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The Potential of Guafit Powder Capsules as an ACE-2 Inhibitor

(An in Silico Study)

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***Abstract*—Batu City in Indonesia is an agricultural area with excellent commodities, one of which is red guava fruit, known to have a higher vitamin C than oranges as an antioxidant. Meanwhile, the farmers often encounter abundant crop obstacles; it is usually processed into fruit chips. However, the manufacturing heating process can degrade its nutrients. Packaging of guava powder in capsules can become an answer to process natural products, preserve their nutrition and add more value to the commodity. In addition, during the Covid-19 pandemic, the health supplements product rich in vitamin C and antioxidants are necessitated to help stimulate healing and enhance immunity. Objective: To identify drug candidates from bioactive compounds in guava fruit through virtual screening and test potential inhibitors of ACE-2 activity. Methods: Samples were prepared from several bioactive compounds of guava fruit: ascorbic acid, gallic acid, quercetin, and chlorogenic acid. They were then analyzed using the drug-likeness test, molecular docking, and chemical bond interaction and molecular visualization analysis. Result: Quercetin and chlorogenic acids in guava can trigger ACE-2 inhibition by binding to the active site with weak bond interactions. Conclusion: The bioactive of guava fruit is proven in silico to inhibit the activity of ACE-2.**

Keywords—red guava, antioxidant, ACE-2 inhibitor, medicinal plants, supplements products

I. INTRODUCTION

Indonesia is a country that has numerous plants that can be utilized as traditional medicine, one of which is Red Guava (Psiidium *javanicum*) that is rich in tannins, phenols, triterpenes, flavonoids, saponins, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit is rich in pectin and has a high vitamin C content [1]. The vitamin C content of red guava fruit is higher than that of white and orange guava (80mg of vitamin C in 100g of fruit) and contains high amounts of vitamin A l with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside and flavonoids, guaijavarinig and quercetin [2]. The role of vitamin C in the hydroxylation process of the amino acids proline and lysine to form hydroxyproline and hydroxyzine. Both compounds are collagen-forming components important in wound healing and are also very important to provide immunity against infections, including viruses.

Guava fruit is a commodity that is quickly perished so that without proper handling, it can only be stored for a few days if stored at room temperature [3]. Traditional medicine has been recognized for supporting the modern medical system. Along with modernization that demands practicality and convenience in the use of traditional medicines, various efforts are continuously made to develop the use of guava plants as medicinal plants in practical dosage forms. In order for traditional medicines to be more readily accepted and used by the community, they are made with various preparations; the most widely circulating in the community are powders, pills, capsules and tablets [4]. The advantages of capsule products are practical, cover taste and odour, easy to swallow, quickly disintegrate and are absorbed by the intestines. Currently, there is no research and the product of red guava powder in capsules.

This study aims to prove the potential of guava fruit using study in silico as a support for research and development of Guafit capsule products (guava fruit powder packaged in capsules).

II. METHODOLOGY

A. Sample Preparation

All bioactive compounds consisting of ascorbic acid, gallic acid, quercetin, and chlorogenic acid are the content of guava fruit with 3D structure information with structure data format (SDF), formula, and CID obtained from PubChem (HTTPS:// pubchem.ncbi.nlm.nih.gov/). Then the OpenBabel plugin was used to generate a protein databank (PDB) format and ligand structure minimization [5]. The angiotensin-converting enzyme 2 (ACE-2) structure was obtained from RCSB PDB (https://www.rcsb.org/), then water molecules and ligand contaminants were removed from the target protein structure with PyMol 2.5 for protein preparation.

B. Maintaining the Integrity of the Specifications

Prediction of drug-like molecules in guava fruit compounds was carried out according to the Lipinski Rule of Five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). It aims to determine the effectiveness of a chemical compound when it becomes a drug by following parameters consisting of molecular weight, high lipophilicity (LogP), hydrogen donor bonds, acceptors, and molar refractivity. Natural ingredient-based drug candidate compounds with drug-like molecule properties are predicted to show a high success rate in inducing target protein activity [6].

C. Molecular Docking

Binding simulation of guava fruit compounds with target proteins was carried out using the blind docking method. This method aims to identify the binding energy of drug candidate ligands when interacting with the protein domain and the interaction pattern in the molecular complex formed [7]. The degree of influence of ligand binding on the target protein is defined by binding affinity, which is the energy formed when the ligand and protein interact. PyRx 0.8 software with the VinaWizard plugin was used in this study to simulate molecular docking.

