The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

A network pharmacology and molecular docking approach in the exploratory investigation of the biological mechanisms of Lagundi (*Vitex negundo* L.) compounds against Covid-19

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

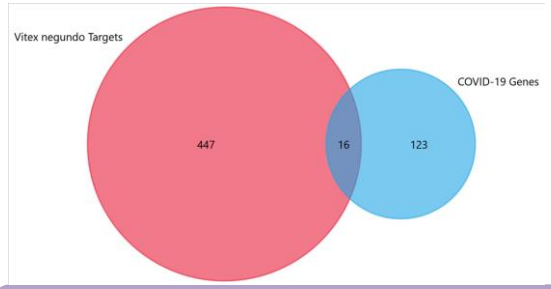
Robertson G. Rivera[1*], Patrick Junard S. Regidor[1], Eric John V. Allanigue[2], Melanie V. Salinas[3], Edwin C. Ruamero Jr[1]

[1] Pharmaceutical Chemistry Department, College of Pharmacy, University of the Philippines Manila, Taft Avenue cor. Pedro Gil St., Ermita, Manila 1000;
[2] Pharmacology Department, College of Medicine, University of the Philippines Manila, Taft Avenue cor. Pedro Gil St., Ermita, Manila 1000
[3] Medical Device-Clinical Research, Safety and Medical Affairs Department, Clinchoice Inc.

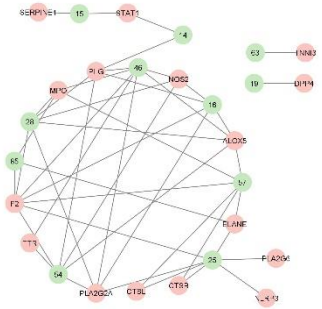
* Corresponding author: rdrivera5@up.edu.ph

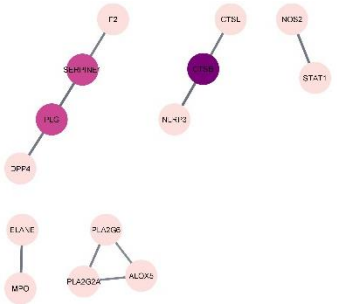
A network pharmacology and molecular docking approach in the exploratory investigation of the biological mechanisms of Lagundi (*Vitex negundo* L.) compounds against Covid-19

Graphical Abstract

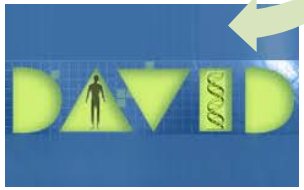


VN compounds review and target identification



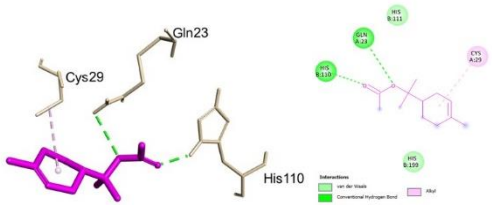


Network construction (compound target and protein-protein interaction)



Gene Ontology and KEGG Pathways Enrichment Analysis

Targets of Ashitaba Compounds:
cathepsin B, plasminogen activator inhibitor-1, and plasminogen



Molecular Docking

ECMC 2022
The 8th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2022 | ONLINE

Abstract

Covid-19 is an inflammatory and infectious disease caused by SARS-CoV-2 virus with a complex pathophysiology. While Covid-19 vaccines and boosters are available, treatment of the disease is primarily supportive and symptomatic. Several research have suggested the potential of herbal medicines as an adjunctive treatment for the disease. A popular herbal medicine approved in the Philippines for the treatment of acute respiratory disease is *Vitex negundo* L. In fact, the Department of Science and Technology of the Philippines has funded a clinical trial to establish its potential as an adjunctive treatment for Covid-19. Here, we utilized network pharmacology and molecular docking in determining pivotal targets of *Vitex negundo* compounds against Covid-19. The results showed that significant targets of *Vitex* negundo compounds in Covid-19 are CSB, SERPINE1 and PLG which code for cathepsin B, plasminogen activator inhibitor-1, and plasminogen, respectively. Molecular docking revealed that α-terpinyl acetate and geranyl acetate have good binding affinity in cathepsin B; 6,7,4-trimethoxyflavanone, 5,6,7,8,3',4',5'-heptamethoxyflavone, artemetin, demethylnobiletin, gardenin A, geranyl acetate in plasminogen; and 7,8,4-trimethoxyflavanone in plasminogen activator inhibitor-1. While the results are promising, these are bound to the limitations of computational methods and further experimentation are needed to completely establish the molecular mechanisms of *Vitex negundo* against Covid-19.

Keywords: lagundi, *Vitex negundo,* Covid-19, herbal medicine, network pharmacology

Introduction

Covid-19

- an inflammatory disease with a complicated pathophysiology; has a systemic effect in the body and physiological functions.
- Up to date, there are no medications which can cure Covid-19 and the clinical care is mostly supportive in nature and are done to alleviate symptoms/
- Several research have pointed out the possible utilization of herbal medicines to help in the management of Covid-19.

Clinical management of COVID-19: Living guideline, 23 June 2022. Accessed September 13, 2022. https://www.who.int/publications-detail-redirect/WHO-2019-nCoV-clinical-2022-1

Demeke CA, Woldeyohanins AE, Kifle ZD. Herbal medicine use for the management of COVID-19: A review article. *Metabolism Open*. 2021;12:100141. doi:10.1016/j.metop.2021.100141

Silveira D, Prieto-Garcia JM, Boylan F, et al. COVID-19: Is There Evidence for the Use of Herbal Medicines as Adjuvant Symptomatic Therapy? *Frontiers in Pharmacology*. 2020;11. Accessed September 13, 2022. https://www.frontiersin.org/articles/10.3389/fphar.2020.581840

Introduction

Lagundi (*Vitex negundo)* (abbreviated as VN)

- Approved herbal medicine in the Philippines for the treatment of mild to moderate cough and has been shown to elicit bronchodilating effects
- There is an ongoing clinical trial on the potential of VN against Covid-19 under the Philippine's Department of Science and Technology.
- However, up to date, the Philippine COVID-19 Living Clinical Practice Guidelines has not approved the use of VN as an adjunctive treatment for Covid-19
  - its molecular mechanism has not been elucidated yet
  - Only few studies have explored the biological effects of VN compounds in Covid 19: in silico screening of VN compounds against SARS-CoV-2 Mpro receptor and against the papain-like protease of SARS-CoV-2

