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RESEARCH ARTICLE

In silico screening of mint leaves compound (*Mentha piperita L.*) as a potential inhibitor of SARS-CoV-2

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Abstract

Introduction: The novel coronavirus in Wuhan, China, was identified at the end of December 2019 and resulted in a global outbreak. Therefore, it is necessary to perform screening of compounds in herbal plants with antiviral potential against COVID-19. Mint leaves (*Mentha piperita L.*) were reported as one of the proposed samples, and this study was performed *in silico* to evaluate the antiviral activity of the content. **Methods:** The proposed mechanism of action includes the inhibition of SARS-CoV-2 proteins from binding with the receptor. Subsequently, several receptors associated with SARS-CoV-2 were validated, and the one with the code PDB 5R7Y and an RMSD value of 1.9974 Å was obtained using the YASARA application. This study was performed on 15 virtual mint leaves and five previously studied comparison compounds with inhibitory capacity. Therefore, docking started with the PLANTS application, and the results were visualised using PyMol to further identify the amino acids contained in the ligand, while the statistical t-test was used for comparison. **Results**: The study results showed the existence of active compounds in mint leaves, including rutin, hesperidin, and isorhoifolin.

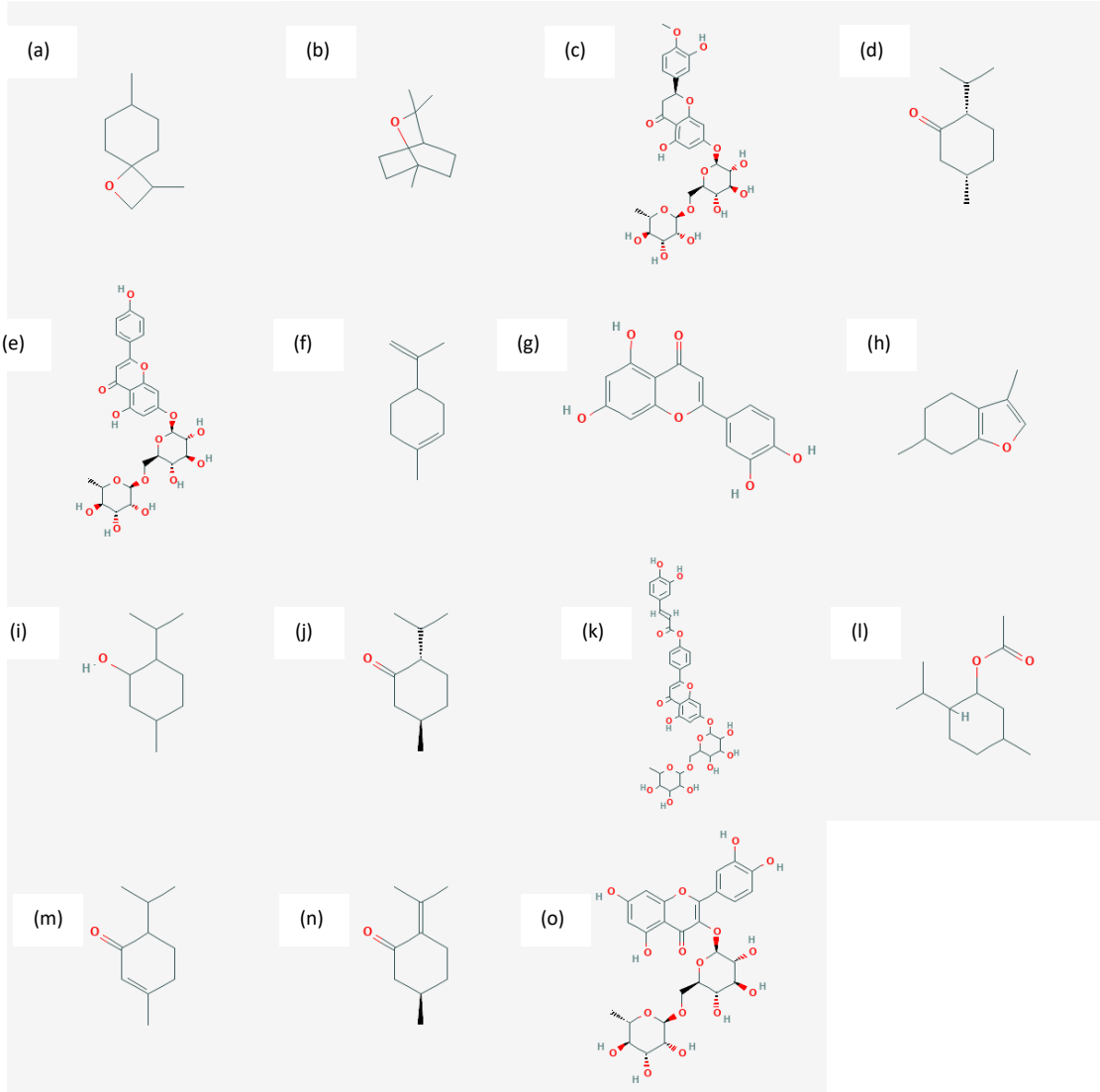
Introduction

Infectious disease is transmitted from one person to another, either directly or through an intermediary. This phenomenon is influenced by three principal factors, i.e., the host, agent (cause), and environment. The agent factors are possibly grouped into viral groups (influenza, trachoma, smallpox, and others), rickets (typhus), bacterial (dysentery), and protozoa (malaria, filaria, Schistosoma, and others) (Masriadi, 2016). The outbreak of mysterious pneumonia in Wuhan, China, in December 2019, spread to numerous surrounding cities and expanded globally. The virus responsible for this disease was later identified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the World Health Organization (WHO) termed the disease Coronavirus Disease 2019 (COVID-19). A total of 28,276 confirmed cases with 565 deaths involving at least 25 countries were documented by the WHO as of 6 February 2020. The incidence of person-to-person transmission potentially occurs through droplet or contact transmission and is facilitated