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Screening of *Plectranthus amboinicus* against COVID-19 — *in silico* approach

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ARTICLE INFO	ABSTRACT
	The present study aimed to investigate the reported bioactives from *Plectranthus amboinicus* as the inhibitor of novel coronavirus (COVID-19) targets, i.e., 3CLPro, PLpro, and spike protein, via *in silico* molecular docking and other computational approaches. The recorded bioactives were evaluated for their druglikeness characteristics using MolSoft based on the Rule of Five and the probable targets of each compound were identified using DIGEP-Pred. The bioactives were docked against 3CLPro, PLpro, and spike protein using AutoDock 4.0. In addition, the enrichment analysis of regulated proteins was carried out using STRING. Plectranthol B scored the highest druglikeness score. Additionally, Plectranthol A and B were predicted as the lead hits based on the molecular docking study. Similarly, the combined synergy of the bioactives identified the modulation of SUMOylation of intracellular receptors and the nuclear receptor transcription pathway. Furthermore, the study revealed the utilization of the system biology approach to identify the lead molecules from *P. amboinicus* against COVID-19 management from the traditional medicinal system.

INTRODUCTION

Recently, the novel coronavirus (COVID-19) has led to millions of deaths throughout the world (WHO, 2019b). Multiple preventive approaches have been suggested to avoid the infection of COVID-19, like social distancing, regular cleaning the hands with sanitizer, using masks to cover mouth and nose in a crowd, and regular health check-ups (WHO, 2019a). Furthermore, investigations are ongoing in order to identify new drug molecules in the management of COVID-19, which may still take time as the drug development process is quite complicated and is time-consuming. Hence, it is necessary to identify alternative sources against COVID-19 that can be processed via traditional data mining.

Plectranthus amboinicus is a Mexican mint that belongs to the family Lamiaceae and has been reported for its multiple pharmacological activities, including anti-viral

activities (Arumugam *et al*., 2016). Further, multiple extracts of *P. amboinicus* have been investigated against herpes and HIV (Arumugam *et al*., 2016). Likewise, *P. amboinicus* is also used to manage respiratory problems and is also a potent anti-inflammatory agent (Arumugam *et al*., 2016; Gurgel *et al*., 2009) that possesses anti-inflammatory activity in virus-infected tissues. The literature studies suggest the necessity of investigating *P. amboinicus* against COVID-19 as this infection is associated with respiratory problems and inflammation.

Hence, the present study utilized *in silico* tools to investigate the reported bioactives from *P. amboinicus* against three reported targets of COVID-19, i.e., PLpro, 3clpro, and spike protein, followed by the effect of bioactives on probably regulated pathways using the Gene ontology (GO) analysis of possibly modulated protein-based targets.

MATERIALS AND METHODS

Compounds and their targets

Phytoconstituents present in *P. amboinicus* were retrieved from the ChEBI database (https://www.ebi.ac.uk/chebi/) along with their simplified molecular-input line-entry system (SMILES). The 2D structure of retrieved compounds is shown

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in Figure 1. The 3D structures of each compound were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in a .sdf format and converted into a .pdb format using Discovery Studio 2019 (Dassault Systèmes BIOVIA, 2019). The protein-based target of each compound was retrieved from DIGEP-Pred (Lagunin et al., 2013) at the cutoff value of 0.7 probable activity.

Druglikeness score of compounds

The druglikeness score of each bioactives was predicted using MolSoft (https://molsoft.com/mprop/) which was based on molecular weight, the number of hydrogen bond donors/acceptors, and MolLogP as explained by Lipinksi's Rule of Five (Lipinski, 2004).

In silico molecular docking

Ligand preparation

The .sdf 3D files of the ligand were converted into .pdb files using Discovery Studio 2019 (Dassault Systèmes BIOVIA, 2019). The energy of individual molecules was minimized using Merck Molecular Force Field 94 (mmff94) (Halgren, 1996) and then converted into a .pdbqt format.

Macromolecule preparation

The 3D structures of 3CLpro (PDB: 6LU7), PLpro (PDB: 4M0W), and spike proteins [homology modeled target, accession number: AVP78042.1 as query sequence, and PDB: 6VSB as a template using the Swiss Model (Schwede et al., 2003)] were obtained from the RCSB database which was in a complex with other heteromolecules. These heteromolecules were removed and saved in .pdb files using Discovery Studio 2019 and then converted into a .pdbqt format as a macromolecule for docking.

Molecular docking

Molecular docking was carried out using AutoDock 4 (Morris et al., 2009). After docking, 10 different ligand poses were obtained. The ligand pose scoring the lowest binding energy (kcal/mol) was chosen to visualize the ligand–protein interaction using Discovery Studio 2019.

Enrichment analysis

The combined list of modulated genes was queried in STRING (Szklarczyk et al., 2019) and the modulated pathways were identified by checking the Reactome database (www.reactome.org). The interaction of bioactives, their targets, and respective pathways were constructed using Cytoscape (Shannon et al., 2003). The node size and the map were used to analyze the network interaction using a network analyzer.

