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Original Article

Bioactive compounds screening of *Rafflesia* sp. and *Sapria* sp. (Family: *Rafflesiaceae*) as anti-SARS-CoV-2 via tetra inhibitors: An *in silico* research

[Cribado de compuestos bioactivos de *Rafflesia* sp. y *Sapria* sp. (Familia: *Rafflesiaceae*) como anti-SARS-CoV-2 vía tetra inhibidores: Una investigación *in silico*]

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Abstract

Context: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread, causing a global pandemic with diverse symptoms and increased risk of mortality. Various symptoms and comorbidities contribute to a higher likelihood of death in patients. Additionally, existing antiviral drugs have shown incomplete efficacy. *Rafflesia* sp. and *Sapria* sp. are parasitic plants with potential medical applications as anti-SARS-CoV-2 agents.

Aims: To evaluate the bioactive compounds derived from *Rafflesia* sp. and *Sapria* sp. as dual inhibitors against SARS-CoV-2.

Methods: Ligand samples were obtained from the PubChem database. Target proteins essential for SARS-CoV-2 entry were obtained from the RCSB PDB. The antiviral potential of the bioactive compounds was evaluated using the Pass Online webserver. The bioactivity and inhibitory potential of selected ligands were analyzed using the SwissADME and Molinspiration web servers. In addition, a specific docking method was performed using PyRx software to determine binding activity and molecular interactions.

Results: Computational analysis revealed that leucoanthocyanidin, ellagic acid, and catechin functioned as dual inhibitors, targeting angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), furin, and cathepsin L for antiviral activity. However, valrubicin and diminazene, serving as control drugs for ACE2 and furin, respectively, demonstrated the most effective results through this mechanism. Further studies are required to validate these findings.

Conclusions: The combination of bioactive compounds derived from *Rafflesia* sp. and *Sapria* sp. shows potential antiviral activity through a dual inhibitor mechanism involving leucoanthocyanidin, ellagic acid, and catechin, which target SARS-CoV-2 proteins, namely ACE2, TMPRSS2, furin, and cathepsin L.

Keywords: antiviral; bioactive compounds; *Rafflesia*; *Sapria*; SARS-CoV-2.

Resumen

Contexto: El síndrome respiratorio agudo grave por coronavirus 2 (SRAS-CoV-2) se ha propagado rápidamente, causando una pandemia mundial con síntomas diversos y mayor riesgo de mortalidad. Diversos síntomas y comorbilidades contribuyen a una mayor probabilidad de muerte en los pacientes. Además, los medicamentos antivirales existentes han mostrado una eficacia incompleta. *Rafflesia* sp. y *Sapria* sp. son plantas parásitas con potenciales aplicaciones médicas como agentes anti-SARS-CoV-2.

Objetivos: Evaluar los compuestos bioactivos derivados de *Rafflesia* sp. y *Sapria* sp. como inhibidores duales contra el SARS-CoV-2.

Métodos: Las muestras de ligandos se obtuvieron de la base de datos PubChem. Las proteínas diana esenciales para la entrada del SARS-CoV-2 se obtuvieron del RCSB PDB. El potencial antiviral de los compuestos bioactivos se evaluó utilizando el servidor web Pass Online. La bioactividad y el potencial inhibidor de los ligandos seleccionados se analizaron utilizando los servidores web SwissADME y Molinspiration. Además, se realizó un método de acoplamiento específico utilizando el software PyRx para determinar la actividad de unión y las interacciones moleculares.

Resultados: El análisis computacional reveló que la leucoantocianidina, el ácido elágico y la catequina funcionaban como inhibidores duales, dirigiéndose a la enzima convertidora de angiotensina 2 (ACE2), proteasa transmembrana serina 2 (TMPRSS2), furina y catepsina L para la actividad antiviral. Sin embargo, la valrubicina y el diminazeno, que sirvieron como fármacos de control para la ACE2 y la furina, respectivamente, demostraron los resultados más eficaces a través de este mecanismo. Se requieren más estudios para validar estos hallazgos.

Conclusiones: La combinación de compuestos bioactivos derivados de *Rafflesia* sp. y *Sapria* sp. muestra una potencial actividad antiviral a través de un mecanismo inhibidor dual en el que intervienen leucoantocianidina, ácido elágico y catequina, que actúan sobre las proteínas del SARS-CoV-2, concretamente ACE2, TMPRSS2, furina y catepsina L.

Palabras Clave: antiviral; compuestos bioactivos; *Rafflesia*; *Sapria*; SARS-CoV-2.

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Abbreviations: ACE2: angiotensin-converting enzyme 2; CTL: cathepsin L; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; TMPRSS2: transmembrane protease serine 2.

INTRODUCTION

Coronaviruses are highly contagious respiratory pathogens that belong to the *Coronaviridae* family (Antonius et al., 2023; Rahman and Haris, 2022; Reviono et al., 2022; Shu et al., 2020). They have the ability to infect various animals, including bats, camels, civets, and humans (Zhou et al., 2020). Previous outbreaks of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus caused severe respiratory illnesses transmitted from civets and camels to humans (Baby et al., 2021; Shu et al., 2020). A new outbreak known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, in late December 2019 and rapidly spread worldwide, leading the World Health Organization to declare it a pandemic (Padmi et al., 2022). Symptoms of SARS-CoV-2 infection include varying degrees of fever, cough, anosmia, and sore throat. Patients with comorbidities such as diabetes mellitus, hypertension, and asthma have a higher risk of death compared to non-comorbid patients. Controlling the spread of COVID-19 requires infection prevention, vaccination, and supportive treatments (Baby et al., 2021; Hsieh et al., 2022; Khuluq et al., 2022; Nugrahaningsih et al., 2022; Santosa et al., 2021).

