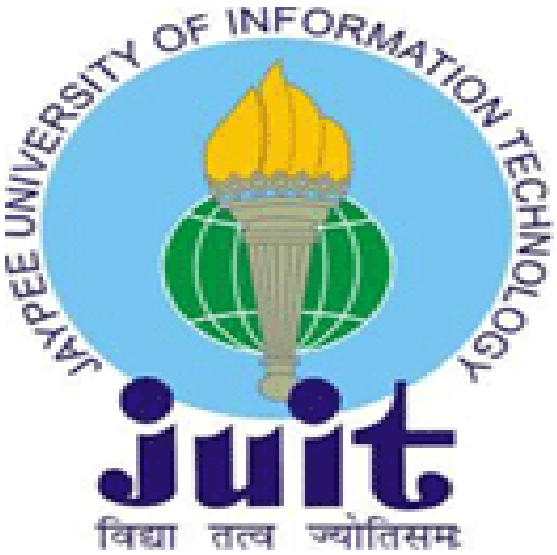
IN SILICO STUDIES TO IDENTIFY COMMERCIALLY AND MEDICINALLY IMPORTANT PHYTOCHEMICALS OF *BAUHINIA VARIEGATA*

Project Thesis

Submitted to

Department Of Biotechnology and Bioinformatics



In partial fulfilment for the degree of

M. TECH INTEGRATED IN BIOTECHNOLOGY

Project by

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Supervised by

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(Associate Professor, JUIT)

DECLARATION

I hereby pronounce that the work presented in this thesis named "**In Silico Studies to Identify Commercially and Medicinally Important Phytochemicals of *Bauhinia variegata***" in partial fulfilment of the requirements for grant of the degree of Integrated M.Tech in the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, 173234, India is an original document of my work completed over a period from July 2020 to May 2021 under the management of Dr. Hemant Sood as guide.

The matter expressed in the thesis has not been submitted for the honor of any other degree or diploma.

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Sunainy Ajrawat (161842)

CERTIFICATE

Date: 15 June 21

This is to confirm that the work named "In Silico Studies to Identify Commercially and Medicinally Important Phytochemicals of *Bauhinia variegata*", submitted by 'Sunainy Ajrawat, enrollment no. 161842' to Jaypee University of Information Technology, Waknaghat, 173234, India; in partial fulfilment for grant of the degree of Integrated M. Tech has been completed under my custody. This work has never been submitted partially or entirely to whichever university or institute for the honor of any other degree or diploma.

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ABSTRACT

Bauhinia variegata has been taken into consideration due to its high pharmacological and commercial significance. This plant is known to be enriched with phytochemicals, that constitute many secondary metabolites such terpenoids, flavanoids, tannins, steroids, reducing sugars and cardiac glycosides, which can help in treatment of various diseases such as cancer, diabetes, obesity, ulcers, cardiovascular diseases and many more.

This study concentrates on in silico study of the plant in order to identify potential bioactive compounds which can be used in treatment of infections caused by the group of corona viruses. Various bioinformatic tools such as molecular docking and ADMET were used to find and study the bioactive compounds against the SARS-CoV-2 target proteins, namely, main protease, receptor binding domain and human furin protease. However, still today, this plant is unexplored for the scientific evidences that authenticate its pharmacological properties.