RESULTS

Compounds and their targets

Five different bioactives, i.e., 11, 20-dihydroxysugiol, 11-hydroxysugiol, plectranthol A, plectranthol B, and abietatriene, were recorded as major bioactives in the ChEBI
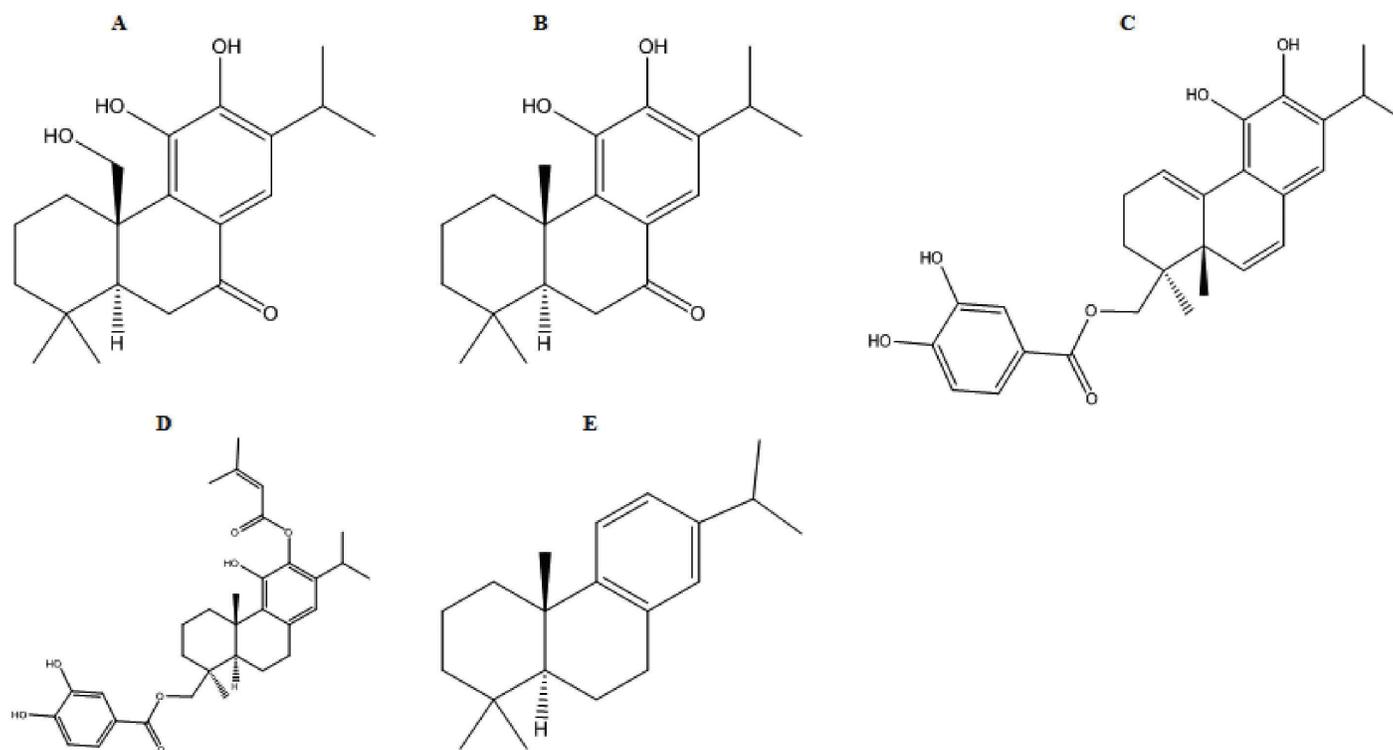
Figure 1. 2D structure of (a) 11,20-dihydroxysugiol, (b) 11-hydroxysugiol, (c) plectranthol A, (d) plectranthol B, and (e) abietatriene.

database for the keyword "*P. amboinicus*". These molecules were identified to downregulate multiple proteins, i.e., vitamin D3 receptor (VDR) was downregulated by all compounds, retinoic acid receptor alpha (RARα) by plectranthol B and CD83 by abietatriene.

Druglikeness score of compounds

Plectranthol B was predicted to possess the highest druglikeness score, i.e., 1.47, with a molecular weight of 536.28, the number of hydrogen bond acceptors (7) and donors (3), and MolLogP of 7.18. The details of each compound for their druglikeness score are summarized in Table 1.

In silico molecular docking

The molecular docking process identified plectranthol A and plectranthol B to possess the highest binding affinities, i.e., −7.9 kcal/mol with PLpro, with an equal number of hydrogen bond interactions. Although plectranthol B showed the highest binding affinity (−8.9 kcal/mol) with 3CLpro, plectranthol A shared the highest number of hydrogen bond interactions, i.e., eight with 3CLpro. Similarly, plectranthol A was predicted to possess the highest binding affinity (−8.4 kcal/mol) and hydrogen bond interactions with spike protein. The binding affinity of an individual ligand with each compound is summarized in Table 2–4. The 3D interactions of each ligand molecule with each PLpro, 3CLpro, and spike protein are shown in Figures 2–4 respectively.