Alegado-Bagaoisan DM, Castro MCR, Purificacion JM. A Systematic Review on Vitex negundo (NIRPROMP formulations) for the Treatment of Acute Cough of Mild to Moderate Severity in Pediatric Patients. *Acta Medica Philippina*. 2020;54(1). doi:10.47895/amp.v54i1.1096

Philippine COVID-19 Living Recommendations -. Published June 18, 2022. Accessed September 13, 2022. https://www.psmid.org/philippine-covid-19-living-recommendations-3/

Cayona R, Creencia E. Discovery of a "Cocktail" of Potential SARS-COV-2 Main Protease Inhibitors through Virtual Screening of Known Chemical Components of Vitex negundo L. ("Lagundi"). *Med Chem*. 2022;18(3):364-381. doi:10.2174/1573406417666210618132003

Mitra D, Verma D, Mahakur B, et al. Molecular docking and simulation studies of natural compounds of Vitex negundo L. against papain-like protease (PLpro) of SARS CoV-2 (coronavirus) to conquer the pandemic situation in the world. *J Biomol Struct Dyn*. 2022;40(12):5665-5686. doi:10.1080/07391102.2021.1873185

Introduction

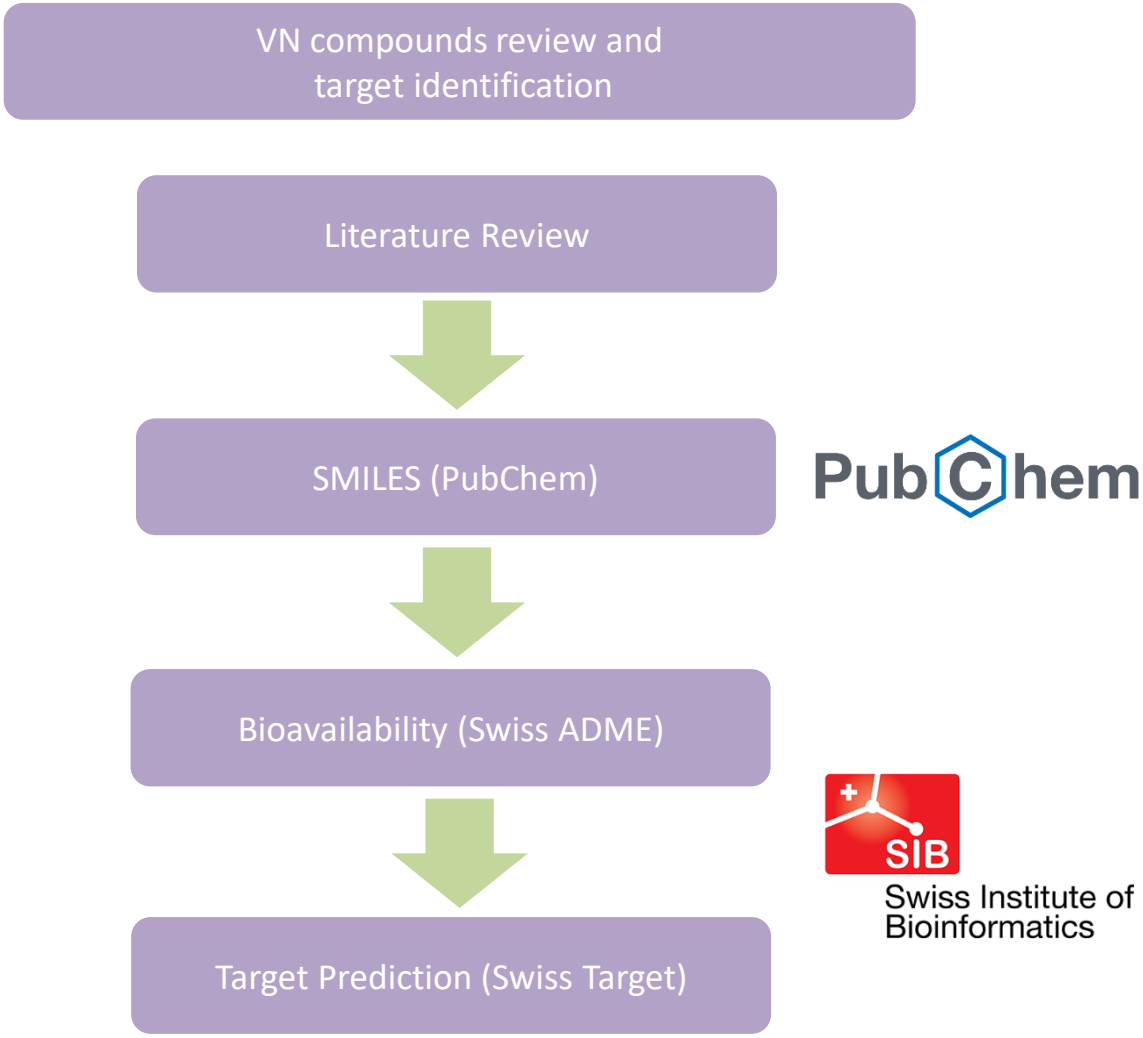
Network Pharmacology

- Integration of biology and polypharmacology via computational methods
- Compound-target-pathway networks analysis

Chandran U, Mehendale N, Patil S, Chaguturu R, Patwardhan B. Network Pharmacology. *Innovative Approaches in Drug Discovery*. Published online 2017:127-164. doi:10.1016/B978-0-12-801814-9.00005-2

Nogales C, Mamdouh ZM, List M, Kiel C, Casas AI, Schmidt HHHW. Network pharmacology: curing causal mechanisms instead of treating symptoms. *Trends in Pharmacological Sciences*. 2022;43(2):136-150. doi:10.1016/j.tips.2021.11.004

Methods



L. Zhang *et al.*, "Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking," *Computational Biology and Chemistry*, vol. 94, p. 107396, Oct. 2021, doi: 10.1016/j.compbiolchem.2020.107396.