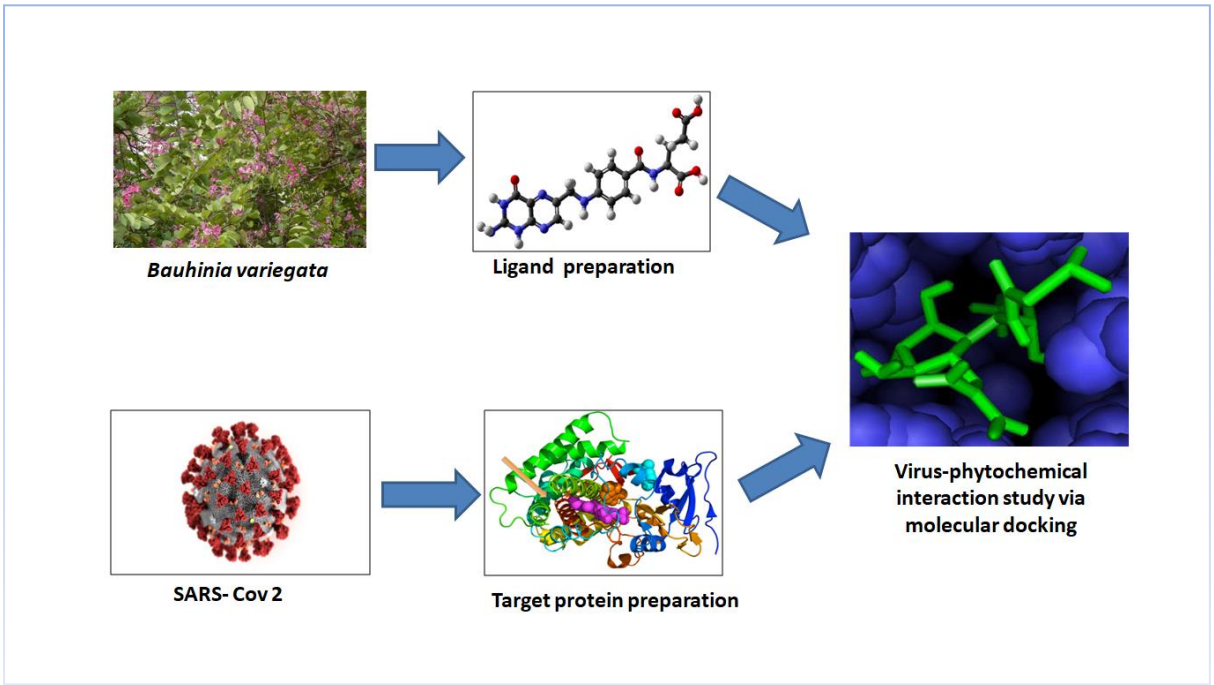

Fig 1-Graphical abstract

Keywords: bauhinia variegata, kachnar, phytochemicals, bioactive compounds, molecular docking, SARS-CoV-2, SARS-CoV-2 target proteins, potential ligands, reference drugs, secondary metabolities, virus-phytochemical interaction

CHAPTER 1: INTRODUCTION

Bauhinia variegata, commonly known as Kachnar, belongs to the Leguminosae family. It is mainly found in tropical and warm regions of the globe [1]. It is well known for its ornamental value and is spread all over India, rising to an altitude of 1300 in Himalayas [1, 2]. The trees are deciduous and medium sized and grows most excellent in full moon or partial shade [1]. Despite the fact that frost kills leaves of saplings and seedlings, they recoup during summers. Leaves are broad and 10-15 cm (length) plus the flowers are large, fragrant, and white or purplish in colour, come out when the tree is leafless that is in the months of January- April [1, 2]. As per studies, the phytochemical screening revealed the presence of wide range of secondary metabolites in B. variegata such as terpenoids, flavanoids, steroids, saponins, tannins, reducing sugars and cardiac glycosides [3]. These metabolites make *B. variegata* useful in treatment of various health problems such as cancer, ulcer, inflammation, bacterial infections and many more [3].

https://www.google.com/imgres?imgurl=https%3A%2F%2Fpaudhshala.com

https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.indiamart.com

Fig 2- a) Bauhinia variegata tree b) Bauhinia variegata flower

Propagation of *B. variegata* by cutting is difficult so it is commercially propagated by seed. Micropropagation of the plant needs to be studied to develop an efficient protocol of propagation and for in vitro production of commercially important phytochemicals [9]. In vitro propagation can be done using the shoot apex, buds, nodal cutting and explants by optimizing the novel plant tissue culture media. Various parameters such as choice of solvent,

pH, temperature, etc. need to be optimized for desirable results. However, in this study, we could not carry out the propagation of this plant as we were not accessible to the lab and university due to Covid-19 problems. Hence, we have attempted to mainly focus of in silico studies with the help of reference sources and bioinformatic tools such as molecular docking and structure visualization softwares. This study is carried out with the objective to find out different phytochemicals present in this plant with medicianal significance.

The recent coronavirus outbreak, provisionally known as 2019-nCoV, has lead to respiratory diseases that are often severe [18]. Since, this plant is loaded with phytochemicals of medicinal significance, we carried out in silico studies to identify potential phytochemicals which may actively bind with the corona virus proteins and may help to inhibit its mode of action.

Till now, there are no evidences that aim at interaction of bioactive compounds from *B. variegata* with SARS-CoV-2 target proteins, namely, SARS-CoV-2 main protease, receptor binding domain and human furin protease.

