EVALUATION OF BIOACTIVE COMPOUNDS AND INSILICO ANALYSIS FROM POLY HERBAL FORMULATION FOR PROPHYLACTIC OF SARS Co-V-2

Submitted in partial fulfilment of the requirements for professional training under the Bachelor of Technology Degree in Biotechnology

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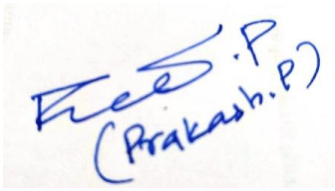
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Jyothiikaa M D (37230032), Gnana Madhurima Kollipara (37230019)**, who carried out the project entitled **"Evaluation of bioactive compounds and Insilco analysis from poly herbal formulation for prophylactic of SARS Co-V-2"** under our supervision from September 2020 to March 2021.



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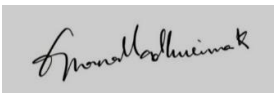
I, **Jyothiikaa M D**, **Gnana Madhurima Kollipara,** hereby declare that the Project Report entitled **"Evaluation of bioactive compounds and insilico analysis from poly herbal formulation for prophylactic of SARS Co-V-2"** done by me under the guidance of **Dr. Valli Nachiyar,** and **Dr. P Prakash**, a Centre for Drug Discovery and Development, Col. Dr. Jeppiaar Research is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

DATE: 15.04.2021 **1)**



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ABSTRACT

India is currently fighting against this deadly contagion in all possible means. Though many antivirals are available in mainstream treatment for respiratory viral infections, so far, no target specific antiviral has been identified and vaccine trials are on the anvil. In the present scenario, Hydroxychloroquine is considered a choice of drug for COVID 19. COVID 19 has created a crisis that requires immediate global attention. Traditional systems of medicine in particular the Siddha system of medicine which have its origin in southern parts of India have scopes to bridge the gaps in treatment methods and the time lag between vaccine developments. There may be chances of the emergence of mutation of the virus, drug resistance etc. So far there is a scarcity of research evidence for Siddha medicines which has been the reason for its non-acceptance by the scientific community. So, an insilico approach is taken to assess the potential use of polyherbal volatile compounds (Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa L, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo, Allium sativum) as prophylactic for COVID 19. The formulation is indicated for cough due to aggravation of Phlegm, fever and sore throat. As a result, the possibility of repurposing the medication for COVID 19 would generate substantiate testing data, and the drug will be used to treat COVID 19 in its early stages, stopping the virus from entering the throat. An In-silico approach is taken to predict the toxicity, pharmacokinetics and the binding to the Spike protein, of volatile constituents of this herbal formulation in order to determine their potential to act as drug candidates for SARS-Co-V-2.

Keywords: Herbal formulation, SARS-CoV2, in-silico, prophylactic, polyherbal volatile compounds.

CONTENTS

No.

LIST OF ABBREVIATIONS

SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2

COVID 19 - Coronavirus disease

GC-MS - Gas chromatography–mass spectrometry

ACE 2 - Angiotensin-converting enzyme 2

RBD - Receptor-binding domain

RNA - Ribonucleic acid

mRNAs - Messenger RNA

IBS - Irritable bowel syndrome

US - United States

vol - Volume

WHO - World Health Organization

FDA - Food and Drug Administration

et al. - And others

mAbs - Monoclonal antibodies

ESV - Epitope-specific vaccines

PDB ID - Protein data bank identification code

ADME - Absorption Distribution Metabolism, and Excretion

3CLpro - 3-chymotrypsin-like cysteine protease

SMILES - Simplified molecular-input line-entry system

TPSA - Topological polar surface area

3D - Three-dimensional

sdf - Spatial Data File

pdb - Protein Data Bank

2D - Two-dimensional

log P - Partition coefficient

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CHAPTER - 1 INTRODUCTION

1.1 AN OVERVIEW

In 2019, there was an outbreak of a lethal variation of coronavirus that is now known as Coronavirus disease 2019 (COVID-19) or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The outbreak led to it becoming a pandemic by March 2020. Its symptoms were rather variable and it ranges from no symptoms to a life-threatening condition. The severity of the situation calls for global attention and there is an urgent need for a promising medicine that can stop the spread of the disease in the body of the individual.

Hydroxychloroquine was used as a possible drug to treat the disease but still, there was no specific drug used to treat the disease as there are several mutated variants of it emerging. Traditional systems of medicine, particularly the Siddha system of medicine, which has its origin in southern parts of India have scopes to bridge the gaps in treatment methods and the time lag between vaccine distributions. So far there is a scarcity of research evidence for Siddha medicines which has been the reason for not its non-acceptance by the scientific community.

So, an in-silico approach is taken to assess the potential use of polyherbal volatile compounds (Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo, Allium sativum) as prophylactic for COVID 19. Each of these plants is known to have some medicinal properties individually against respiratory infections so they have been considered for this particular study. To test the potential of the mixture against the coronavirus, we use in-silico methods like toxicity analysis and molecular docking. To perform the in-silico studies, we need a list of compounds to work from so a list of nearly 100 compounds at least should be taken from literature. To get the compounds from the plant mixture, extraction is done with equal quantities of all the plant leaves and then the sample is to be sent for GC-MS analysis to get a list of volatile compounds.

1.2 SARS CoV-2 TRANSMISSION AND PATHOGENESIS

Covid 19 is an extremely contagious and pathogenic viral infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that caused a

global pandemic that resulted in a catastrophic loss of human life. SARS-CoV-2 is phylogenetically similar to SARS-like (SARS-like) bat viruses, according to genomic research (Sheeran et. al., 2020).

Due to the higher reproductive number of SARS-CoV-2 compared to SARS-CoV-1, it spreads more quickly. Some characteristics such as structural alterations in the surface proteins enable its easy invasion into the cells and also a better affinity with the ACE 2 (Cevik M. et. al., 2020)

1.2.1 Transmission

SARS-CoV-2, like other coronaviruses, is transmitted mainly by infectious respiratory droplets, with viral transmission arising by direct or indirect contact with nasal, conjunctival, or oral mucosa. The epithelium of the human respiratory tract, including the oropharynx and upper airway, contains the majority of target host receptors. Infection may also spread via the conjunctiva and gastrointestinal tract, which may act as transmission hubs (Hui KPY et. al., 2020).

As previously stated, transmission risk is determined by factors such as communication pattern, climate, infectiousness of the host, and socioeconomic factors. Near range communication accounts for the majority of transmission, and spread is particularly successful within households via gatherings among family and friends. In comparison to outdoor conditions, the risk of infection is significantly higher in enclosed areas. During extended stays in cramped, poorly ventilated indoor environments, aerosol transmission can also be a concern (Cevik M. et. al., 2020).

1.2.2 Pathogenesis

SARS-CoV-2 is an enveloped β-coronavirus that shares an 80 percent genetic code with SARS-CoV-1 and 96.2 percent with the bat coronavirus RaTG13. The viral envelope of the SARS-CoV-2 includes the spike (S) protein, the envelope (E) protein and the membrane (M) protein. The S protein acts as the means of binding and entry of the virus. The receptor binding domain is present in the S1 subunit of the S protein and it binds to the angiotensin-converting enzyme 2 (ACE 2); to the

peptidase domain specifically (Cevik M. et. al., 2020). The S protein's conformational alteration helps viral envelope fusion with the cell membrane through the endosomal pathway. The essential lysine 31 residue on the human ACE2 receptor recognises the 394-glutamine residue in the RBD region of SARS-CoV-2. Following that, SARS-CoV-2 injects RNA into the host cell. Viral replicase polyproteins pp1a and 1ab that are encoded into genome RNA get translated, which is then cleaved into small products by viral proteinases. By discontinuous transcription, the polymerase creates a sequence of sub genomic mRNAs and finally, they are converted into viral proteins that are useful (Shereen et. al., 2020).

1.3 TRADITIONAL KNOWLEDGE OF THE THERAPEUTIC PROPERTIES OF THE HERBS

Coleus amboinicus is a big succulent aromatic perennial herb that grows to a height of 30–90 cm and has thick fleshy leaves and stems. The leaves of this branched, fleshy, strongly aromatic pubescent herb have a unique smell. It is a medicinal herb with antibacterial and antimicrobial properties. Coleus is commercially grown for the production of herbal medicines used to treat illnesses such as cough, glaucoma, cancer, skin infections, cardiac problems, and eczema (Nisar et. al., 2020).

Citrus limon is a well-known cough remedy. Citrus limonoids are tetranortriterpenoids compounds found primarily in citrus fruits such as oranges, lemons, grapefruits, and others. Citrus limonoids have been shown to have anti-bacteria, antiviral, anti-tumour, antioxidative, anti-inflammatory, and other bioactivities. Antiviral activities were shown by citrus limonoids against various viruses such as HIV-1, hepatitis B virus, and HTLV-1 though it was at the high micromolar level potency (Shi Y-S. et. al., 2020).

Antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive, and cytotoxic properties have been observed for Leucas aspera. Flowers and leaves are inhaled through the nose in order to treat migraines. In addition, two drops of flower juice can be used as a nasal drop. The leaves' juice is used as a topical treatment for psoriasis, chronic skin eruptions, and chronic rheumatism (Vimalanathan et. al., 2009).

Curcuma longa is a therapeutic plant that is widely used in Ayurvedic, Unani, and Siddha medicine as a home remedy for a various disease such as anorexia, hepatic disorders, biliary disorders, cough, sinusitis. As its widely used traditionally, and its relatively mild side effects, the main phenolic compound of Curcuma longa L. , curcumin has been used various antimicrobial studies (Moghadamtousi et. al., 2014).

The common cold, indigestion, flatulence, menstrual pains, diarrhoea, nausea, muscle and nerve pain, depression-related anxiety, and IBS are some of the conditions that Mentha piperita is used to treat. Peppermint contains antiviral substances that are similar to those found in lemon balm, and a variety of other mint plants also contain antiviral compounds (Herrmann et. al., 1967).

Sweet basil, or Ocimum basilicum, has long been used as a classical medicine plant to treat headaches, coughs, worms, diarrhoea, constipation, warts, and kidney problems. The various components of Ocimum basilicum are used to treat a variety of ailments, including viral ocular, respiratory, and hepatic infections. The essential oils of Ocimum basilicum have been shown to have antibacterial, antifungal, and antiparasitic properties (Chiang L-C. et. al., 2005).

Ocimum gratissimum is an emetic and a treatment for haemorrhoids. Rheumatism, coma, epilepsy, high fever, diarrhoea, sunstroke, influenza, gonorrhoea, and mental illness are all treated with the plant. The leaves were used as a general tonic and anti-diarrheal treatment in traditional medicine, as well as for treating conjunctivitis by instilling straight in the eye; the leaf oil, when combined with alcohol, is applied as a lotion for skin infections, and used internally for bronchitis (Ayisi N.K. et. al., 2003).

Vitex negundo extracts have been used as an anti-arthritic, anthelmintic, anti-fungal, anti-inflammatory, expectorant, tranquiliser, antispasmodic, and antipyretic in the Unani system of medicine. It is best known for its ability to influence cellular processes such as apoptosis, cell cycle, sperm motility, polycystic ovary cancer, and the menstrual cycle (Nisar S. et. al., 2020).

Allium sativum acts as a functional food as it’s uses go beyond nutritional value. It displays anti-inflammatory, anti-mutagenic, anti-microbial and anti-tumour

properties. Its antiviral effectiveness was also confirmed. Any of the plant's constituents have been shown to be effective against protozoan parasites. In this context, it tends to reverse the majority of immune system dysfunctions seen in COVID-19 infection patients (Donma M.M. et. al., 2020). Garlic is a good medicinal plant because of its antitumoral and antioxidant properties, as well as its ability to prevent cardiovascular diseases by controlling blood pressure, decreasing blood sugar and cholesterol levels, and being effective against bacterial, viral, fungal, and parasite infections.

Thus, these plants are taken as a mixture and are tested to see if their volatile compounds have any potential of stopping the spread of coronavirus in the body at the early stage or even as a preventive measure.

Fig: 1.1: Some of the plants used in the research, a) Curcuma longa, b) Citrus limon, c) Ocimum gratissimum , d) Vitex negundo

CHAPTER - 2 LITERATURE SURVEY

Pasdaran A. et. al., (2016) mentions that, herbal plants provide a huge range of secondary metabolites that have the potential to become new drugs. Respiratory infections are often challenging to treat with the current treatment options available opening up an opportunity to explore the therapeutic potential of volatile natural compounds from essential oils. This review focuses on traditional remedies involving natural volatile compounds that aid with respiratory infections with regard to their antibacterial, antiviral and anti-inflammation activities. It's important to give more attention to the chemical constituents of these essential oils to help interpret the results in a better way. A relation was established between the plant family and the therapeutic for better understanding. Volatile compounds belonging to the Asteraceae, Pinaceae, Cupressaceae, Scrophulariaceae, Rutaceae, Myrtaceae, Fabaceae, and Lamiaceae show promise for development of therapeutic compounds against respiratory infection.

Shyr Z.A. et. al., (2020) states that, for the treatment of COVID-19, researchers are looking for an appropriate vaccine as well as successful therapeutics. Drug repurposing screens are a useful and efficient way of detecting possible SARS-CoV-2 therapeutics. Drug repurposing screens are a useful and efficient way of detecting possible SARS-CoV-2 therapeutics. For example, the US Food and Drug Administration has licenced the experimental drug remdesivir, which was initially designed to cure Ebola virus infections, as an emergency treatment for COVID-19. However, this drug's effectiveness and toxicity may be improved further. In this study, we address recent studies on coronavirus pathology as well as medication targets for COVID-19 therapy. Inhibitors that are specific to SARS-CoV-2 as well as anti-coronavirus drugs against SARS-Co-V that have a broad-spectrum, d SARS-CoV-2, and Middle East respiratory syndrome coronavirus would be beneficial inclusions to this arsenal. For treating the extreme COVID-19, a multitarget treatment technique using synergistic drug formulations with various modes of action may be a viable therapeutic option.