Enrichment analysis

The combined GO analysis of modulated targets identified two pathways to be majorly regulated with special reference to the Reactome pathway database, i.e., SUMOylation of intracellular receptors (HSA-4090294) and nuclear receptor transcription pathway (HSA-383280): modulated RARα and VDR. The interaction of each bioactive with its targets and modulated protein is shown in Figure 5.

DISCUSSION

This study aimed to investigate the bioactives from *P. amboinicus* against the three targets of COVID-19, i.e., PLpro, 3CLpro, and spike protein, and their possible role in modulating multiple pathways. Furthermore, this study also calculated the probable druggability characteristics based on the Rule of five. Traditional medicines possess an important role in the management of infectious (Pinn, 2001) and non-infectious

diseases (Duyu *et al.*, 2020; Hughes *et al.*, 2015; Khanal and Patil, 2019; Khanal and Patil, 2020a, 2020b; Patil *et al.*, 2019; Rodrigues *et al.*, 2020a, 2020b). Multiple approaches have been made to investigate the bioactives from traditional medicines using *in silico* approaches which often help to identify the probable lead hit molecules (Duyu *et al.*, 2020; Khanal *et al.*, 2019a, 2019b, 2019c; Patil *et al.*, 2019; Rodrigues *et al.*, 2020a, 2020b; Ternikar *et al.*, 2020). Nonetheless, approaches have been made to investigate the probable anti-viral agents against COVID-19 using molecular docking, network pharmacology, and enrichment analysis (Balkrishna *et al.*, 2020; Khanal *et al.*, 2020a, 2020b, 2020c). Hence, in the present study, we aimed to investigate the bioactives of *P. amboinicus* against COVID-19.

Initially, the bioactives of *P. amboinicus* were retrieved from the ChEBI database and their proteins and targets were predicted, which were further queried in STRING; they also identified the SUMOylation of intracellular receptors (HSA-4090294) and nuclear receptor transcription pathway (HSA-383280) as modulated pathways. The previous report identified the involvement of viral infections with the SUMOylation of IRF3 and IRF7, leading to the downregulation of type I Interferon gene expression (Kubota *et al.*, 2008). In the present study, the combined action of 11,20-dihydroxysugiol, 11-hydroxysugiol, plectranthol A, plectranthol B, and abietatriene was identified to regulate the SUMOylation of intracellular receptors by modulating RARα and VDR. Furthermore, the controversial reports with vitamin D and associated genes in viral infection (Lee, 2020) need to be investigated.

In the present study, all the bioactives were identified to downregulate RARα, except abietatriene. However, previous reports reflect the inhibition of retinoid-associated receptors that may lead to prone viral infections (Song *et al.*, 2018). Furthermore, a previous study reflects the anti-viral potency of *P. amboinicus* (Arumugam *et al.*, 2016); however, anti-viral efficacy of individual bioactives needs to be still investigated.

Table 2. Binding energy with PLpro (PDB: 4M0W).

Ligand	Binding energy (Kcal/mol)	Number of hydrogen bonds	Hydrogen bond residues
11,20-dihydroxysugiol	−6.8	2	TYR265, GLU168
11-hydroxysugiol	−7	1	TYR274
Plectranthol A	−7.9	2	GLU204, ARG167
Plectranthol B	−7.9	2	ARG167, THR171
Abietatriene	−7.3	–	–

Table 1. Druglikeness score of bioactives from *P. amboinicus*.

| Phytoconstituents	Molecular formula	Molecular weight	Number of HBA	Number of HBD	MolLogP	MolLogS Log (moles/l)	MolLogS mg/l	MolPSA (A²)	MolVol (A³)	Druglikeness score
Plectranthol A	$C_{27}H_{30}O_6$	450.20	6	4	5.99	−5.52	1.37	84.99	491.44	1.11
Plectranthol B	$C_{32}H_{40}O_7$	536.28	7	3	7.18	−5.84	0.77	89.74	598.82	1.47
11,20-dihydroxysugiol	$C_{20}H_{28}O_4$	332.20	4	3	4.11	−4.2	20.83	61.40	373.53	0.42
11-hydroxysugiol	$C_{20}H_{28}O_3$	316.20	3	2	5.19	−4.8	4.97	44.29	368.61	0.13
Abietatriene	$C_{20}H_{30}$	270.23	0	0	6.52	−6.16	0.19	0.00	337.73	−0.12

Table 3. Binding energy with 3CLpro (PDB: 6LU7).