D. Chemical Bond Interaction Analysis and Molecular Visualization

The interaction of the ligand-protein complex formed from the docking simulation results was identified through the Discovery Studio 2017 software. The software was used to determine the position and type of interactions such as hydrogen, hydrophobic, Van der Waals, pi, and electrostatic that contribute to forming the target protein's biological response [8]. The 3D ligand-protein complex was displayed via PyMol 2.5 software.

III. Results

According to Benet et *al.* [6], Lipinski Rule of Five consists of molecular mass > 500 Dalton, high lipophilicity < 5, hydrogen bond donor < 5, hydrogen bond acceptor < 10. All compounds in guava fruit can perform as drug-like molecules or good candidate drug molecules because they meet more than 2 Lipinski rules. These natural compounds are predicted to reach intracellular target proteins by passing through cell membranes and induce specific activities when forming molecular complexes with target proteins. The overall 3D structure of the compound from the guava fruit is displayed using PyMol 2.5 software with structural selection based on sticks and specific carbon chain colouring (Figure 1). Result of drug-like molecule test of red guava fruit compound and molecular docking result of bioactive compound in red guava can be seen in Table 1 and Table 2.

TABLE I. Result of Drug-Like Molecule Test of Red Guava Fruit Compound

Compound	MW	LOGP	HBD	HBA	MR
Ascorbic acid	176,000	-1,407	4	6	35,256
Gallic acid	170,000	0,501	4	5	38,395
Quercetin	302,000	2,010	5	7	74,050
Chlorogenic acid	354,000	-0,645	6	9	82,518

*MW: Molecular Weight; LOGP: High lipophilicity HBD: Hydrogen Bond Donor HBA: Hydrogen Bond Acceptor MR: Molar Refractivity

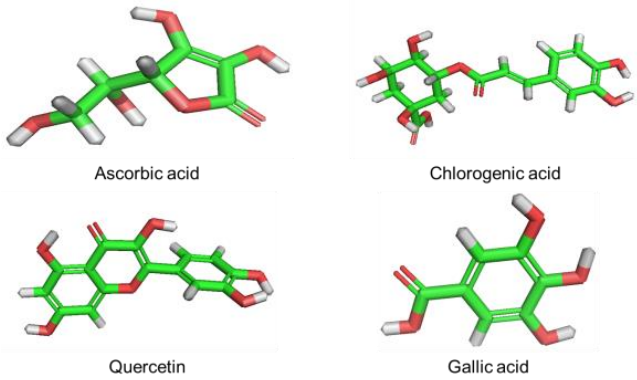
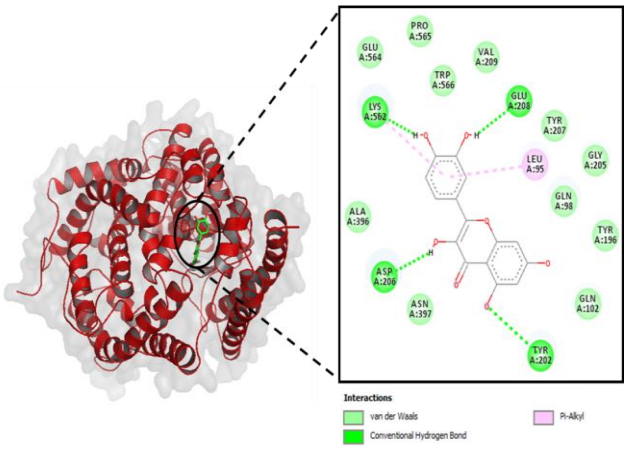


Fig. 1. The visualization of the 3D structure of chemical compounds in guava fruit compounds is composed of hydrogen (grey), oxygen (red), and carbon (green) atoms.

TABLE II. Molecular Docking Result of Bioactive Compound In Red Guava

Compound	CID	Formula	Target	Binding Affinity (kcal/mol)
Quercetin	5280343	C15H10O7	ACE-2	-8,0
Chlorogenic acid	1794427	C16H18O9	ACE-2	-7,8
Gallic acid	370	C7H6O5	ACE-2	-5,9
Ascorbic acid	54670067	C6H8O6	ACE-2	-5,3

This study uses a docking grid with binding to the ACE-2 domain (PDB ID: 7DF4) with Center X:162,941 Y:224,533 Z:301,519; Dimensions X:74,115 Y:79,130 Z:77,524 by guava fruit compound. The level of binding affinity indicates the binding activity of the ligand to the target protein. If it has a negative value, it can induce specific biological responses such as inhibition [5,9]. The results of the molecular docking simulation confirm that there is chemical compound quercetin containing guava fruit with the most negative binding affinity value of -8.0 when interacting with the ACE-2 domain. The ligand-protein molecular complex is shown with the red (ACE-2), transparent surfaces (grey), and ligand (green) cartoons structures using PyMol software (Figure 2).