Though, B. variegata is a very important and valued plant of Indian Himalayan regions, but it not much mentioned commercially. Basically, this plant is yet unexplored for the scientific evidences that validate its medicinal significance.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction to *Bauhinia variegata*

Bauhinia variegata L., also recognized as the mountain ebony, Kachnar, orchid tree, camel's foot, and Napoleon's hat, is a part of the Leguminosae family [1,3]. It is spread all over India, rising to an altitude of around 1300 in the Himalayas [1]. It is well known for its ornamental value and generally grown in tropical as well as sub-tropical regions with hot and arid summers, also mild winters. Full sun or partial shade is good for tree growth and trees are rather resistant to drought but at risk to fire. Despite the fact that frost kills leaves of saplings along with seedlings, they recoup through summers. The trees are small to medium sized growing to 10-12 m (height) and diameter of 50 cm [2]. Leaves are broad and 10-15 cm in length. The flowers are large, fragrant, white or purplish in colour, come out when the tree is leafless that is in the months of January- April [1,2].

B. variegata along with *Albizia lebbeck* and *Grewia optiva* is seen as the most suitable trees for plantation in the regions of Garhwal Himalayas. It is popularly recognized fodder variety of mid hill area and has a function in production of paper and cellulose, round log and sawn wood, wood wool board, gum and fibre as well as recovery of degraded areas [2]. Various parts of the species such as stem, bark, seeds, roots, leaves, flowers and flower buds are utilized in traditional system of medicines for the treatment of variety of diseases such as bronchitis, leprosy and tumors [1, 2, 3]. Stem bark is utilized as an astringent, tonic, anthelmintic as well as antidiabetic. Herbal blend of leaves is beneficial as a laxative and for piles as well. Dried buds are used too for cure of tumors, diarrhea, piles and infestations [3]. Numbers of compounds are isolated from B. Variegata such as kaempferol galactoside, malvidin glucoside, cyanidin glucoside and peonidin glucoside, to prevent development and spread of a variety of cancers of liver, lung, mouth, larynx and malignant ascites [2].

As per previous studies, the photochemical screening of *B. variegata* exposed that a wide range of secondary metabolites are present such as terpenoids, flavanoids, , reducing sugars, tannins, steroids, saponins and cardiac glycosides. And the pharmacological studies revealed that *B. variegata* is useful as antioxidant, anti-inflammatory, anticancer, antimicrobial, antiulcer, hypolipidemic, nephroprotective, immunomodulating, hepatoprotective and wound healing agent [3].

2.2 Phytochemical constituents of *B. variegata*

The phytochemical analysis of constituents of B. variegata flowers, that is n-hexane ethyl acetate, methanolic fractions and chloroform, exposed the occurance of terpenoids, flavanoids, reducing sugars, tannins, steroids, and cardiac glycosides. [20]

Non woody aerial parts showed the occurrence of six flavanoids that is kaempferol, ombuin, kaempferol 7,4 –dimethylether-3-o-β-D-glucopyranoside, isorhamnetin-3-o-β-D-glucopyranoside and hespheridin along with one triterpene caffeate, 3β trans-(3,4 dihydroxycinnamoyloxy)olean-12-en-28-oic acid. [21]

Analysis of stem bark revealed the presence of 5,7 dihydroxy and 5,7 dimethoxy flavanone-4-O-L rhamnopyrosyl-β-D-glycopyranosides, Kaempferol-3-glucoside, lupeol and betasitosterol.

B. variegata leaves constitutes lupeol, alkaloids, fat glycoside, oil, phenolics, tannins, saponins, terpinoids, lignin, β-sitosterol, kaempferol-3- glucoside, apigenin, quercitrin, quercetin, rutin, amides, carbohydrates, reducing sugars, apigenin-7-O-glucoside, protein, fibres, vitamin C, phosphorus and calcium. [3]

The seeds yielded 41.9 ±1.6% total proteins, 18 ±0.9% total oils, 28.4 ±1.6% carbohydrates and 0.6 ± 0.1% free fatty acids. The fatty acids content of oil (%) included palmitic 22.1 ±1.5, palmitoleic 0.4± 0.1, margaric 0.3 ±0.04, stearic 17.5 ±1.7, oleic (C18: 1cis 9) 13.4±0.8, oleic (C18: 1cis 7) 0.5± 0.1, linoleic 42.1±1.8, linolenic (C 18: 3 n-3)0.6±0.4, linolenic (C18: 3 n-6)0.5±0.1, archidic 1.3±0.6, behenic 0.5±0.2, eicosapentaenoic 0.2±0.4 and nervonic acid 0.6±0.6. From the overall lipid content, the content of polyunsaturated fatty acids was 43.2%, saturated fatty acid was 41.7% and monounsaturated fatty acid was 15.1%. [19]

