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

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**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
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 Lipinski’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-dihydroxyisugiol, 11-hydroxyisugiol, plectranthol A, plectranthol B, and abietatriene, were recorded as major bioactives in the ChEBI.

![Figure 1. 2D structure of (a) 11,20-dihydroxyisugiol, (b) 11-hydroxyisugiol, (c) plectranthol A, (d) plectranthol B, and (e) abietatriene.](image-url)
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.

| Phytoconstituents | Molecular formula | Molecular weight | Number of HBA | Number of HBD | MolLogP | MolLogS | MolPSA | MolVol | Druglikeness score |
|-------------------|-------------------|------------------|---------------|---------------|---------|---------|--------|--------|-------------------|
| Plectranthol A    | C_{20}H_{22}O_{5} | 450.20           | 6             | 4             | 5.99    | 5.52    | 1.37   | 84.99  | 598.82            |
| Plectranthol B    | C_{32}H_{40}O_{7} | 536.28           | 7             | 3             | 7.18    | 5.84    | 0.77   | 89.74  | 373.53            |
| 11,20-dihydroxysugiol | C_{20}H_{28}O_{4} | 332.20           | 4             | 3             | 4.11    | 4.2     | 20.83  | 61.40  | 1.11              |
| 11-hydroxysugiol  | C_{27}H_{30}O_{6} | 316.20           | 3             | 2             | 5.19    | 4.8     | 4.97   | 44.29  | 368.61            |
| Abietatriene      | C_{20}H_{30}      | 270.23           | 0             | 0             | 6.52    | 6.16    | 0.19   | 0.00   | 337.73            |

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