Ligand	Binding energy(Kcal/mol)	Number of hydrogen bonds	Hydrogen bond residues
11,20-dihydroxysugiol	−6.6	2	GLU14, GLY120
11-hydroxysugiol	−6.7	2	LEU271, LEU287
Plectranthol A	−8.7	8	GLY143, SER144, HIS163, THR25, THR45, THR24
Plectranthol B	−8.9	3	PHE294, ARG105, GLN107
Abietatriene	−7.3	–	–

Table 4. Binding energy with spike protein.

Ligand	Binding energy (Kcal/mol)	Number of hydrogen bonds	Hydrogen bond residues
11,20-dihydroxysugiol	−7.1	2	GLN825
11-hydroxysugiol	−7.1	2	TRP609
Plectranthol_A	−8.4	5	LYS807, CYS812, PHE805, ASP802, ALA803
Plectranthol_B	−8.3	3	ASN932, PHE805, CYS812
Abietatriene	−7.6	–	–
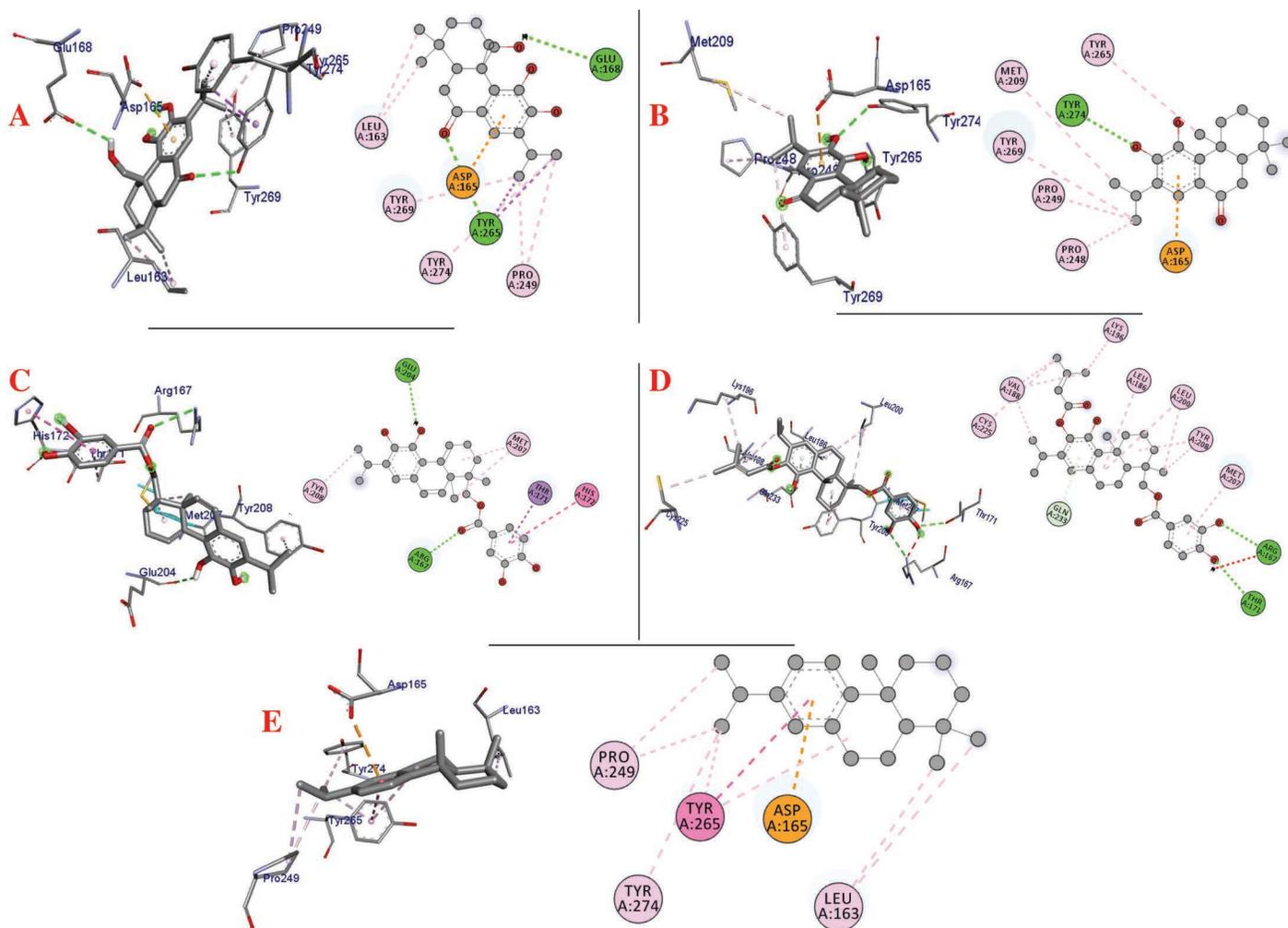
Figure 2. Interaction of (a) 11,20-dihydroxysugiol, (b) 11-hydroxysugiol, (c) plectranthol A, (d) plectranthol B, and (e) abietatriene with PLpro (PDB: 4M0W).
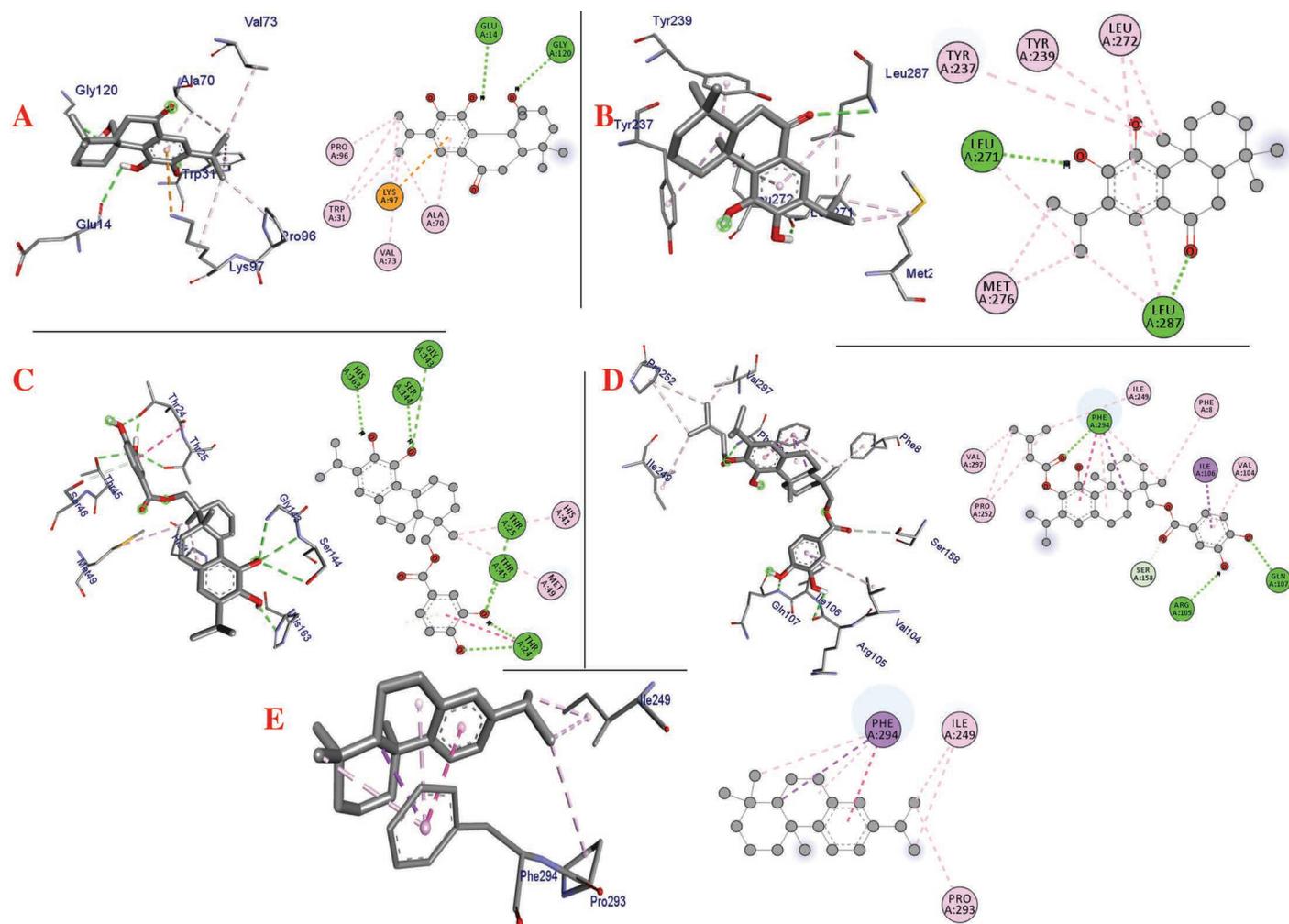
Figure 3. Interaction of (a) 11,20-dihydroxysugiol, (b) 11-hydroxysugiol, (c) plectranthol A, (d) plectranthol B, and (e) abietatriene with 3CLpro (PDB: 6LU7).