Specific interactions between various receptors in human cells play a crucial role in viral entry. Angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), furin, and cathepsin L (CTL) are responsible for the entry of SARS-CoV-2 by interacting with the spike glycoprotein (Hoffman et al., 2020; Wu et al., 2020; Zhao et al., 2021). Following the interaction, SARS-CoV-2 may enter host cells through endocytosis and initiate infection (Hou et al., 2020). Currently, licensed antiviral agents such as remdesivir, lopinavir, and favipiravir have not shown complete efficacy (Padmi et al., 2022). Therefore, *in silico* drug discovery is recommended for rapid viral therapy screening (Antonius et al., 2017; Kharisma et al., 2021). Promising drug candidates can then be further assessed in clinical trials.

Southeast Asian countries harbor high biodiversity, including a unique holoparasite flora. *Rafflesia* sp., *Sapria* sp., and *Rhizanthes* sp. are extremely small vegetative-bodied parasites belonging to the *Rafflesiaceae* family (Mursidawati and Irawati, 2017; Nikolov et al., 2014). These plants develop large red flowers as distinctive features to attract insects for pollination. Consequently, conserving this family poses numerous challenges due to its rare population in the wild and difficulties in propagation (Wicaksono et al., 2022). Certain regions in the Malaysian Peninsular have utilized *Rafflesia* sp. for various purposes, including the treatment of internal bleeding, fever, and backaches, as well as energy drinks and fertility supplements for women (Kanchanapoom et al., 2007; Wicaksono et al., 2022). Additionally, *Sapria* sp. flowers have been claimed by local people to alleviate fever and liver disorders (Wangchuk et al., 2011). Previous studies have identified bioactive compounds in *Rafflesia* and *Sapria* (Iwashima et al., 2020; Kanchanapoom et al., 2007; Sofiyanti et al., 2008). *In silico* analysis has been employed in previous studies targeting various human disease proteins, including antiviral targets (Ansori et al., 2021; Wicaksono et al., 2022). However, no studies have explored the potential of bioactive compounds from these plants in animal and human clinical trials, particularly as anti-SARS-CoV-2 agents. Therefore, this study employs *in silico* analysis to evaluate the bioactive compounds from *Rafflesia* sp. and *Sapria* sp. against ACE2, TMPRSS2, furin, and CTL, aiming to discover new anti-SARS-CoV-2 drugs.

MATERIAL AND METHODS

Sample preparation of *in silico* anti-SARS-CoV-2 activity

A total of 22 bioactive compounds from *Rafflesia* sp. and *Sapria* sp. were collected from PubChem and HMDB databases in .sdf format as ligands (Iwashina et al., 2020; Kanchanapoom et al., 2007; Sofiyanti et al., 2008; Wicaksono et al., 2022). Valrubicin (CID: 10349017), lopinavir (CID: 92727), diminazene (CID: 2354), and amantadine (CID: 2130) were chosen as control drugs for this study (Baby et al., 2021; Wu et al., 2020; Zhao et al., 2021). Ligand minimization was performed using PyRx v.0.9.9 software. The target proteins, including ACE2 (PDB: 1R4L), TMPRSS2 (PDB: 7MEQ), furin (PDB: 5JXH), and CTL (PDB: 5MQY), were obtained from the RCSB Protein Data

Bank. Water and native ligands were sterilized using PyMol version 2.4.1 (Schrödinger Inc, USA) (Christina et al., 2021; Putra et al., 2020).

Antiviral probability prediction

The antiviral probability of the compounds was predicted using the PASS Online web server (Antonius et al., 2022). The predicted compounds were assigned a probability of activity (Pa), which needed to be greater than the probability of inhibition (Pi). A Pa value higher than 0.3 indicated favorable results for computational evidence in the *in silico* analysis (Tamam et al., 2022).

Bioactivity and inhibitory analysis

Bioactivity analysis was performed using the SwissADME web server to investigate pharmacological similarities among the drugs. The prediction of bioactivity analysis was based on Lipinski's rules, which include criteria such as molecular mass <500 Da, partition coefficient in water-lipid (miLogP) ≤5, the number of hydrogen donors attached to oxygen and nitrogen (nOHNH) ≤10, and the number of hydrogen bond acceptors (nON) ≤5 (Umar et al., 2022). Additionally, inhibitory prediction analysis of protease and enzyme activities was conducted using the Molinspiration 2018.03 web server. A positive value for the ligand indicated its potential as a specific inhibitor (Wahyuni et al., 2022).

Virtual screening and visualization

Molecular docking simulations were performed using PyRx v.0.9.9 software to determine the inhibitory activities of the ligands against the target proteins as antivirals. Specific docking methods were employed in this study (Table 1). The interaction was assessed based on the binding affinity value, with lower values indicating more favorable results due to increased stability (Wargasetia et al., 2021). Interaction visualization was carried out for the compound with the lowest binding affinity. Furthermore, the compounds were visualized and compared with the control drugs using BIOVIA Discovery Studio 2016 16.1.0 software (Dassault Systèmes, France) (Widyananda et al., 2021; Aini et al., 2022a).