by the absence of any strict infection control measures or proper personal protective equipment for the first-line health workers at risk. There is currently no validated treatment, and the therapeutic strategy adopted is a symptomatic approach and supportive care by maintaining vital signs, oxygen saturation, blood pressure, and treating complications, including secondary or secondary infections or organ failure (Wu Y, 2019). Therefore, the performance of further research on the discovery of antiviral drugs is necessary (Tornery, 2020), including the use of medicinal plants expected to have antiviral activity. A virtual compound of the *Mentha piperita* L. plant, commonly known as peppermint/mint leaves, was used in this investigation. Furthermore, these samples have a high antioxidant content estimated to portray antimicrobial, anti-tumour, and anti-allergenic characteristics (Handayani, 2020). This research applied bioinformatics and molecular docking to

identify compounds of mint leaves with antiviral activity against COVID-19 using the *in silico* method. Also, samples with the best docking score data were obtained from the interaction between the active compounds and the receptor's 5R7Y protein. The results of the *in silico* screening provided insight into the activity of compounds on SARS-CoV-2 receptors, showing that some of them have antiviral effects against the novel coronavirus.

Material and method

The virtual structure of compounds in mint leaves *(Mentha piperita L.)* is shown in Figure 1 (3,7-dimethyl-1-oxaspiro (3,5) nonane, eucalyptol, hesperidin, isomenthone, isorhoifoline, limonene, luteolin, menthofuran, menthol, menthone, menthoside, menthyl acetate, piperitone, pulegone, rutin). The comparison compounds were arbidol, darunavir, chloroquine, lopinavir, and remdesivir. The virtual structure of the 5R7Y receptor was also obtained.



(a) 3,7-dimethyl-1-oxaspiro (3,5) nonane; (b) eucalyptol; (c) hesperidin; (d) isomenthone; (e) isorhoifoline; (f) limonene; (g) luteolin; (h) menthofuran; (i) menthol; (j) menthone; (k) menthoside; (l) menthyl acetate; (m) piperitone; (n) pulegone; (o) rutin

Figure 1: The structure of compounds in mint leaves

Receptor (protein) preparation

The protein complex demonstrated in the format (.pdb) was obtained from the Protein Data Bank (PDB) (RSCB, 2020) before subsequent preparation with the YASARA program. This procedure yielded three files, including

the protein. mol2, ref_ligand.mol2 and ligand.mol2 (Chowdhury, 2021).