2.3 Pharmacological studies of B. variegata

2.3.1 Anticancer impacts

The ethanolic fractions of *B. variegata* have anticancer impacts in DLA cells (Dalton's Lymphoma Ascites cells) and EAC mouse cell lines. It also protects liver against the cytotoxic cause of diethyl nitrosamine. The methanolic fractions of stem bark of *B. variegata* (500 and 1000 mg/kg bw dose) also possess antitumor impacts in skin warts against 7,12-dimethybenz(a) anthracene and also against the skin carcinogenesis induced in mice by croton oil. The rate of tumor occurrence and papillomas frequency was decreased. The exhausted level of glutathione was restored in groups treated with bark extract of *B. variegata*.[22]

Ethanolic fractions of the stem of *B.variegata* possess chemoprevention and cytotoxic impact against liver tumor induced experimentally by N-nitrosodiethylamine in rats at dose of 200 mg/kg, and on human cell lines as well. The suppression of tumor caused by decreased level of N-nitrosodiehylamine lead to increase in level of serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, total bilirubin, lipid peroxidise, alkaline phosphatise, gamma glutamate transpeptidase and glutathione-S-transferase. . The ethanolic fraction proved out to be cytotoxic towards human epithelial larynx tumor and breast cancer. [3]

2.3.2 Antioxidant impacts

Antioxidant properties were demonstrated by the crude fractions of *B. variegata* and with the help of DPPH radical scavenging assay. In general, chloroform fraction demonstrates the lowest antioxidant action. Methanol, ethyl acetate and n-hexane were evaluated with moderate scavenging action in comparison to standard querceitin. The ethanolic and aqueous fractions of *B. variegata* were extracted to evaluate antioxidant action, in vitro, with the help of a range of techniques such as scavenging of free radicals (like DPPH), total reducing power, nitric oxide, super oxide and hydrogen peroxide. Antioxidant action for reducing power was observed to be $P \geq 0.01$ and for DPPH scavenging, nitric oxide, super oxide and hydrogen peroxide was $P \geq 0.001$. [20, 23]

2.3.3 Anti-inflammatory impacts

Non woody aerial parts of the species, when analyzed, showed the occurrence of six flavanoids along with one triterpene caffeate. Anti-inflammatory action was shown by these compounds by inhibiting the lipopolysaccharides and interferon γ stimulated nitric oxide as well as the cytokines. [24]

2.3.4 Antimicrobial impacts

The antimicrobial impacts of the ethanolic fractions of B. variegata were studied in vitro. The extracts appeared to be more effective against the gram positive than the gram negative bacteria. The chloroform and mehanolic fractions appeared to be effective against *Staphylococcus aureus, Bacillus subtilis* and *Klebsiella*.[20]

The alcoholic fractions of leaves demonstrated greater antimicrobial activity in comparison to petroleum ether and chloroform fractions. [3]

2.3.5 Antiulcer impacts

The ethanolic fractions of *B. variegata* reduce the volume of gastric secretion, ulcer index and total free acidity resulting from the gastric ulcer induced by pyloric ligation and aspirin induced ulcer model in rats. [25]

2.3.6 Wound healing

The aqueous and ethanolic fractions of root of *B. variegata* produced considerable effect in wound healing by excision and incision wound models, which were comparable to the standard (framycetin) of excision wound model. [27]

2.3.7 Anti obesity impacts

To investigate the anti obesity impacts of methanolic fractions of stem and root barks of B. variegata, female rats were fed with hypercaloric diet. The extracts showed a hypolipidemic impact and hence reduced the obesity. The feed intake and body weight was also observed to be reduced. Treatment of obesity in animals with methanolic extracts resulted in increased level of serotonin and high density lipoprotein and decrease in level of total cholesterol, low density lipoprotein and triglycerides. The study concluded the presence of β- sitosterol in stems and affinity of extracts to reduce the lipid profile and release of serotonin in brain. Serotonin acts as an appetite suppressant that helps manage appetite and control excessive weight gain or obesity. [4]

2.4 Obesity and interaction of associated protein with the quercetin

Obesity is a multifaceted disorder which comprises an excessive accumulation of body fat. It isn't just a cosmetic concern but also could increase risk of several health problems such as hypertension, diabetes and heart diseases. Treatment for obesity may involve lifestyle changes such as making exercise, dieting and weight reduction as a part of life. Weight reduction drugs are also available; however, these drugs come along with risks of dangerous heart and lung side effects such as hypertension and tachycardia. Side effects of these drugs also involve constipation, restlessness, and dry mouth, insomnia and withdrawal effects.