Data analysis

Data analysis was conducted at each step of the research. Bioactive compounds that exhibited Pa > Pi and Pa > 0.3, indicating favorable antiviral probability activities, were given preference (Tamam et al., 2022). Each selected bioactive compound was subjected to bioactivity analysis using Lipinski rules. Compounds that did not violate the criteria for molecular weight, water-lipid partition coefficient (miLogP), number of hydrogen donors attached to oxygen and nitrogen (nOHNH), and number of hydrogen bond acceptors (nON) were chosen for inhibitory analysis of protease and enzyme activities (Umar et al., 2022; Wahyuni et al., 2022). Positive results from the bioactive compounds were further examined through molecular docking (Wargasetia et al., 2021). The compound with the most negative binding affinity for each target protein was then compared to the control drugs, and 3D and 2D ligand-target protein visualizations were presented in figures and tables.

Table 1. Catalytic sites binding positions and grid for specific docking.

Protein	Key residues	Reference	Grid	
			Center	Dimension
ACE2	Arg166, His373, Met376, Trp477, and Lys481	Towler et al., 2004; Madhavi Sastry et al., 2013	X: 40.233 Y: 2.545 Z: 20.250	X: 17.359 Y: 43.475 Z: 24.117
TMPRSS2	His296, Asp345, and Ser441	Fraser et al., 2022	X: 9.483 Y: -6.708 Z: 13.996	X: 55.032 Y: 46.280 Z: 50.240
Furin	Asp=153, His194, Trp254, Pro256, Asn295, and Ser368	Dahms et al., 2016	X: 44.795 Y: -38.048 Z: -10.739	X: 28.440 Y: 31.826 Z: 51.490
CTL	Gly19, Cys25, Gly67, Gly68, Leu69, Asp162, Ala135, and Ala214	Kuhn et al., 2017	X: 51.651 Y: 49.473 Z: 11.018	X: 26.112 Y: 27.762 Z: 38.242

Table 2. Results of ligand sample preparation.

Compound	ID	Chemical database	Genera	Reference
Caffeine	2519	PubChem	*Rafflesia* sp.	Sofiyanti et al., 2008; Zulkkfle et al., 2014
Nicotine	89594	PubChem	*Rafflesia* sp.	Sofiyanti et al., 2008
Catechin	9064	PubChem	*Rafflesia* sp.	Sofiyanti et al., 2008
Salicylic acid	23361	PubChem	*Rafflesia* sp.	Sofiyanti et al., 2008
Leucoanthocyanidin	3081374	PubChem	*Rafflesia* sp.	Sofiyanti et al., 2008
1,6-digalloylglucose	118431216	PubChem	*Rafflesia* sp., *Sapria* sp.	Iwashina et al., 2020
1,2,4,6-tetra-O-galloyl-β-D-glucopyranoside	HMDB0039191	HMDB	*Rafflesia* sp., *Sapria* sp.	Kanchanapoom et al., 2007; Iwashima et al., 2020
1,2,6-tri-O-galloyl-β-D-glucopyranoside	HMDB0039182	HMDB	*Rafflesia* sp., *Sapria* sp.	Kanchanapoom et al., 2007; Iwashima et al., 2020
1,4,6-tri-O-galloyl-β-D-glucopyranoside	HMDB0039185	HMDB	*Rafflesia* sp., *Sapria* sp.	Kanchanapoom et al., 2007; Iwashima et al., 2020
Cyanidin 3-O-glucoside	90659011	PubChem	*Sapria* sp.	Iwashima et al., 2020
Cyanidine 3-O-xyloside	HMDB0037984	HMDB	*Sapria* sp.	Iwashima et al., 2020
Quercetin 3-O-glucoside	5280804	PubChem	*Sapria* sp.	Iwashima et al., 2020
Quercetin 7-O-glucoside	5282160	PubChem	*Sapria* sp.	Iwashima et al., 2020
Quercetin 3-O-glucoronide	HMDB0029212	HMDB	*Sapria* sp.	Iwashima et al., 2020
Isorhamnetin 3-O-glucoside	5318645	PubChem	*Sapria* sp.	Iwashima et al., 2020
Ellagic acid	5281855	PubChem	*Sapria* sp.	Iwashima et al., 2020
Gallic acid	370	PubChem	*Sapria* sp.	Iwashima et al., 2020
Ethyl gallate	13250	PubChem	*Sapria* sp.	Iwashima et al., 2020
Syringin	5316860	PubChem	*Rafflesia* sp.	Kanchanapoom et al., 2007
1,2,4,6-tetragalloylglucose	11297287	PubChem	*Sapria* sp.	Iwashima et al., 2020
1,4,6-trigalloylglucose	129650490	PubChem	*Sapria* sp.	Iwashima et al., 2020
1,2,6-trigalloylglucose	440308	PubChem	*Sapria* sp.	Iwashima et al., 2020

RESULTS

Antiviral activity of bioactive compounds from *Rafflesia* sp. and *Sapria* sp.

