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Original Article

IN SILICO STUDY OF SOME FLAVONOID COMPOUNDS AGAINST ACE-2 RECEPTORS AS ANTI-COVID-19

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ABSTRACT

Objective: The coronavirus disease 2019 (COVID-19) pandemic has become a global concern today. As a receptor that plays an important role in viral entry, inhibition of angiotensin-converting enzyme-2 (ACE-2) activity could prevent severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Quercetin is one of the flavonoid compounds reported to have activity as an ACE-2 inhibitor via interaction with the hydroxyl group at ring B positions 3' and 4'. The aims of this research to analyze the binding interaction of some flavonoid compounds into ACE-2 receptor to predict their activity as an anticovid-19.

Methods: An *in silico* approach via molecular docking simulations was conducted, and the selection of potential compounds was based on Lipinski's rules, prediction of absorption, distribution, metabolism, and toxicity (ADMET).

Results: The results showed that nepetin was the most potent compound, with a bond energy of-4.71 kcal/mol and an inhibition constant of 355.62 µM. The compound is bound to amino acid residues Asp30, His34, Glu35, and Thr27, which are important amino acid residues of the ACE-2 receptor.

Conclusion: The nepetin compound complies with all Lipinski rules and has a better ADMET profile compared to other compounds.

Keywords: ACE-2, COVID-19, Flavonoid, *In silico*

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INTRODUCTION

Since the first case in Wuhan, cases of Coronavirus Disease-19 (COVID-19) have been increasing every day. Based on data from World meter, as of May 10, 2021, it was reported that the number of positive cases of COVID-19 and mortality worldwide reached 158,974,260 and 3,306,830, respectively (1). In Indonesia, the number of positive cases of COVID-19 has reached 1,713,684 with 47,012 deaths (case fatality rate/CFR = 2.7%) [1].

COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), which is transmitted from human to human through droplets released by an infected person's coughs or sneezes, which are inhaled or through contact with contaminated objects in the vicinity of the infected person [2]. Clinical manifestations of COVID-19 usually appear within 3-14 d after exposure. Common symptoms of COVID-19 include fever, cough, and shortness of breath. However, a person exposed to SARS-CoV-2 may not show any symptoms (asymptomatic) and can still transmit the virus to others [3].

Various types of synthetic drugs have been used as therapy in patients with COVID-19 to reduce the case fatality rate (CFR), one of which is chloroquine. However, research has reported that the side effects are greater than the effectiveness [4]. Therefore, further research is needed to find active compounds that can be used in COVID-19 therapy.

In the search for active compounds, a phytochemical study was carried out by screening compounds that have the potential for COVID-19. Flavonoids (fig. 1) are compounds that can be found in many plants and have bioactivity that is beneficial to health, such as anti-inflammatory, antioxidant, antimicrobial, and antiviral [5]. This bioactive compound has the potential to be developed as an anti-COVID-19 drug by considering its mechanism of action as an inhibitor of the Angiotensin Converting Enzyme-2 (ACE-2) receptor as well as chloroquine.

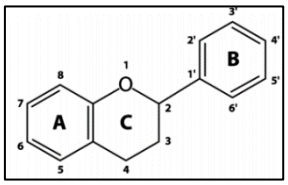

Fig. 1: Structure of flavonoids [6]

ACE-2 is an integral type 1 membrane protein and a functional receptor for SARS-CoV-2, playing an important role in virus transmission into alveolar cells [7, 8]. Inhibition of ACE-2 activity could be promising in preventing SARS-CoV-2 infection due to its role in viral entry. Quercetin is one of the flavonoid compounds reported to have activity as an ACE-2 inhibitor, with two hydroxyl groups in ring B (positions 3' and 4') of the quercetin structure (fig. 2) playing a role in the inhibition [9]. Therefore, it is postulated that other flavonoid compounds with structures similar to quercetin could provide similar activity.

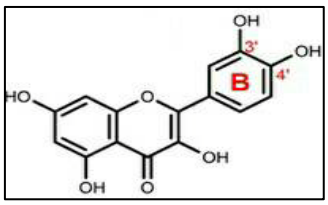

Fig. 2: Quercetin [9]

Delphinidine, eriocitrin, eriodictyol, gossypetin, hyperoside, luteolin, monoxerutin, myricetin, nepetin, nepitrin, orientin, rhamnetin,

robinetin, rutin, and tricetin are flavonoid compounds having a similar structure to quercetin [6]. Molecular docking simulations were used to determine the interaction of these compounds with the ACE-2 receptor. In drug discovery and development, it is necessary to identify the pharmacokinetic profile and toxicity of these compounds. Therefore, compounds that meet Lipinski's rules were further tested for pharmacokinetics and toxicity using pre-ADMET and vNN programs.