This result reflects the controversial investigation of lead bioactives against viral infection treatment from *P. amboinicus*. The outcome of this controversial result could be due to two main reasons. Firstly, the secondary metabolites from *P. amboinicus* are not sufficient enough to regulate the multiple genes/targets. Secondly, the cutoff point of target prediction could be high against the viral infection as the study is set at a minimum 70% similarity index.

Additionally, 3CLpro, PLpro, and spike protein are involved in the manipulation of the ubiquitin system, processing of pp1a and pp1ab into the replicase proteins, and utilization angiotensin-converting enzyme 2 as a receptor to enter inside the cell (Bhoj and Chen, 2009; Li *et al.*, 2003; Lindner *et al.*, 2005). Based on the binding affinity of bioactives from *P. amboinicus* against these three bioactives, we identified all the phytoconstituents to act over those proteins, except abietatriene as it did not possess any hydrogen bond interactions compared to the rest of the molecules. This reflects the abietatriene-3CLpro/PLpro/spike protein complex as unstable compared to other bioactives in the biological system. In the docking studies, the highest binding affinity (−7.9 kcal/mol) of plectranthol A

and B was predicted with PLpro via the two hydrogen bond interactions, i.e., -OH...GLU204, =O...ARG167 for plectranthol A and -OH...ARG167, -O...THR171 for plectranthol B. Likewise, plectranthol B (−8.9 kcal/mol) was predicted to have the interaction with 3CLpro via =O...PHE294, -OH...ARG105, and -O...GLN107. Furthermore, plectranthol A (−8.4 kcal/mol) was predicted to have the highest binding affinity with spike protein by interacting with -O...LYS807, -OH...CYS812, -O...PHE805, -OH...ASP802, and -O...ALA803. The compound abietatriene had no any hydrogen bond interactions with PLpro, 3CLpro, and spike protein which could be due to the absence of a functional group in the molecules.

The calculation of the druglikeness score helps to understand the oral bioavailability of drug-like compounds if administered orally. This theory was proposed by Lipinksi, which is based on molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, and LogP value of the compounds (Lipinski, 2004). Furthermore, *P. amboinicus* belongs to the traditional medicine system and is consumed orally. Hence, we attempted to investigate the ChEBI-recorded bioactives for druglikeness score in which 80% of bioactives secured positive
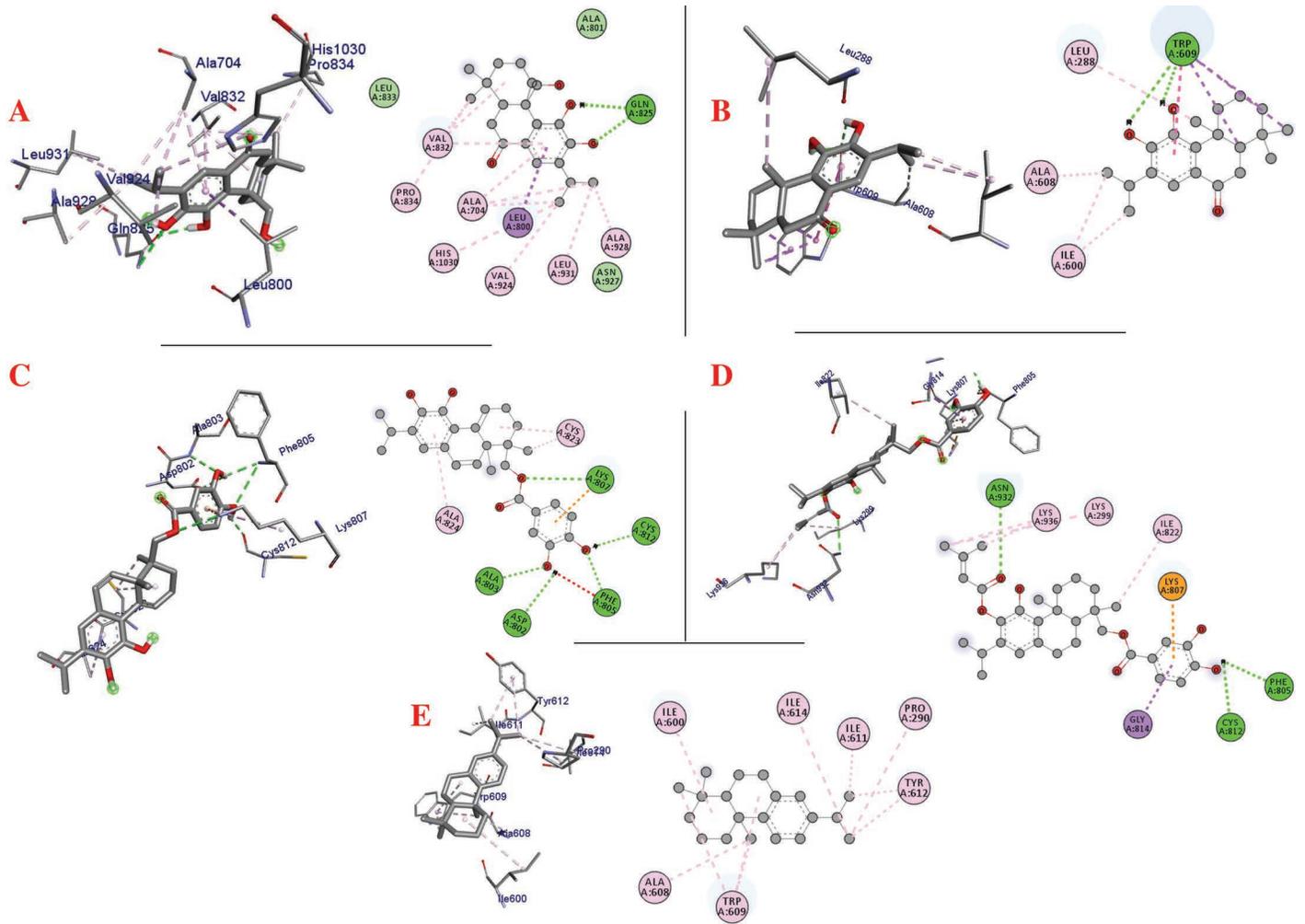
Figure 4. Interaction of (a) 11,20-dihydroxysugiol, (b) 11-hydroxysugiol, (c) plectranthol A, (d) plectranthol B, and (e) abietatriene with Spike protein.
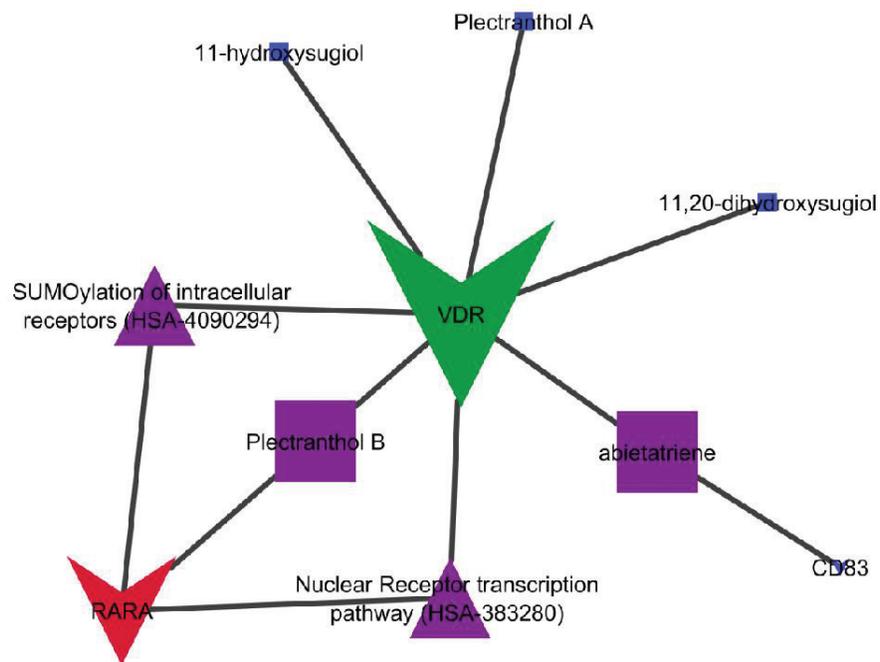
Figure 5. Network interactions of bioactives with respected proteins and modulated pathways.