A total of 22 bioactive compounds from *Rafflesia* sp. and *Sapria* sp. were collected from PubChem and HMDB, and their corresponding identification numbers (IDs), chemical databases, genera, and references are provided in Table 2. Positive or drug control compounds included valrubicin (CID: 10349017), lopinavir (CID: 92727), diminazene (CID: 2354), and amantadine (2130). In this study, ACE2 (PDB: 1R4L), TMPRSS2 (PDB: 7MEQ), furin (PDB: 5JXH), and CTL (PDB: 5MQY) were utilized as receptors. Furthermore, the PASS Online server was utilized to predict the antiviral potential of the compounds, considering their Pa > Pi and Pa > 0.3 values (medium confidence). Thirteen compounds demonstrated theoretical antiviral activity, aligning with the research objective (Table 3).

Antiviral and anti-inflammatory activity of a combination compound derived from herbs

Subsequently, the 13 identified bioactive compounds were subjected to bioactivity testing using SwissADME, based on the Lipinski rules. Criteria such as molecular mass, coefficient partition in water-lipid, hydrogen donors, and hydrogen receptors were employed to evaluate the drug-likeness of the compounds derived from *Rafflesia* and *Sapria* (Table 4). Following the assessment, eligible bioactive compounds were further analyzed to determine their inhibitory activities against the target proteins. Among them, three bioactive compounds displayed inhibitory effects, indicating their potential as inhibitors (Table 5).

Table 3. Results of antiviral probability prediction.

Compound	Antiviral probability	Result
Caffeine	0.218	×
Nicotine	-	×
Catechin	0.378	√
Salicylic acid	0.187	×
Leucoanthocyanidin	0.347	√
1,6-digalloylglucose	0.601	√
1,2,4,6-tetra-O-galloyl-β-D-glucopyranoside	0.406	√
1,2,6-tri-O-galloyl-β-D-glucopyranoside	0.398	√
1,4,6-tri-O-galloyl-β-D-glucopyranoside	0.407	√
Cyanidin 3-O-glucoside	0.272	×
Cyanidine 3-O-xyloside	-	×
Quercetin 3-O-glucoside	0.372	√
Quercetin 7-O-glucoside	0.341	√
Quercetin 3-O-glucoronide	0.262	×
Isorhamnetin 3-O-glucoside	0.290	×
Ellagic acid	0.322	√
Gallic acid	0.342	√
Ethyl gallate	0.283	×
Syringin	0.291	×
1,2,4,6-tetragalloylglucose	0.406	√
1,4,6-trigalloylglucose	0.395	√
1,2,6-trigalloylglucose	0.398	√

Bioactivity analysis is indicated by a check mark (√) for compounds that met the criteria and a cross mark (×) for compounds that did not meet the criteria.

Table 4. Bioactivity or drug-likeness analysis of selected bioactive compounds.

Compound	MW (<500)	miLogP (≤5)	nON (≤10)	nOHNH (≤5)	Result
Catechin	290.27	1.37	6	5	√
Leucoanthocyanidin	322.27	0.09	8	7	√
1,6-digalloylglucose	484.36	-3.31	14	11	×
1,2,4,6-tetra-O-galloyl-β-D-glucopyranoside	788.57	1.81	22	13	×
1,2,6-tri-O-galloyl-β-D-glucopyranoside	636.47	0.86	18	11	×
1,4,6-tri-O-galloyl-β-D-glucopyranoside	636.47	0.64	11	3	×
Quercetin 3-O-glucoside	464.38	-0.36	12	8	×
Quercetin 7-O-glucoside	464.38	-0.10	12	8	×
Ellagic acid	302.19	0.94	8	4	√
Gallic acid	170.12	0.59	5	4	√
1,2,4,6-tetragalloylglucose	788.57	1.81	22	13	×
1,4,6-trigalloylglucose	636.47	-4.01	18	14	×
1,2,6-trigalloylglucose	636.47	0.86	18	11	×

Bioactivity analysis is indicated by a check mark (√) for compounds that met the criteria and a cross mark (×) for compounds that did not meet the criteria.

Table 5. Inhibitor activities of selected bioactive compounds.

Compound	Inhibitor activity		Result
	Protease	Enzyme	
Catechin	0.26	0.47	√
Leucoanthocyanidin	0.11	0.39	√
Ellagic acid	-0.18	0.17	√
Gallic acid	-0.94	-0.17	×

Inhibitor activity is indicated by a check mark (√) for compounds that met the criteria and a cross mark (×) for compounds that did not meet the criteria.

Table 6. Binding affinities of herb ligand combinations.

Compound	Binding affinity (kcal/mol)			
	ACE2	TMPRSS2	Furin	Cathepsin L
Valrubicin (inhibitor of ACE2)	**-9.2**	-	-	-
Lopinavir (inhibitor of TMPRSS2)	-	**-6.5**	-	-
Diminazene (inhibitor of furin)	-	-	**-8.3**	-
Amantadine (inhibitor of cathepsin L)	-	-	-	**-4.4**
Catechin	-8.3	-7.1	-7.6	**-7.4**
Leucoanthocyanidin	**-8.9**	-6.9	-7.4	-7.3
Ellagic acid	-8.7	**-7.6**	**-8.0**	-6.7

Revealing selected bioactive compounds as dual inhibitors and investigating their molecular interactions

This study employed molecular docking simulations to assess the binding activities of compounds derived from *Rafflesia* sp. and *Sapria* sp. with ACE2, TMPRSS2, furin, and CTL based on their respective binding affinities. The compound exhibiting the most negative binding activity is presumed to have a significant role. The findings indicated that leucoanthocyanidin from *Rafflesia* sp. (-8.9 kcal/mol), ellagic acid from *Sapria* sp. (-7.6 kcal/mol against TMPRSS2 and -8.0 kcal/mol against furin), and catechin from *Rafflesia* sp. (-7.4 kcal/mol) exhibited lower binding affinities. However, despite demonstrating the best interactions with ACE2 and furin, the binding affinities of leucoanthocyanidin and ellagic acid remained lower than those of the control drugs valrubicin and diminazene (-9.2 and -8.3 kcal/mol, respectively). On the other hand, ellagic acid and catechin exhibited the strongest interactions with TMPRSS2 and CTL compared to other compounds and the control drug. These optimal compounds may synergistically initiate a response to the target proteins (Table 6).

