plants growing wild in Croatia. Using Ellman's colorimetric assay all tested ethanolic extracts and their hydroxycinnamic acid constituents demonstrated *in vitro* AChE inhibitory properties in a dose dependent manner. The extracts of *Mentha x piperita, M. longifolia, Salvia officinalis, Satureja montana, Teucrium arduini, T. chamaedrys, T. montanum, T. polium* and *Thymus vulgaris* at 1 mg/mL showed strong inhibitory activity against AChE. The antioxidant potential of the investigated Lamiaceae species was assessed by $DPPH^{\bullet}$ scavenging activity and total antioxidant capacity assays, in comparison with hydroxycinnamic acids and trolox. The extracts differed greatly in their total

hydroxycinnamic derivatives content, determined spectrophotometrically. Rosmarinic acid was found to be the predominant constituent in most of the investigated medicinal plants (by RP-HPLC) and had a substantial influence on their AChE inhibitory and antioxidant properties, with the exception of *Teucrium* species. These findings indicate that Lamiaceae species are a rich source of various natural AChE inhibitors and antioxidants that could be useful in the prevention and treatment of Alzheimer's and other related diseases.

In a study conducted by Rai *et.al.*, (2013), the members of Lamiaceae family including aromatic plants that are being used in traditional medicine for various disorders were investigated. The therapeutic application of these plants is attributed to the presence of secondary metabolites or phytochemicals such as alkaloids, saponins, flavonoids, glycosides and phenols. The study was a preliminary screening of phytochemicals of *Leucas linifolia*, *Coleus aromaticus*, *Pogestemon patchouli*, which belong to Lamiaceae family. Methanol, ethanol and chloroform extracts of each plant were subjected to both qualitative and quantitative phytochemical screening. The alkaloids, saponins, total phenols, tannins, flavonoids, steroids and proteins were quantified in the extracts by standard spectrophotometric methods. The results of the qualitative analysis of the study plants, revealed the presence of steroids and absence of terpenoids, amino acids in all the leaf extracts. In addition, methanol extracts were positive for alkaloids, saponins, tannins and phenols, flavonoids, carbohydrates and glycosides. Whereas, the ethanol extracts showed fewer constituents in different combination in all the three study plants. However, chloroform is a good solvent for extraction of steroids from these plants since only steroids were present in chloroform extract. The concentration of total phenols and tannins was higher in methanol extract. *Pogestemon patchouli* had the highest concentration of flavonoids more so in ethanol than either methanol or chloroform extracts. Although, protein concentrations were higher in the ethanol extracts of *Leucas linifolia* and *Coleus aromaticus*, methanol extract of Pogestemon patchouli had more protein than ethanol extract. However, chloroform which extracted steroids, is not a good choice for extraction of proteins from these plants. In conclusion, for further studies on the phytochemical and biological structure-function relationship, the use of appropriate solvent for extraction and purification of the specific phytochemical from plants is one of the crucial steps.

4. MATERIALS AND METHODS

4.1. Ligand Screening

For the initial Ligand screening purposes, a web-based tool named SwissADME (https ://www.swiss adme.ch/) was used to eliminate a few compounds according to Lipinski's rule of five parameters. For a compound to qualify as ligand it should Have <500 Da molecular weight, a high lipophilicity i.e., value of Log P being less than 5, hydrogen bond acceptors being less than 10 and H-bond donors less than 5. Any compound with more than 2 violations was ruled out for further study (Lipinski, 2004).

4.2. Protein Preparation and Active site Determination

Required protein in PDB format was downloaded from the website rcsb.org, commonly known as the Protein Data Bank. 3D conformers of the ligand were downloaded from PubChem.

Using PyMOL (Version 2.4.1) software water molecules as well as native ligands from the protein were removed, defined as cleaning/purification of the protein for further application. Using a web server called Deep Site Active Pockets of the proteins were calculated. The results calculated by the web server were in the form of different ids, centers and scores.

Scoring In deep site was using neural networking based on following instructions using DCNN architecture. Center values for the grid were selected keeping score greater than 0.98.

UCSF Chimera (Version 1.14) was used to prepare the receptor using the DockPrep function. Dock Prep prepared structures for Docking using these functions:

- deleting water molecules
- repairing truncated sidechains
- adding hydrogens
- assigning partial charges
- writing files in Mol2 format

4.2.1. In silico Docking Using Auto dock Vina

Auto dock Vina (Version 1.1.2) along with UCSF Chimera (Version 1.14) was used for molecular Docking Studies. Centre values and size of the grid of different scores were used from DEEPSITE calculations done above.

Following Parameters were set in auto dock vina.

4.2.2. Receptor options –

- Add hydrogens in Chimera (true/false) – whether to add hydrogens in Chimera before calling the script. The receptor prep script will check for hydrogens and add them if they are missing. AutoDock Vina needs the polar (potentially H-bonding) hydrogens to identify atom types for scoring purposes.

- Merge charges and remove non-polar hydrogens (true/false) – note AutoDock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect results except for the presence or absence of nonpolar hydrogens in the processed receptor

- Merge charges and remove lone pairs (true/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the processed receptor (and there may not have been any lone pairs to start with)

- Ignore waters (true/false)

- Ignore chains of non-standard residues (true/false) – ignore chains composed entirely of residues other than the 20 standard amino acids.