The role of SHC1 protein was identified by the scientists in causing obesity as it has been found abundantly in fatty tissues. Hence, with the help of protein-ligand interaction studies, out of 80 potential ligands, four best compounds were identified which follow the Lipinski's rule and showed good docking scores while binding with the SHC1 protein. The four compounds found were:

- Resveratrol

- **6-hydroxy-2-(3-methoxyphenyl)chromen-4-one**
- **Cis-dihydroquercetin**
- 2-(3,4-dimethoxyphenyl)benzo[h]chromen-4-one

After the bioactivity assessment and ADMET studies of these ligands, 6-hydroxy-2-(3-methoxyphenyl)chromen-4-one and cis-dihydroquercetin were found out to be potent ligands. This way it was concluded that this ligand can be used as SHC1 inhibitor and hence, could be useful for controlling the obesity. [8]

As per my observation, structural similarity was seen between 6-hydroxy-2-(3-methoxyphenyl)chromen-4-one and cis-dihydroquercetin with quercetin. And, since 6-hydroxy-2-(3-methoxyphenyl)chromen-4-one and cis-dihydroquercetin inhibits the SHC1 protein successfully, quercetin may too.

However, this no evidence for anti-obesity action of quercetin till now.

2.5 Phytochemicals against SARS-Cov-2

Coronaviruses are viral elements which constitute spicules on their outer envelope and give them a crown like structure under the electron microscope. These viruses are capable of affecting humans as well as animals by causing serious respiratory problems. Three main target sites of this virus are:

- Furin (proprotein convertase)
- Receptor Binding Domain of SAR-CoV-2 spike protein
- SAR-CoV-2 main protease

Plants have always actively helped in treatment and cure of various pathologies since millennia. According to research, nine compounds were extracted from Djiboutian therapeutic plants *(Acacia seyal, Indigofera caerulea and Cymbopogon commutatus)*, namely, Catechin, β-sitosterol, Quercetin, Lupeol, Kaempferol, Gallic Acid, Piperitone, Limonene and Rutin. These compounds and two reference drugs were tested in opposition to the target sites of the SARS-CoV-2. The main goal was to find out the energies of molecule-target interaction, ADMET properties and probable toxicities produced from these compounds. The research resulted that the phenolic compounds such as rutin, kaempferol and catechin have superior binding affinity compared to two reference drugs, whatever the active site taken. Quercetin also showed very promising anti-viral effects which may be helpful in vaccine development. [10]

These molecules give the best preliminary outcomes with minimized docking scores with the three target sites [10]. Hence, these could be considered potential phytochemicals which are also available in the *Bauhinia vaiegata,* which may make this plant an active source of phytochemicals required against the development of treatment of coronavirus infections.

Considering this research, we can assume that Bauhinia variegata maybe a potential source of bioactive compounds such as kaempferol, rutin, quercetin, gallic acid, lupeol and β-sitosterol, which seems to be useful against the coronavirus infection.

Available drugs for SARS CoV-2 which could be considered as reference for docking studies

Ivermectin is broadly employed for the treatment and cure of numerous tropical illnesses which are commonly neglected. This drug is proved to be excellent as far as safety is concerned as greater than 2.5 billion doses have already reached the population over previous 30 years. According to Caly et al., it has been reported, ivermectin is a competent inhibitor of SARS-CoV-2 replication; hence it has been popularly talked about in global press. [11]

According to Eweas et al., ivermectin and remdesivir have shown excellent binding affinity towards diverse viral proteins. Hence, these may be competent agents to inhibit the action of SARS-CoV-2; yet, clinical trials are necessary, mainly for ivermectin. Ivermectin showed high binding affinity with the viral S protein and human cell surgace receptors (ACE-2 and TMPRSS2). And, remdesivir is an adenosine analog which inhibits the elongation of RNA strand and hence forth inhbiting the viral copying. [12]

Lopinavir, an inhibitor for HIV-1 protease, is used in combination with rotanavir in order to enhance its plasma half life. It is also active with in vitro anti viral inhibition activity for the SARS-CoV-2 strains. Recent studies have shown that lopinavir is not a much effective agent to treat severe cases of SARS CoV-2 infections. However, additional studies of required to estimate the most effective dosage considering along the safety profile of the drug. [13]

Savarino et al first reported that hydroxychloroquine and chloroquine may be potential drugs for the treatment of SARS [14]. Limited in vitro and clinical data in the beginning of pendemic led to use of hydroxychloroquine and chloroquine for treatment of patients

with Covid-19 infection. However, later it was observed that these drugs could lead to certain adverse effects such as cardiac problems when these were used in combination with other agents [15]. Many other considerable evidences came into notice after further researches which make the use of hydroxychloroquine and chloroquine questionable.

