Potential Analysis of Majapahit Fruit Powder (Crescentia cujete L) as Shrimp Immunostimulants using the in Silico Method

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Abstract. Shrimp production is currently constrained by many diseases. These diseases are conventionally prevented by administering chemicals as immunostimulants, with their attendant side effects. Alternative natural ingredients as immunostimulants in shrimp production are being sought, and Majapahit plant (Crescentia cujete L.) is one. In vitro and in silico studies on the plant’s stems, leaves and fruits showed the plant’s desirable effects on the immune system of Vannamei Shrimp, as the plant contains important bioactive compounds (e.g. carotenoids, phenolics, alkaloids, pectin, tannins, flavonoids and terpenoids). The present study determined the ability of Majapahit fruit powder mixed into shrimp feeds in enhancing the immune system of the seafood. Liquid chromatography–mass spectrometry (LC-MS) revealed quercetin flavonoids as the effective active ingredient in the powder, which in silico study proved to be an effective immunostimulant for shrimps.

1. Introduction

Vannamei shrimp is a sea shrimp that can be found widely in Pacific ocean waters starting from Mexico, Central and South America. Life inhabits all water columns, from the bottom to the surface layers, for example in muddy sandy areas, estuaries, and the sea, with a depth of 0 to 70 m [1]. According to [2] Vanamei shrimp (Litopenaeus vannamei) is a type of penaeid shrimp that has a higher endurance compared to tiger species. Even so, in the cultivation of vannamei shrimp, there are still many obstacles that can hamper success, including disease attacks caused by, among others, bacteria and viruses that can cause great losses to farmers.

One effort that can be done to reduce disease is by providing immunostimulant materials that can improve the immune system. Increasing the body's defense or immune system against attacks by disease can be done by feeding with balanced nutrient composition, or by providing immunostimulants in feed, so that it will be directly related to immune system cells that make these cells more active [3]. Majapahit plants are flowering dicot plants originating from South and Southeast Asia including Indonesia, commonly found in tropical and sub-tropical regions. In the leaves of the majapahit plant it contains active ingredients; flavonoids, saponins, tannins, steroids, and triterpenoids. While the stem contains saponins, tannins, steroids and teriterpenoids [4]. According to [5], based on phytochemical tests of Majapahit fruit powder containing saponins, triterpenoids and flavonoids. The results of the analysis using FTIR, UV-VIS Spectrophotometry and GCMS, Majapahit fruit extracts contain active ingredients such as furfural, 1,2,3 Benzentriol, 4H-Pyran-4-one and others which have potential as
antibacterial. Previous research proves that majapahit leaf powder can increase the immune system of sangkuriang catfish (Clarias gariepinus) that are infected with aeromonas bacteria [6], in silico majapahit leaf extract has proven potential as an antibacterial for cannamei shrimp infected with Vibrio harveyi bacteria [7]. Use of Majapahit leaf extract also plays a role in reducing the activity of Aeromonas hydrophila bacteria in tilapia (Oreochromis niloticus) cultivation [8].

According [9] insilico method or bioinformatics test is a test using a computer to determine the potential of a compound. The stages in insilico analysis include; selection and characterization of target molecules, visualizing the structure of target molecules and designing the mechanism of relationships between drug molecules or chemical compounds. This research determines the ability of Majapahit fruit powder mixed into shrimp feed to improve the shrimp's immune system in an insilico manner.

2. Research Methods

2.1. Material
The material used in this study was Majapahit fruit powder (Cresentia cujete L.) which was analyzed using LCMS. The result is a flavonoid type Quercetin-3β-D-glucoside, Quercetin, Chlorogenic Acid and Taxifolin [10].

2.2. Modelling Protein
Modeling of the LGBP protein (Lipopolysaccharide Glucan Binding Protein), Toll like receptor, and Lectin from Penaeus vannamei was done using the Swiss Model webserver (https://swissmodel.expasy.org/). Protein modeling is done because LGBP and Toll like receptor proteins are not yet available in the database, so homology needs to be done with 3D protein models that are already available in the database. LGBP protein construction uses a template with PDB ID (3AZX) with a sequence identity of 33.3%. While the Toll like receptor protein construction uses the PDB ID (5ZSA) protein template with a sequence identity of 21.3%. Proteins with PDB ID 6HUG were used to make Lectin protein with sequence identity of 22.22%.

2.3. Molecular Docking
Docking is done by using Autodock Vina on the PyRx 9.5 Protein program which is used as a target and controls are attached to the Excel data. The PyMol 2.3.1 program is used to visualize the docking results, while the LigPlot 2.1 program is used to see the amino acid interactions that occur.

3. Results and Discussion

3.1. Molecular Docking Analysis
The molecular docking method is also used to explore possible ligand binding modes and the relationship between 3D binding modes and structural features of 2D-molecular ligands so that they can be explored further. Wang et al (2010) [11]. Docking conducted in this study is reverse docking by mimicking the interaction of target proteins and ligands that are considered as controls (peptidoglycan from bacterial walls). Docking is a method for predicting the strength of interactions between receptors and ligands, based on binding affinity values. The more negative the value, the stronger the interaction that occurs between receptors and ligands. Compared to other bioactives, Quercetin-3β-D-glucoside has a stronger interaction with TLR, whereas Taxifolin with GLBP protein.

| Pubchem ID | Compound (as a ligand) | Binding Affinity (kcal/mol) |
|------------|------------------------|---------------------------|
| 5280804    | Quercetin-3β-D-glucoside | -7.9, -8.1, -7.3          |

Table 1. Results of Majapahit Bioactive Docking
Table 1. describes the docking results of LCMS compounds from majapahit fruit powder (*Crescentia cujete* L.) namely Quercetin-3β-D-glucoside, Quercetin, Chlorogenic Acid and Taxifolin, as ligands with proteins that play a role in shrimp immune system, namely LGBP (Lipopolysaccharide Glucan Binding Protein), Toll like receptor, and Lectin as receptor. The value of binding affinity (kcal/mol) of receptors with ligands shows a higher value than control (peptidoglycan) both in GLBP, TLR and Lectin ligands, this shows that the compounds contained in Majapahit fruit powder have stronger affinity binding bonds than controls.