- Ignore all non-standard residues (true/false) – ignore all residues other than the 20 standard amino acids.

4.2.3. For Ligands

- Merge charges and remove non-polar hydrogens (true/false) – note Auto Dock Vina does not use charges or nonpolar hydrogens, so this setting is not expected to affect

results except for the presence or absence of nonpolar hydrogens in the ligand output files

- Merge charges and remove lone pairs (true/false) – note AutoDock Vina does not use charges or lone pairs, so this setting is not expected to affect results except for the presence or absence of lone pairs in the ligand output files (and there may not have been any lone pairs to start with)

4.3. Docking parameters

- Number of binding modes (1-10, 10) – maximum number of binding modes to generate
- Exhaustiveness of search (1-8, 8) – thoroughness of search, roughly proportional to time
- Maximum energy difference (kcal/mol) (1-3,3) – maximum score range; binding modes with scores not within this range of the best score will be discarded.

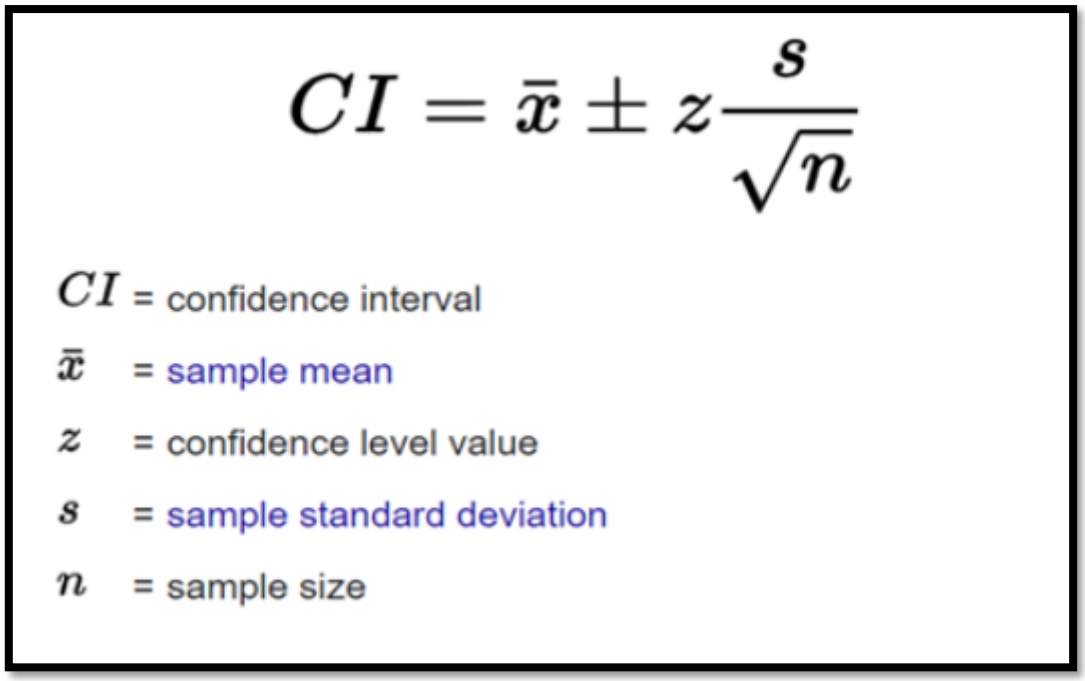
The docking results were calculated by Auto dock vina using its Scoring function and results were displayed in the form of Scores and RMSD values. Docking results with the highest value score accompanied by negative sign and least RMSD values were chosen for further studies.

4.4. Residue Analysis

PyMOL was used for visualization of interactions of the docked structure at the ligand sites. Discovery Studio 2020 was used to study the ligand interactions and total number of residues. It was also used to plot the 2D structure of the interactions and residues.

4.5. Statistical Analysis

Descriptive, estimation and Hypothesis testing with confidence interval of 95% was applied to data using formula 1 given below.



Formula 1: used for calculation of confidence interval

5. RESULTS

5.1 Ligand Screening

The following ligands were selected:

- Alpha Cardinol
- Beta Caryophyllene
- Camphene
- Carvacrol
- Eugenol
- Linalool
- Nepetalactone
- Rosmarinic Acid
- Thymol
- Ursolic Acid

The ligands were screened using SwissADME. The various chemical properties of the ligands are displayed in Table 5.1.

5.2. Protein Preparation and Active site Determination

The structure of the receptor protein -6YCZ was obtained from PDB (Protein Data Bank). The structure of the protein is shown in Fig. 5.1. The structure of the ligands is shown in Table 5.2.