The 3D structures resulting from the molecular docking simulations were visualized using BIOVIA Discovery Studio 16.1.0 software. Homologous staining was applied to highlight the target protein, while the ligands were represented in red color. The specific chemical interactions between each ligand and the target protein were observed through 3D visualization and further depicted in 2D format. The positions of the chemical bonds were annotated based on the identified interactions (Figs. 1-4).

The examination of molecular interactions through the docking simulation revealed distinct types of chemical bonds formed within the binding pocket of the target protein. These bonds included hydrophobic interactions, hydrogen bonds, and unfavorable interactions (Table 7). Valrubicin and leucoanthocyanidin exhibited similar interactions involving several amino acids in hydrogen and hydrophobic bonding. Leucoanthocyanidin and lopinavir showed adverse interactions against ACE2 and TMPRSS2, although no interactions were observed with catalytic residues in both proteins. Diminazene displayed multiple interactions with key residues of furin, as did ellagic acid. The chemical interactions of these ligands involved hydrophobic interactions, van der Waals (vdw) forces, and hydrogen bonds to provide stabilization. On the other hand, neither amantadine nor catechin formed electrostatic or key residual interactions.

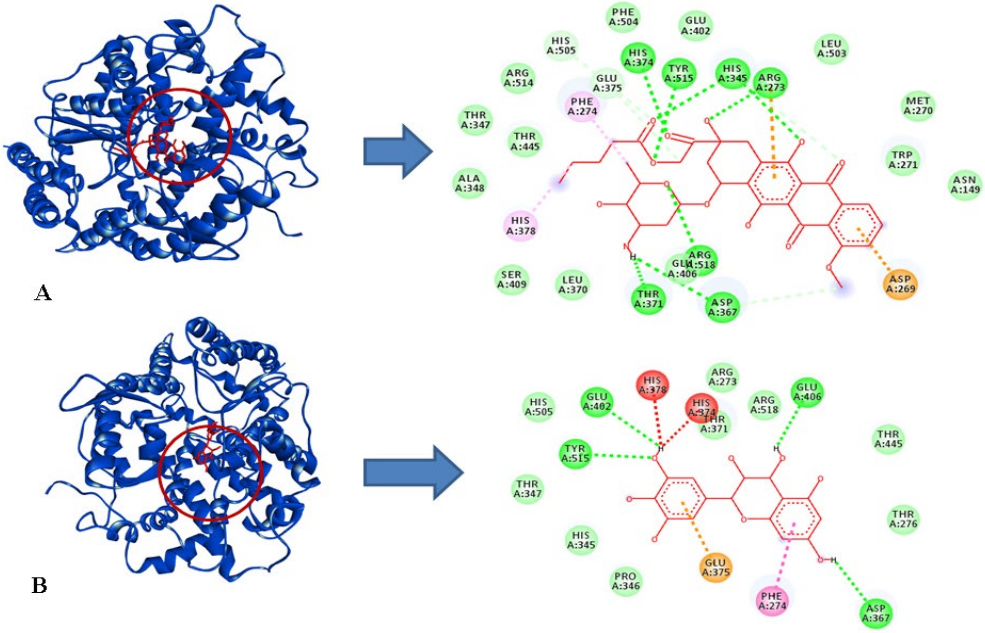

Figure 1. Interaction between ligand and ACE2 in 3D and 2D structures: **(A)** valrubicin and **(B)** leucoanthocyanidin.