Methods

Covid-19 Genes (MalaCards)

Venn Diagram with VN Targets

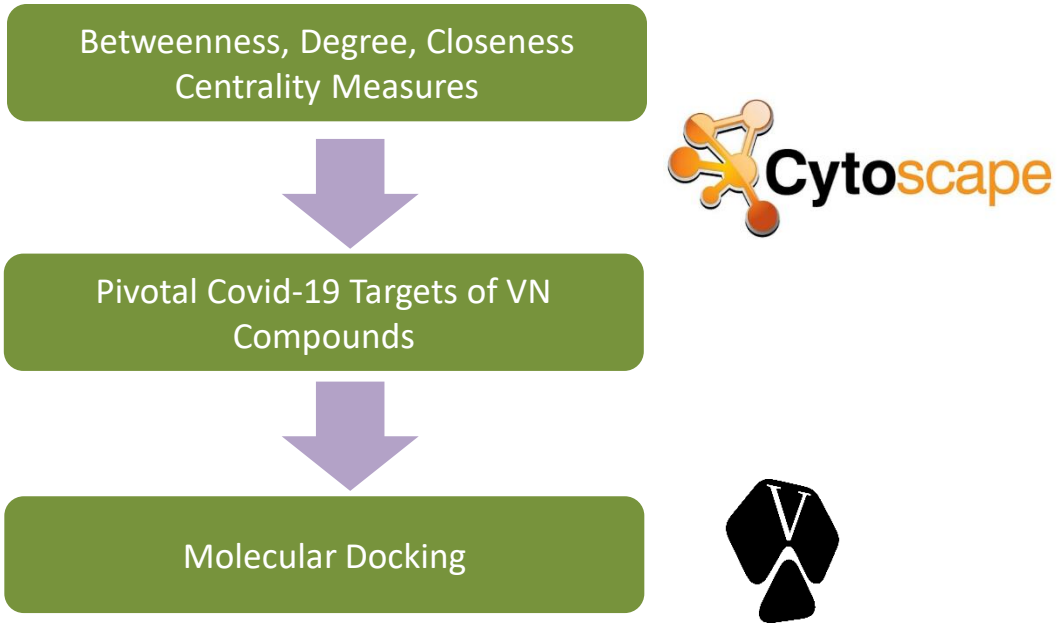
Compound-Target Network Construction

Protein-Protein Interaction Analysis

Gene Ontology and KEGG Pathway Enrichment Analysis

L. Zhang *et al.*, "Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking," *Computational Biology and Chemistry*, vol. 94, p. 107396, Oct. 2021, doi: 10.1016/j.compbiolchem.2020.107396.

Methods



L. Zhang *et al.*, "Exploring the mechanism of Cremastra Appendiculata (SUANPANQI) against breast cancer by network pharmacology and molecular docking," *Computational Biology and Chemistry*, vol. 94, p. 107396, Oct. 2021, doi: 10.1016/j.compbiolchem.2020.107396.

Results and discussion

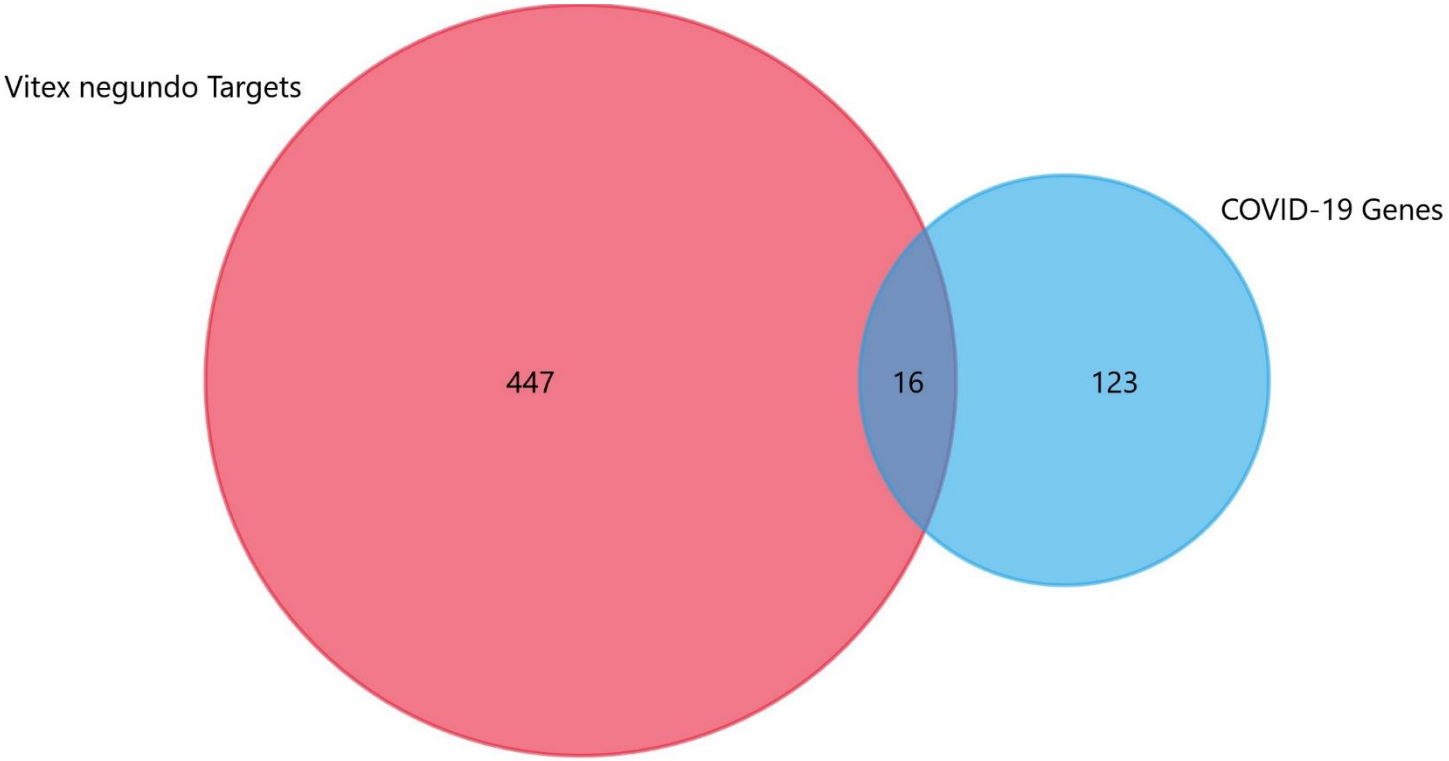


Figure 1. Venn diagram showing the intersection of the Covid-19 genes and the *Vitex negundo* L. compound targets