MATERIALS AND METHODS

Materials

The hardware used in this study was a Lenovo laptop (model 80XU) with the Microsoft Windows 10 Pro 64-bit operating system. It was equipped with an AMD A9-9420 RADEON R5 processor, which had 5 COMPUTE CORES 2C+3G and a speed of 3.00 GHz. Additionally, it had 4.00 GB of RAM. The software used included AutoDockTools 1.5.6, BIOVIA Discovery Studio Visualizer 2016, and Chem Office 2016.

The materials used in this study consisted of a macromolecule ACE-2 (downloaded from the Protein Data Bank with a resolution of 2.45 Å) and the three-dimensional structures of flavonoids, which were described using the software Chem Office 2016. The flavonoid compounds used as ligands were: quercetin, delphinidine, eriocitrin, eriodictyol, gossypetin, hyperoside, luteolin, monoxerutin, myricetin, nepetin, nepitrin, orientin, rhamnetin, robinetin, rutin, and tricetin. Chloroquine was used as the positive control.

Method

Molecular docking simulation

The test and comparison ligands were prepared by converting them into a three-dimensional structure using the Chem3D program. The energy was minimized, and Gasteiger charge and torque parameters were added. Grid parameters were then created by specifying the grid box and selecting the map type. A molecular docking parameter was created by adding Lamarckian Parameters and setting the Number of GA Runs to 100 repetitions. The file was saved in. dpf format [10]. The interactions and bond energies between the comparison drug, chloroquine, the test flavonoid compounds, and the ACE-2 receptor were simulated using the ADT program.

Selection of compounds using lipinski's rule

The website http://scfbio-iitd.res.in/software/drugdesign/lipinski.jsp was used to view the parameters in Lipinski's rules. For an active compound to be used as an oral drug candidate, it must meet no more than one of the Lipinski rule parameters, which include a hydrogen bond donor<5, a hydrogen bond acceptor<10, a molecular weight<500 Da, and a log P<5 [11].

Prediction of absorption, distribution, metabolism and toxicity

Analysis of the pharmacokinetic properties of the test flavonoid compounds can be carried out using pre-ADMET and vNN programs. The parameters analyzed were Human Intestinal Absorption (HIA) and Caco-2 cells for absorption, Plasma Protein Binding (PPB) and Blood Brain Barrier (BBB) for distribution, Cytochrome P450 (CYP) inhibitors for metabolism, and mutagenicity and carcinogenicity for toxicity [12].

RESULTS AND DISCUSSION

Molecular docking simulation

In docking molecules, a grid box is needed to determine the active site coordinates of the ACE-2 receptor. Parameters that need to be considered are the size of the grid box and the center (initial position of the ligand to be docked). The determination of the grid box was carried out through a literature study to obtain the grid box size of 40 x 40 x 40, space of 0.375, and center coordinates of x =-36.126, y = 32.573, and z = 3.383 [13].

Table 1: Simulation results of molecular docking to ACE-2 receptor

Compound	ΔG (kcal/mol)	Ki (μM)	Interaction with amino acids		
			Hydrogen	Hydrophobic	Other interactions
Chloroquine	-4.34	653.69	Asp30	His34	-
Quercetin	-4.58	441.46	Glu35, Thr27	His34	Asp30, Lys31
Delphinidine	-4.51	495.27	Asp30, Thr27, Glu35	His34	-
Eriocitrin	-3.02	6090	Asp30, His34, Glu35	Lys31	-
Eriodictyol	-4.71	350.23	Thr27, Lys31, Glu35	His34	Asp30
Gossypetin	-4.47	523.74	Thr27, Glu35	-	His34, Asp30
Hyperoside	-3.94	1290	Asp30, Lys31, Glu35, Thr27	-	His34
Luteolin	-4.79	308,41	Asp30, Thr27, Glu35	His34	Lys31
Monoxerutin	-2.78	9160	Asp30, Thr27, Lys31	-	His34
Myricetin	-4.51	490.88	Thr27, Glu35	-	Asp30
Nepetin	-4.71	355.62	Asp30, Thr27, Lys31, Glu35	His34	-
Nepitrin	-3.59	2330	Asp30, Glu35, Lys31, Lys353, His34	-	-
Orientin	-3.25	4110	Asp30, Glu35	His34, Lys31	-
Rhamnetin	-4.17	872.26	Glu35	His34, Lys31	Asp30
Robinetin	-4.53	477	Glu35, Thr27	His34	Asp30, Lys31
Rutin	-3.12	5150	Asp30, Lys31, Glu35	-	His34
Tricetin	-4.55	465.08	Asp30, Thr27, Glu35, Lys31	His34	-