Abd El-Aziz et. al., (2020) states that, the novel coronavirus epidemic was declared a global pandemic by the World Health Organization (WHO) in March 2020. Despite extensive global control and quarantine measures, COVID-19 continues to spread, with over 1,948,617 laboratory-confirmed cases and 121,846 deaths reported worldwide. Governments and pharmaceutical firms are battling to develop a coronavirus-fighting vaccine quickly. We summarise the current state of understanding about COVID-19, available drugs, and treatment alternatives in this article. Favilavir is an antiviral drug that has been approved in Japan for the treatment of general influenza and is now approved in China for the treatment of COVID-19 symptoms. The US Food and Drug Administration (FDA) is currently investigating chloroquine and hydroxychloroquine as COVID-19 treatments. By the end of the year, the first COVID-19 vaccine is expected to be available for clinical trials. Drug development for SARS-CoV-2 is a difficult but necessary task. Furthermore, the advancement of anti-virus vaccines is critical in combating this outbreak. As a result, more structural biology information as well as information on the 2019-nCoV life cycle are needed. These findings are expected to hasten the progress of anti-SARS-CoV-2 medicines and/or vaccines.

Kulkarni R. et al. (2008) states that, ayurveda recommends Vitex negundo for the treatment of arthritic conditions. The aim of this study was to determine the plant's antioxidant ability and anti-inflammatory function. In terms of total polyphenols, the plant's total methanol extract was standardised. When tested for anti-inflammatory activity using the carrageenan-induced rat paw edoema procedure, the standardised extract in a dosage of 100 mg/kg triggered a similar reduction in edoema as diclofenac sodium (25 mg/kg). The findings clearly conclude that radical quenching is one of the pathways behind its anti-inflammatory properties. V. negundo's ethnomedical use as a treatment for inflammatory and arthritic conditions may be due to its potent anti-inflammatory and antioxidant properties. The anti-inflammatory and antioxidant functions of V. negundo were shown to be highly correlated in the current study. One of the pathways responsible for the plant's anti-inflammatory effect may thus be the avoidance of oxidative harm to tissue. The anti-

inflammatory function of this plant has been confirmed in animal models, which adds to the plant's common application for inflammatory disorders.

Donma M.M. et. al., (2020) states that, the seriousness of coronavirus disease 2019 (COVID19) infection ranges widely, with symptoms ranging from asymptomatic to serious acute respiratory infection. The most common general symptoms include, dry cough, shortness of breath, muscle pain, fever, nausea, reduced sense of smell and taste . The signature characteristics reduction in number of immune system cells such as T helper cells, suppressed T regulatory cells, natural killer cells, with an increase in proinflammatory cytokines. Compounds obtained from Allium sativum (garlic) have the ability to lower proinflammatory cytokine expression and reverse immunological defects to more appropriate amounts. Until being contaminated with the SARS-CoV-2 virus, Allium sativum is recommended as a helpful prevention measure. To summarise, Allium sativum can be an effective COVID-19 infection prevention strategy by boosting immune system cells and suppressing the synthesis and secretion of cytokines that are proinflammatory, as well as the proinflammatory hormone leptin extracted from adipose tissue.

Hussai et. Al., (2020) mentions that, the advancement of alternative therapeutic platforms is critical as the cases of the recently discovered SARS-CoV2 virus continues to rise every day. Since the receptor-binding domain (RBD) of SARS-CoV-2 and SARS-CoV are so close, it seems critical to test anti-SARS-CoV monoclonal antibodies (mAbs) for cross-reactivity with SARS-CoV2 spike (S)-protein. Indeed, designing mAbs that target the SARS-CoV2 S-protein RBD could open up new avenues for the fast and responsive production of epitope-specific vaccines (ESV). In this paper, we provide an outline of recent CoV discovery, as well as an explanation of the SARS-CoV2 S-protein RBD site. Following that, the mechanism of RBD's association with various mAbs was demonstrated, and it was proposed that one of the SARS-CoV-specific human mAbs, CR3022, may have the highest binding affinity with SARS-CoV2 S-protein RBD. Lastly, some current issues and potential opportunities for therapeutic mAbs targeting S-protein RBD were

addressed. Finally, it is possible that this study will lay the foundation for the identification of RBD and other mAbs in order to improve future therapeutic ESV.

Oyuntsetseg et. al., (2014) mentoins that in herbal medicine, the herbal formulation of Deva-5 is applied for treating acute infectious diseases. Hypecoum erectum L., Momordica cochinchinensis L., Gentiana decumbens L., Polygonum bistorta L., and Terminalia chebula Retz are the five herbs that make up Deva-5. In vitro antiviral function of its five constituents against avian influenza A virus subtype H3N8 was investigated. At concentrations up to 2%, extracts of G. decumbens, H. erectum, P. bistorta, and Deva-5 showed no substantial toxicity, while extracts of T. chebula and M. cochinchinensis were well received by Madin-Darby canine kidney cells at concentrations up to 1%. Plaque reduction neutralisation testing showed that none of the extracts examined were able to prevent plaque formation by 90%. At low dilutions of 1:3 to 1:14, three extracts, H. erectum, T. chebula, and M. cochinchinensis, were able to prevent plaque formation by more than 50%. The inhibitory activity of the T. chebula extract was concentration dependent. The extracts of H. erectum, T. chebula, and M. cochinchinensis were shown to have a consistent direct antiviral effect for the first time. When used at elevated concentrations (0.5–1%), these extracts greatly decreased the infectious activity of the H3N8 influenza A virus in vitro. Deva-5 and the rest of its elements, on the other hand, did not display any antiviral activity.

Lan et. al., (2020) states that, siddha Medication is a valuable treatment option that has traditionally been used to cure viral respiratory infections. This medical concept has been shown to include antiviral compounds. The aim of the research is to conduct in-silico studies of phytochemical constituents in the official Siddha formulation Kabasura Kudineer and a novel herbal formulation known as JACOM, these are widely used in the treatment of repiratory tract infections and viral diseases and may be effective against the novel strain, SARS-CoV-2. For molecular docking experiments against the spike protein SARS-CoV-2, Cresset Flare programme was used (PDB ID: 6VSB). Using the free pkCSM and SwissADME web servers, and in-silico studies were carried out to predict the pharmacokinetic

properties (ADME) and the safety profile in order to find the best drug candidates. A total of 37 compounds were tested, and 9 of them had a high binding affinity for the SARS-CoV-2 spike protein. None of these phytochemical compounds displayed carcinogenicity or tumorigenicity. A new formulation “SNACK–V” was suggested based on these findings.

Kallingal et. al., (2020) stated that, the latest emergence of the COVID-19 virus has sparked a worldwide epidemic due to the lack of any vaccine or treatment that can efficiently and deterministically combat it, making scientists looked into various possibilities (including herbal remedies of proven medicinal value). The molecular components (proteins) of COVID-19 have already been identified, allowing for a rigorous clinical review of herbal medicines in particular and any medication in general. Mpro or 3CLpro, the main protease of the COVID-19 virus, is a central CoV enzyme and a promising drug target because it is involved in viral replication and transcription. In this analysis, 3CLpro is used to analyse drug:3CLpro interactions and, as a result, whether any or any of Tinospora cordifolia's major chemical constituents (e.g., berberine, -sitosterol, coline, tetrahydropalmatine, and octacosanol) can be employed as an antiviral drug for SARS-CoV-2.The in-silico research, which used network pharmacology, molecular docking, and molecular dynamics, revealed that berberine, out of all the phytochemicals studied in Tinospora cordifolia, can regulate 3CLpro protein function and thus control viral replication due to its ease of inhibition. Tinospora cordifolia was chosen because its key constituents are considered to have antiviral properties and to be useful in the treatment of jaundice, rheumatism, diabetes, and other ailments.

CHAPTER 3 - AIM AND SCOPE OF THE PRESENT INVESTIGATION

3.1 AIM

To evaluate the bioactive compounds from a herbal formulation as prophylactic of SARS Co-V-2 using an Insilco approach.

3.2 OBJECTIVES

1. To extract various bioactive volatile compounds from the herbal formulation of 9 herbs; Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo, and Allium sativum.
2. To evaluate secondary metabolites by GC MS analysis
3. To conduct Insilico analysis for toxicity, ADME, molecular docking studies

3.3 SCOPE OF THE PROJECT

To look at the possibility of developing a herbal formulation of volatile compounds as a prophylactic of SARS Co-V-2 using various bioinformatic tools. An Insilico approach is taken so as to determine the toxicity, drug likeness and the efficacy of the compounds extracted from the herbal formulation with that of the SARS Co-V-2 Spike protein. This study could act as the preliminary foundation for further studies for development of an effective drug against SARS Co-V-2.

CHAPTER 4 - EXPERIMENTS, MATERIALS AND METHODS

4.1 SELECTION AND PREPARATION OF VIRAL TARGET PROTEIN

The spike protein plays a major part in the infection cycle of SARS CoV2 as it is the means of entry into the cell, so this was chosen as the target for the insilico studies. The structure of the S protein (PDB ID: 6VXX) was gathered from the protein data bank. The protein was prepared by removal of the prebound ligands (2-acetamido-2-deoxy-beta-D-glucopyranose) so as to interfere with the docking of the desired ligands. Visualization was done using BIOVIA Discovery Studio Visualizer.

4.2 SELECTION AND PREPARATION OF LIGANDS

Initially the compounds were obtained from the present literature data. Around ten compounds were selected from each plant based on their occurrence and activity. The compounds were selected from extractions using various types of solvents such as methanol, water, ethanol etc.

Water extraction of the herbal formulation using all the nine plant samples was also carried out using Soxhlet apparatus to isolate volatile compounds and then analysed through GC-MS. The compounds obtained from the GC MS results were screened for toxicity and ADME. The screened compounds were then considered for further docking studies.

4.2.1 Extraction of Volatile compounds and GC-MS Analysis

The plant samples (Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo, Allium sativum) were collected and cured. The leaf part was taken for Coleus amboinicus, Citrus limon, Leucas aspera, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo. The bulbs were chosen for Allium sativum

and the root part was chosen for Curcuma longa. The samples were first thoroughly washed with tap water followed by distilled water and the water was allowed to dry out. The samples were taken in equal weight i.e., 5g of each plant sample was weighed and a mixture of all the 9 samples (total weight 45g) was taken for extraction. The sample mixture was ground using a mortar and pestle until a paste like consistency is obtained. The ground sample was then mixed with 100ml of distilled water and transferred into the Soxhlet apparatus for volatile compound extraction. The volatile compounds were extracted using the Soxhlet apparatus at 60° C (Liang C. et. al., 2020). The extracted compounds were then sent for GC-MS analysis. The list of volatile compounds present in the herbal formulation were obtained from GCMS analysis results.

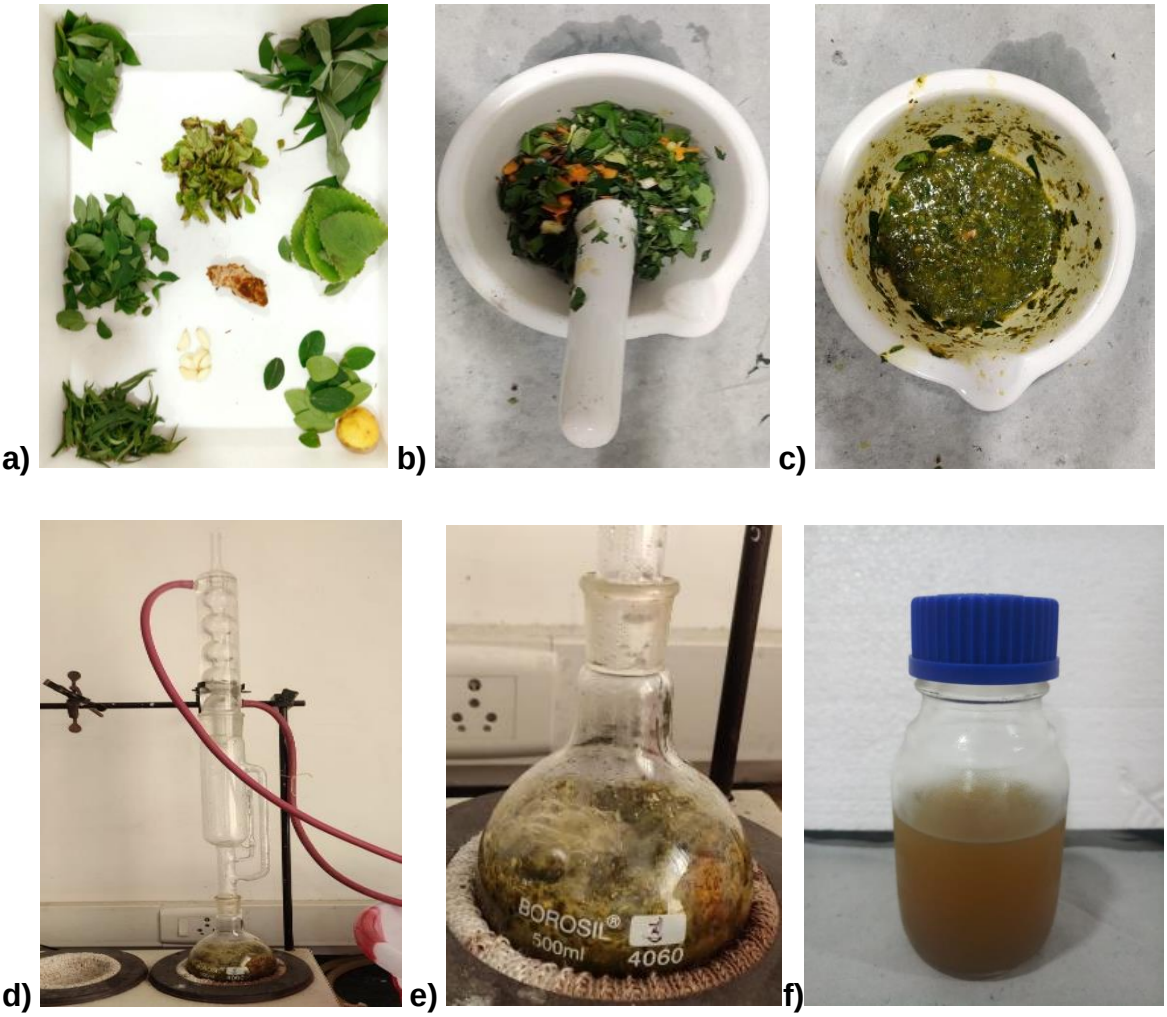


Fig: 4.1: Extraction of plant sample as a mixture, a) Curated samples from each plant taken, b) plant samples as a mixture before grinding in the mortar and pestle, c) plant samples as a mixture after grinding in the mortar and

pestle, d) & e) Soxhlet apparatus set up for extracting the volatile compounds from the plant mixture, f) collected extract

4.2.2 Toxicity screening

The structures and the SMILES notation for the list of compounds was obtained from PubChem. The compounds were then screened for toxicity based on a toxicity prediction tool, OSIRIS Property Explorer, that predicts whether a compound could be mutagenic, tumorigenic, an irritant and its reproductive effects. This toxicity data was collected and correlated with literature data and the compounds that displayed toxicity were eliminated. Those compounds that passed the toxicity screening were considered for further Insilco studies.