Results and discussion

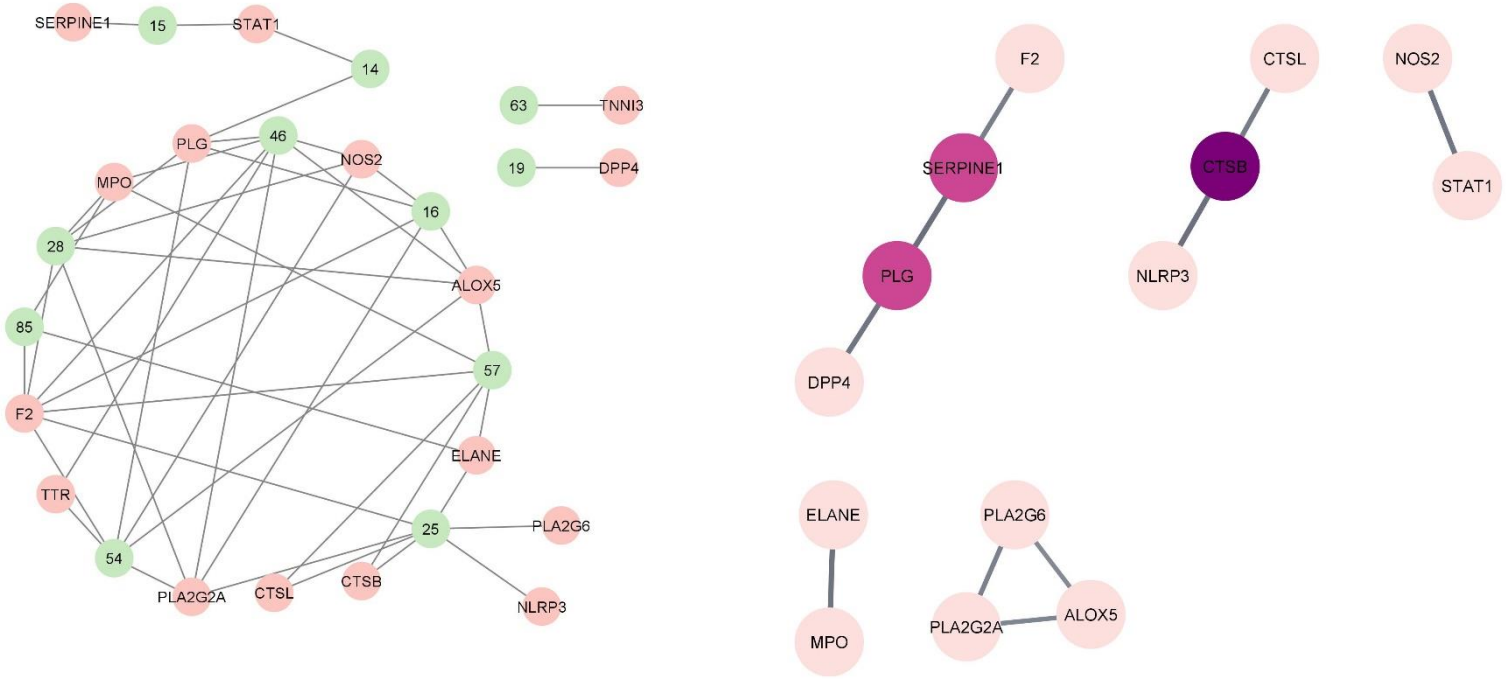


Figure 2. Compound-target network (left): Represented in each node are the compounds (green) and the targets (pink). Each line represents the non-directional interaction between compounds. (right): Protein-protein interaction of the Covid-19 targets of Vitex negundo compounds. The darker the color, the higher is the degree centrality.