There are three parameters used in determining the affinity of the test compound to the receptor, namely binding energy (ΔG), inhibition constant (Ki), and interaction with amino acids. The negative value of ΔG indicates that interactions occur spontaneously [14]. The estimated minimum effective concentration is represented by the value of Ki, which is well-correlated to IC_{50} in the experimental assay [15]. The more negative the bond energy value and the lower value of the inhibition constant, the higher and more stable the affinity of the ligand to the receptor [16, 17]. Based on table 2, luteolin (-4.79 kcal/mol; 308.41 μM), eriodictyol (-4.71 kcal/mol; 350.23 μM), and nepetin (-4.71 kcal/mol; 355.62 μM) have higher bond energy values with lower inhibition constants than chloroquine (-4.34 kcal/mol; 653.69 μM) and quercetin (-4.58 kcal/mol; 441.46 μM).

Han *et al.* identified an ACE-2 residue that directly interacts with the Receptor Binding Domain (RBD) of the SARS-CoV spike protein. The residues involved were Asp30, His34, Lys353, Thr27, Glu35, Gln24, Tyr41, Gln42, Met82, and Lys353 [18, 19]. It is important to identify the amino acid residues that interact with the test compounds, as the more similar amino acids, the more similar the mode of interaction will be established [20, 21].

Based on table 2, chloroquine, as the comparison compound, forms hydrogen bonds with Asp30 and hydrophobic interactions with His34. Meanwhile, quercetin, as a guide compound, forms hydrogen bonds with Glu35, Thr27, and hydrophobic interactions with His34. All test compounds interact with important amino acid residues at the active site of the ACE-2 receptor. However, based

on the three parameters used to determine the affinity of the test compound for the receptor, luteolin, eriodictyol, and nepetin are the best test compounds (fig. 3). This indicates that the test compounds can form stronger interactions than chloroquine and quercetin, making them potential candidates to inhibit the ACE-2 receptor.