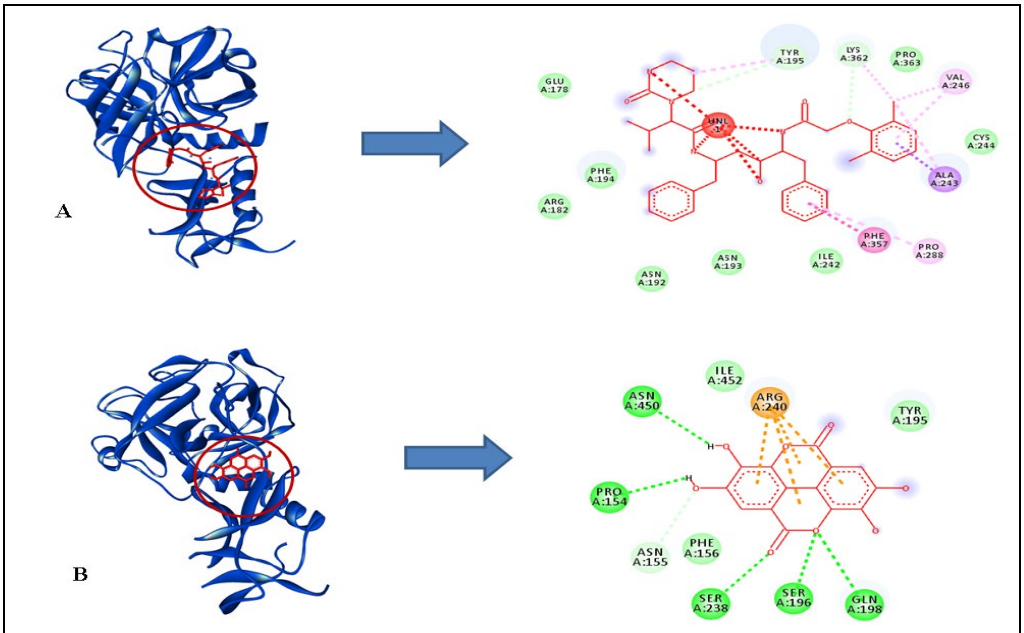

Figure 2. Interaction between ligand and TMPRSS2 in 3D and 2D structures: **(A)** lopinavir and **(B)** ellagic.

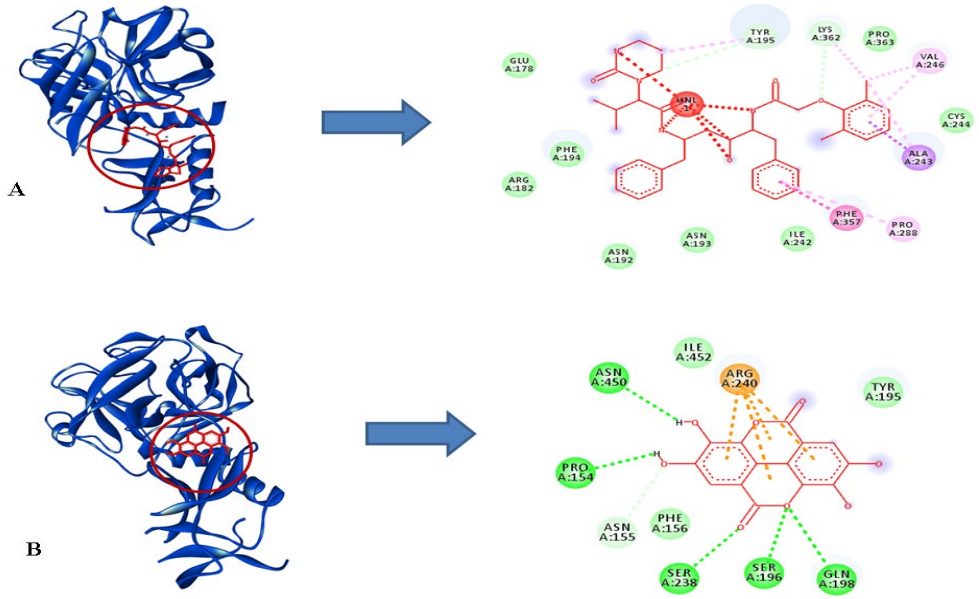

Figure 3. Interaction between ligand and furin in 3D and 2D structures: **(A)** diminazene and **(B)** ellagic.

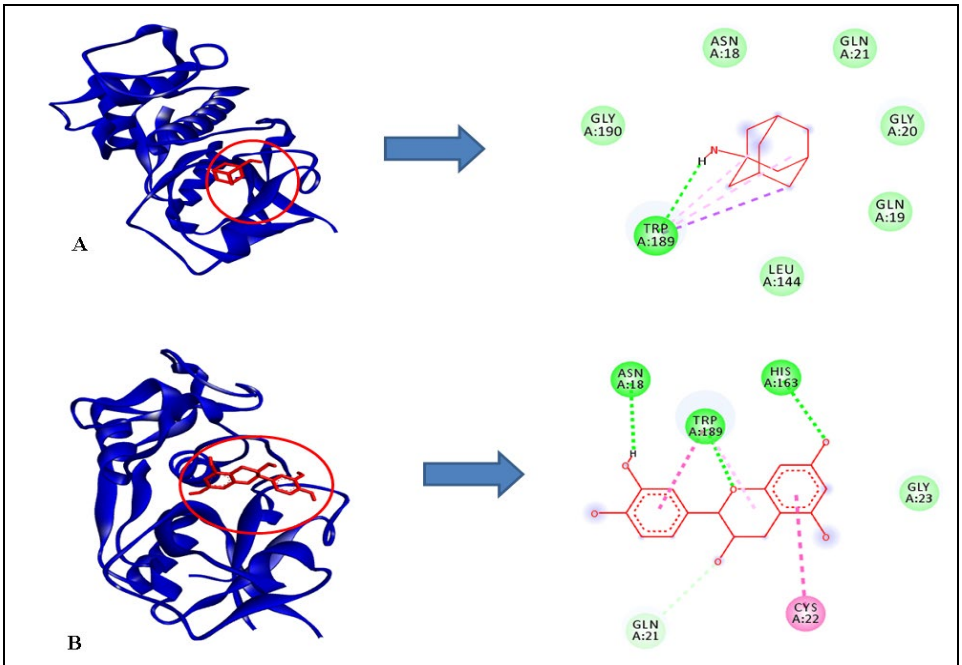

Figure 4. Interaction between ligand and CTL in 3D and 2D structures: **(A)** amantadine and **(B)** catechin.