4.2.3 ADME screening

Those compounds that passed the toxicity screening were considered for ADME studies. Swiss ADME an online tool was used for evaluating the drug likeness, ADME parameters, and pharmacokinetics of a particular compound. The SMILES notation of the compound to be evaluated is submitted for analysis. Lipinski's rule of five for drug likeness of a compound was considered for the screening after the SwissADME results were obtained.

The following Lipinski’s rules were taken into account for screening of the compounds:

1. Molecular weight should below 500 Daltons.
2. Consensus Log P value should be below 5.
3. There should be less than 5 hydrogen bond donors.
4. There should be less than 10 hydrogen bond acceptors.
5. Molar refractivity must fall between 40-130.
6. Rotatable bonds should be less than 10.
7. TPSA should be less than 140.
8. There should be at least one hydrogen bond acceptor or donor.

Those compounds that passed the drug likeness screening were considered for further Insilco studies.

4.2.4 Docking studies

The viral target protein (spike protein) was obtained from protein data bank and modified as mentioned above and the modified protein was obtained as a pdb format file using the software open babble.

The ligands are the final compounds that are left after the above-mentioned screenings are done. The 3D conformer of each ligand molecule was downloaded in the sdf format from PubChem and it was converted to a pdb format using the open babble software.

Once all the pdb files of the protein and the ligand molecules were converted, the files were then uploaded to Patchdock for each ligand with the same protein molecule to check if there is interaction between the molecules in the desired location. The pdb file of the modified spike protein and pdb file of each of the chosen ligand was added to Patchdock individually under the default docking parameters and the docking was run. The results were obtained through email approximately 3-4hrs from the time input was given.

Discovery studio software was used to visualize the results of the docking, to see the interaction between the molecules in 3D and 2D format.

CHAPTER - 5 RESULTS AND DISCUSSION

5.1 GCMS RESULTS

The GC-MS results of volatile compounds from each herb was obtained from literary search while awaiting the GC-MS analysis of the extracted volatile compounds from the herbal formulation. The Fig 5.1 represents the GC-MS spectrum obtained from the analysis of the extracted herbal mixture. The compounds from the water extracted GC-MS compounds are listed in the Tables 5.1 to 5.6, and the compounds obtained from literary search and are listed in the Table 5.7 and Table 5.8.

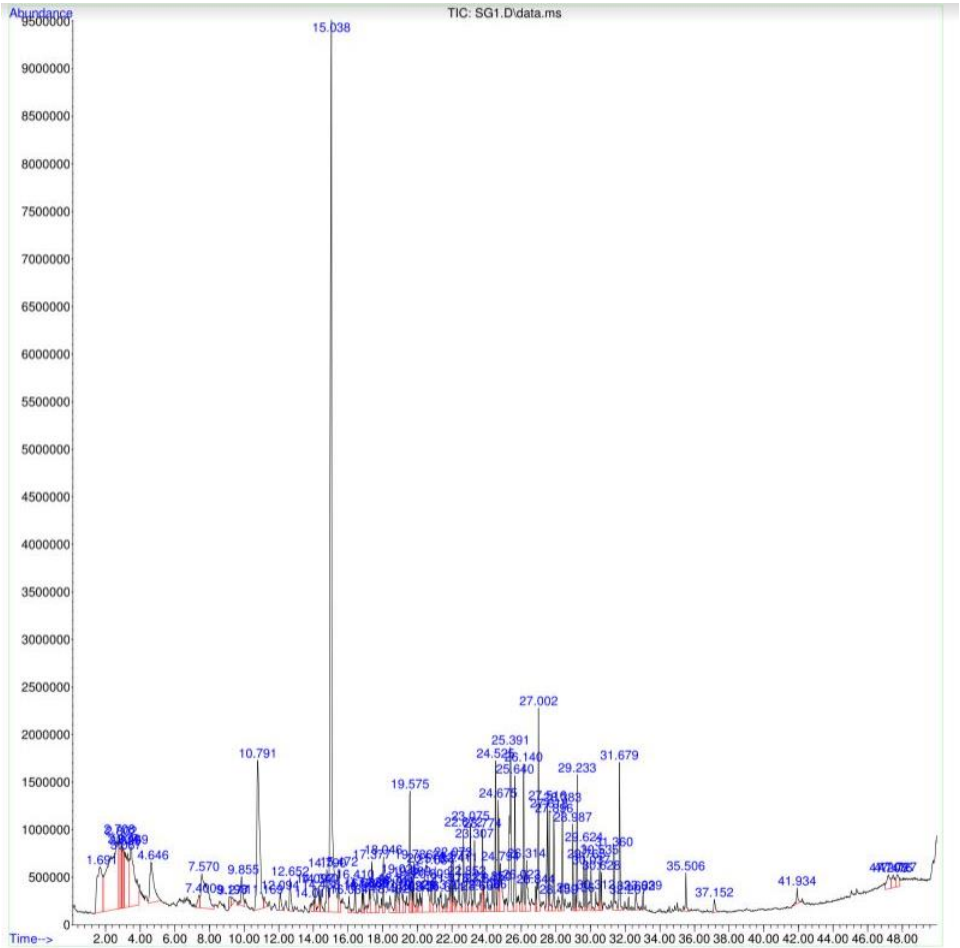


Fig:5.1: The GC-MS spectrum obtained from the analysis of the extracted volatile compounds from the herbal mixture.

Table 5.1: List of compounds (1-29) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
1	Propanoic acid, 2-chloro-, methyl ester	1.682	2.79
2	Carbonochloridic acid, 4-nitrophenyl ester	1.682	2.79
3	Bis(2-chloroethyl) sulfone	1.682	2.79
4	Ethane, 1,1-bis(ethylthio)-	2.718	8.19
5	Cyclopentanecarboxylic acid, 4,4-dimethyl-3-trimethylsilylmethylene-, methyl ester	2.718	8.19
6	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-	2.801	1.66
7	Trimethylsilyl ethaneperoxoate	2.925	0.68
8	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	3.029	1.01
9	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-	3.091	2.81
10	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5-dimethyl-	3.091	2.81
11	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-isopropyl-2,4'-dimethyl-9,11-bis(2-met	3.091	2.81
12	1,4-Eicosanediol	3.463	3.61
13	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	3.463	3.61
14	Methoxydi(tert-butyl)silane	3.463	3.61
15	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	4.644	1.95
16	3-Furaldehyde	4.644	1.95
17	Furfural	4.644	1.95
18	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	7.398	0.28
19	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	7.398	0.28
20	2-Thiophenecarboxylic acid hydrazide	7.398	0.28
21	2-Furancarboxaldehyde, 5-methyl-	7.564	2.43
22	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	9.2	0.2
23	Dibutyl 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))diacetate	9.2	0.2
24	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-	9.283	0.19
25	1,2-Phenylene bis(mesitylsulfonate)	9.283	0.19
26	o-Cymene	9.283	0.19
27	Benzeneacetaldehyde	9.863	0.7
28	2,5-Furandicarboxaldehyde	10.795	5.03
29	Orcinol	10.795	5.03

Table 5.2: List of compounds (30-61) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
30	2,4-Diaminophenol	10.795	5.03
31	4H-Pyran-4-one, 3,5-dimethyl-	11.167	0.25
32	5-Methyl-2-pyrazinylmethanol	11.167	0.25
33	6-Methyl-2-pyrazinylmethanol	11.167	0.25
34	Dimethyl dl-malate	12.099	0.32
35	3-Acetoxy-3-hydroxypropionic acid, methyl ester	12.099	0.32
36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.659	0.58
37	2,4-Difluoroanisole	12.659	0.58
38	1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide	12.659	0.58
39	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-	14.005	0.25
40	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-	14.005	0.25
41	Cyclohexanecarboxylic acid, 4-butyl-, 2,3-dicyano-4-(pentyloxy)phenyl ester	14.005	0.25
42	2-Tetradecene, (E)-	14.088	0.33
43	3-Tetradecene, (Z)-	14.088	0.33
44	2-Pentene-1,4-dione, 1-(1,2,2-trimethylcyclopentyl)	14.44	0.34
45	Cyclohexane, (1,2-dimethylpropyl)-	14.44	0.34
46	Cyclohexane, 1-ethyl-2,4-dimethyl-	14.44	0.34
47	1H-Inden-5-ol, 2,3-dihydro-	14.792	0.84
48	Benzaldehyde, 4-ethyl-	14.792	0.84
49	Benzaldehyde, 3,4-dimethyl-	14.792	0.84
50	5-Hydroxymethylfurfural	15.04	16.48
51	2-Fluorobenzyl alcohol	15.04	16.48
52	Cyclohexane, (4-methylpentyl)-	15.475	0.69
53	Cyclohexane, hexyl-	15.475	0.69
54	Undecane, 2,4-dimethyl-	16.055	0.19
55	Tetradecane, 4-ethyl-	16.055	0.19
56	Sulfurous acid, hexyl octyl ester	16.055	0.19
57	Tridecane, 1-iodo-	16.407	0.35
58	Nonadecane	16.407	0.35
59	Dodecane, 4,6-dimethyl-	16.407	0.35
60	3,4-Difluorobenzoic acid, 3-pentadecyl ester	16.821	0.22
61	3,4-Difluorobenzoic acid, 4-pentadecyl ester	16.821	0.22

Table 5.3: List of compounds (62-91) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
62	2,4-Difluorobenzoic acid, 4-pentadecyl ester	16.821	0.22
63	2-Undecanone	16.904	0.23
64	4-Heptenal	17.235	0.4
65	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-	17.235	0.4
66	Phthalic anhydride	17.38	1.26
67	1,2-Benzenedicarboxylic acid	17.38	1.26
68	Dodecane, 2,6,10-trimethyl-	17.691	0.4
69	Tetratetracontane	17.691	0.4
70	Benzenemethanol, 3-fluoro-	17.691	0.4
71	Phthalic acid, monoamide, N-ethyl-N-(3-methylphenyl)-, pentyl ester	18.043	0.61
72	Phthalic acid, propyl 2-tert-butyl-6-methylphenyl ester	18.043	0.61
73	Phthalic acid, monoamide, N-ethyl-N-(3-methylphenyl)-, octyl ester	18.043	0.61
74	Cyclooctacosane	18.437	0.34
75	Cyclohexane, 1,2,4,5-tetraethyl-	18.437	0.34
76	N-Isopropoxy-2-carbomenthyloxyaziridine	18.437	0.34
77	Methoxy(methyl)chlorosilane	18.747	0.28
78	Urea, N-(4-hydroxy-2-methylcyclohexyl)-N'-(4-hydroxyphenyl)-	18.747	0.28
79	3-Pyridinol, 6-methyl-	18.747	0.28
80	1-Docosanol, methyl ether	18.851	0.5
81	Cyclotetradecane	18.851	0.5
82	2-Cyclohexylpiperidine	19.016	0.74
83	DL-Proline, 5-oxo-, methyl ester	19.182	0.37
84	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	19.182	0.37
85	LISDEXAMFETAMINE	19.182	0.37
86	1-Nonadecene	19.576	1.2
87	1-Tetradecene	19.576	1.2
88	1H-Pyrazole-4-carbothioamide, 5-amino-	19.679	0.39
89	Benzene, 1-chloro-4-methoxy-	19.679	0.39
90	Benzene, 1-chloro-2-methoxy-	19.679	0.39
91	Dodecane	19.783	0.54

Table 5.4: List of compounds (92-122) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
92	9-Eicosene, (E)-	19.969	0.25
93	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane	19.969	0.25
94	Hexadecane	20.156	0.35
95	Tetradecane	20.156	0.35
96	Boric acid, ethyl-, didecyl ester	20.156	0.35
97	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide	20.528	2.54
98	t-Butyl 1-thio-.alpha.-D-glucopyranoside	20.528	2.54
99	Lethane	20.528	2.54
100	2(1H)-Naphthalenone, octahydro-, trans-	20.818	0.51
101	Decalin, anti-1-methyl-, cis-	20.818	0.51
102	Decalin, syn-1-methyl-, cis-	20.818	0.51
103	Cyclohexane, octyl-	21.025	0.56
104	1-Heneicosyl formate	21.647	0.22
105	2-Butenedioic acid (Z)-, monododecyl ester	21.647	0.22
106	2-Bromo dodecane	21.978	0.57
107	Pentadecane, 2,6,10-trimethyl-	21.978	0.57
108	Docosane	21.978	0.57
109	2-Acetylthiazole	22.081	0.51
110	Propanamide, N-methyl-	22.081	0.51
111	Phenol, 2,5-bis(1,1-dimethylethyl)-	22.413	0.69
112	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	22.661	0.91
113	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	22.661	0.91
114	Pyrrole, 4-ethyl-2-methyl-	22.661	0.91
115	Thiocyanic acid, 1H-indol-3-yl ester	22.848	0.49
116	Glycine, N,N-bis(trimethylsilyl)-, trimethylsilyl ester	22.848	0.49
117	Phthalimide, N-(1-hydroxy-2-propyl)-	22.848	0.49
118	Benzene, (1-butylhexyl)-	23.076	1.04
119	Benzene, (1-butyloctyl)-	23.076	1.04
120	Benzene, (1-propylheptyl)-	23.303	0.8
121	Benzene, (1-propylnonyl)-	23.303	0.8
122	Methoxyacetic acid, tetradecyl ester	23.655	0.33