Results and discussion

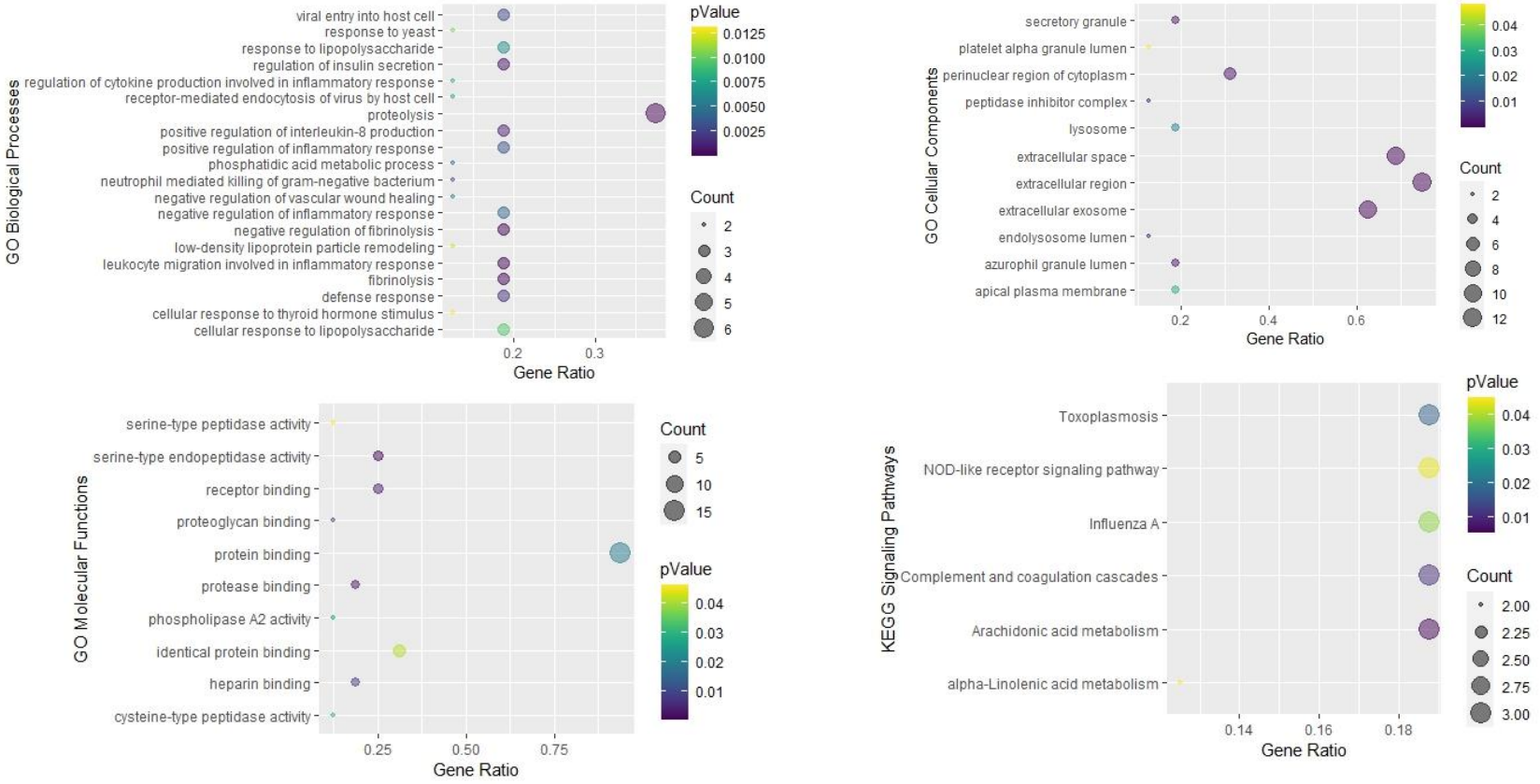


Figure 3. Bubble plot of gene ontology and KEGG pathways enrichment analysis showing the biological processes, cellular components, molecular functions and KEGG pathways which are associated with the Covid-19 proteins targeted by VN. The most enriched pathways are the arachidonic acid metabolism and complement and coagulation pathways.

Results and discussion

There are 229 documented compounds of VN; 89 of which are found either in leaves or in the unspecified parts of the plant; 20 good oral bioavailability and have passed the druglikeness rules; 447 total VN compounds targets; 123 Covid-19 associated genes

16 genes associated with Covid-19 targeted by VN compounds

In the present study, network pharmacology was utilized to determine the targets of VN compounds in Covid-19. Through this method, it was identified that **CTSB, SERPINE1 and PLG which codes for cathepsin B, plasminogen activator inhibitor-1 and plasminogen, respectively** are the pivotal genes targeted by VN in Covid-19. Moreover, molecular docking was conducted to determine the possible binding of VN compounds towards these identified targets.

Results and discussion

Table 1. Calculated binding energies of the different VN compounds when docked to the different pivotal proteins.

Protein	Ligand	Binding Energy (kcal/mol)
PLG-Plasminogen (PDB ID: 1qrz)	6,7,4-trimethoxy- flavanone	-6.3
	5,6,7,8,3',4',5'-hepta-methoxyflavone	-5.5
	artemetin	-6.3
	demethyl-nobiletin	-5.7
	gardenin A	-5.6
	geranyl acetate	-5.4

Results and discussion

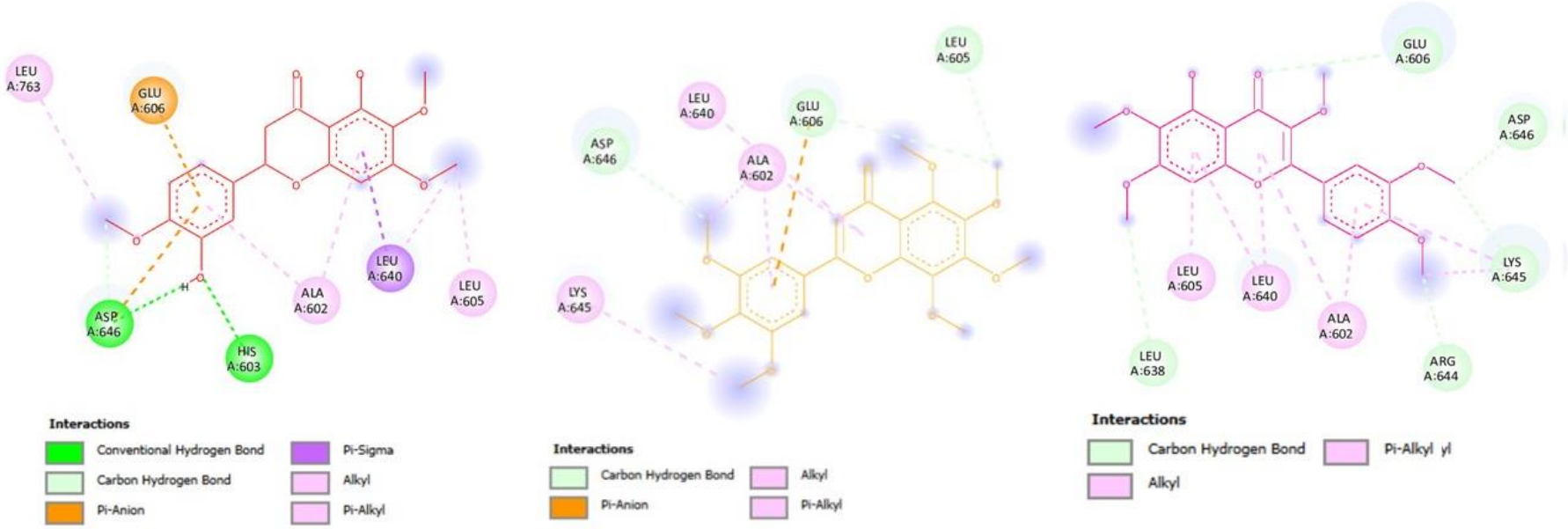


Figure 4A. Protein-ligand interactions between plasminogen (PDB ID:1qrz) and 6,7,4-trimethoxyflavanone (left), 5,6,7,8,3`,4`,5`-heptamethoxyflavone (center) and artemetin (right)