Till date, remdesivir and ivermectin are known to be the highest potential drugs for the treatment of Covid-19 patients. [12]

2.6 Molecular docking

Molecular docking approach could be used to search for bioactive compounds present in extracts of B. variegata that exhibit pharmacological effects with specific target plus selective inhibition mechanism [5]. Molecular docking could be performed by various softwares such as Autodock, Autodock Vina, ArgusLab 4.0.1, PyRx software, Open babel and so on. ArgusLab is the most common and freely available software for docking [5,6,7]. We used Autodock software for conducting molecular docking.

We need to check whether Bauhinia variegate is a potential source of phytochemicals in development of treatment against SAR-CoV-2. This could be done by docking the various phytochemicals from the plants with the three target sites of SAR-CoV-2.

CHAPTER 3: RATIONALE AND OBJECTIVE OF STUDY

3.1 Rationale of Study

- *Bauhinia variegata* is a very valuable plant of Indian Himalayan regions for its medicinal significance and therefore, it is widely used in tribal, homeopathic and ayurvedic medicine systems. But it is not much commercially mentioned due to lack of scientific evidences for its pharmacological importance.
- Since, we are focusing on identification of significant phytochemicals from this plant that can potentially interact with SARS-CoV-2 active protein sites and inhibit its mode of action, I would like to state that there have not been any studies yet that has mentioned the interaction of stated phytochemicals from *B. variegata* with SARS-CoV-2 target proteins, which justifies the novelty and authenticity of our research.

3.2 Objective of Study

- To identify different phytochemicals present in *Bauhinia variegata* against SARS-CoV-2.
- To identify the marker compounds which are effective for treatment of SARS-CoV-2.

CHAPTER 4: MATERIALS AND METHODS

4.1 Protein selection and preparation

Three proteins were chosen as target sites of the corona virus. These are: main protease (PDB ID- 5R84), human furin protease (PDB ID - 5MIM) and receptor binding domain (PDB ID - 6VW1). These proteins were procured from online database Protein Data Bank (https://www.rcsb.org) as pdb files. [10]

Since the proteins had multiple binding sites, the structures were edited in BIOVIA Discovery Studio 2021 to eliminate multiple target sites. Further, the structures were minimized in structure visualization software "UCSF Chimera molecular visualization application".

Steps involved are:

i. Open PDB in browser and type protein PDB ID and save protein in pdb format.
ii. Then open UCSF chimera, file --> open-> protein.
iii. Now we have our protein structure built.
iv. Go to tools -> structure editing -> minimize structure (set steepest descent steps-1000, conjugate gradient steps- 1000) -> minimize.
v. Add hydrogen will pop-up, click OK.
vi. Assign charges to minimize will pop-up -> select gasteiger -> click OK.
vii. Net charge is displayed, click OK.
viii. Save protein to current directory (save as .pdb).

4.2 Ligand selection and preparation

Seven ligands were selected along with two reference drugs and verified for Lipinski's rule. Christopher Lipinski and his co-workers studied the physio-chemical properties of greater than 2,000 drugs as well as candidate drugs in clinical trials plus found out that there are greater chances of a compound to be absorbed by the compound if it satisfies the following criteria [17]:

- Molecular weight <500 kcal/mol
- LogP < 5
- Hydrogen-bond donar groups <5

- Hydrogen-bond acceptor groups <10

Ligands are: gallic acid, β-sitosterol, kaempferol, lupeol, kaempferol-3-glucoside, quercetin, rutin and the two reference drugs are ivermectin and lopinavir. These compounds were procured from online database PubChem (https://pubchem.ncbi.nlm.nih.gov/) in form of smiles string and further, these were minimized in the UCSF Chimera following same procedure as for the proteins.

Table 1- Lipinski's rule for molecular properties of ligands

S. no	Compound	Molecular weight (g\mol) <500	H-bond donar <5	H-bond acceptor <10	Log P <5	Pubchem Cid	Molecular formula
1	Gallic acid	170.12	4	5	0.7	370	$C_7H_6O_5$
2	B-sitosterol	414.7	1	1	9.34	222284	$C_{29}H_{50}O$
3	Kaempferol	286.24	4	6	2	5280863	$C_{15}H_{10}0_6$
4	Lupeol	426.7	1	1	9.23	259846	$C_{30}H_{50}O$
5	Kaempferol-3-glucoside	448.4	7	11	0.16	5282102	$C_{21}H_{20}O_{11}$
6	Quercetin	302.23	5	7	1.48	5280343	$C_{15}H_{10}O_7$
7	Rutin	610.5	10	16	-0.33	5280805	$C_{27}H_{30}O_{16}$
8	Remdesivir	602.6	4	13	1.91	121304016	$C_{27}H_{35}N_6O_8P$
9	Hydroxychloroquine	335.9	2	4	3.58	3652	$C_{16}H_{26}ClN_3O$

4.3 Molecular docking

Molecular docking was conducted between the selected proteins with set of selected ligands and reference drugs using Autodock 4.2.6 (http://autodock.scripps.edu/). The purpose of docking proteins with ligands is to understand possible mechanism of protein-ligand binding in comparison to the reference drugs [10].