Table 5.5: List of compounds (123-154) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
123	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	23.655	0.33
124	Sulfurous acid, octadecyl 2-propyl ester	23.655	0.33
125	Benzene, (1-ethyloctyl)-	23.78	0.72
126	Benzene, (1-ethylundecyl)-	23.78	0.72
127	Benzene, (1-ethyldecyl)-	23.78	0.72
128	Dodecane, 1-fluoro-	23.842	0.29
129	Triacontane, 1,30-dibromo-	23.842	0.29
130	5-Eicosene, (E)-	23.842	0.29
131	Dodecane, 1,1-dimethoxy-	24.07	0.36
132	Undecanal dimethyl acetal	24.07	0.36
133	Hexadecane, 1,1-dimethoxy-	24.07	0.36
134	Diethyl Phthalate	24.36	0.46
135	1-Octadecene	24.525	1.34
136	7-Hexadecene, (Z)-	24.525	1.34
137	E-15-Heptadecenal	24.525	1.34
138	Benzene, (1-methylnonyl)-	24.67	1.36
139	Benzene, (1-methyldecyl)-	24.67	1.36
140	Tetracosane, 1-bromo-	24.794	1.22
141	Dodecane, 2,6,11-trimethyl-	24.794	1.22
142	Pentadecane, 2,6,10,14-tetramethyl-	24.794	1.22
143	Benzene, (1-butylheptyl)-	25.395	2.98
144	Benzene, (1-propyloctyl)-	25.644	1.43
145	2-Cyclohexylnonadecane	26.016	0.4
146	Cyclohexane, decyl-	26.016	0.4
147	Heptylcyclohexane	26.016	0.4
148	Benzene, (1-ethylnonyl)-	26.141	1.46
149	8-Pentadecanone	26.306	0.92
150	Heptadecane, 9-octyl-	26.845	0.27
151	Benzene, (1-methylundecyl)-	27.01	2.05
152	Benzene, (1-pentylheptyl)-	27.507	1.13
153	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	28.149	0.24
154	Nonahexacontanoic acid	29.144	0.14

Table 5.6: List of compounds (155-188) characterized through GC-MS analysis

S.No.	Compound name	RT (min)	Area %
155	Tritetracontane	29.144	0.14
157	Benzene, (1-hexylheptyl)-	29.62	0.65
158	Benzene, (1-butylnonyl)-	29.765	0.44
159	Benzene, (1-propylheptadecyl)-	30.034	0.47
160	Benzene, (1-propyldecyl)-	30.034	0.47
161	Phthalic acid, butyl tetradecyl ester	30.303	0.31
162	Phthalic acid, isobutyl undecyl ester	30.303	0.31
163	Phthalic acid, butyl 4-methylpent-2-yl ester	30.303	0.31
164	Hexadecane, 2,6,10,14-tetramethyl-	30.635	0.35
165	2-(p-Tolyl)ethylamine	31.359	0.77
166	2-Benzoyl-1,2-dihydro-1-isoquinolinecarbonitrile	31.359	0.77
167	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-	31.359	0.77
168	Pentadecanoic acid, 14-methyl-, methyl ester	31.67	1.55
169	Hexadecanoic acid, methyl ester	31.67	1.55
170	1,2-Benzenedicarboxylic acid, decyl octyl ester	32.209	0.14
171	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	32.209	0.14
172	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	32.209	0.14
173	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	32.623	0.27
174	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	32.623	0.27
175	Z-5-Nonadecene	33.037	0.14
176	Methyl stearate	35.501	0.41
177	Heptadecanoic acid, 16-methyl-, methyl ester	35.501	0.41
178	Pimpinellin	37.158	0.23
179	7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-	37.158	0.23
180	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	41.942	0.26
181	Glycerol 1-palmitate	41.942	0.26
182	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	41.942	0.26
183	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	47.202	0.58
184	Tris(tert-butyldimethylsilyloxy)arsane	47.202	0.58
185	Cyclotrisiloxane, hexamethyl-	47.202	0.58
186	Tetrasiloxane, decamethyl-	47.472	0.46
187	4-Methyl-2-trimethylsilyloxy-acetophenone	47.472	0.46
188	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	47.472	0.46

Table 5.7: List of compounds (1-60) obtained through literature studies

S.No.	Compound name	S.No.	Compound name
1	Carvacrol	31	Ar-tumerone
2	Methyl eugenol	32	Tumerone
3	Thymol	33	Curlone
4	β-selinene	34	Terpinolene
5	Patchoulane	35	Ar-tumerol
6	14-methyl-8-hexadecyn-1-ol	36	Ar-curcumene
7	dioctyl phthalate	37	α-Humulene
8	Camphene	38	Menthol
9	myrcene	39	Menthone
10	limonene	40	Menthyl Acetate
11	citronellal	41	1,8-cineole
12	Alpha-terpineol	42	Isomenthone
13	neral	43	Menthofuran
14	Nonanal	44	Neomenthol
15	sabinene	45	β-caryophyllene
16	(E)-β-ocimene	46	Pulegone
17	4-terpineol	47	3-Octanol
18	citronellol	48	trans-Sabinene hydrate
19	Longifolene	49	Terpinen-4-ol
20	Naphthalene	50	Germacrene D
21	Isocaryophyllene	51	beta-Pinene
22	Beta-Santalol	52	Isomenthol
23	Tetracosane	53	2,3-Dihydrothiophene 1,1-dioxide
24	Heptacosane	54	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6methyl-
25	Spiro	55	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-
26	Octadecane	56	6-O-alpha-D-Galactopyranosyl-alpha-D-Glucopyranose
27	Pentadecanal	57	10, 13-Octadecadienoic acid, methyl ester
28	α-Phellandrene	58	3-Hydroxybenzoic acid
29	Eucalyptol	59	Ledol
30	Caryophyllene	60	1,4-Dimethyl-7-(1-hydroxy-1-methylethyl)azulene

Table 5.8: List of compounds (61-105) obtained through literature studies

S.No.	Compound name	S.No.	Compound name
61	2-Octadec-9-enoxyethanol	84	**γ**-cadinene
62	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol	85	α-cadinol
63	Aromadendrene oxide-(1)	86	ocimene
64	n-Hexadecanoic acid	87	α-pinene
65	Phytol	88	camphor
66	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	89	p-cymene
67	12-Bromo-13-hydroxy-2,5,9,13-tetramethyltetradeca-4,8-dienoic acid, methyl ester	90	sabinene hydrate
68	trans β-ocimene	91	germacrene-D
69	linalool	92	β-bisabolene
70	borneol	93	α-Bulnesene
71	α-terpineol	94	Sulfide, allyl methyl
72	methyl chavicol	95	1,3,5-Trithiane
73	geraniol	96	Disulfide dimethyl
74	geranial	97	Diallyl sulfide
75	bornyl acetate	98	Allyl methyl disulfide
76	eugenol	99	2-Oxazolidinethione
77	α-copaene	100	Dimethyl trisulfide
78	β-elemene	101	Diallyl disulfiide
79	trans-caryophyllene	102	Allyl methyl trisulfide
80	trans-alpha-bergamotene	103	2-vinyl-4H-1,3-dithiin
81	α-guaiene	104	Allitridin
82	epi-bicyclo-sesquiphellandrene	105	Tetrasulfide
83	**δ**-guaiene		

5.2 TOXICITY RESULTS

The compounds obtained through GC-MS analysis of extracted herbal mixture and the through literature studies were check for toxicity. The toxicity prediction was done using the software Osiris Property Explorer the results obtained were then correlated with pre-existing research and the compounds known to be toxic were eliminated. The Fig.5.2 depicts the toxicity prediction result of Thymol. From literature data, 53 compounds out of 105 compounds were found to be non-toxic. From the extracted mixture, 107 compounds out of 188 compounds were found to be non-toxic. The compounds that were found to be non-toxic from the literature data are listed in the Table 5.9 and compounds found to be non-toxic from the extracted herbal mixture are listed in Table 5.10 and Table 5.11 respectively.

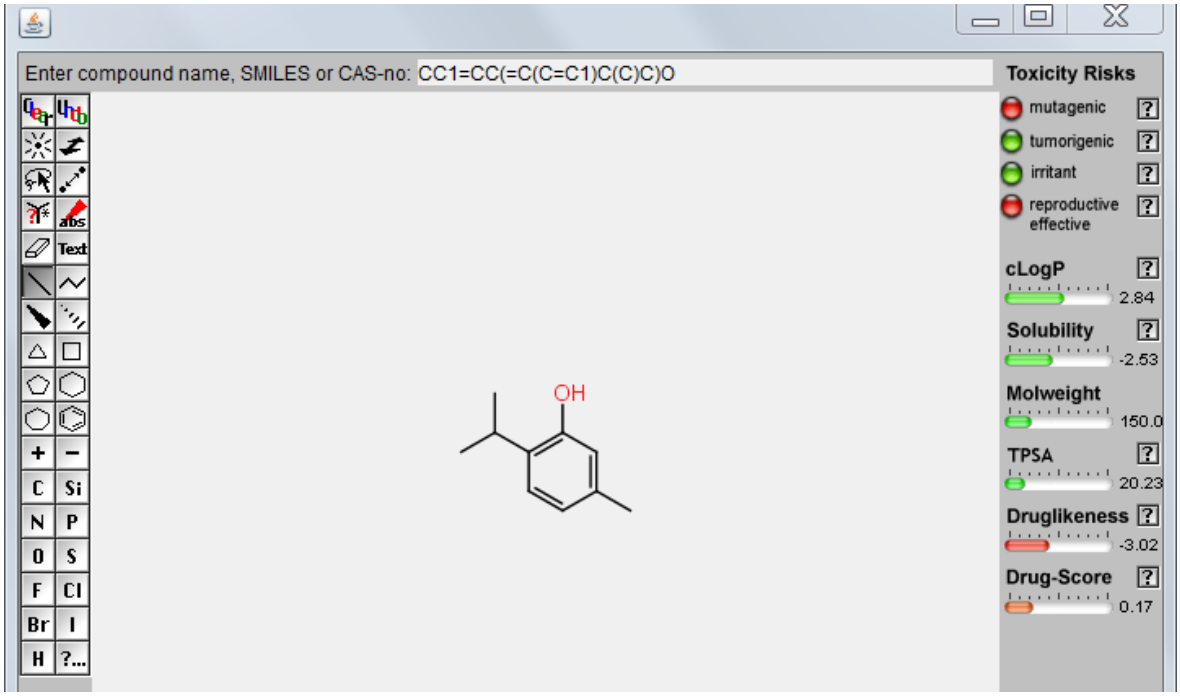


Fig: 5.2: Example of toxicity analysis result obtained using OSIRIS Property Explorer; result displays toxicity prediction of the compound thymol obtained from literature studies.

Table 5.9: List of compounds obtained from literature data that were found to be non-toxic; compounds 1-53

S.No.	Compound Name	S.No.	Compound Name
1	α-humulene	28	2-Octadec-9-enoxyethanol
2	β-selinene	29	(7α-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol
3	β-caryophyllene	30	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
4	Terpinolene	31	trans β-ocimene
5	sabinene	32	bornyl acetate
6	(E)-β-ocimene	33	β-elemene
7	geraniol	34	trans-caryophyllene
8	Alpha-terpineol	35	α-guaiene
9	Longifolene	36	epi-bicyclo-sesquiphellandrene
10	Isocaryophyllene	37	**δ**-guaiene
11	Phytol	38	**γ**-cadinene
12	Beta-Santalol	39	α-cadinol
13	Tetracosane	40	ocimene
14	Heptacosane	41	sabinene hydrate
15	Spiro	42	β-caryophyllene
16	Octadecane	43	germacrene-D
17	Caryophyllene	44	β-bisabolene
18	Ar-tumerol	45	α-Bulnesene
19	Menthofuran	46	Sulfide, allyl methyl
20	trans-Sabinene hydrate	47	Allyl methyl disulfide
21	Germacrene D	48	Dimethyl trisulfide
22	2,3-Dihydrothiophene 1,1-dioxide	49	Diallyl disulfiide
23	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-	50	Allyl methyl trisulfide
24	6-O-alpha-D-Galactopyranosyl-alpha-D-Glucopyranose	51	2-vinyl-4H-1,3-dithiin
25	10, 13-Octadecadienoic acid, methyl ester	52	Allitridin
26	Caryophyllene	53	Tetrasulfide
27	1,4-Dimethyl-7-(1-hydroxy-1-methylethyl)azulene		

Table 5.10: List of compounds (1-29) obtained from extracted herbal mixture that were found to be non-toxic.