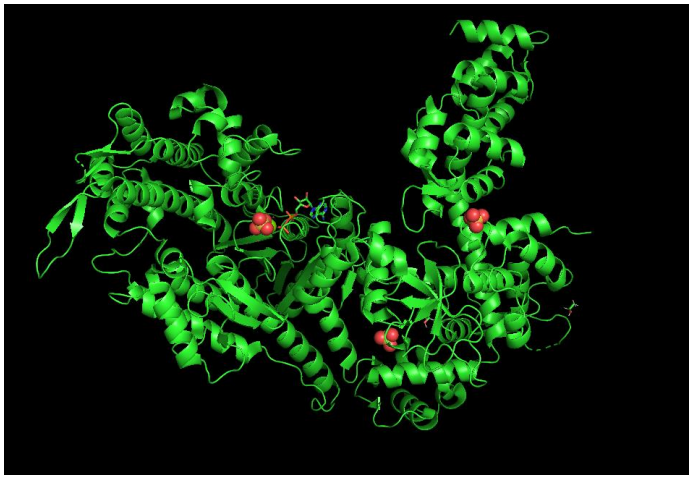


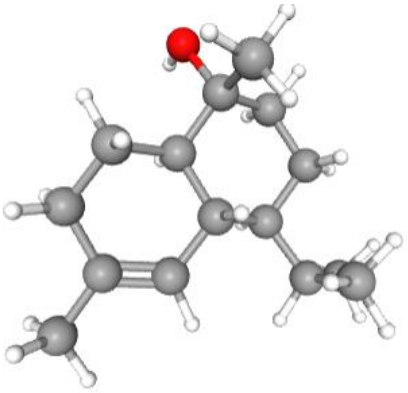
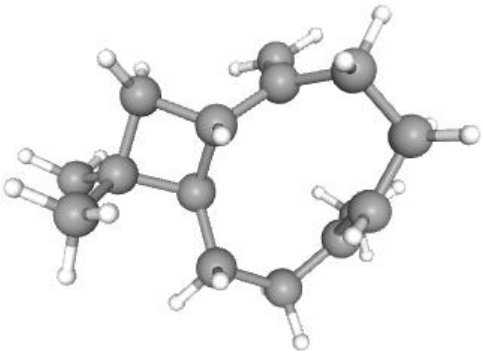
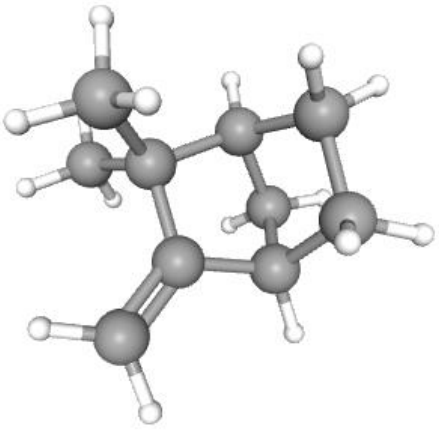
Fig. 5.1: Structure of 6YCZ receptor protein.

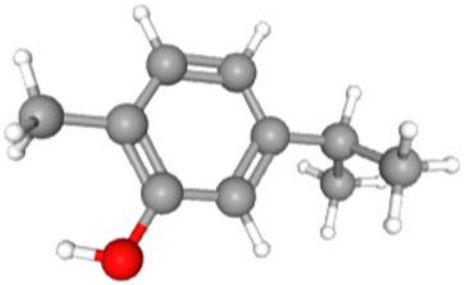
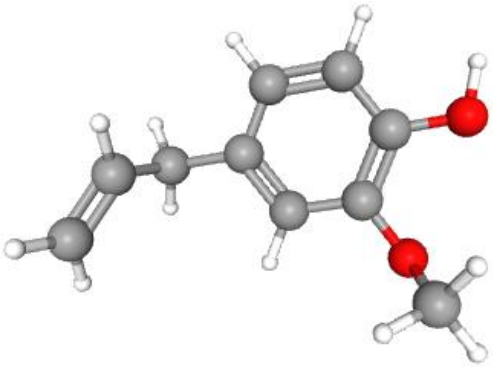
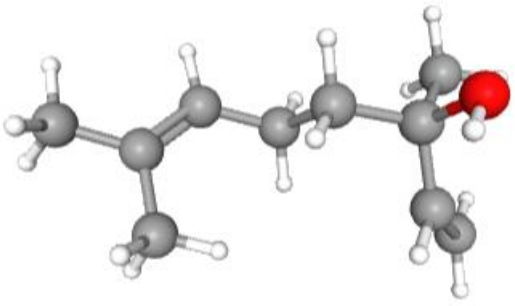
PHYTOCHEMICAL	PHYSIOCHEMICAL PROPERTIES			LIPOPHILICITY	Water Solubility		Pharmacokinetics		Drug likeness		Medicinal Chemistry	
	FORMULA	MOLECULAR WEIGHT	TPSA	Consensus Log P(o/w)			GI absorption	Log Kp (skin permeation)	Lipinski	Bioavailability Score	Leadlikeness	Synthetic accessibility
ALPHA CARDINOL	C15H26O	222.37 g/mol	20.23 Å²	3.43	Log S (ESOL)	-3.26	High	5.29 cm/s	Yes; 0 violation	0.55	No; 1 violation: MW<250	4.29
					Solubility	1.23e-01 mg/ml; 5.54e-04 mol/l						
					Class	Soluble						
BETA CARYOPHYLLENE	C15H24	204.35 g/mol	0.00 Å²	4.24	Log S (ESOL)	-3.87	Low	4.44 cm/s	Yes; 1 violation: MLOGP>4.15	0.55	No; 2 violations: MW<250, XLOGP3>3.5	4.51
					Solubility	2.78e-02 mg/ml; 1.36e-04 mol/l						
					Class	Soluble						
CAMPHENE	C10H16	136.23 g/mol	0.00 Å²	3.43	Log S (ESOL)	-3.34	Low	-4.3 cm/s	Yes; 1 violation: MLOGP>4.15	0.55	No; 2 violations: MW<250, XLOGP3>3.5	3.5
					Solubility	6.18e-02 mg/ml; 4.54e-04 mol/l						
					Class	Soluble						