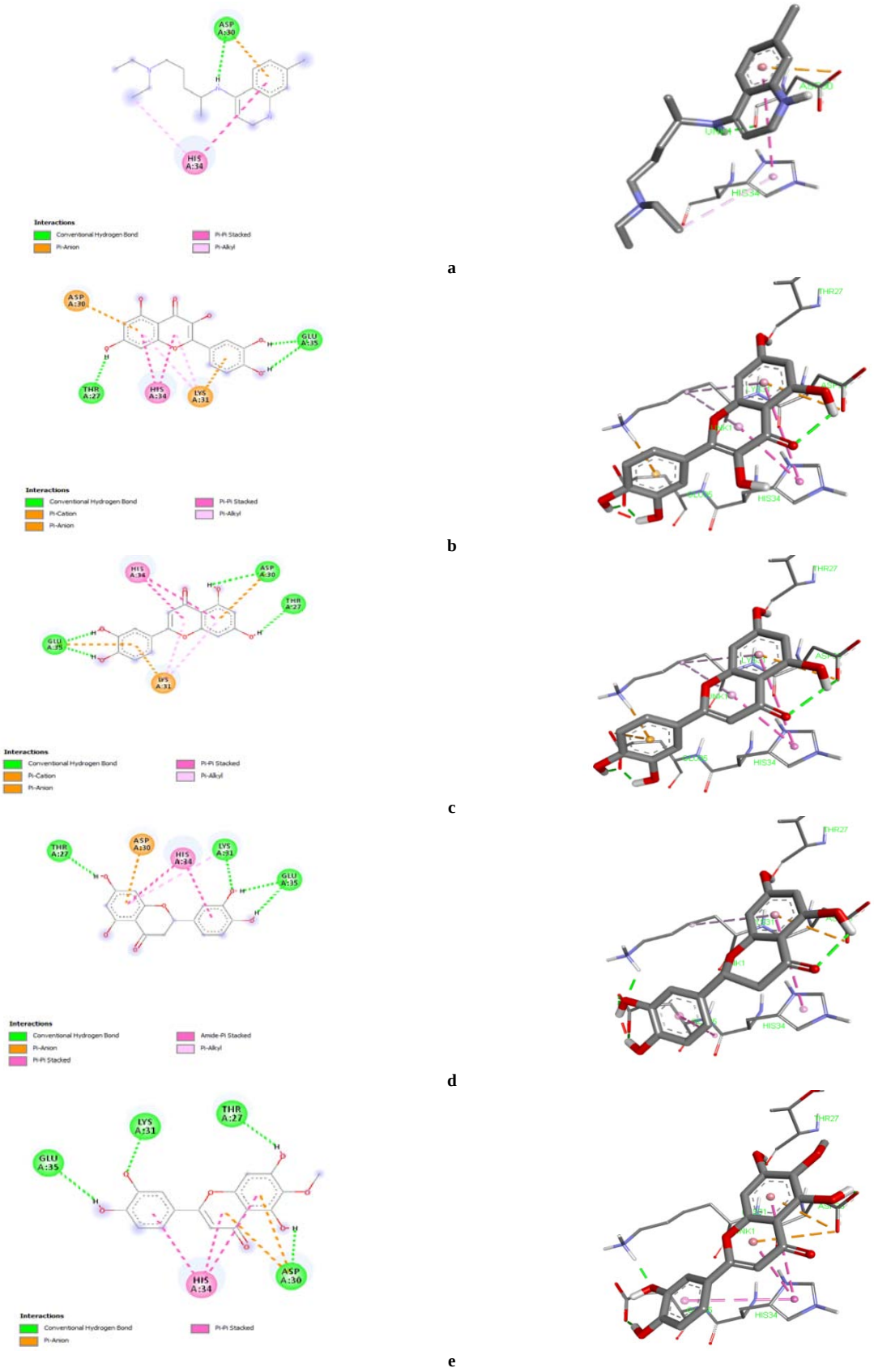

Fig. 3: Interaction between (a) Chloroquine, (b) Quercetin, (c) Luteolin, (d) Eriodictiol, (e) Nepetin with ACE-2 receptors

Selection of compounds using lipinski's rule

As an oral drug, an orally dissolving tablet (ODT) is the most preferred dosage form [22]. Therefore, the compatibility of the test drugs to be formulated in an oral dosage form was also investigated. Active compounds used as oral drug candidates must comply with Lipinski's rules to determine whether these compounds can penetrate biological membranes and have good permeability [23, 24]. Lipinski parameters of the flavonoid compounds tested are shown in table 2.

Based on table 2, luteolin, eriodictyol, and nepetin are the best compounds complying with Lipinski's rules without any violations. Thus, these compounds can be further investigated to determine their absorption profile, distribution, metabolism, and toxicity.

Table 2: Lipinski rule parameters of Flavonoids

No.	Compound	Molecular weight	Log P	Hydrogen bond	
				Donor	Acceptor
1.	Quercetin	302	2	5	7
2.	Delphinidin	303	2.61	6	7
3.	Eriocitrin	596	-1.46	9	15
4.	Eriodictyol	288	2.21	4	6
5.	Gossypetin	318	1.71	6	8
6.	Hyperoside	464	-0.73	8	12
7.	Luteolin	286	2.12	4	6
8.	Monoxerutin	654	-2.21	10	17
9.	Myricetin	318	1.71	6	8
10.	Nepetin	316	2.13	4	7
11.	Nepitrin	478	-0.39	7	12
12.	Orientin	448	-0.36	8	11
13.	Rhamnetin	316	2.31	4	7
14.	Robinetin	302	1.18	5	7
15.	Rutin	610	-1.87	10	16
16.	Tricetin	302	1.8	5	7

Prediction of absorption, distribution, metabolism and toxicity

Prediction of the pharmacokinetic profile of drug candidates could minimize inappropriate decisions on suitable drugs for oral dosage form [25–27]. Table 3 shows the absorption, distribution, and toxicity profiles of the tested flavonoid compounds. Parameters used to view absorption profiles include Human Intestinal Absorption (HIA) and Caco-2 cell permeation values; distribution profiles include Plasma Protein Binding (PPB) and Blood Brain Barrier (BBB); and toxicity profiles include mutagen city and carcinogenicity.

The HIA value indicates the predicted percentage of drugs that can be absorbed by the human intestine [28]. A compound is categorized as well absorbed if the % HIA value is in the range of 70-100%, sufficiently absorbed if in the range of 20-70%, and poorly absorbed if in the range of 0-20% [29, 30]. Based on table 3, luteolin, eriodictyol, and nepetin have HIA values of 77-79%, indicating that these compounds can be well absorbed by the intestines.