Results and discussion

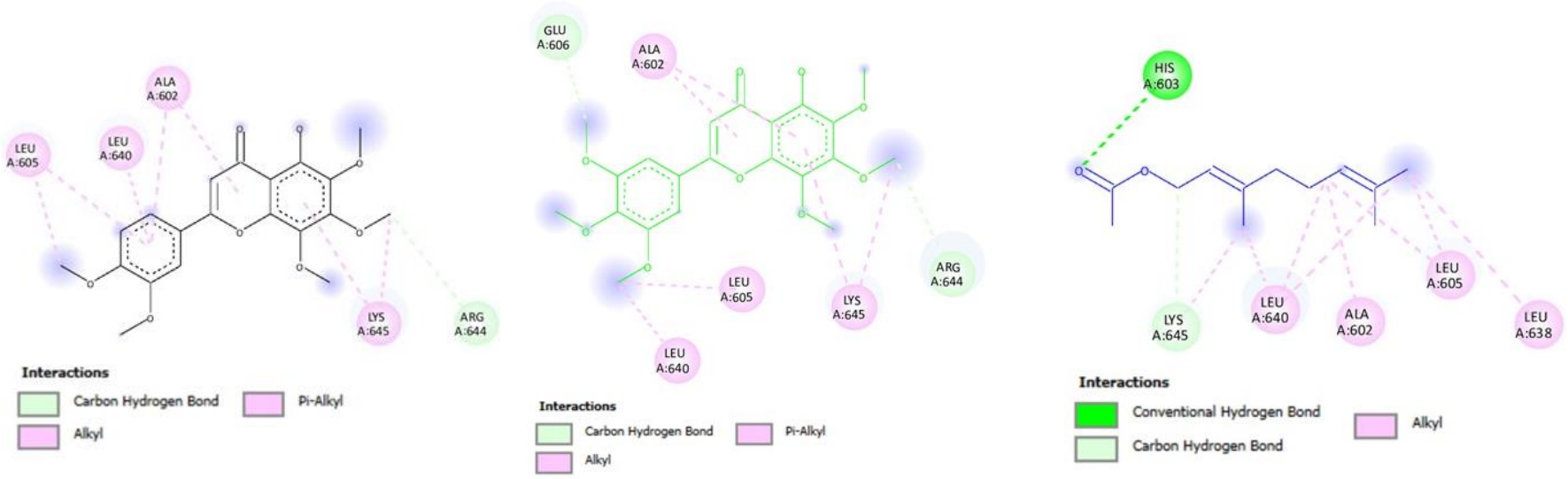


Figure 4B. Protein-ligand interactions between plasminogen (PDB ID:1qrz) and demethylnobiletin (left), gardenin A (center) and geranyl acetate (right)

Results and discussion

PLG codes for the protein **plasminogen** which is the precursor of plasmin, that in turn functions in **clot dissolution and extracellular matrix protein degradation**. Moreover, plasmin may activate intracellular signaling pathways which include **expression of proinflammatory genes.**

- **plasminogen and plasmin contribute to susceptibility to COVID-19** and that in vitro experiments revealed that plasmin cleaves protein S of SARS-CoV allowing it to penetrate the cellular host

In the present study, six VN compounds were shown to have favorable binding affinity towards plasminogen with calculated binding energies ranging from -5.4 to -6.3 kcal/mol

Didiasova M, Wujak L, Wygrecka M, Zakrzewicz D. From Plasminogen to Plasmin: Role of Plasminogen Receptors in Human Cancer. *Int J Mol Sci*. 2014;15(11):21229-21252. doi:10.3390/ijms151121229
Ji HL, Zhao R, Matalon S, Matthay MA. Elevated Plasmin(ogen) as a Common Risk Factor for COVID-19 Susceptibility. *Physiological Reviews*. 2020;100(3):1065-1075. doi:10.1152/physrev.00013.2020

Results and discussion

Table 1. (Cont.)

Protein	Ligand	Binding Energy (kcal/mol)
CTSB-Cathepsin B (PDB ID: 1csb)	α-terpinyl acetate	-5.5
	geranyl acetate	-5.3
	EP048*	-7.4
CTSB-cathepsin B (PDB ID: 1huc)	α-terpinyl acetate	-5.5
	geranyl acetate	-5.1

Results and discussion

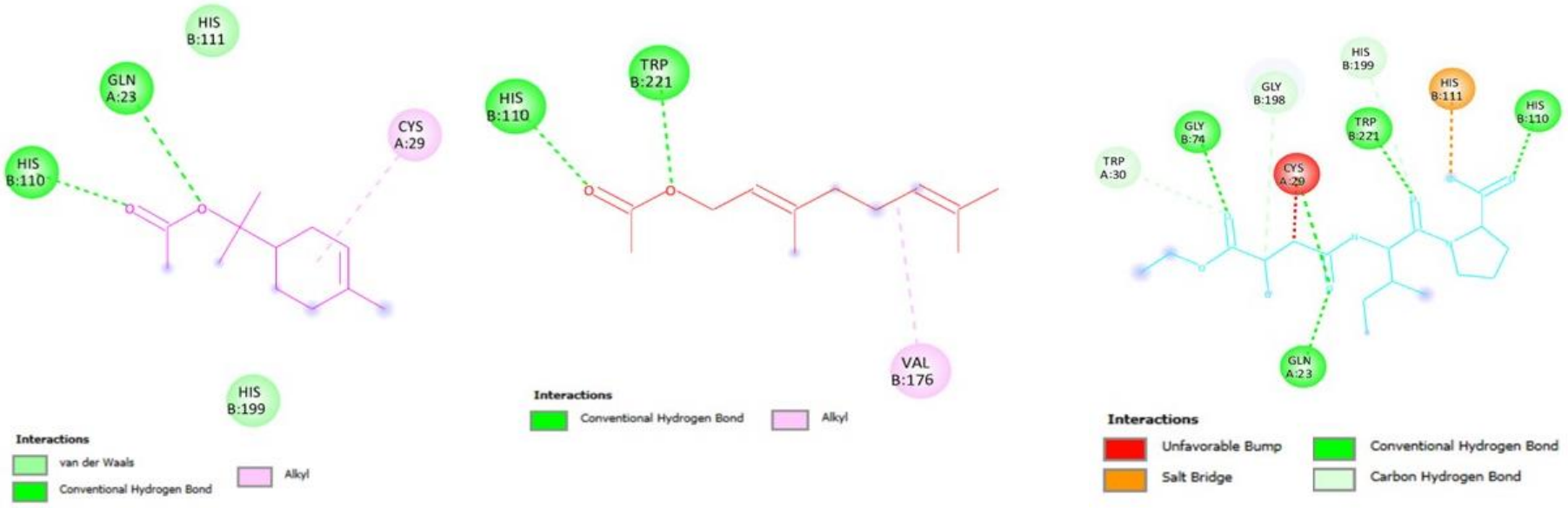


Figure 5A. Protein-ligand interactions between cathepsin B (PDB ID:1csb) and α-terpinyl acetate (left), geranyl acetate (center), and the co-crystallized ligand EP048 (right).