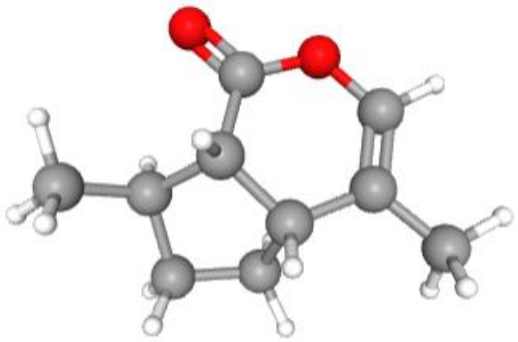
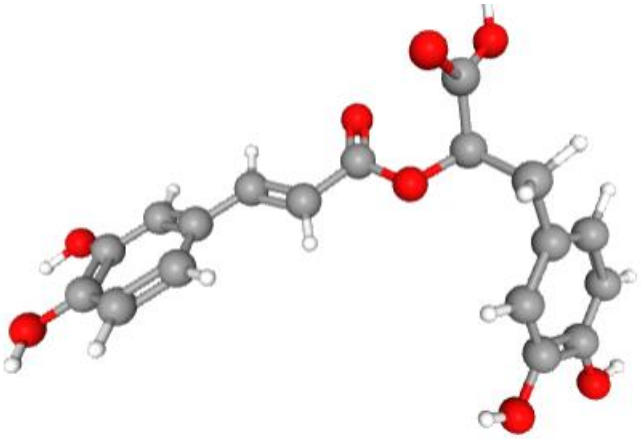
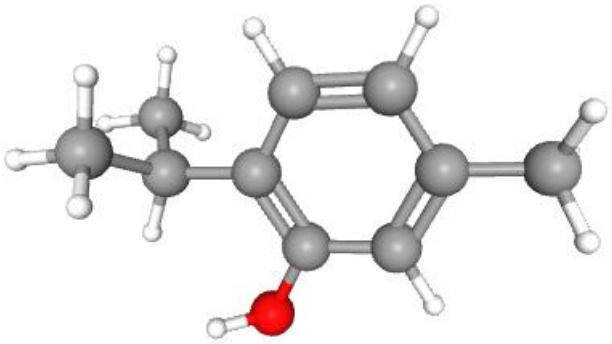
CARVACROL	C10H14O	15O.22 g/mol	20.23 Å²	2.82	Log S (ESOL)	-3.31	High	-4.74 cm/s	Yes; 0 violation	0.55	No; 2 violations: MW<200, Heteroatoms<2	1
					Solubility	7.40e-02 mg/ml; 4.92e-04 mol/l						
					Class	Soluble						
EUGENOL	C10H12O2	164.20 g/mol	29.46 Å²	2.25	Log S (ESOL)	-2.46	High	-5.69 cm/s	Yes; 0 violation	0.55	No; 1 violation: MW<250	1.58
					Solubility	5.69e-01 mg/ml; 3.47e-03 mol/l						
					Class	Soluble						
LINALOOL	C10H18O	154.25 g/mol	20.23 Å²	2.66	Log S (ESOL)	-2.4	High	-5.13 cm/s	Yes; 0 violation	0.55	Yes; 0 violation	2.74
					Solubility	6.09e-01 mg/ml; 3.95e-03 mol/l						
					Class	Soluble						
NEPETALACTONE	C10H14O2	166.22 g/mol	26.30 Å²	2.06	Log S (ESOL)	-2.07	High	-5.96 cm/s	Yes; 0 violation	0.55	No; 1 violation: MW<250	3.98
					Solubility	1.40e+00 mg/ml; 8.44e-03 mol/l						
					Class	Soluble						
ROSMARINIC ACID	C18H16O8	360.31 g/mol		1.58	Log S (ESOL)	-3.44	Low	-6.82 cm/s		0.56	No; 1 violation	3.38

			144.52 Å²		Solubility	1.31e-01 mg/ml; 3.63e-04 mol/l			Yes; 0 violation		: MW>350	
					Class	Soluble						
THYMOL	C10H14O	150.22 g/mol	20.23 Å²	2.8	Log S (ESOL)	-3.19	High	-4.87 cm/s	Yes; 0 violation	0.55	No; 1 violation : MW<250	1
					Solubility	9.74e-02 mg/ml; 6.49e-04 mol/l						
					Class	Soluble						
URSOLIC ACID	C30H48O3	456.7 g/mol	144.52 Å²	1.05	Log S (ESOL)	-2.46	High	-4.74 cm/s	Yes; 0 violation	0.56	No; 1 violation : MW>350	3.59
					Solubility	5.69e-01 mg/ml; 3.47e-03 mol/l						
					Class	Soluble						

Table 5.1: Chemical Properties of Ligands.