druglikeness scores, reflecting their probability to get absorbed in the gastrointestinal tract.

CONCLUSION

The present study investigated the reported bioactives in the ChEBI database against three targets of COVID-19 using *in silico* molecular docking and system biology approach which identified plectranthol A and B as lead hits. However, the findings of the presents are based on computer simulations which need to be further investigated via experimental studies.

CONFLICT OF INTEREST

All the authors declare that they have no conflicts of interest for this work.

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None.

REFERENCES

Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules, 2016; 21(4):369.

Balkrishna A, Subarna P, Singh J, Varshney A. Withanone from withania somnifera may inhibit novel coronavirus (COVID-19) entry by disrupting interactions between viral S-protein receptor binding domain and host ACE2 receptor. Res Sq. 2020;

Bhoj VG, Chen ZJ. Ubiquitylation in innate and adaptive immunity. Nature, 2009; 458(7237):430–7.

Dassault Systèmes BIOVIA. Discovery studio. Dassault Systèmes, San Diego, CA, 2019.

Duyu T, Khatib NA, Khanal P, Patil BM, Hullatti KK. Network pharmacology-based prediction and experimental validation of Mimosa pudica for Alzheimer's disease. J Phytopharmacol, 2020; 9(1):46–53.

Gurgel AP, da Silva JG, Grangeiro ARS, Oliveira DC, Lima CMP, da Silva ACP, Oliveira RAG, Souza IA. *In vivo* study of the anti-inflammatory and antitumor activities of leaves from *Plectranthus amboinicus* (Lour.) Spreng (Lamiaceae). J Ethnopharmacol, 2009; 125(2):361–3.

Halgren TA. Merck molecular force field. I. basis, form, scope, parameterization, and performance of MMFF94. J Comput Chem, 1996; 17:490–519.

Hughes GD, Aboyade OM, Beauclair R, Mbamalu ON, Puoane TR. Characterizing herbal medicine Use for noncommunicable diseases in Urban South Africa. Evid Based Complement Alternat Med, 2015; 2015:736074.