Preparation of protein, comparison, and test ligands

The protein, comparative, test compound ligands were prepared using the MarvinSketch at pH 7.4 and saved as ligand_2D.mrv. Then, the conformational search was selected and the result saved with file type .mol2. This procedure was conducted for every single sample.

Optimise protein and set RMSD value

The prepared native ligands were then optimised with the protein crystal structure, using the PLANTS program to obtain a score. The best was then selected and stored in the form of a mole file 2. Subsequently, the optimisation result in terms of RMSD amount was calculated with reference to the experimental results or protein crystal structure using the YASARA program.

Docking comparison ligands

Docking was performed on the three files obtained from the protein preparation conducted later, using the PLANTS program. This approach aimed to obtain the best score for ligands of the comparison compound, which was consequently contrasted with test samples with topmost values.

Docking the test ligand against the receptor

The docking between each test compound ligand was performed using the PLANTS program. Then, the best score was contrasted with the top value for the comparison compound.

Visualisation of ligand and receptor interactions

The respective docking result files were created using the YASARA program (file type.pdb). These outcomes were subsequently visualised and interpreted to determine the initiated interactions using the VMD application.

Results and discussion

Analysis of the receptors used

COVID-19 is a recently discovered disease caused by SARS-CoV-2 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). The infection in humans requires some receptors, which serve as an entry route. In addition, the virtual structure was obtained from the Protein Data Bank (RSCB, 2020), while the receptors for docking provided an RMSD value (Root Mean Square Deviation) less than 2.0 Å after validation.

Subsequently, a protein with the PDB code 5R7Y was used after authenticating several SARS-CoV-2 receptors, based on the results from PLANTS and YASARA applications, and an RMSD value of 1.9974 Å was obtained. These proteins were used because the validation results met the requirements, at less than 2 Å, preventing a far shift of the ligand position binding the protein (active side) because the conversion from 2 Å equals 0.2 mm, according to the diameter size range of an atom, measuring about 0.1 mm. In addition, smaller RMSD implies better ligand position prediction, which results from the relative closeness to the original conformation.

Analysis software used

The software used in this study was downloaded for free and separately to attain the desired docking simulation, comprising a combination of several different applications. Moreover, PLANTS is a docking software benchmarked internally in the Vrije Universiteit Amsterdam, medical chemistry research group with GOLD, a paid docking software routinely used at medicinal chemistry laboratories in Europe and the USA. The other supporting software used were YASARA for protein preparation and RMSD calculation, MarvinSketch for ligand preparation, and PyMol for bond visualization between amino acids and the active representative compounds.

Docking simulation results at the 5R7Y receptor

In this study, some already studied medicinal compounds were used for their antiviral activity against SARS-CoV-2, such as arbidol, darunavir, chloroquine, lopinavir, and remdesivir (Costanzo, 2020). The comparison compound was used as a measure for the ability of the test compound to act on the target receptor. Good activation ability was seen from a low or negative docking score. The docking score for each comparison compound was negative (arbidol: -89.2994, darunavir: -105,304, chloroquine: -81,3629, lopinavir: -98,3046, and remdesivir: -93.0524), showing that the five comparison compounds have a good affinity for the inhibition of the 5R7Y code enzyme used.

The tests were performed on 15 compounds in mint leaves (*Mentha piperita L.*) (Trevisan, 2017) and expected to demonstrate inhibition tendency against the protein coded for PDB 5R7Y. The affinity was also assessed using the molecular docking method performed based on the PLANTS application, with scores determined based on a ChemPLP value. This

factor is calculated with reference to the Gibbs free energy, where the smaller values for the test compound in contrast with the comparison indicates good receptor bond affinity. Moreover, the samples evaluated were obtained using the MarvinSketch application, and docking was performed using the PLANTS software to obtain the respective ChemPLP value.