Table 7. Chemical interactions of selected bioactive compounds and control drugs against target proteins.

Protein	Ligand	Chemical interaction	Amino acid
ACE2	Valrubicin	Hydrogen bond	**Arg273, His345**, **Asp367**, **Thr371**, His374, Tyr515, **Arg518**
		Electrostatic	Asp269, **Arg273**
		Hydrophobic interaction	Phe274, **Glu375**, His378, **His505**
		Van der Waals	Asn149, Met270, Trp271, **Thr347**, Ala348, Leu370, **Glu402**, **Glu406**, Ser409, **Thr445**, Leu503, Phe504, Arg514
	Leucoanthocyanidin	Hydrogen bond	**Asp367**, **Glu402**, **Glu406**, Tyr515
		Electrostatic	**Glu375**
		Hydrophobic interaction	Phe274
		Van der Waals	**Arg273**, Thr276, **His345**, Pro346, **Thr347**, **Thr371**, **Thr445**, **His505**, **Arg518**
		Unfavourable bond	His374, His378
TMPRSS2	Lopinavir	Hydrophobic interaction	**Tyr195**, Ala243, Val246, Pro288, Phe357, Lys362
		Van der Waals	Glu178, Arg182, Asn193, Phe194, Tyr195, Ile242, Cys244, Pro363
		Unfavourable bond	Unl1 (3 bonds)
	Ellagic acid	Hydrogen bond	Pro154, Ser196, Gln198, Ser238, Asn450
		Electrostatic	Arg240
		Hydrophobic interaction	Asn155, Phe156
		Van der Waals	**Tyr195**, Ile452
Furin	Diminazene	Hydrogen bond	**Leu227**, **Pro256**, Ala292
		Electrostatic	**Asp258**, Asp306, Glu331
		Hydrophobic interaction	**His194**, **Trp254**, **Gly255**, **Leu227**, Ala292
		Van der Waals	**Asp154**, Asn192, Asp228, Gly229, **Ser253**, Gly255, Trp291, Ser293, **Gly294**, **Asn295**, Thr309, **Thr367**, **Ser368**
		Unfavourable bond	Un1 (3 bonds)
	Ellagic acid	Hydrogen bond	**His194**, **Gly255**, **Pro256**, **Asp258**, **Ser368**
		Hydrophobic interaction	**Leu227**
		Van der Waals	**Asp154**, **Trp254**, **Gly294**, **Ser253**, **Thr367**
		Unfavorable bond	**Asn295**
Cathepsin L	Amantadine	Hydrogen bond	**Trp189**
		Hydrophobic interaction	**Trp189**
		Van der Waals	**Asn18**, Gln19, Gly20, **Gln21**, Leu144, Gly190
	Catechin	Hydrogen bond	**Asn18,** His163, **Trp189**
		Hydrophobic interaction	**Gln21**, Cys22, Gly23, **Trp189**
		Van der Waals	Gly23

Bold amino acids indicate shared interactions between specific ligands and several amino acids of the respective proteins.

DISCUSSION

This computational study assessed the antiviral potential of bioactive compounds from *Rafflesia* sp. and *Sapria* sp., targeting ACE2 and TMPRSS2. A total of 13 compounds met the computational antiviral probability criteria based on the PASS Online Server (Pa > Pi, Pa > 0.3). However, further *in vitro* and *in vivo* tests are necessary to confirm the antiviral properties of these compounds (Ansori et al., 2022). As mentioned earlier, the filtered compounds were also assessed for compliance with Lipinski's rules. Compounds that exhibited no more than one violation and

were expected to interact well with the target and pass through the cell membrane were considered drug-like molecules (Umar et al., 2022). Four selected compounds successfully passed the drug-likeness analysis. Furthermore, the selected compounds were evaluated for their inhibitory activities against proteases and enzymes to determine their potential as specific inhibitors against SARS-CoV-2 entry. Three bioactive compounds met the protease and enzyme inhibitory activity criteria, suggesting their potential as inhibitors (Wahyuni et al., 2022).

ACE2 plays a crucial role in facilitating the entry of SARS-CoV-2 into host cells (Hoffman et al., 2020). The interaction occurs specifically in the S2 domain, which is being investigated for its fusion of functional elements (Shen et al., 2017). Inhibiting this interaction is essential to prevent viral entry, particularly using naturally occurring bioactive compounds found in plants. Molecular docking analysis revealed that leucoanthocyanidin exhibited the strongest inhibitory effect on ACE2, although valrubicin demonstrated greater inhibitory activity as a control drug. Previous studies by Madhavi Sastry et al. (2013) and Towler et al. (2004) identified key residues of ACE2, including Arg166, His373, Met376, Trp477, and Lys481. However, neither valrubicin nor leucoanthocyanidin showed interactions with these key residues. Compared to valrubicin, leucoanthocyanidin exhibited fewer chemical interactions and unfavorable bumps, resulting in lower stability and less ideal docking results (Aini et al., 2022b; Kharisma et al., 2020). Additionally, blocking ACE2 with a bioactive compound like leucoanthocyanidin may impede the activity of angiotensin-2 (Ang-2) on ACE2, preventing ACE2 degradation, vasoconstriction, inflammation and promoting positive feedback through p38 activation and upregulation of a disintegrin and metalloproteinase 17 (ADAM17) (Cao et al., 2020; Gheblawi et al., 2020; Yu et al., 2018; Zipeto et al., 2020).