Khanal P, Duyu T, Patil BM, Dey Y, Pasha I, Kavalapure RS. *In silico* screening of JAK-STAT modulators from the antiviral plants of Indian traditional system of medicine with the potential to inhibit 2019 novel coronavirus. Res Sq, 2020a.

Khanal P, Mandar BK, Magadum P, Patil BM, Hullatti KK. *In-silico* docking study of Limonoids from *Azadirachta indica* with pfpk5: a novel anti-malarial target for *Plasmodium falciparum*. Indian J Pharm Sci, 2019a; 81(2):326–32.

Khanal P, Mandar BK, Patil BM, Hullatti KK. *In silico* antidiabetic screening of borapetoside C, cordifolioside A and magnoflorine. Indian J Pharm Sci, 2019c; 81(3):550–55.

Khanal P, Patil BM. Gene set enrichment analysis of alpha-glucosidase inhibitors from ficus benghalensis. Asian Pac J Trop Biomed, 2019; 9(6):263–70.

Khanal P, Patil BM, Mandar BK, Dey YN, Duyu T. Network pharmacology-based assessment to elucidate the molecular mechanism of

anti-diabetic action of Tinospora cordifolia. Clin Phytosci, 2019b; 5(1):35.

Khanal P, Patil BM, Chand J, Naaz Y. Anthraquinone Derivatives as an Immune Booster and their Therapeutic Option Against COVID-19. Nat Prod Bioprospect. 2020;10(5):325–335.

Khanal P, Patil BM, Pasha I, Dey YN, Chand S. Withanolides from *Withania somnifera* as an immune booster and their therapeutic option against COVID-19. Res Sq, 2020b.

Khanal P, Patil BM. Gene ontology enrichment analysis of α-amylase inhibitors from duranta repens in diabetes mellitus. J Diabetes Metab Disord, 2020b.

Khanal P, Patil BM. α-Glucosidase inhibitors from Duranta repens modulate p53 signaling pathway in diabetes mellitus. Adv Tradit Med, 2020a; 20:427–38.

Kubota T, Matsuoka M, Chang TH, Tailor P, Sasaki T, Tashiro M, Kato A, Ozato K. Virus infection triggers SUMOylation of IRF3 and IRF7, leading to the negative regulation of type I interferon gene expression. J Biol Chem, 2008; 283(37):25660–70.

Lagunin A, Ivanov S, Rudik A, Filimonov D, Poroikov V. DIGEP-pred: web service for *in silico* prediction of drug-induced gene expression profiles based on structural formula. Bioinformatics, 2013; 29:2062–3.

Lee C. Controversial effects of vitamin D and related genes on viral infections, pathogenesis, and treatment outcomes. Nutrients, 2020; 12(4):962.

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensinconverting enzyme 2 is a functional receptor for the SARS coronavirus. Nature, 2003; 426(6965):450–4.

Lindner HA, Fotouhi-Ardakani N, Lytvyn, Lachance P, Sulea T, Ménard R. The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. J Virol, 2005; 79(24):15199–208.

Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol, 2004;1(4):337–41.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ AutoDock4 and autoDockTools4: automated docking with selective receptor flexibility. J Comput Chem, 2009; 30:2785–91.

Patil VS, Biradar PR, Attar V, Khanal P. *In silico* docking analysis of active biomolecules from cissus quadrangularis L. against PPAR-γ. Indian J Pharm Educ. 2019; 53(3S):S332–7.

Pinn G. Herbal medicine in infectious disease. Aust Fam Physician, 2001; 30(7):681–4.

Rodrigues J, Hullatti KK, Jalalpure S, Khanal P. *In-vitro* Cytotoxicity and *in silico* molecular docking of alkaloids from Tiliacora acuminata. Indian J Pharm Educ, 2020a; 54(2S):S295–300.

Rodrigues J, Hullatti KK, Khanal P. *In silico* and *in vitro* cytotoxicity profile of hydroalcoholic extract/fraction(s) of Pachygone ovata. J Appl Pharm Sci, 2020b; 10(05):135–41.

Schwede T, Kopp J, Guex N, Peitsch MC. SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res. 2003; 31(13):3381–5.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003; 13:2498–504.

Song M, Sun Y, Tian J, He W, Xu G, Jing Z, Li W. Silencing retinoid X receptor alpha expression enhances early-stage hepatitis B virus infection in cell cultures. J Virol, 2018; 92(8):e01771–17.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, Simonovic M, Doncheva NT, Morris JH, Bork P, Jensen LJ, Mering CV. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res, 2019; 47:D607–13.

Ternikar SG, Patil MB, Pasha I, Khanal P. Gene set enrichment analysis of α-amylase and α-glucosidase inhibitors of Cassia glauca. J Diabetes Metab Disord, 2020.

WHO. Coronavirus disease (COVID-19) advice for the public. 2019a. Available via: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public. (Accessed 03 June 2020).

WHO. Rolling updates on coronavirus disease (COVID-19). 2019b Available via: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen. (Accessed 03 June 2020).