The research identified three compounds from mint leaves (*Mentha piperita L.*,) with an affinity for inhibiting the SARS-CoV-2 code 5R7Y protein receptor (Gervasoni, 2020). These include Rutin, Hesperidin, and Isorhoifolin. Additionally, the respective ChemPLP values were lower than that of the chloroquine, darunavir, lopinavir, and remdesivir, while Rutin and Hesperidin specifically had lower values than arbidol. The findings indicate a more significant direct inhibition activity of rutin and hesperidin against SARS-CoV-2 protease enzyme receptors compared to others (Bellavite, 2020; Huynh, 2020). This also signifies a similarity in action mechanism between hesperidin, arbidol, and chloroquine. Therefore, both mint leaf (*Mentha piperita L.) in silico* constituents have the potential to be developed into antiviral drugs against COVID-19, although further in vivo research is also needed (Khan, 2020).

Statistic analysis

The ChemPLP docking score data showed higher activity in all three compounds compared to the five comparison samples at the protease receptor coded 5R7Y PDB. Furthermore, data analysis was performed using a statistical two-tailed paired T-test with paired samples from the same unit or group of units via Microsoft Excel. This approach was used to ascertain the suitability between the sample pair data tested. Based on the p-value, samples above 0.05 indicate the absence of any significant differences against the comparison. Conversely, the inverse is reported at $p < 0.05$.

Table I shows the different test and comparison compound pairs within the required $p < 0.05$, including Hesperidin-Chloroquine (0.0005), Hesperidin-Lopinavir (0.0153), Rutin-Lopinavir (0.0062), Rutin-Remdesivir (0.0159), and Isorhoifolin-Chloroquine (0.0240). This finding indicates significant differences between these five pairs, while others tend to not vary substantially.

Table I: Statistics results of active representative compounds

Test compound	Best score docking with the 5R7Y receptor	Comparison of compound	Best score docking with the 5R7Y receptor	*p*-value
Hesperidin	-91.2724	Arbidol	-89.2994	0.5607
		Darunavir	-105.304	1.1625
		Chloroquine	-81.3629	0.0005
		Lopinavir	-98.3046	0.0153
		Remdesivir	-93.0324	0.0808
Rutin	-90.0029	Arbidol	-89,2994	0,3473
		Darunavir	-105.304	2,1162
		Chloroquine	-81.3629	4.0763
		Lopinavir	-98.3046	0.0062
		Remdesivir	-93.0324	0.0159
Isorhoifolin	-84.5769	Arbidol	-89.2994	2.2113
		Darunavir	-105.304	4.3193
		Chloroquine	-81.3629	0.0240
		Lopinavir	-98.3046	4.1460
		Remdesivir	-93.0324	1.1469

Elucidation of the mode of binding of the representative compound active on the active site of the 5R7Y receptor

The amino acid bond visualisation in the binding pocket of the SARS-CoV-2 receptor protease enzyme was performed using PyMol software. This finding was described in three dimensions (3D), and the bonding distance between the active compound and the receptor was determined.

Table II and Table III show the analysis result using PyMol, where the amino acids obtained were thought to play an important role in the compound's affinity at the SARS-CoV-2 receptor, including GLN189 (glutamine), THR25 (threonine), ARG188 (arginine), CYS44, and CYS145 (cysteine). The residues produced demonstrated inhibitory characteristics, while the results of bond distance determination identified ligand-bound forms in hesperidin, Rutin, and isorhoifolin. The bonding generally occurs at a distance

of 1-5 Å, which is within the requirements for all samples. Furthermore, the developed bonds are similar to hydrogen bonds.

Table II: The interaction between test compounds and binding pocket protein with code 5R7Y

Compound name	Types of amino acids	Amino acid amount in the binding site
Rutin	HIS163, ASN142, GLN189, GLY143, THR26, CYS44, SER144, CYS145	8
Hesperidin	HIS163, THR25, GLN189, CYS44, ARG188	5
Isorhoifolin	GLU166, ASN142, GLN189, SER144, GLY143, CYS44, PRO39, CYS145, ARG188	9

Table III: The bond distance between the active representation compound and the bound amino acid