S.No.	Compound name
1	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-
2	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-
3	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-
4	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5-dimethyl-
5	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-isopropyl-2,4'-dimethyl-9,11-bis(2-met
6	1,4-Eicosanediol
7	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-
8	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-
9	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane
10	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide
11	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one
12	Dibutyl 2,2'-(2,2'-oxybis(ethane-2,1-diyl)bis(oxy))diacetate
13	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-
14	1,2-Phenylene bis(mesitylsulfonate)
15	o-Cymene
16	4H-Pyran-4-one, 3,5-dimethyl-
17	5-Methyl-2-pyrazinylmethanol
18	6-Methyl-2-pyrazinylmethanol
19	Dimethyl dl-malate
20	3-Acetoxy-3-hydroxypropionic acid, methyl ester
21	1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide
22	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-
23	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-
24	Cyclohexanecarboxylic acid, 4-butyl-, 2,3-dicyano-4-(pentyloxy)phenyl ester
25	3-Tetradecene, (Z)-
26	Cyclohexane, (1,2-dimethylpropyl)-
27	Cyclohexane, 1-ethyl-2,4-dimethyl-
28	Benzaldehyde, 3,4-dimethyl-
29	Cyclohexane, (4-methylpentyl)-

Table 5.11: List of compounds (30-60) obtained from extracted herbal mixture that were found to be non-toxic

S.No.	Compound name
30	Undecane, 2,4-dimethyl-
31	Tetradecane, 4-ethyl-
32	Sulfurous acid, hexyl octyl ester
33	Dodecane, 4,6-dimethyl-
34	3,4-Difluorobenzoic acid, 3-pentadecyl ester
35	3,4-Difluorobenzoic acid, 4-pentadecyl ester
36	2,4-Difluorobenzoic acid, 4-pentadecyl ester
37	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-
38	Tetratetracontane
39	Cyclooctacosane
40	Cyclohexane, 1,2,4,5-tetraethyl-
41	1-Docosanol, methyl ether
42	Cyclotetradecane
43	2-Cyclohexylpiperidine
44	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-
45	1H-Pyrazole-4-carbothioamide, 5-amino-
46	Benzene, 1-chloro-4-methoxy-
47	9-Eicosene, (E)-
48	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane
49	Boric acid, ethyl-, didecyl ester
50	6,8-Dioxa-3-thiabicyclo(3,2,1)octane 3,3-dioxide
51	t-Butyl 1-thio-.alpha.-D-glucopyranoside
52	2(1H)-Naphthalenone, octahydro-, trans-
53	Decalin, anti-1-methyl-, cis-
54	Cyclohexane, octyl-
55	1-Heneicosyl formate
56	Pentadecane, 2,6,10-trimethyl-
57	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol
58	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-
59	Pyrrole, 4-ethyl-2-methyl-
60	Phthalimide, N-(1-hydroxy-2-propyl)-

Table 5.12: List of compounds (61-90) obtained from extracted herbal mixture that were found to be non-toxic

S.No.	Compound name
61	Benzene, (1-butylhexyl)-
62	Benzene, (1-butyloctyl)-
63	Benzene, (1-propylheptyl)-
64	Benzene, (1-propylnonyl)-
65	Methoxyacetic acid, tetradecyl ester
66	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-
67	Sulfurous acid, octadecyl 2-propyl ester
68	Benzene, (1-ethyloctyl)-
69	Benzene, (1-ethylundecyl)-
70	Benzene, (1-ethyldecyl)-
71	5-Eicosene, (E)-
72	Dodecane, 1,1-dimethoxy-
73	Undecanal dimethyl acetal
74	Hexadecane, 1,1-dimethoxy-
75	7-Hexadecene, (Z)-
76	Benzene, (1-methylnonyl)-
77	Benzene, (1-methyldecyl)-
78	Dodecane, 2,6,11-trimethyl-
79	Benzene, (1-butylheptyl)-
80	Benzene, (1-propyloctyl)-
81	2-Cyclohexylnonadecane
82	Cyclohexane, decyl-
83	Benzene, (1-ethylnonyl)-
84	8-Pentadecanone
85	Heptacosane
86	Heptadecane, 9-octyl-
87	Benzene, (1-methylundecyl)-
88	Benzene, (1-pentylheptyl)-
89	Nonahexacontanoic acid
90	Tritetracontane

Table 5.13: List of compounds (91-107) obtained from extracted herbal mixture that were found to be non-toxic

S.No.	Compound name
91	Benzene, (1-pentyloctyl)-
92	Benzene, (1-hexylheptyl)-
93	Benzene, (1-butylnonyl)-
94	Benzene, (1-propylheptadecyl)-
95	Benzene, (1-propyldecyl)-
96	Hexadecane, 2,6,10,14-tetramethyl-
97	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-
98	Pentadecanoic acid, 14-methyl-, methyl ester
99	2H-1-Benzopyran-2-one, 4,7-dimethoxy-
100	Z-5-Nonadecene
101	Methyl stearate
102	Heptadecanoic acid, 16-methyl-, methyl ester
103	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
104	Glycerol 1-palmitate
105	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-
106	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
107	4-Methyl-2-trimethylsilyloxy-acetophenone

5.3 SWISSADME RESULTS

The compounds that passed the toxicity screening were selected for SwissADME analysis, to determine their pharmacokinetics and their drug likeness. The filtering of the compounds from SwissADME was done based on the Lipinski's rules of drug likeness. For example; The Fig.5.3 represents a compound that satisfies Lipinski's rule such as the consensus log P value, the molecular weight, number of rotatable bonds etc. Whereas Fig. 5.4 violated 2 of Lipinski's rules; the consensus log P value is way above 5 and the compound has both hydrogen bond donors and acceptors as 0. From the literature data, 11 compounds out of the 53 that were found to be non-toxic, showed druglike pharmacokinetic properties. From the extracted herbal mixture, 27 out of the 107 that were found to be non-toxic, showed druglike pharmacokinetic properties. Table 5.12 and 5.13 represent compounds that passed the ADME screening from literature data, and the extracted herbal mixture respectively.

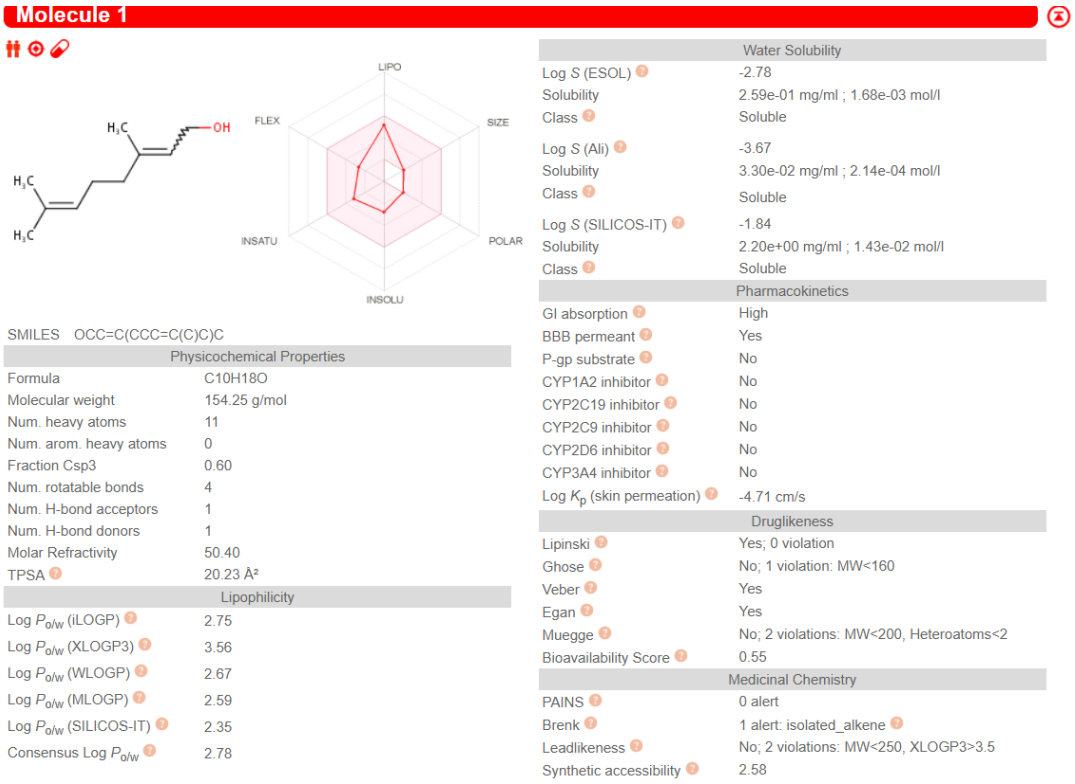


Fig: 5.3: SwissADME results for a compound whose parameters satisfy all the Lipinski's rules.

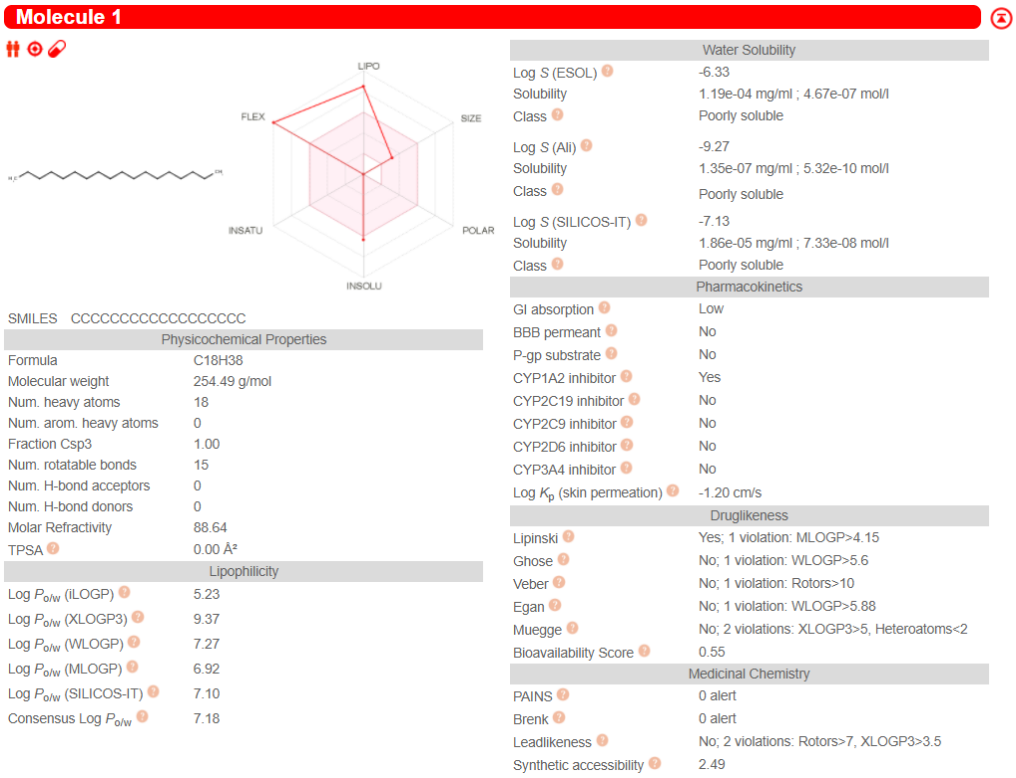


Fig: 5.4: SwissADME results for a compound whose parameters does not satisfy all the Lipinski's rules.

Table 5.14: List of compounds from literature data that showed drug likeness.

S.No	Compound Name
1.	geraniol
2.	Alpha-terpineol
3.	2H-Spiro[benzofuran-3,4'-piperidine]
4.	Ar-tumerone
5.	trans-Sabinene hydrate
6.	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-
7.	1,4-Dimethyl-7-(1-hydroxy-1-methylethyl)azulene
8.	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol
9.	bornyl acetate
10.	α-cadinol
11.	sabinene hydrate

Table 5.15: List of compounds(1-20) from extracted from herbal mixture that showed drug likeness.

S.No.	Compound Name
1.	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-
2.	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4- [(2-methoxyethoxy)methoxy] - 5, 9 - dimethyl-
3.	Spiro[3,5-dioxatricyclo[6.3.0.0(2,7)]undecan-6-one-4,2'-cyclohexane], 1'-isopropyl-2, 4'-dimethyl-9, 11-bis(2-met
4.	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy] - 2 - methyl-
5.	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-
6.	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane
7.	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide
8.	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one
9.	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl-
10.	1,2-Phenylene bis(mesitylsulfonate)
11.	Cyclohexanone, 3-ethyl-3,5,5-trimethyl-
12.	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, sulfite (2:1), [1R-[1.alpha.(1R*,2S*,5R*),2.beta.,5.alpha.]]-
13.	Benzaldehyde, 3,4-dimethyl-
14.	3H-Pyrazol-3-one, 2,4-dihydro-4,4,5-trimethyl-
15.	2-Cyclohexylpiperidine
16.	beta.-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-
17.	t-Butyl 1-thio-.alpha.-D-glucopyranoside
18.	2(1H)-Naphthalenone, octahydro-, trans-
19.	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol
20.	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-

Table 5.16: List of compounds (21-27) from extracted from herbal mixture that showed drug likeness.

S.No.	Compound Name
21.	Phthalimide, N-(1-hydroxy-2-propyl)-
22.	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-
23.	Propiohydrazide, 2,2-dimethyl-N2-(1-methyl-3-oxo-3-phenylpropylideno)-
24.	2H-1-Benzopyran-2-one, 4,7-dimethoxy-
25.	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-
26.	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
27.	4-Methyl-2-trimethylsilyloxy-acetophenone

5.4 MOLECULAR DOCKING RESULTS AND INTERPRETATION

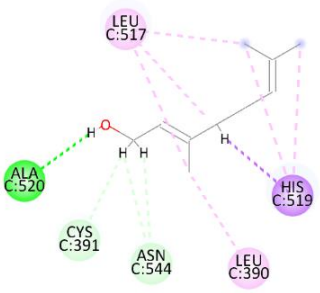
The compounds that passed the ADME screening were considered for molecular docking. The software Patchdock was used and the results obtained were visualized using Discovery Studio Visualizer. The results were screened for those compounds that showed binding inside or close to the Receptor Binding Domain (RBD) of the spike protein lies within the S1 subunit from amino acid 319- 544, ligands that bind to this site or close to this site are desirable. (Akisawa K., et. al., 2021).