Quercetin_ACE-2

Fig. 2. Visualization of the 3D structure of the Quercetin_ACE-2 molecular docking simulation.

Quercetin is predicted to affect the inhibitory activity of ACE-2 because it binds to the active site with glutamic acid (Glu) and glutamine (Gln) residues [10]. The chemical bond interactions formed consist of Van der Waals, hydrogen, and alkyl.

SARS coronavirus is a single- stranded RNA virus, which uses ribosome sites to encode 2 replicase glycoproteins, PP1a and PP1b, which mediate viral replication. Once these precursor glycoproteins are synthesized, 3C- like protease (3CLpro) plays a critical role in the lytic release of its replicates [11]. Quercetin and its metabolites, inhibition of recombinant human ACE2 (rhACE2) activity has been reported *in vitro.* The rhACE2 activity was then inhibited by rutin, quercetin-3-O-glucoside, tamarixetin, and 3,4-dihydroxyphenylacetic acid by 42–48%. With an IC50 of 4.48 μM, quercetin was the most potent rhACE2 inhibitor tested in this study. The herbal extracts investigated here contain high amounts of these plant metabolites [12].

Quercetin, identified as one of the constituents of Pichia pastoris displayed good inhibition toward 3CLpro with an IC50 value of 73 M. Quercetin-3- O-β- galactoside binds to SARS-Cov 3 Cl protease and inhibits its proteolytic activity. Based on current studies, Quercetin may be one of the key flavonoids that inhibits the coronavirus infective cycle with pleiotropic functions and low toxicity [11]. These findings have indicated that Quercetin could be an antiviral agent against SARS-CoV-2 [13].

The results might support the previous findings on inflammation in virus-related acute injured kidneys. Increasing evidence shows that inflammatory and cytokine storm are associated with the severity of COVID-19 disease, for example, the level of Serum IL-6 and IL-10 are significantly higher in severe confirmed cases than in the mild group. Previous studies have verified that Quercetin could play multiple regulatory roles in halting inflammation during acute kidney injury [14].

Vitamin C is an essential vitamin with known antiviral properties which is under investigation for its beneficial effects during the stress response in sepsis and critically ill patients. Co-administration of Quecertin (12.5 mg/kg/week) and vitamin C and B3 in a murine model of exercise-induced susceptibility to influenza H1N1 prolonged time-to-death and improved survival. Clinical trial identified the combination of flavonoids and ascorbic acid (1:1 ratio) as beneficial for respiratory infection (200 mg thrice a day). There is evidence that vitamin C and Quecertin exerts a synergistic antiviral action due to overlapping antiviral and immunomodulatory properties and the capacity of ascorbate to recycle Que, increasing its efficacy. Safe, cheap interventions which have a sound biological rationale should be prioritized for experimental use in the current context of a global health pandemic. The use of vitamin C and Que has also been suggested both for prophylaxis in high-risk populations and for the treatment of COVID-19 patients as an adjunct to promising pharmacological agents such as Remdesivir or convalescent plasma [11].

Coronavirus appears to be susceptible to the inhibitory actions of zinc, which may prevent viral entry into cell and Natural Product Communications appears to reduce coronavirus virulence. Quecertin also functions as a zinc ionophore and has been shown to facilitate the transport of zinc across lipid membranes. This could, theoretically, enhance the antiviral actions of zinc [13].

IV. Conclusion

The bioactive of guava fruit is proven in silico to affect the activity of the target protein. Quercetin compounds can induce ACE-2 inhibition by binding to the active site with weak bond interactions such as hydrogen, Van der Waals, pi, and alkyl. Further research is needed to determine the mechanism of action inhibition of ACE-2 activity by the active compound of guava fruit. In addition, it is also necessary to conduct a quantitative test to determine the levels of antioxidants and vitamin C in Guafit Capsule Powder.

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