LIGAND	STRUCTURE
Alpha Cardinol	
Beta Caryophyllene	
Camphene	

Carvacrol	
Eugenol	
Linalool	

Nepetalactone	
Rosmarinic Acid	
Thymol	

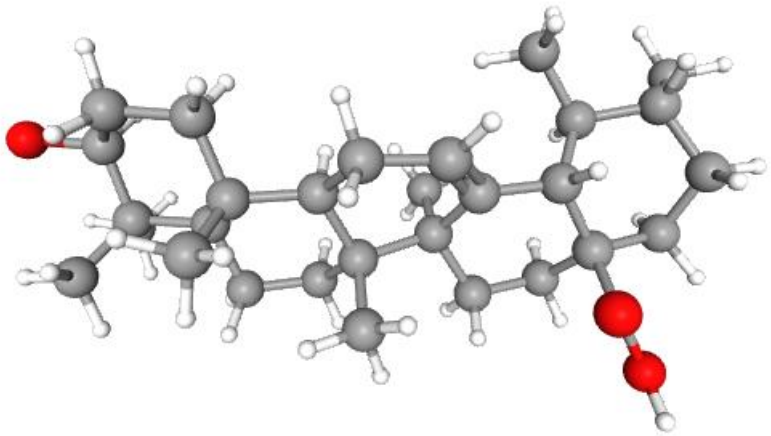
Ursolic Acid	

Table 5.2: Structure of Ligands obtained from PubChem

5.2 Docking Scores

For 6YCZ, two active sites were selected out of which the 2th active site was selected with a Deep site score of 0.991. The selection was made on the basis of the highest binding energy of the ligand-receptor.

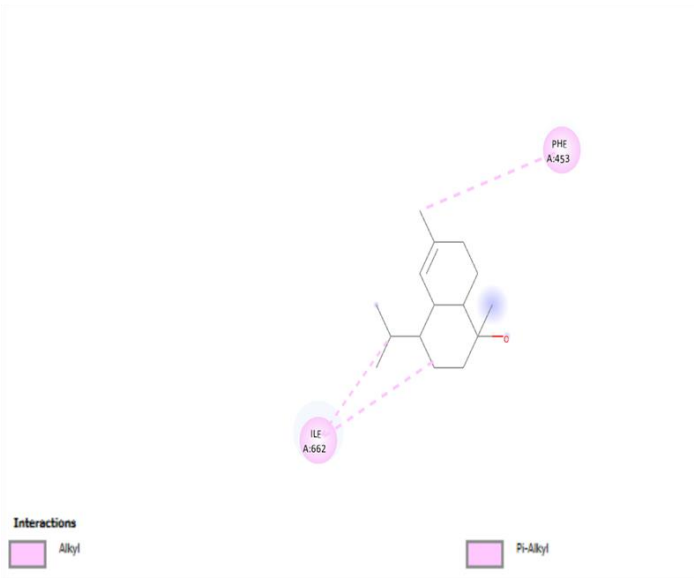
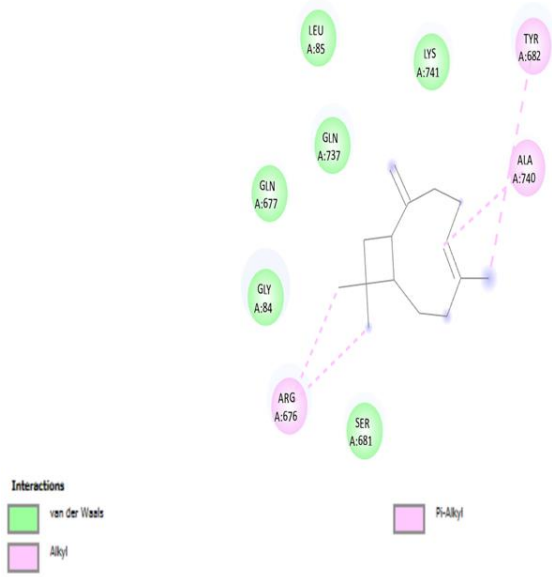
Docking scores for the above 10 ligands with the receptor protein 6YCZ was obtained by using the software AutoDock vina. The docking scores obtained are shown in Table 5.3 with respect to the ligands.