5.4.1 Docking results for compounds obtained from literature data

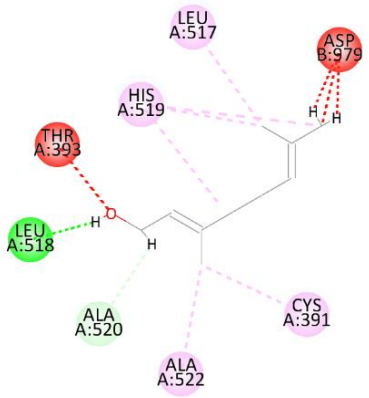
The results of molecular docking of the compounds obtained from literature data showing desirable binding with the spike protein are listed in the Table 5.17. 6 compounds out of the 11 from literature data showed druglike properties, showed desirable binding. The ligand interactions are depicted in the 2D form from the Fig 5.5 to Fig 5.10.

Table 5.17: List of compounds obtained from literature data that showed desirable binding with the spike protein; the docking score and the amino acids involved in hydrogen bonding are also listed.

S. no.	Compound Name	Fig	Score	Amino acids involved in hydrogen bonding
1	Geraniol	5.4 a)	3630	ALA(C-520), CYS(C-391), ASN(C-544)
		5.4 b)	3514	LEU(A-518), ALA(A-520)
		5.4 c)	3500	THR(B-573)
2	α-terpineol	5.5	16920	HIS(A-519)
3	2H-Spiro[benzofuran-3,4'-piperidine]	5.6	21592	ILE(B-312) GLN(B-314)
4	Trans-Sabinene hydrate	5.7 a)	3528	LEU(B-518)
		5.7 b)	3410	HIS (B-519)
		5.7 c)	3398	ASN(B-544)
5	(7α-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	5.8 a)	4158	GLN(A-314) SER(A-596)
		5.8 b)	3976	PHE(B-377)
6	Sabinene hydrate	5.9 a)	3528	LEU(B-518)
		5.9 b)	3410	HIS(B-519)
		5.9 c)	3398	ASN(A-544)



a)



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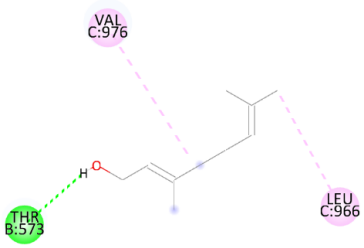


c)

Fig:5.5: Geraniol docking result; a), b), c) each showing a different ligand interaction with the spike protein with the docking scores 3630, 3514 and 3500 respectively

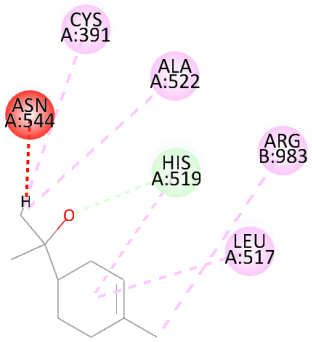


Fig:5.6: α-terpineol docking result showing ligand interactions with a docking score of 16920

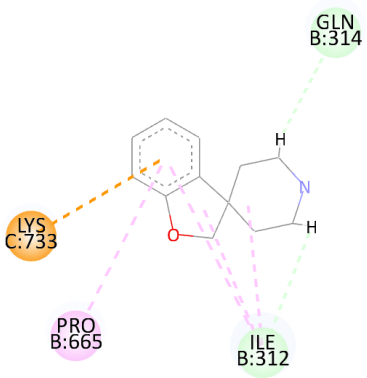
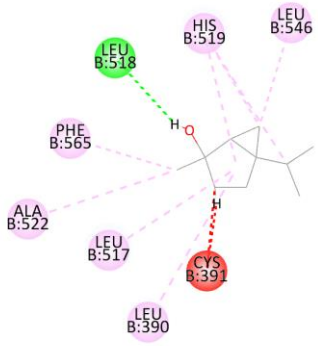
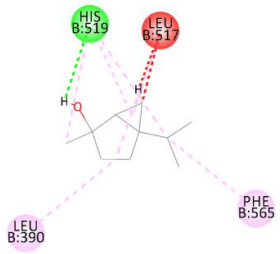


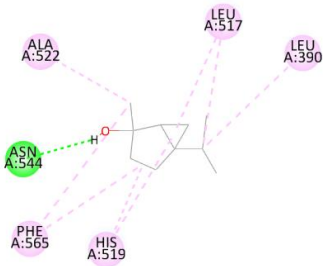
Fig:5.7: 2H-Spiro[benzofuran-3,4'-piperidine] docking result showing ligand interaction with spike protein with a docking score of 21592



a)

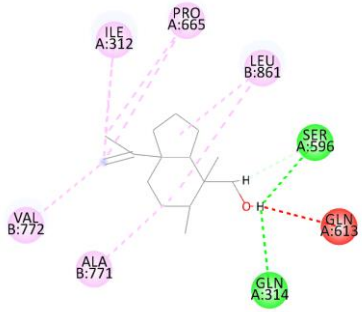


b)

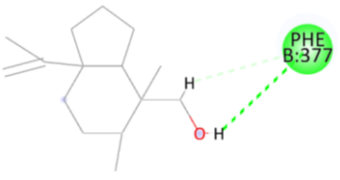


c)

Fig:5.8 : Trans - sabinene hydrate docking result; a), b), and c) each showing different ligand interactions with spike protein with a docking score of 3528, 3410, and 3398 respectively

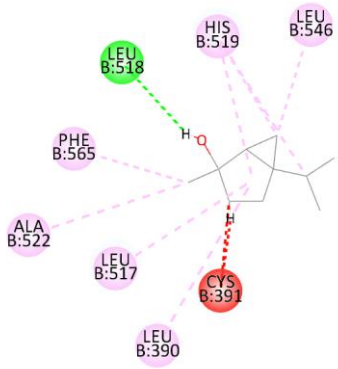


a)

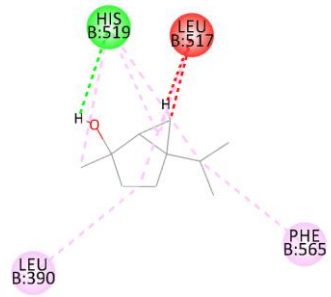


b)

Fig:5.9 : (7α-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 4158, and 3976 respectively



a)



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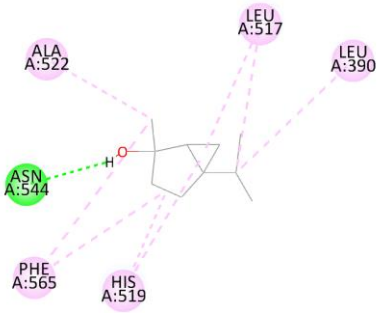


c)

Fig :5.10 : Sabinene hydrate docking result; a), b), and c) each showing different ligand interactions with spike protein with a docking score of 3528, 3410, and 3398 respectively

5.4.2 Docking results for compounds extracted from herbal mixture

The results of molecular docking of the extracted compounds from the mixture showing desirable binding with the spike protein are listed in the Table 5.18 to Table 5.20. From the extracted herbal mixture, out of the 27 compounds that showed drug likeness, 18 showed desirable binding. The ligand interactions are depicted in the 2D form from the Fig 5.11 to Fig 5.28., the hydrogen n binding (represented in green) and the amino acids involved are shown.

Table 5.18 : List of compounds (1-6) obtained from extracted herbal that showed desirable binding with the spike protein; the docking score and the amino acids involved in hydrogen bonding are also listed.

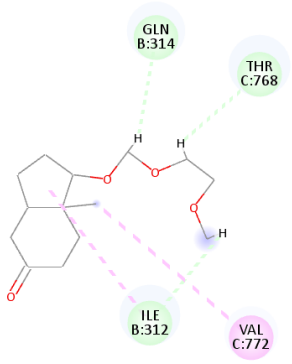
S.No.	Compound Name	Fig	Score	Amino acids involved in hydrogen bonding
1	Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-	5.10 a)	4514	GLN (B:314)
		5.10 b)	4446	ILE (C:312)
		5.10 c)	4420	THR (B:549), THR (B:573)
		5.10 d)	4412	TYR (C:369), THR (C:376), PHE (C:377), THR (B:415)
		5.10 e)	4408	THR (B:549)
2	Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-	5.11 a)	4904	PHE (A:377)
		5.11 b)	4874	CYS (C:379), SER (C:383), GLY (B:416) PRO (C:384),
		5.11 c)	4866	PHE (C:377), TYR (C:369), SER (C:383), ARG (B:408)
		5.11 d)	4846	THR (B:573)
		5.11 e)	4844	ARG (C:403), PHE (A:377), TYR (A:369)
3	Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-	5.12 a)	4778	GLN (C:314)
		5.12 b)	4634	ASP (C:568), THR (C:572)
		5.12 c)	4602	TYR (C:369)
		5.12 d)	4582	GLN (B:314)
4	1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-	5.13 a)	3124	ASN (B:544), GLY (B:545)
		5.13 b)	3002	LEU (A:517)

Table 5.19: List of compounds (7-15) obtained from extracted herbal that showed desirable binding with the spike protein; the docking score and the amino acids involved in hydrogen bonding are also listed.

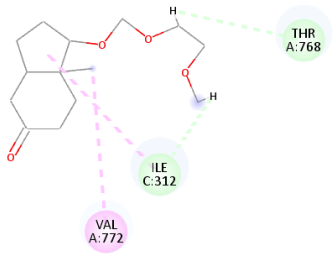
S.No.	Compound Name	Fig	Score	Amino acids involved in hydrogen bonding
5	6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane	5.14 a)	2726	CYS (C:391)
		5.14 b)	2706	CYS (B:391), LEU (B:518), ASN (B:544)
6	N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide	5.15 a)	4366	THR (C:573)
		5.15 b)	4334	THR (B:573)
7	7-Diethoxymethylbicyclo[3.2.0]heptan-2-one	5.16 a)	4108	THR (C:573)
		5.16 b)	4094	THR (A:573)
8	1,2-Phenylene bis(mesitylsulfonate)	5.17 a)	6046	THR (C:572), THR (C:549)
		5.17 b)	6040	THR (B:572), THR (B:549)
		5.17 c)	5974	GLY (B:548), THR (B:572)
		5.17 d)	5880	THR (A:572)
		5.17 e)	4726	THR (A:549), THR (A:573)
9	Benzaldehyde, 3,4-dimethyl-	5.18 a)	3384	LEU (B:518)
		5.18 b)	3196	LEU (C:518), ALA (C:520)
		5.18 c)	3122	LEU (A:518)
10	2-Cyclohexylpiperidine	5.19 a)	3654	ASN(C-544)
		5.19 b)	3548	CYS(B-391) LEU(B-517) LEU(B-518)
		5.19 c)	3548	ILE(B12)
		5.19 d)	3532	HIS(C-519)
		5.19 e)	3526	LEU-(A-517)
11	β-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-	5.20 a)	5616	THR (C-549) PHE(A-855)
		5.20 b)	5338	LYS(C-304) GLN(C-314) SER(A-758) THR(A-761) THR(A-768)

Table 5.20 : List of compounds (16-18) obtained from extracted herbal that showed desirable binding with the spike protein; the docking score and the amino acids involved in hydrogen bonding are also listed.

S.No.	Compound Name	Fig	Score	Amino acids involved in hydrogen bonding
12	t-Butyl 1-thio-.alpha.-D-glucopyranoside	5.21	4088	ILE(A-312) GLN(A-314) SER(A-596)
13	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	5.22	4060	GLN(C-314) LYS(A-733)
14	8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-	5.23	2846	LEU(A-517)
15	Phthalimide, N-(1-hydroxy-2-propyl)-	5.24 a)	3700	GLN(B-314) SER(B-596)
		5.24 b)	3632	ALA(A-520) ASN(A-544)
		5.24 c)	3604	ARG(C-355)
16	Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-	5.25 a)	4718	THR(A-573) ASN(B-856) ASN(B-978)
		5.25 b)	4668	THR(B-549) ASP(C-745)
		5.25 c)	4602	PHE(B-377) SER(B-383) THR(A-415)
17	2H-1-Benzopyran-2-one, 4,7-dimethoxy-	5.26	3692	GLN(B-314) SER(B-596) ILE(B-666) ASP(C-775)
18	Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)-	5.27 a)	4546	GLN(C-314) SER(C-596) PRO(A-862)
		5.27 b)	4506	THR(A-547) THR(A-573)



a)



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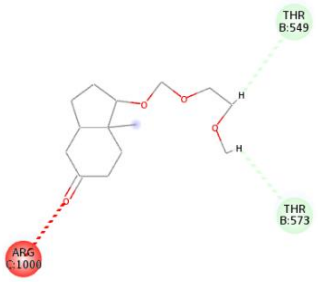

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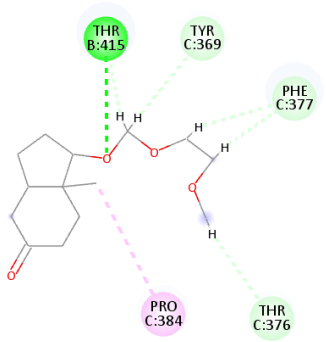

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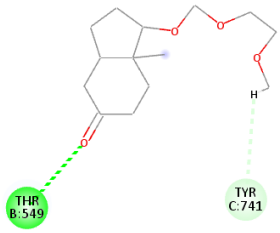
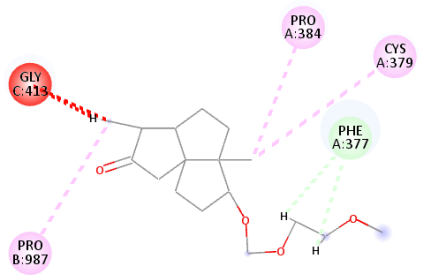
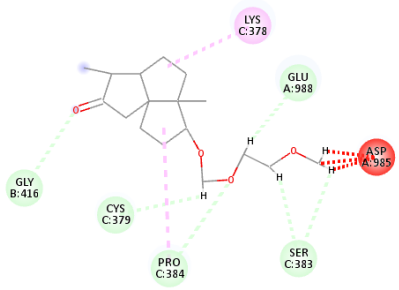


e)