We found interactions from Autodock in terms of binding energy, electrostatic energy and intermolecular energy [8].

Steps followed:

i. Open Autodock 1.5.6.\
ii. Go to file-> preferences -> set a directory of folder.
iii. File-> read molecule-> protein. Protein structure is displayed.
iv. Change the visual representation of protein to ribbon for more clarity.
v. Change color selection to ss element type-> secondary structure.

vi. Go to edit-> delete water.

vii. Again, edit --> hydrogens -> add -> all hydrogens -> OK.

viii. Again, edit -> charges -> add Kollman charges (Total Kollman charges added appear) -> OK.

ix. Again, edit--> charges ->compute Gasteiger(Total Gasteiger charges added appear) -> OK.

x. Again, edit-> atoms-> assign AD4 type.

xi. File-> save-> write PDBQT-> OK.

xii. Ligand-> input-> open. Switch to all files and select the ligand. Setup ligand name pop-up box appear, click OK.

xiii. Ligand-> output-> save as PDBQT.

xiv. Go to grid -> macromolecule-> choose-> select your protein/macromolecule. Initializing protein.pdb pop-up box appear, click OK. Another pop-up appears: do you want to replace-> click yes.

xv. Go to grid-> grid box:

-Adjust grid to the binding site residue.

-Set the number of x,y,z dimensions.

-Set the spacing accordingly.

xvi. File-> close saving current.

xvii. Grid -> output -> save GPF(grid.gpf).

xviii. Go to run-> autogrid.

Program pathname: go to browse-> this PC-> C drive --> program files(x86)--> the scripps research institute -> autodock -> 4.2.6-> autogrid4

Parameter file: go to browse-> grid.gpf

Log file name: go to browse-> grid.glg

Click launch.

xix. After the autogrid process is complete, a glg file is generated in the working directory.

xx. Then, go to docking-> macromolecule-> set rigidname-> select protein.pdbqt.

xxi. Again, go to docking-> ligand-> choose-> ligand-> accept.

xxii. Again, go to docking-> output-> Lamarckian-> save as dock.dpf.

xxiii. Go to run-> autodock.

Program pathname: go to browse-> this PC-> C drive--> program files(x86)--> the scripps research institute-> autodock -> 4.2.6-> autodock4

Parameter file: go to browse-> dock.dpf
Log file name: go to browse-> dock.dlg

Click launch.

xxiv. A dlg file is generated in the working directory in notepad format.

xxv. Analyze the dlg file for binding energies, intermolecular energies and electrostatic energies.

CHAPTER 5: RESULTS AND DISCUSSION

This research aims at identification of potential bioactive compounds from *Bauhinia variegata* on the basis of their molecular docking results with the target proteins of SARS-CoV-2. SARS-CoV-2 main protease, receptor binding domain and human furin protease are promising targets for antiviral drugs. Hence, targeting these proteins to discover antiviral drugs against corona viruses is a good strategy. [5]

Molecular interaction of potential phytochemicals of *Bauhinia variegata* with corona virus target proteins choosen have been studied using Autodock software, and results are **as shown in Table-2** [16]. Among the number of interactions between the bioactive compounds and target protein site., the hydrogen bonding with the active site residues is most critical and the affinity of this bond is estimated in terms of binding energy(Kcal/mol). Lower the binding energy (more negative), better is the affinity of the bond between the two bonding molecules. [10]

SARS-CoV-2 main protease: lupeol> β-sitosterol> ivermectin> ombuin> quercetin> kaempferol> lopinavir>kaempferol-3-glucoside> gallic acid> rutin

SARS-CoV-2 receptor binding domain: lupeol> ivermectin> β-sitosterol> kaempferol> quercetin> ombuin> kaempferol-3-glucoside> gallic acid> lopinavir> rutin

SARS-CoV-2 human furin protease: lupeol> β-sitosterol> kaempferol> ombuin> quercetin> ivermectin> kaempferol-3-glucoside> gallic acid> lopinavir> rutin

Table 2- Molecular docking results of selected ligands and reference drugs against corona virus target proteins