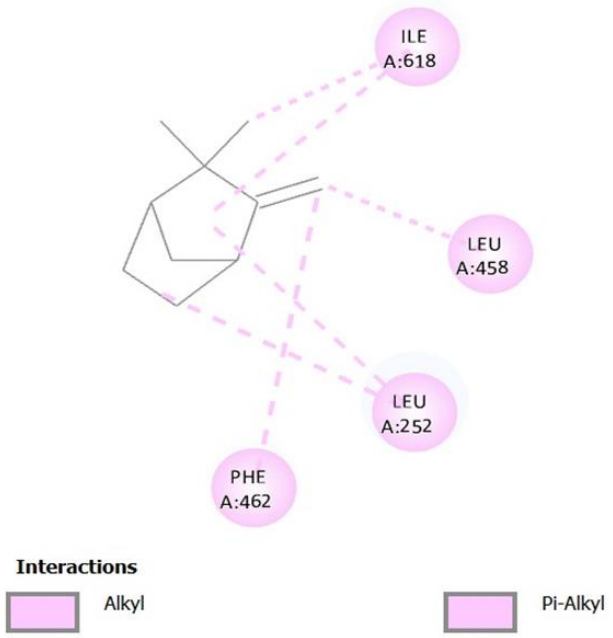
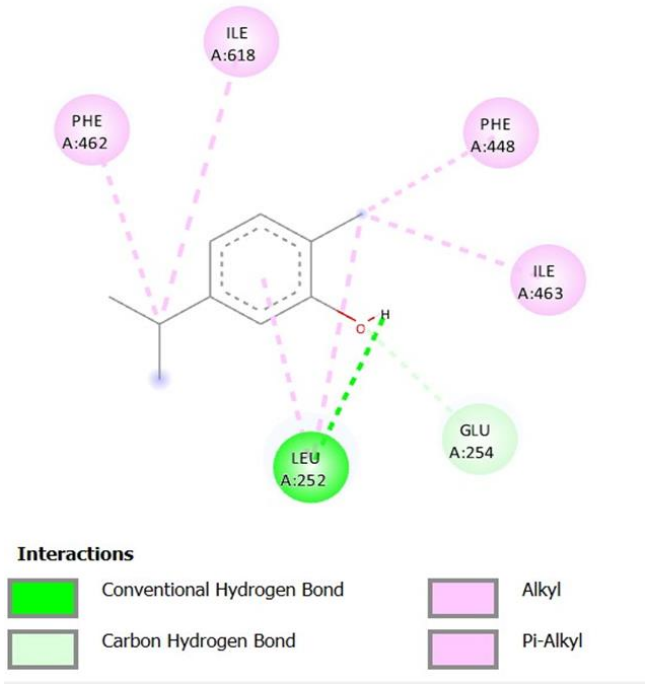
LIGANDS	DOCK SCORE
alpha cardinol	-6.7
Beta caryophyllene	-6.8
Camphene	-6
Carvacrol	-6.7
Euegenol	-6.4
Linalool	-5.6
Nepetalactone	-6.7
Rosmarinic acid	-8.1
Thymol	-5.6
Ursolic acid	-9.7

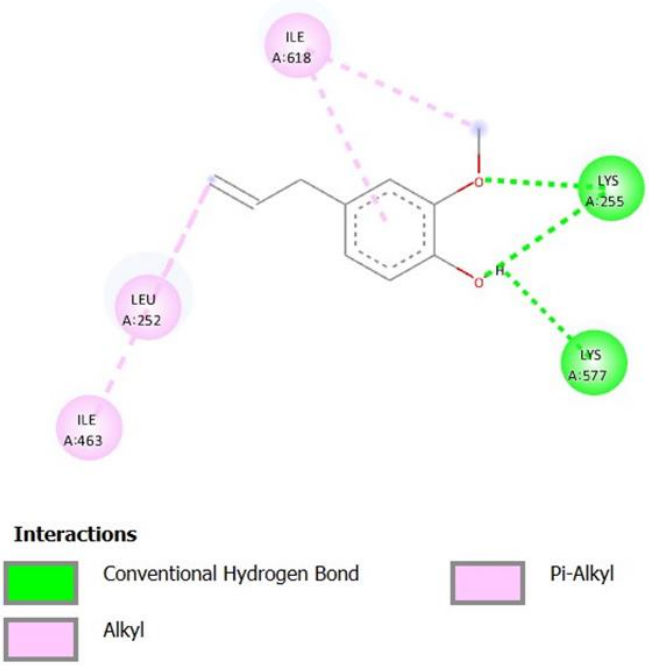
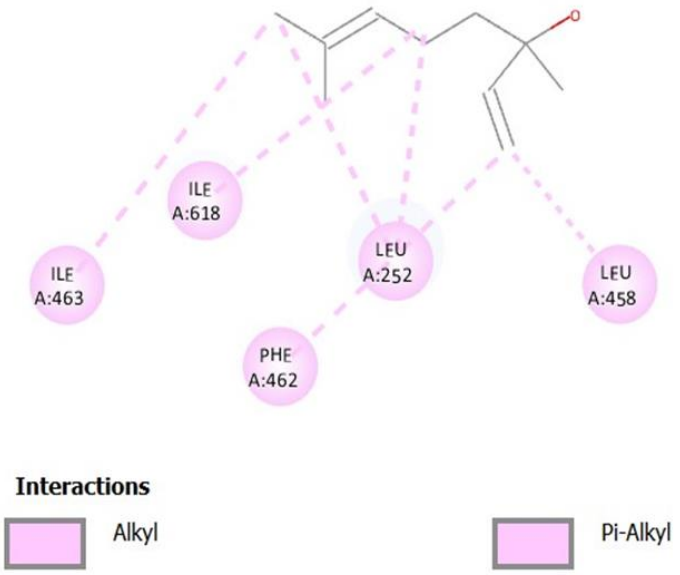
Table 5.3: Docking Scores of Ligands.