TMPRSS2 is another cell membrane protein receptor essential for SARS-CoV-2 infection (Hoffman et al., 2020). It interacts with the S1 functional domain of the S-protein of SARS-CoV-2, which is responsible for the binding activity (Shen et al., 2017). Multiple cleavage sites are present at the S1/S2 and S2 boundaries, and the interaction with TMPRSS2 is required to cleavage and activate the SARS-CoV-2 S-protein (Thunders and Delahunt, 2020). Ellagic acid demonstrated effective inhibition of TMPRSS2 compared to lopinavir. Various chemical interactions may contribute to structural alterations in the target protein, resulting in functional modifications (Padmi et al., 2022). Among the filtered bioactive compounds from *Rafflesia* sp. and *Sapria* sp., lopinavir only exhibited hydrophobic interactions and unfavorable bumps, interacting with the Unl1 residue and yielding a more positive binding affinity (Wang and Wu, 2008; Yang et al., 2016). Furthermore, lopinavir and ellagic acid did not interact with key residues such as His296, Asp345, and Ser441 in the active sites of TMPRSS2 (Fraser et al., 2022; Gaurav et al., 2022).

Furin belongs to the calcium-dependent proprotein/prohormone convertase (PC) family (Thomas, 2002) and is located in the trans-Golgi network, activated by acidic pH (Dahms et al., 2016; Feliciangeli, 2006). It is involved in various viral infections, including SARS-CoV-2 (Seidah and Prat, 2012). Furin recognizes the S1/S2 and S2 cleavage sites of the SARS-CoV-2 S-protein (Tay et al., 2012; Wu et al., 2020). Therefore, inhibiting furin using bioactive compounds is an effective strategy against SARS-CoV-2. Docking results between furin and ligands revealed that ellagic acid exhibited a high binding affinity (-8.0 kcal/mol). This bioactive compound formed four hydrogen bonds (His194, Pro256, and Ser368) and van der Waals interactions (Trp254). The stability of this compound was projected to be higher due to the increased number of hydrogen bond interactions, targeting active sites and blocking viral entry (Wu et al., 2020). Conversely, diminazene exhibited the strongest binding affinity (-8.3 kcal/mol) in this interaction, forming hydrogen bonds, hydrophobic interactions, and van der Waals interactions with key residues (His194, Trp254, Pro256, Asn295, and Ser368). Electrostatic interactions also play a role in catalytic reactions and protein stabilization (Nakamura, 1996; Ongko et al., 2022; Tan et al., 2022).

CTL is a lysosome-cysteine protease that functions in the cytoplasmic endosome to proteolyze pathogen endocytosis protein (Fujishima et al., 1997; Gomes et al., 2020). Previous research has shown that CTL is involved in S-protein and membrane interaction, relying on proteolysis and membrane fusion (Cevik et al., 2020; Hoffman et al., 2020; Zhao et al., 2021). Among the compounds tested, catechin exhibited the lowest binding affinity towards CTL compared to other compounds and the control drug. The hydrogen bonds and hydrophobic interactions formed by this ligand were more diverse than those of amantadine, but no interactions were observed with active sites. The presence of hydrogen bonds and other hydrophobic interactions, such as carbon-hydrogen (CH), pi-pi stacked, and amide-pi stacked interactions, contribute to the stability and turnover between the ligand and target protein in biological processes (Chia et al., 2023; Kharisma et al., 2021). This result suggests that catechin may potentially block the activity of CTL in COVID-19 infection.

Proposing drugs from different disease targets as anti-SARS-CoV-2 treatments may have implications

for intracellular and physiological functions, affecting metabolism (Albasri et al., 2023; Baby et al., 2021). However, trials combining several synthetic medicines have shown limited benefits for severe SARS-CoV-2 patients (Cao et al., 2020; Hung et al., 2020). Promoting bioactive compounds from plants, including *Rafflesia* sp. and *Sapria* sp., could serve as an alternative treatment for this condition. Leucoanthocyanidin, ellagic acid, and catechin have been identified as potential compounds targeting ACE2, TMPRSS2, furin, and CTL using *in silico* approaches. *In vitro* and *in vivo* analyses are necessary to further validate the inhibitory potential of these compounds as anti-SARS-CoV-2 agents.

CONCLUSION

The bioactive compounds derived from *Rafflesia* and *Sapria* exhibit promising potential as anti-SARS-CoV-2 agents due to their ability to inhibit the interactions of SARS-CoV-2 with ACE2, TMPRSS2, furin, and CTL. Among these compounds, leucoanthocyanidin, ellagic acid, and catechin have demonstrated notable efficacy in targeting ACE2, TMPRSS2, furin, and CTL. However, it is important to note that leucoanthocyanidin and ellagic acid are not as effective as the control drugs lopinavir and diminazene. Further *in vitro* and *in vivo* analyses are required to substantiate the inhibitory effects of these compounds as anti-SARS-CoV-2 agents.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x	x	x	x	x
Literature search	x	x	x		x							
Experimental studies	x	x	x	x	x							
Data acquisition	x	x	x		x							
Data analysis	x	x	x		x	x	x	x	x	x	x	
Manuscript preparation	x	x		x						x	x	x
Manuscript editing	x	x		x		x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x

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