Results and discussion

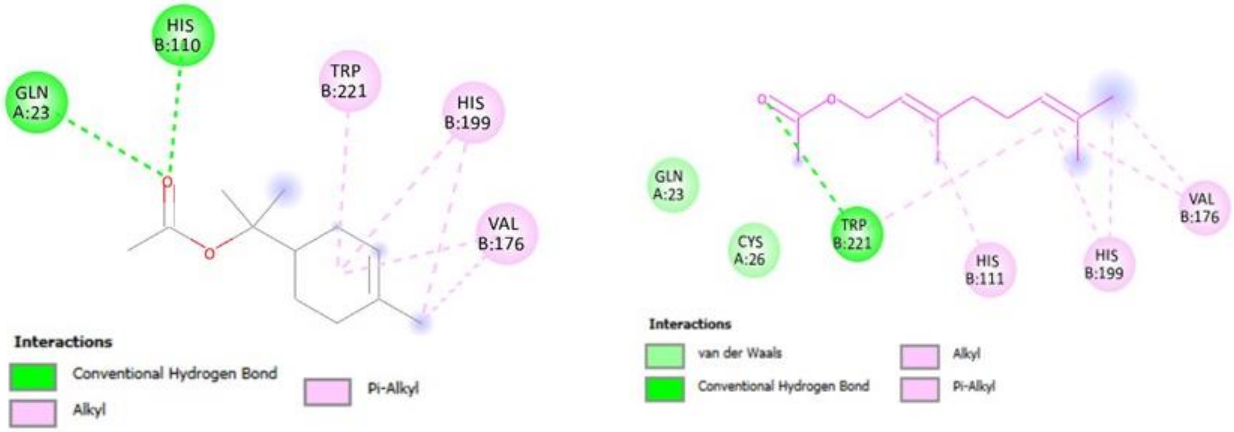


Figure 5B. Protein-ligand interactions between cathepsin B (PDB ID:1huc) and α-terpinyl acetate (left), and geranyl acetate (right).

Results and discussion

Cathepsin B which is a member of cysteine proteases that exhibits both endopeptdase and exopeptidase activity. **In COVID-19, CTSB is upregulated.**

- It also interacts with proteins that are involved in antigen processing, presentation, and inflammatory responses.
- CTSB is involved in the entry of the COVID-19 virus within cells via the endosomal pathway by cleaving and activating protein S.
- Expression of CTSB in COVID-19 may also lead to an increased risk of COVID-19 infection44

In our current work, two VN compounds have been found to bind favorably to cathepsin B which include geranyl acetate and α-terpinyl acetate with a little over 5.0 kcal/mol binding energies

Cavallo-Medved D, Moin K, Sloane B. Cathepsin B. *AFCS Nat Mol Pages*. 2011;2011:A000508.

Ding X, Ye N, Qiu M, et al. Cathepsin B is a potential therapeutic target for coronavirus disease 2019 patients with lung adenocarcinoma. *Chemico-Biological Interactions*. 2022;353:109796. doi:10.1016/j.cbi.2022.109796

Prasad K, AlOmar SY, Almuqri EA, Rudayni HA, Kumar V. Genomics-guided identification of potential modulators of SARS-CoV-2 entry proteases, TMPRSS2 and Cathepsins B/L. *PLoS One*. 2021;16(8):e0256141. doi:10.1371/journal.pone.0256141

Results and discussion

Table 1. (Cont.)

Protein	Ligand	Binding Energy (kcal/mol)
SERPINE1-Plasminogen activator inhibitor-1 (PDB ID: 4aqh)	7,8,4-trimethoxy-flavanone	-6.9
	TB71*	-8.9
SERPINE1-Plasminogen activator inhibitor-1 (PDB ID: 7aqf)	7,8,4-trimethoxy-flavanone	-6.5
	RV2401*	-7.1

Results and discussion

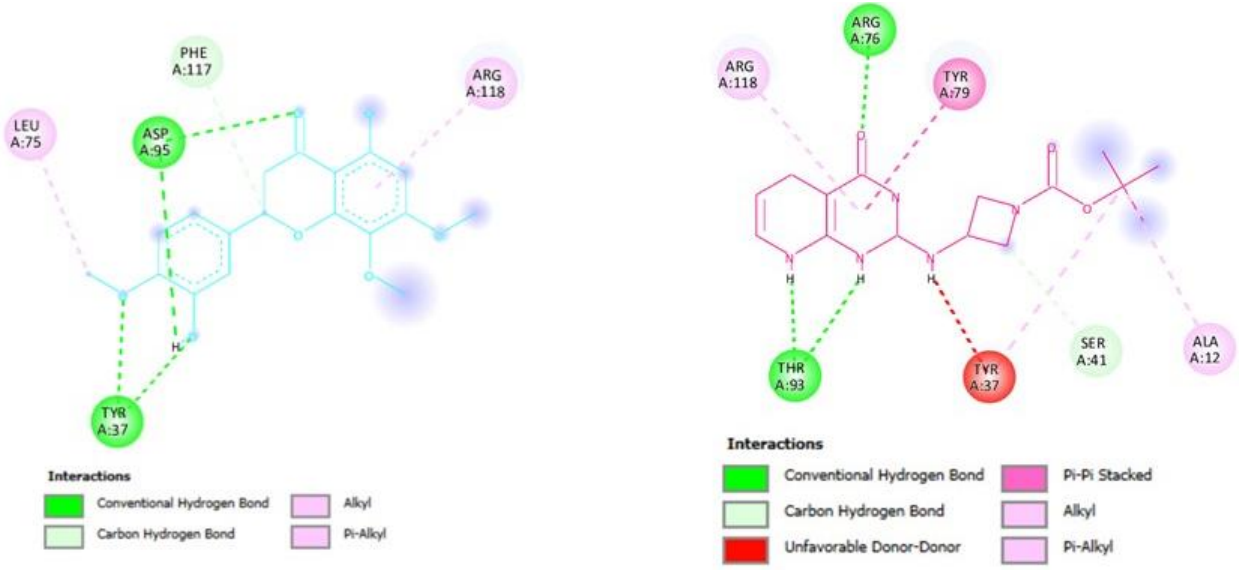


Figure 6A. Protein-ligand interactions between plasminogen activator inhibitor-1 (PDB ID:4aqh) and 7,8,4-trimethoxyflavanone (left), and the co-crystallized ligand TB71(right).