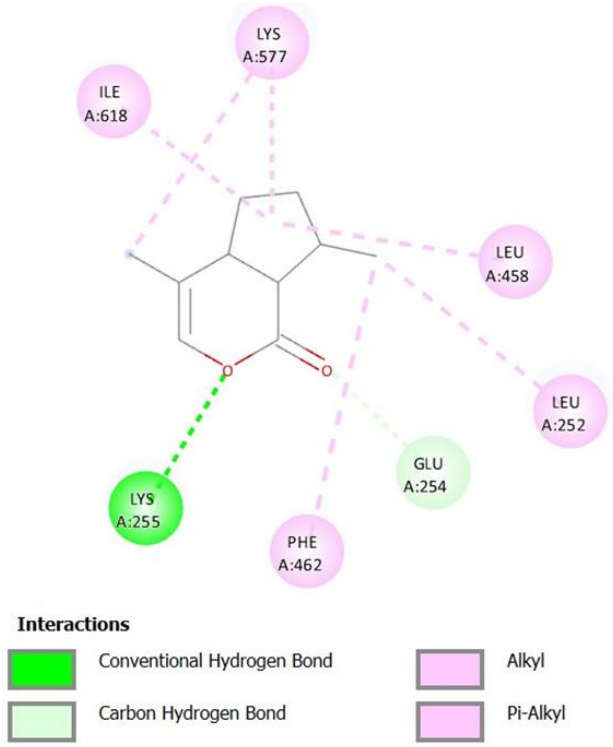
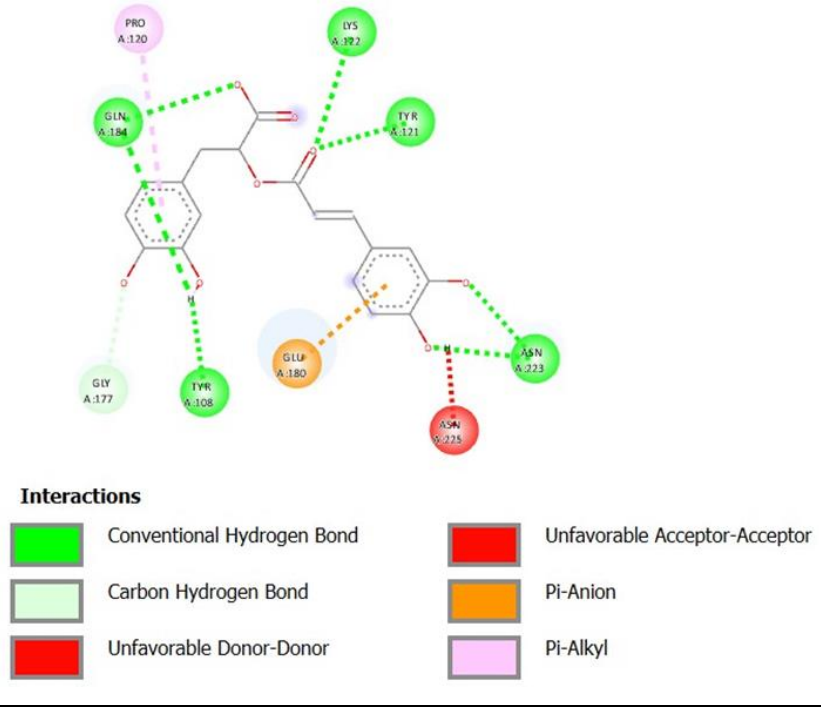
5.3 Residue and Statistical Analysis

The residue and statistical analysis were performed using Discovery Studio 2020. The results produced are shown in Table 5.4.

LIGAND	DOCK SCORE	INTERACTION
Alpha Cardinol	-6.7	
Beta Caryophyllene	-6.8	

Camphene	-6.0	
Carvacrol	-6.7	

Eugenol	-6.4	
Linalool	-5.6	

Nepetalactone	-6.7	
Rosmarinic Acid	-8.1	

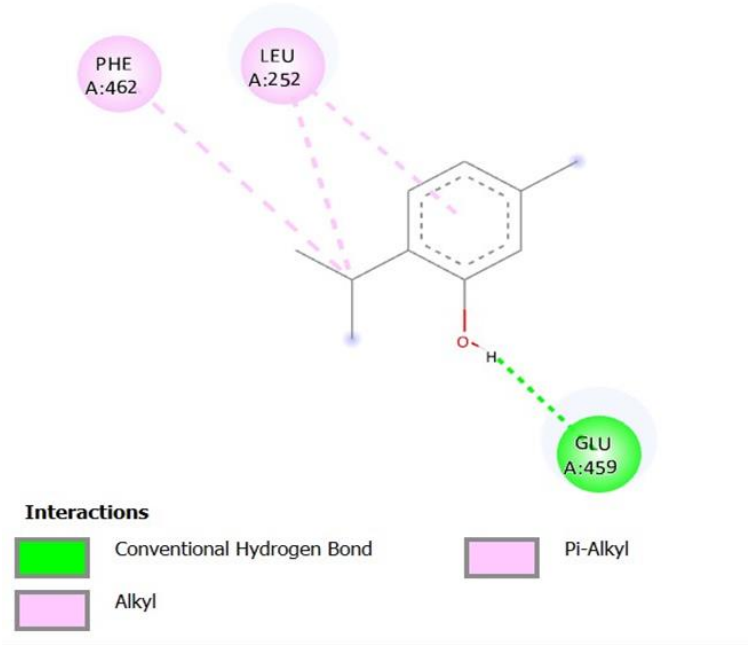
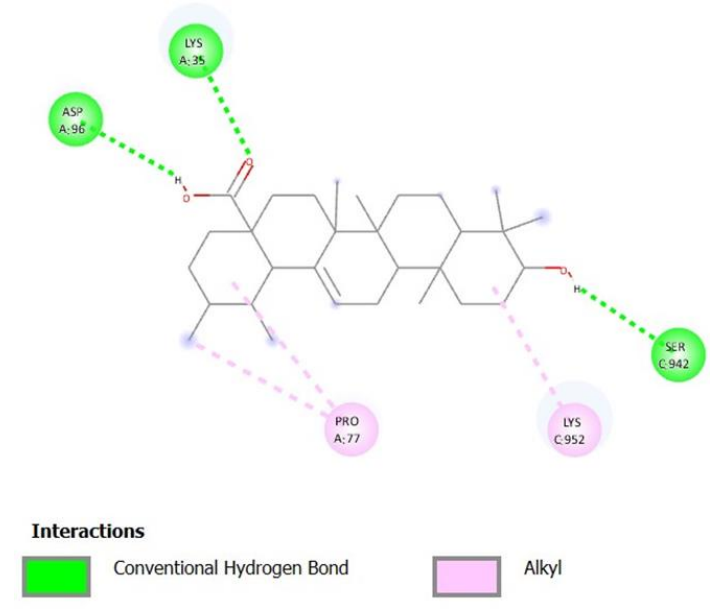
Thymol	-5.6	
Ursolic Acid	-9.7	