Compound name	Types of amino acids	Bond distance (Ã)
Rutin	GLN189	1.9
	CYS44	2.1; 1.8
	CYS145	2.4
`Hesperidin	THR25	2.3; 2.7
	GLN189	1.7; 2.1
	CYS44	1.9
	ARG188	2.3
Isorhoifolin	GLN189	1.9
	CYS44	2.1
	CYS145	2.1; 2.3
	ARG188	2.0

Visualisation of representative active compounds

Figure 2 shows a total of five amino acid residues at binding site of hesperidin. Meanwhile, the other four bind to the test ligand and are thought to play an important role in the compound's affinity towards SARS-CoV-2 receptor. The amino acids include THR25 with a distance of 2,3 Ã and 2,7 Ã, GLN189 at 1.7 Ã and 2,1 Ã, CYS44 at 1.9 Ã, and ARG188 at 2,3 Ã.

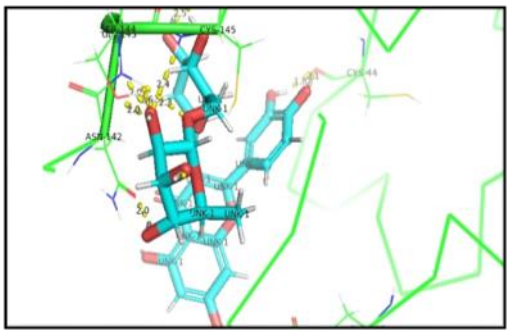


Figure 2: Hesperidin's 3D pose with bound amino acids

Figure 3 is an illustration of a total of eight amino acid residues at the binding site of Rutin compounds. Three were interacted with the test ligand and are thought to play an important role in the affinity towards SARS-CoV-2 receptor. In addition, the amino acids observed include GLN189 with a distance of 1.9 Ã, CYS44 at 1.8 Ã and 2.1 Ã, as well as CYS145 at 2.4 Ã.

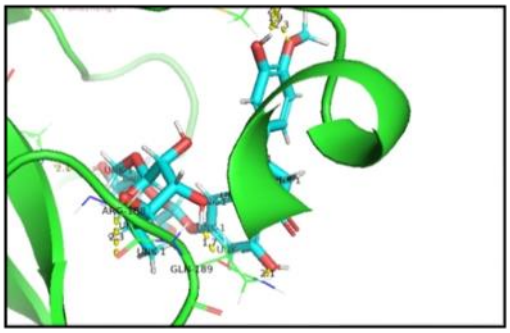


Figure 3: 3D pose of Rutin with bound amino acids

Figure 4 is an illustration of Isorhoifolin compound with a total of nine amino acid residues present at the binding site. Four were interacted with the test ligand, and are assumed to play an important role in the affinity aspect towards the SARS-CoV-2 receptor. In addition, the amino acids identified include GLN189 with a distance of 1.9 Ã, CYS44 at 2.1 Ã, CYS145 at 2.1 Ã and 2,3 Ã, as well as ARG188 at 2.0 Ã.

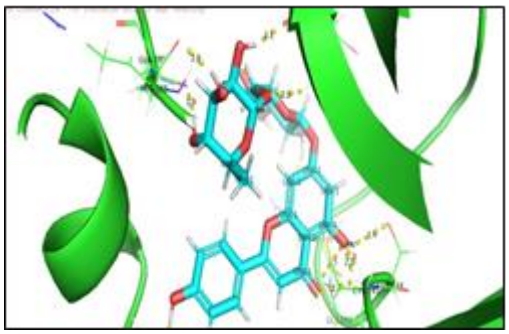


Figure 4: 3D pose of Isorhoifolin with bound amino acids

Conclusion

The research conducted *in silico* on virtual compounds of mint leaves (*Mentha piperita* L.) and molecular docking revealed the activity of these compounds and the potential for the development of antiviral agents to inhibit SARS-CoV-2. The three intrinsic representative active compounds in mint leave compounds, namely Hesperidin, Rutin, and Isorhoifolin, can individually inhibit proteases contained in the SARS-CoV-2 virus protease component. Further research experimental in vitro research on representative active compounds is recommended for the consequent development into SARS-CoV-2 antiviral compounds.

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