According to [12] Ligands are substances that form complexes with biomolecules to serve biological purposes. In protein-ligand binding, ligands are usually molecules that produce signals by binding to sites on the target protein (receptors). For example the binding of DNA-ligands, ligands can be small molecules, ions, or proteins. Besides the bonding relationship between ligands and receptors in the form of charge functions, hydrophobic, and molecular structure.

Ligands binding to receptor proteins can change conformations by affecting the orientation of three-dimensional shapes. The binding level is called affinity, (binding affinity) which indicates the tendency or strength of the effect. Binding / affinity bonding is actualized not only by host-guest interactions, but also by the effect of solvents which can play a dominant steric role that encourages non-covalent binding in solution. Besides the binding occurs by intermolecular forces, such as ionic bonds, hydrogen bonds and Van der Waals [13]. Figures 2, 3 and 4 show the visualization of docking results between ligands (Quercetin-3β-D-glucoside, Quercetin, Chlorogenic Acid and Taxifolin) and receptors (LGBP (Lipopolysaccharide Glucan Binding Protein), Toll-like receptors, and Lectin).

![Figure 1](image1.png)

**Figure 1.** a) Visualization of TLR docking results. Red (Control), Green (Quercetin-3β-D-glucoside) b). Visualization of TLR docking results. Red (Control), Green (Quercetin-3β-D-glucoside), Cyan (Chlorogenic Acid), Pink (Taxifolin), Orange (Quercetin)

![Figure 2](image2.png)

**Figure 2.** a) Visualization of GLBP docking results. Red (Control), Pink (Taxifolin), b) Visualization of GLBP docking results. Red (Control), Green (Quercetin-3β-D-glucoside), Cyan (Chlorogenic Acid), Pink (Taxifolin), Orange (Quercetin)
After knowing the value of binding affinity, it is necessary to do further analysis, namely the interaction of amino acids between proteins and ligands, using the LigPlot program. Based on LigPlot analysis there are several amino acids that are the same between bioactive and ligand control, which means that predicted bioactive Majapahit can interact with the target protein similar to the interaction between the target protein and the peptidoglycan used as control. The following table shows a visualization of the results of the Lig Plot.

**Table 2. LigPlot Visualization Results**

| Proteins   | Hydrophobic Bonds                  | Hydrogen Bonding                      |
|------------|------------------------------------|---------------------------------------|
| **TLR**    |                                    |                                       |
| Control    | ILE725, LEU694, ARG681, LEU722, THR691 | ARG727, ARG726, ARG690, ASP692         |
| Quercetin-3β-D-glucoside | THR691, ARG690, ILE725, LEU722, ARG681, ASP692, ARG678, LEU718, LEU694 | ALA729, ARG726, SER724 |
| Quercetin  | LEU718, LEU694, ARG678, LEU722, ILE725, ASP692 | ARG726, SER724, VAL689 |
| Chlorogenic Acid | LEU718, ARG681, LEU694, LEU722, ILE725 | ARG690, VAL689, SER724 |
| Taxifolin  | ASP692, LEU722, ILE725, THR691      | ARG690, VAL689, SER724 |
| **GLBP**   |                                    |                                       |
| Control    | SER188, GLY203, PHE307, VAL222, TYR201 | THR220, HIS221, ARG189, VAL222         |
| Quercetin-3β-D-glucoside | GLU223, ARG189, SER188, PHE307, GLY203, VAL222 | ASN191, TYR201, GLU187, ASN199, THR220, HIS221 |
| Quercetin  | GLU223                              | ASN199, ARG189, TYR201, HIS221         |
| Chlorogenic Acid | TYR201, GLY203, PHE307, GLU223, HIS221 | ARG189, ASN191, THR220, GLU187, THR205 |
| Taxifolin  | SER188, GLU187, GLY203, HIS221, PHE307, LEU652, THR655, LYS654, ASP662, SER649, THR648, TYR657 | TYR201, ARG189, THR205, VAL222, THR220 |
| **LECTIN** |                                    |                                       |
| Control    |                                    |                                       |
| Quercetin-3β-D-glucoside | LEU645, ASP662, PHE658, LYS659, TYR657, THR648, LEU652 | LYS654, SER649, PRO653, THR655 |
| Quercetin  | SER649, LEU645, LEU665,             | LYS654, THR648, LYS659                |
From table 2. it is known hydrophobic bonds and hydrogen bonds between ligands and receptors and bonds on control. Amino acids that bind to control are also present in ligand compounds, this shows that ligand compounds also work like controls that play a role in the shrimp immune system. Based on its affinity bond, the Taxifolin compound binds best with GLBP WITH VALUE -8 kcal / mol, cholargen acid compound binds best with TLR at -8.1 kcal / mol, whereas quercetin compound binds best with TLR and GLBP at -7.8 kcal / mol, while quercetin 3β-D-glucoside compounds are best bound to TLRs of -8.1 kcal / mol. Figure 5 is a visualization of the bonds that occur between compounds as ligands with amino acids both hydrophobic and hydrophilic bound.

**Figure 4.** a) Visualization of 2D Lectin with taxifolin, b) TLR with Quercetin, c) TLR with quercetin 3β-D-glucoside

4. Conclusions
Quercetin-3β-D-glucoside is predicted to be bioactive which has the best potential to influence immunity in shrimp.

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