Table 5.4: Results of Residue and Statistical Analysis

6. DISCUSSION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. Malaria is caused by a parasite known as Plasmodium, which is normally spread through infected mosquitoes. *Plasmodium falciparum* is the main causative organism of Malaria in humans. In the study conducted, the receptor protein of P. *falciparum* was identified and the receptor degradation was studied, using phytochemicals of Lamiaceae family as ligands.

The major phytochemicals in the representatives of Lamiaceae family were selected and plants common in India such as *Ocimum basilicum, Leucas aspera* etc were taken into consideration. The Lamiaceae (Labiatae) is one of the most diverse and widespread plant families in terms of ethnomedicine and its medicinal value is based on the volatile oil concentration (Venkateshappa *et.al.,* (2013).

The interaction of the ligand with the protein is studied through the software AutoDock vina, which produces a docking score with respect to the ligand and the receptor protein. The scores are statistically analysed in Discovery Studio 2020. The residue analysis is also performed. In computational repurposing of ligands, the ligand structures are obtained from PubChem. The receptor protein structure is obtained from PDB.

Drug repurposing is performed either experimentally or computationally. The latter approach is also called 'in silico drug repurposing', which belongs to the area of computational pharmacology. In silico drug repurposing is classified into discovering new indications for an existing drug (drug-centric) and identifying effective drugs for a disease (disease-centric) and has the common strategy of similarity assessment between drugs and or diseases (Park *et.al.,* (2019).

The results of the present study showed that from the ten phytochemicals selected Ursolic acid and rosmarinic acid showed the best results. Ursolic (UA), oleanolic (OA) and rosmarinic (RA) acids are bioactive metabolites found in *Lepechinia caulescens* that have generated interest for their health benefits, which include antimicrobial, antioxidant, antimutagenic, gastroprotective, antidiabetic, antihypertensive and anti-inflammatory properties, among others (Vergara *et.al.,* (2021).

The ligands which produced the highest docking scores can be selected to produce a potential drug to cure Malaria. The ligands which showed the highest scores include - Ursolic Acid, Rosmarinic Acid, Alpha Cardinol, Beta Caryophyllene and Carvacrol which showed best docking results with the protein 6YCZ.

The main strategies to control malaria globally are vector control with long-lasting insecticide-treated bed nets, early diagnosis and treatment with artemisinin-based combination therapies (ACTs), and chemoprevention in pregnant women and young children. After decades of research, a malaria vaccine (RTS, S/AS01) received a positive opinion from the European Medicines Agency under Article 58 in 2015; however, it is only moderately efficacious and has an uncertain future. Its introduction is unlikely to lessen the demand for antimalarial drugs (Ashley & Phyo *et.al.*, (2018).

7. SUMMARY & CONCLUSION

Malaria is one of the most common diseases found in developing countries. Malaria is caused by the infection of *Plasmodium falciparum*. The infection spreads at rapid rates during monsoon season as the Mosquitoes of the genus Anopheles transmit malaria parasites.

The study aimed to identify ligands of Lamiaceae family which could be used as a potential cure for malaria. The receptor protein of *Plasmodium falciparum* was identified as 6YCZ and its structure was obtained from PDB. The ligands selected from the Lamiaceae family are - Alpha Cardinol, Beta Caryophyllene, Camphene, Carvacrol, Eugenol, Linalool, Nepetalactone, Rosmarinic Acid, Thymol and Ursolic Acid.

All ten ligands were studied using bioavailability radar. The results of the present study proposed that Ursolic acid, Rosmarinic acid, Alpha Cardinol, Beta Caryophyllene and Carvacrol showed best docking results with 6YCZ. Moreover, Ursolic acid and Rosmarinic acid showed the best docking results with the protein 6YCZ. To find the effectiveness and to propose the exact mechanism, *in-vitro* studies can be encouraged on these ligands by targeting proteins of organisms responsible for malaria that are discussed above to understand the mechanism and a potential cure for Malaria.

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