Results and discussion

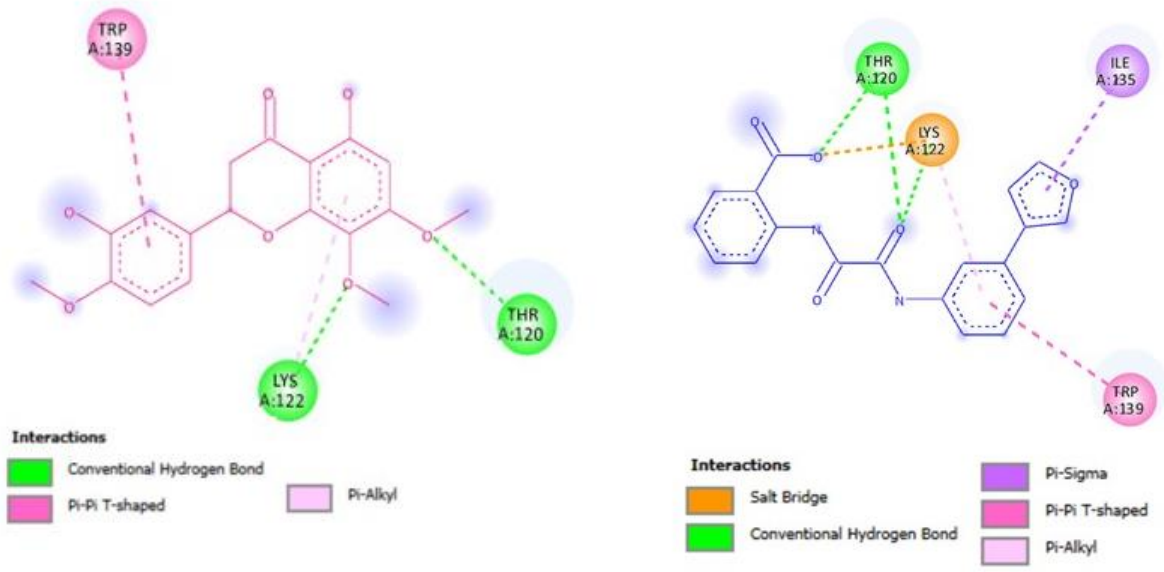


Figure 6B. Protein-ligand interactions between plasminogen activator inhibitor-1 (PDB ID:7aqf) 7,8,4-trimethoxyflavanone (left), and the co-crystallized ligand RV2401 (right).

Results and discussion

SERPINE1 codes for the protein plasminogen activator inhibitor 1 (PAI-1) that inhibits the activation of the tissue-type plasminogen activator, hence preventing the fibrinolytic process.

- **Abnormalities in the interplay between plasminogen activators and impairment in fibrinolysis has been seen in Covid-19 patients leading to thrombotic events and coagulopathies.**
- The level of PAI-1 is increased in Covid-19 patients and is associated with worse respiratory status and poor clinical outcomes.

Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. Plasminogen Activator Inhibitor-1 and Thrombotic Cerebrovascular Diseases. *Stroke*. 2012;43(10):2833-2839. doi:10.1161/STROKEAHA.111.622217

Zuo Y, Warnock M, Harbaugh A, et al. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. *Sci Rep*. 2021;11(1):1580. doi:10.1038/s41598-020-80010-z

Whyte CS, Simpson M, Morrow GB, et al. The suboptimal fibrinolytic response in COVID-19 is dictated by high PAI-1. *J Thromb Haemost*. Published online July 21, 2022:10.1111/jth.15806. doi:10.1111/jth.15806

Results and discussion

Here, 5,3'-dihydroxy-7,8,4-trimethoxyflavanone binds favorably to PAI-1 with a calculated binding affinity of -6.9 kcal/mol.

- However, the amino acid residues involved in the protein ligand interactions of inhibitors (TB701 and RV2401) and PAI-1 are not found in the protein ligand interactions of the flavanone and the said receptor.
- the presence of thiophene rings with bulky or hydrophobic substituents and a carboxylic acid moiety make the compound a potent PAI-1 inhibitor
- **These structural features are not present in the flavanone structure. This may indicate that the flavanone binds in a different conformation than that of the inhibitors and may or may not exhibit an activity**

Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. Plasminogen Activator Inhibitor-1 and Thrombotic Cerebrovascular Diseases. *Stroke*. 2012;43(10):2833-2839. doi:10.1161/STROKEAHA.111.622217

Zuo Y, Warnock M, Harbaugh A, et al. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. *Sci Rep*. 2021;11(1):1580. doi:10.1038/s41598-020-80010-z

Whyte CS, Simpson M, Morrow GB, et al. The suboptimal fibrinolytic response in COVID-19 is dictated by high PAI-1. *J Thromb Haemost*. Published online July 21, 2022:10.1111/jth.15806. doi:10.1111/jth.15806

Yamaoka N, Kawano Y, Izuhara Y, Miyata T, Meguro K. Structure-Activity Relationships of New 2-Acylamino-3-thiophenecarboxylic Acid Dimers as Plasminogen Activator Inhibitor-1 Inhibitors. Chem Pharm Bull. 2010;58(5):615-619. doi:10.1248/cpb.58.615

Conclusions

- Pivotal genes in Covid-19 that are targeted by the reported lagundi compounds include CSB, SERPINE1 and PLG which codes for cathepsin B, plasminogen activator inhibitor-1 and plasminogen, respectively.

- Molecular docking revealed that α-terpinyl acetate and geranyl acetate have good binding affinity in cathepsin B; 6,7,4-trimethoxyflavanone, 5,6,7,8,3',4',5'-heptamethoxyflavone, artemetin, demethylnobiletin, gardenin A, geranyl acetate in plasminogen; and 7,8,4-trimethoxyflavanone in plasminogen activator inhibitor-1.

Conclusions

- Limitation of this study relies on the fact that these are computational predictions and further experimentations are needed.

- Moreover, the quantity and potency of these compounds present in Vitex negundo, and the possibility of synergism further complicates the establishment of the potential of VN against Covid-19.