Table 3: Prediction of absorption, distribution, and toxicity of test flavonoid compounds

No	Compound	Absorption		Distribution		Toxicity	
		HIA (%)	Caco2 (nm/sec)	PPB (%)	BBB	Mutagenic	Carcinogenic
1.	Luteolin	79.4	4.54	99.7	0.36	+	+
2.	Eriodiktiol	77.4	4.53	100	0.3	+	+
3.	Nepetin	78.3	2.47	92.9	0.1	+	+

In addition, there is a Caco-2 cell model, which is a model for estimating drug absorption *in vitro* [31]. The value of the Caco-2 cell divides the level of permeability of a compound into three levels, namely<4 nm/sec (low), 4-70 nm/sec (moderate), and>70 nm/sec (good) [32, 33]. Based on table 3, luteolin and eriodictyol have moderate permeability, while nepetin has poor permeability. The value of protein plasma binding (PPB) affects the pharmacokinetic and pharmacodynamic properties of the drug [34]. The PPB value>90% indicates that the drug is strongly bound to plasma proteins, while the PPB value<90% indicates that the drug is weakly bound to plasma protein so that it can be well distributed to its target of action [35, 36]. Eriodictyol showed 100% binding to plasma proteins, so it was not possible to use as a drug compound because only free molecules (not bound to plasma proteins) could interact with the receptor. Nepetin, with the best affinity, has a PPB value of 92.9%, indicating that there are still 7.1% free molecules that can be delivered to the target receptor, ACE-2.

The blood-brain barrier (BBB) value indicates the concentration of a drug that can penetrate the central nervous system (CNS) [37]. A BBB value<0.1 indicates that the drug has a low ability to penetrate the CNS (low absorption to the CNS), middle absorption to the CNS if the BBB value is in the range of 0.1-2.0, and high absorption to the CNS if the BBB value is>2.0 [38]. Luteolin and eriodictyol have a BBB value>2, which means that both compounds have a high potential to penetrate the CNS. Meanwhile, nepetin has an intermediate ability to penetrate the CNS with a BBB value of 0.1. The drug compounds for anti-COVID-19 are not designed to be targeted at the CNS but at the ACE-2 receptor, which is highly expressed in the lung epithelium. Therefore, the ability of drugs to penetrate the CNS needs to be prevented to avoid side effects on the CNS [39].

The toxicity profile was evaluated based on the mutagenicity and carcinogenicity parameters [40]. In table 3, luteolin, eriodictyol, and nepetin have the potential to cause mutations (mutagenic) and cancer (carcinogenic). These properties should not be exhibited by compounds that will be developed into drugs.

The metabolic profile of a drug can be determined based on its inhibitory effect on cytochrome enzymes [41]. Cytochrome P450 (CYP) enzymes play a crucial role in drug elimination through metabolic biotransformation [42]. CYP450 comprises five primary isoforms, namely CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 [43]. Inhibiting the activity of these isoforms can cause drug interactions related to pharmacokinetics, leading to side effects or unwanted drug reactions due to reduced clearance and the accumulation of drugs or drug metabolites [44]. According to table 4, luteolin inhibits CYP1A2, while eriodictyol inhibits both CYP1A2 and CYP2C19. Meanwhile, nepetin was predicted to have no potential for inhibiting cytochrome P450 isoenzymes.

Table 4: Prediction profile of flavonoid compound metabolism test

No.	Compound name	Inhibitor CYP				
		1A2	2C9	2C19	2D6	3A4
1.	Luteolin	Yes	No	No	No	No
2.	Eriodictyol	Yes	No	Yes	No	No
3.	Nepetin	No	No	No	No	No

CONCLUSION

Based on the studies, nepetin has the best interaction with the angiotensin-converting enzyme-2 (ACE-2) receptor, as indicated by the binding energy value ΔG of -4.71 kcal/mol, an inhibition constant of 355.62 μM, and interaction with the important amino acid residues Asp30, His34, Glu35, and Thr27. The absorption, distribution, metabolism, and toxicity profiles of nepetin have been identified. Nepetin is predicted to be well absorbed in the human intestine, as indicated by the human intestinal absorption (HIA) value of 78.3%. While the Caco-2 cell permeability value of 2,467 indicates that nepetin can be well absorbed in the intestine but has a low ability to penetrate membranes. The protein plasma binding (PPB) value of nepetin is 92.9%, and the blood-brain barrier (BBB) value is 0.1%, indicating that there are still 7.1% free molecules that could be delivered to the ACE-2 receptor and have an intermediate ability to penetrate the blood-brain barrier. Meanwhile, for toxicity, nepetin was predicted to be mutagenic and carcinogenic. In addition, it does not inhibit the five main cytochrome P450 enzyme isoforms and thus would not be involved in the inhibition of cytochrome P450 (CYP450) enzyme activity.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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