Fig:5.11 : Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1- methyl- docking result; a), b), c), d), and e) each showing different ligand interactions with spike protein with a docking score of 4514, 4446, 4420, 4412 and 4408 respectively.



a)



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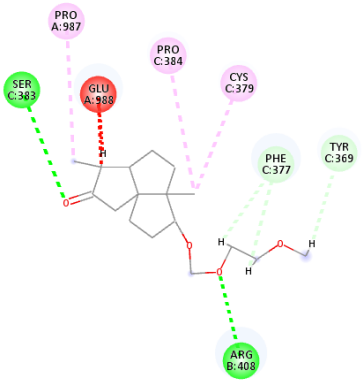

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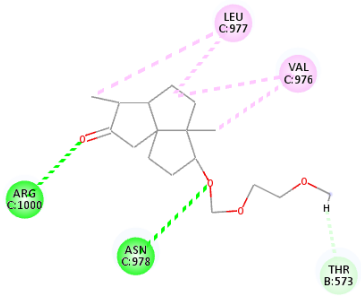

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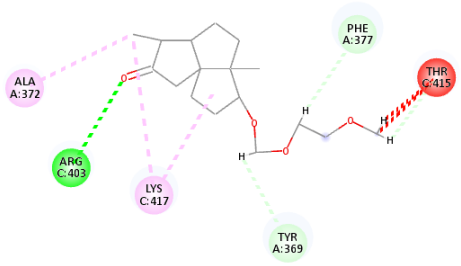
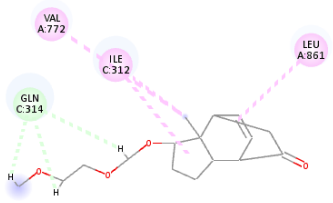
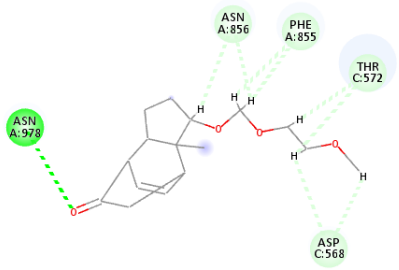


e)

Fig:5.12: Tricyclo [6.3.0.0(1,5)] undecan-10-one, 4-[(2-methoxyethoxy) methoxy]-5, 9-dimethyl- docking result; a), b), c), d), and e) each showing different ligand interactions with spike protein with a docking score of 4904, 4874, 4866, 4846 and 4844 respectively.



a)



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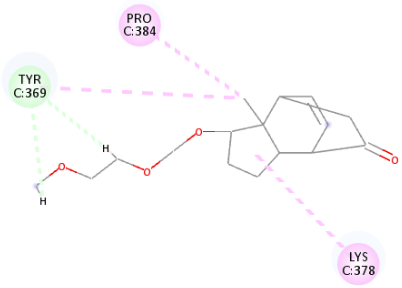
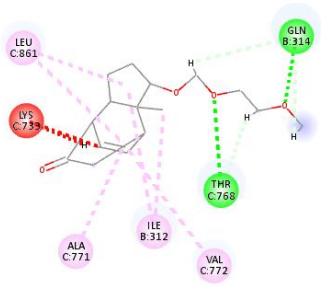
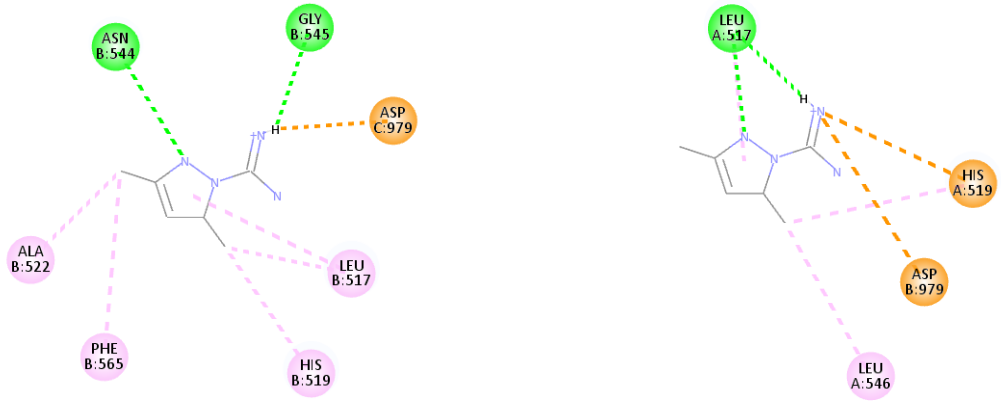


c)



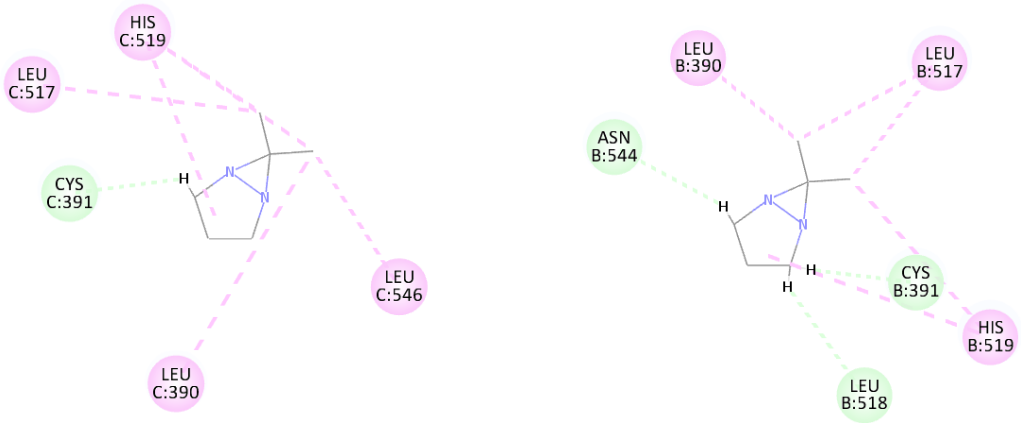
d)

Fig:5.13: Tricyclo [5.2.2.0(2,6)] undec-8-en-11-one, 3-[(2-methoxyethoxy) methoxy]- 2-methyl- docking result; a), b), c) and d) each showing different ligand interactions with spike protein with a docking score of 4778, 4634, 4602, and 4582 respectively.



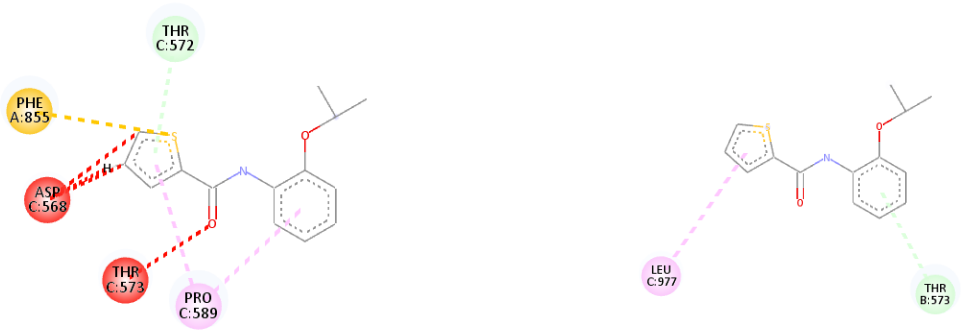
a) ***b)***

Fig:5.14 : 1H-Pyrazole-1-carboximidamide, 3,5-dimethyl- docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 3124 and 3002 respectively.



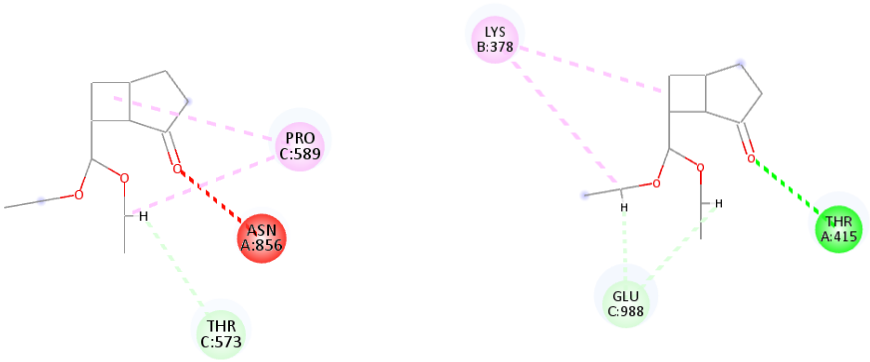
a) ***b)***

Fig:5.15 : 6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 2726 and 2706 respectively.



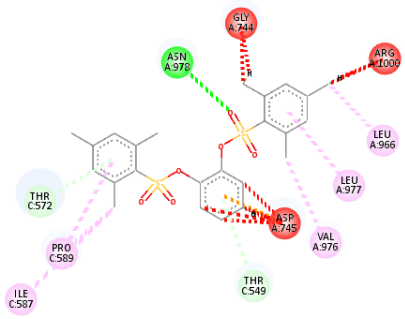
a) ***b)***

Fig:5.16 : N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 4366 and 4334 respectively.

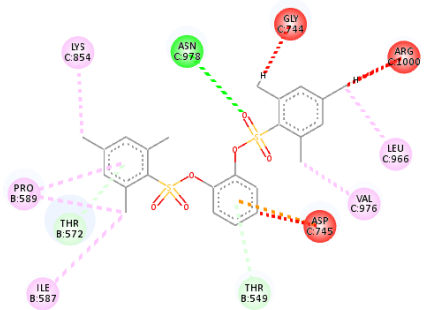


a) ***b)***

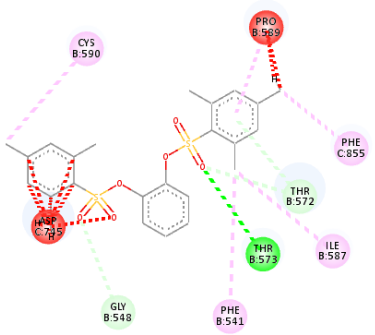
Fig:5.17 : 7-Diethoxymethylbicyclo[3.2.0]heptan-2-one docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 4108 and 4094 respectively.



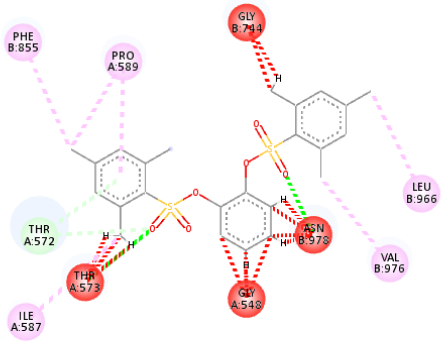
a)



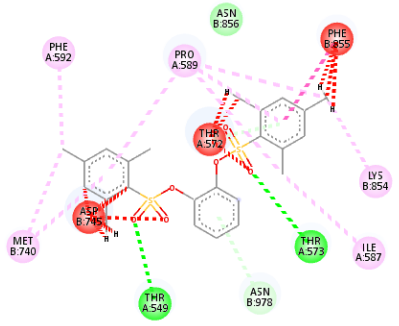
b)



c)

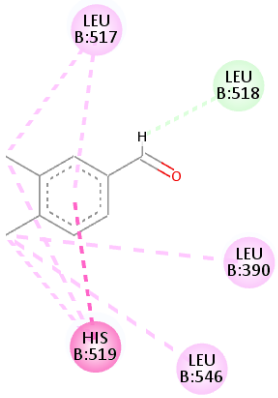


d)

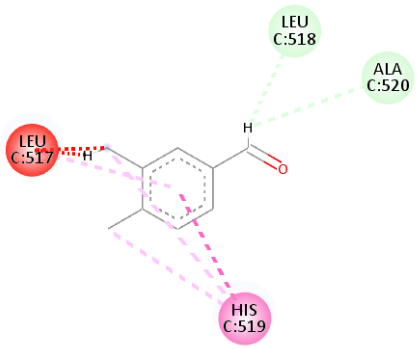


e)

Fig:5.18 : 1,2-Phenylene bis(mesityl sulfonate) docking result; a), b), c), d), and e) each showing different ligand interactions with spike protein with a docking score of 6046,6040,5974, 5880 and 4726 respectively.



a)



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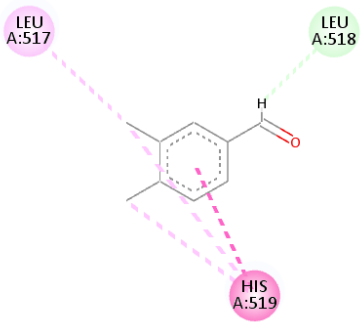
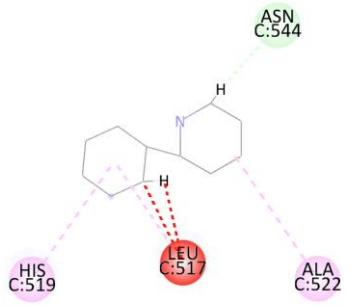
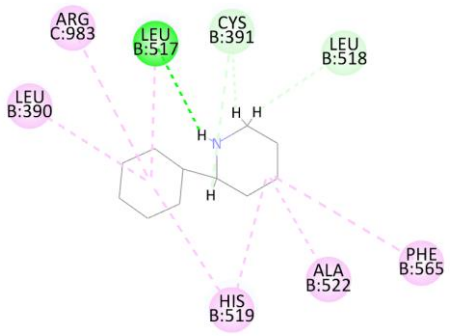


c)

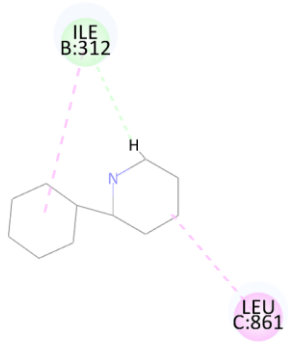
Fig:5.19 : Benzaldehyde, 3,4-dimethyl- docking result; a), b), and c) each showing different ligand interactions with spike protein with a docking score of 3384, 3196, and 3122 respectively



a)



b)