	SARS-CoV main protease			SARS-CoV receptor binding domain			SARS-CoV human furin protease		
Compound	**BE (kcal/mol)**	**EE (kcal/mol)**	**IE (kcal/mol)**	**BE (kcal/mol)**	**EE (kcal/mol)**	**IE (kcal/mol)**	**BE (kcal/mol)**	**EE (kcal/mol)**	**IE (kcal/mol)**
Gallic acid	-4.77	-0.48	-6.26	-4.86	-0.13	-6.35	-4.68	-0.29	-6.17
Quercetin	-5.73	-0.18	-7.52	-5.79	-0.14	-7.58	-6.53	-0.16	-8.32
Kaempferol	-5.72	-0.20	-7.21	-7.04	-0.13	-8.53	-7.13	-0.36	-8.62
B-sitosterol	**-7.88**	-0.11	-9.97	**-7.07**	-0.04	-9.16	**-7.39**	-0.13	-9.48
Lupeol	**-8.00**	-0.04	-8.60	**-8.30**	0.01	-8.90	**-8.75**	+0.02	-9.35
Kaempferol-3-glucoside	-4.37	-0.30	-7.66	-5.20	-0.31	-8.48	-5.54	-0.44	-8.83

Rutin	-1.36	-0.20	-6.13	-1.10	-0.26	-5.87	-2.66	-0.52	-7.43
Ombuin	-6.01	-0.14	-7.80	-5.56	-0.15	-7.35	-6.71	-0.10	-8.50
***Ivermectin**	-6.39	-0.04	-9.68	-7.54	-0.22	-10.82	-5.77	-0.22	-9.05
***Lopinavir**	-5.05	-0.08	-9.82	-4.39	-0.21	-9.16	-3.77	-0.02	-8.54

BE= Binding Energy, EE= Electrostatic Energy, IE= Intermolecular Energy,

*Ivermectin and lopinavir were taken as reference drugs

We noted that, Lupeol has shown the most promising results no matter what the target site. Also, β-sitosterol, kaempferol, ombuin and quercetin have shown remarkable results, atleast better than the reference drug lopinavir, considering all three target sites.

At active site SARS-CoV main protease: lupeol, β-sitosterol, ombuin, quercetin and kaempferol have shown better binding compared to lopinavir. And, Lupeol and β-sitosterol have shown better binding than ivermectin.

At active site SARS-CoV receptor binding domain: lupeol, β-sitosterol, kempferol, quercetin, ombuin, kaempferol-3-glucoside and gallic acid have shown better binding than lopinavir. And, only lupeol has better binding energy than ivermectin.

At active site SARS-Cov human furin protease: lupeol, β-sitosterol, kaempferol, ombuin, quercetin, kaempferol-3-glucoside and gallic acid have shown better binding energies than lopinavir. And, lupeol, β-sitosterol, kaempferol, ombuin and quercetin have shown better binding than ivermectin.

According to Elmi et al., binding energies for reference drugs, remdesivir and hydroxychloroquine, were calculated to be: [10]

Table 3- Binding energies of another two reference drugs

	Binding energy (kcal/mol)		
Reference drug	Main protease	Receptor binding domain	Human furin protease
Remdesivir	-7.194	-7.851	-5.544
hydroxychloroquine	-5.816	-4.828	-4.277

Considering the reference studies, binding energies of two more reference drugs, **as shown in table 3**, can be compared to the binding energies of the choosen ligands for better results:

Compared to remdesivir:

It is noted that only lupeol shows better binding affinity than remdesivir, whatever the binding site taken. And, β-sitosterol shows better binding affinity with target site SARS-CoV main protease as well as human furin protease.

Compared to hydroxychloroquine:

Lupeol, β-sitosterol and ombuin show better binding affinity, whatever the binding site taken. Quercetin, kaempferol, kaempferol-3-glucoside and gallic acid also show better binding affinity with target site SARS-CoV receptor binding domain as well as human furin protease.

CHAPTER 6: CONCLUSION

Bauhinia variegata is a potential source of phytochemicals that constitute terpenoids, flavanoids, tannins, steroids, reducing sugars and cardiac glycosides which could be considered useful for their pharmacological properties such as anticancer, anti-infammatory, antimicrobial, wound healing, antiulcer and antiobesity.

However, significant observation is that, comparing to four reference drugs that are, ivermectin, lopinavir, remdesivir and hydroxychloroquine; bioactive compounds from *Bauhinia variegata*: lupeol and β-sitosterol have shown excellent docking results. Also, ombuin, kaempferol and quercetin have shown considerably remarkable docking results. This states that these phytochemicals are potential inhibitors of corona virus target proteins (main protease, receptor binding domain and human furin protease) and may be helpful in treatment of infections caused by these viruses.

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