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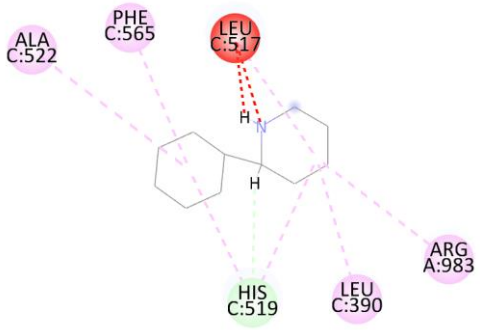

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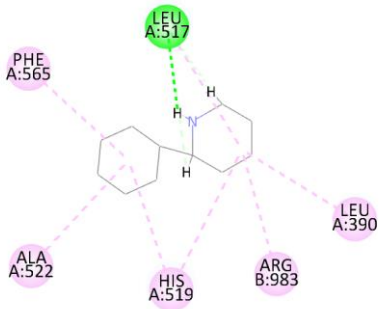
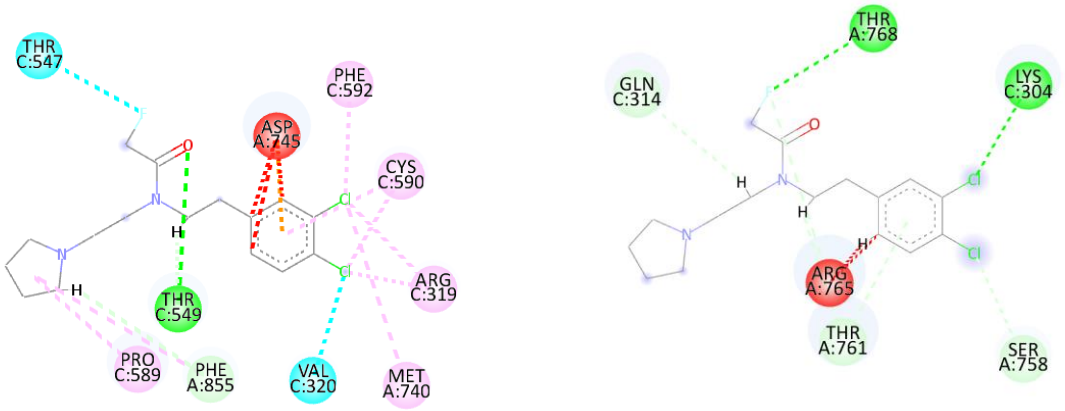


e)

Fig:5.20 : 2-Cyclohexylpiperidine docking result; a), b), c), d), and e) each showing different ligand interactions with spike protein with a docking score of 3654, 3548, 3548, 3532, and 3526 respectively



a) ***b)***

Fig:5.21 : β-(3,4-Dichlorophenyl)ethylamine,N-fluoroacetyl-N-(2-pyrrolidinoethyl)- docking result; a) and b) each showing different ligand interactions with spike protein with a docking score of 5616 and 5338 respectively.

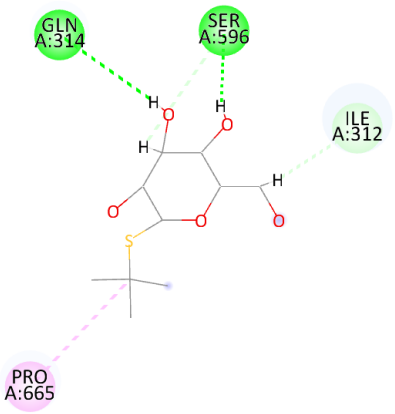


Fig:5.22 : t-Butyl 1-thio-.alpha.-D-glucopyranoside docking result showing ligand interactions with spike protein with a docking score of 4088.

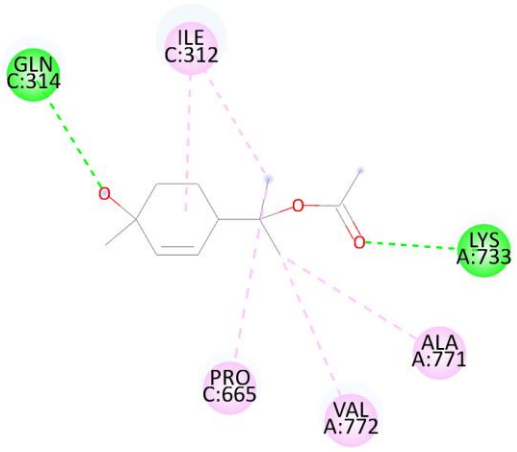


Fig:5.23 : 1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol docking result showing ligand interactions with spike protein with a docking score of 4060.

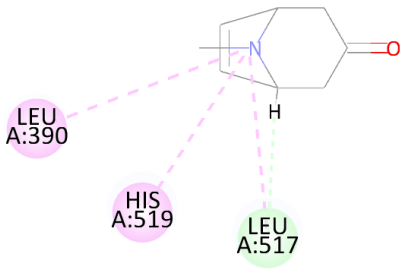
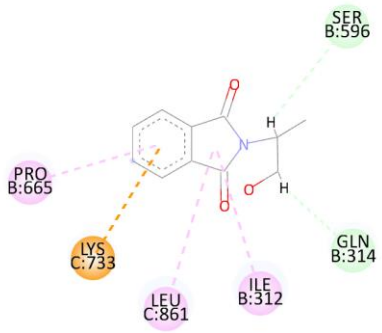
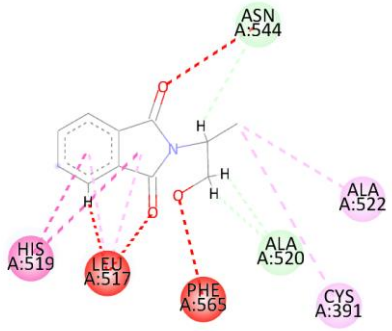


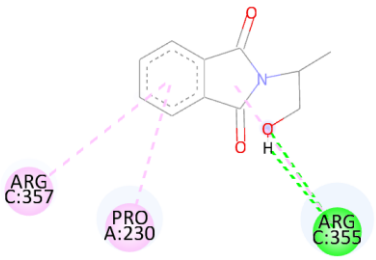
Fig:5.24 : 8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl- docking result showing ligand interactions with spike protein with a docking score of 2846.



a)

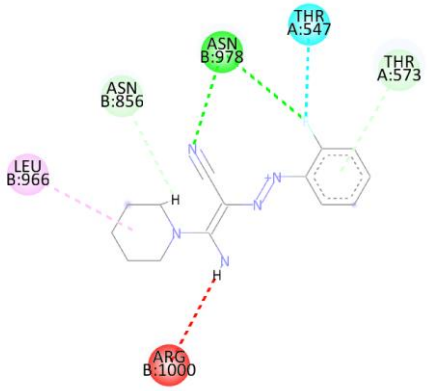


b)

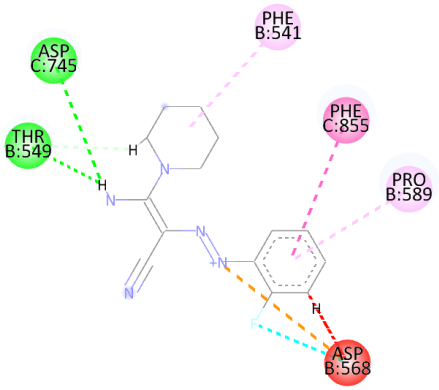


c)

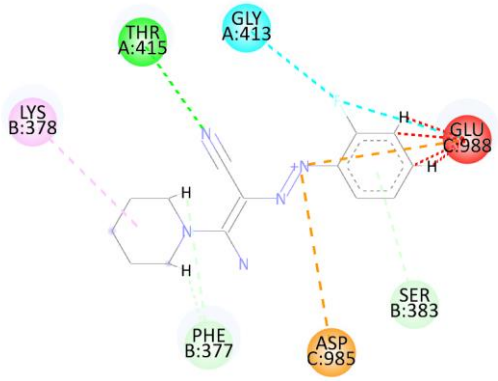
Fig:5.25 : Phthalimide, N-(1-hydroxy-2-propyl)- docking result; a), b), and c) each showing different ligand interactions with spike protein with a docking score of 3700, 3632, and 3604 respectively.



a)



b)



c)

Fig:5.26 : Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)- docking result; a), b), and c) each showing different ligand interactions with spike protein with a docking score of 4718, 4668, and 4602 respectively.

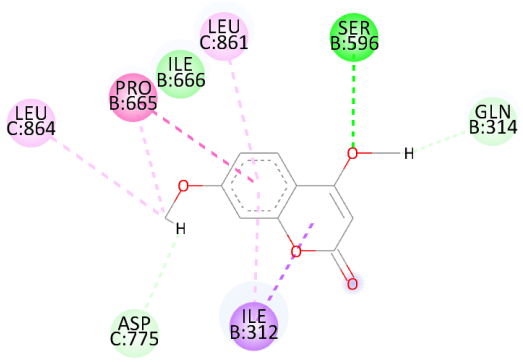
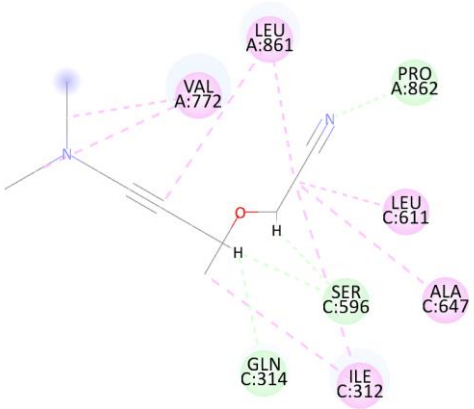
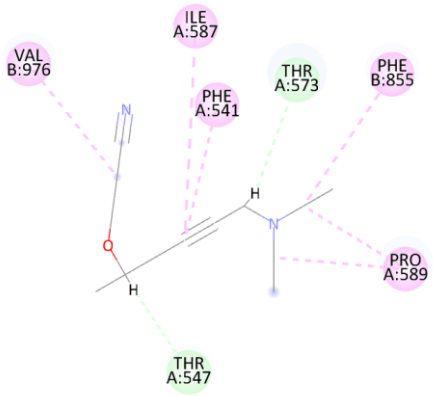


Fig:5.27 : 2H-1-Benzopyran-2-one, 4,7-dimethoxy- docking result showing ligand interactions with spike protein with a docking score of 3692.





a) ***b)***

Fig:5.28 : Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy)- docking result; a) and b)each showing different ligand interactions with spike protein with a docking score of 4546 and 4506 respectively.

CHAPTER -6 SUMMARY AND CONCLUSION

SARS Co-V-2 is a viral infection transmitted by infectious respiratory droplets, what makes this strain more contagious over SARS Co-V-1 is the alterations in the surface proteins, mainly the S protein, rendering it with a higher affinity to the ACE-2 receptor. The spike protein is the main means of entry into the host cell, as it binds to the ACE 2 receptor, therefore it was chosen as a target protein for this study. The ligand molecules were obtained from the volatile water extracts of a herbal formulation consisting of nine herbs, namely *Coleus amboinicus, Citrus limon, Leucas aspera, Curcuma longa L, Mentha piperita, Ocimum basilicum, Ocimum gratissimum, Vitex negundo, Allium sativum*, chosen as they are used in traditional siddha medicine for their antiviral activity, to relieve symptoms similar to that of SARS Co-V-2. Several compounds were also obtained from literature data from each of the mentioned plants for their antiviral activity. An Insilico approach was taken for evaluating these compounds to determine their prophylactic ability.

The results obtained show that 6 compounds (from literature data); *Geraniol, α-terpineol, 2H-Spiro[benzofuran-3,4'-piperidine], Trans-Sabinene hydrate, (7α-Isopropenyl-4,5-dimethyloctahydroinden-4-yl) methanol, Sabinene hydrate,* could potentially inhibit the binding of the Spike protein to the ACE-2 receptor, as they show desirable binding to the Spike protein. The docking result of the water extracted herbal mixture show that 18 compounds; *Bicyclo[4.3.0]nonan-4-one, 9-(2-methoxyethoxymethoxy)-1-methyl-, Tricyclo[6.3.0.0(1,5)]undecan-10-one, 4-[(2-methoxyethoxy)methoxy]-5,9-dimethyl-, Tricyclo[5.2.2.0(2,6)]undec-8-en-11-one, 3-[(2-methoxyethoxy)methoxy]-2-methyl-, 1H-Pyrazole-1-carboximidamide, 3,5-dimethyl-, 6,6-Dimethyl-1,5-diazabicyclo[3.1.0]hexane, N-(2-Isopropoxyphenyl)-2-thiophenecarboxamide, 7-Diethoxymethylbicyclo[3.2.0]heptan-2-one, 1,2-Phenylene bis(mesitylsulfonate), Benzaldehyde, 3,4-dimethyl-, 2-Cyclohexylpiperidine, β-(3,4-Dichlorophenyl)ethylamine, N-fluoroacetyl-N-(2-pyrrolidinoethyl)-, t-Butyl 1-thio-.alpha.-D-glucopyranoside, 1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol, 8-Azabicyclo[3.2.1]oct-6-en-3-one, 8-methyl-, Phthalimide, N-(1-hydroxy-2-propyl)-, Propanenitrile, 2-(2-fluorophenylhydrazono)-3-imino-3-(1-piperidyl)-, 2H-1-Benzopyran-2-one, 4,7-dimethoxy-, Propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy*)-, show desirable binding to the spike protein, i.e. inside and around the receptor binding domain.

This study shows that the herbal formulation containing the 9 herbs shows promise as a potential prophylactic of SARS-Co-V-2. However, these In-Silico studies are only preliminary tools of analysis and they don't have a 100% accuracy, a lot of parameters influence the action of these compounds in-vivo, so they must be considered for further studies.

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