Ethanol extract analysis of steam pineapple (*Ananas comosus*. L) and its application as antibacterial agent: In vitro and silico studies

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**Abstract.** Pineapple contains active compounds, such as phenolics, steroids, flavonoids, terpenoids, saponins, quinines, coumarins, alkaloids, and tannins. These compounds can be used as antioxidants, antibacterial, and antifungals. The purpose of this study was to determine the activity of compounds in pineapple steam in inhibiting the growth of *Staphylococcus aureus*, *Shigella dysentry*, *Salmonella typhi* in vitro and silico. The extraction method was carried out by maceration using 70% ethanol. Analysis of the compound in pineapple extract using LC-MS. In vitro antimicrobial activity test using the disc diffusion method. Toxtree software was used to test the toxicity of compounds. Physicochemical test of compounds using Lipinski Test. Docking molecular using PyRx software. Visualization of docking results using Discovery Studio 2019 Software. The results showed that the yield obtained from the extraction process of pineapple weevils was 79.3%. There were 58 compounds obtained from the LC-MS analysis. Compounds that have antibacterial activity are acetic acid, cinnamic acid, oxalic acid, malic acid, thiamine, niacin, riboflavin, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, quercetin, ananasic acid, subaphyllin, beta carotine, lutein, cyanin, cyaniding 3,5,3'-triglucoside, beta-sitosterol, campesterol, and hydroxysitosterol. Toxicity test results with toxtree software found that all compounds belong to Class I. Class I compound that have low toxicity properties.

1. **Introduction**

Antimicrobial is a substance that can kill or inhibit the growth of microbes (bacteria, fungi, or viruses) by interfering with microbial metabolism. Generally, the substance used as an antibacterial is an antibiotic. However, excessive use of synthetic antibiotics can cause patient allergies or resistance to bacteria. Therefore, research on antibacterial substances derived from natural ingredients continues to be developed.

One of the natural ingredients that have potential as an antimicrobial is pineapple. All parts of pineapple contain active compounds, such as phenolics, steroids, flavonoids, terpenoids, saponins,
quinine, coumarins, alkaloids, and tannins with varying levels [1]. These compounds can be used as antioxidants, antifungal, and antibacterial [2,3].

In vitro and in vivo antibacterial testing methods have been widely used in drug discovery efforts. Currently, in addition to these two methods, it is possible to use servers and software applications for computational studies. This method uses the ability of computers to design drugs as a complement to in vitro and in vivo tests. In silico studies offer the ability to predict the activity, toxicity, and lethal dose of an active compound [2,3].

Drug design is one of the steps used to obtain new drugs by modifying the bond structure between drug compounds and target proteins. This drug design aims to find candidate compounds used as drugs with low toxicity and high bioactivity. Structural modification is carried out by synthesizing several derivatives of the parent compound, identifying the structure, and testing its biological activity [4].

Changes in the structure of the compound will change the physicochemical properties of the compound, including lipophilic, electronic, and steric properties, and changes in these physicochemical properties will cause changes in the biological activity of the compound [5,6]. Molecular modeling also called in silico testing, has a very important role in the field of Medicinal Chemistry in the context of designing, discovering, and optimizing bioactive compounds in the drug development process [7,8]. Based on the above background, in this study, the extraction and analysis of bioactive compounds contained in the ethanolic extract of pineapple hump will be carried out using Liquid Chromatography-Mass Spectrometry (LC-MS). The pineapple weevil extract will also be tested for its antibacterial properties against Staphylococcus aureus, Shigella dysentriae, Salmonella typhi bacteria in vitro, and the interaction of its chemical components in silico will be studied.

2. Materials and methods

2.1. Sample preparation
Pineapple samples were obtained from a pineapple plantation in Ngancar village, Kediri Regency, East Java. The pineapple hump is cleaned and separated from the fruit flesh and then cut into thin strips, then dried in an oven at a temperature of 40-50 °C. The dried bulbs were mashed and then sieved through a 30 mesh sieve. Then for the webservers used are PubChem.NCBI, rscb, PassOnline Way2Drug, and Lipinski. The content of the active compound (ligand) is stored in the form of a 3D structure with the file type.

2.2. Ethanol extract preparation
A total of 100 g of samples that passed a 30 mesh sieve were dissolved in 300 ml of 70% ethanol, then stirred for 2 hours, and allowed to stand (macerated) for 24 hours. Furthermore, filtering and remaceration were carried out 2 times. The filtrate obtained was then concentrated using a rotary vacuum evaporator.

2.3. Antibacterial activity
The antibacterial activity test was carried out using the disc diffusion method [9]. The bacteria used in this study were Staphylococcus aureus, Shigella dysentriae, and Salmonella Typhi. Bacterial inoculants were grown in Nutrient Agar (NA) media, and then one colony was taken to be dissolved in Nutrient Broth (NB) media. The bacterial cultures were incubated at 37 °C for 24 hours. Next, the bacteria were streaked evenly on Mueller Hilton Agar (MHA) media using a sterile swap. Paper discs that had been soaked with ethanol extract were placed on MHA media, then incubated at 37 °C for 24 hours. The same method also applies to the positive control test, where the disc paper is immersed in an antibiotic solution (ciprofloxacin), and the negative control, where the disc paper is immersed in sterile distilled water. The inhibition zone was measured after 24 hours of incubation using a caliper.
2.4. **Ethanol extract analysis using LC-MS**
Analysis of the chemical components contained in the ethanol extract of pineapple cob was carried out using Shimadzu LCMS – 8040 LC/MS. A total of 1 l of ethanol extract was injected into the Shim Pack FC-ODS column with a column temperature of 35 °C and the running process for 60 minutes. The analysis process is carried out at Brawijaya University, Malang.

2.5. **Ligands minimization**
Ligands that have been downloaded to the PubChem database must first be converted from sdf to pdb. In addition, the goal of minimization is to maximize the binding energy between the atoms that make up the ligand so that it becomes more flexible when entering the molecular docking stage. Minimization using the OpenBabel plugin contained in the PyRx software so that we will get a flexible 3D structure with pdb format.

2.6. **Toxsisitas assay**
A toxicity test is a test to determine the level of toxicity of compounds, and the first step is to open the software. Then the next step is to enter the canonical smile obtained on the Pubchem webserver, then place it on the "Chemical identifier," then select the method used in the "Select a decision tree." Then select the Cramer rules parameter, then click "Estimate," then the results will appear after the identification of the results is complete.

2.7. **Lipinski's role**
The Lipinski test is a test to see compounds that have poor solubility and permeability and are predicted using the Lipinski rule of 5, and the test compounds can meet the requirements if they meet the five Lipinski Lipinski parameters, namely: that absorption and permeation are not good when a compound has more than five hydrogen bond donors, molecular weight more than 500, Log P more than 5, molar refractivity between 40-130 and there are more than 10 hydrogen bond acceptors.

2.8. **Molecular docking**
This method is used to interact between molecules with each other to obtain a stable binding energy value that allows the formation of a molecular complex. The chosen binding energy is the most negative binding energy because it can potentially affect the biological activity of a protein. The docking method used is blind docking, so the docking grid will be made maximally comprehensive on all surfaces of the target protein to know the query compounds that have the potential to affect the activity of the target protein. The output of the docking results is a comparison of the binding energy value with mode 0 because the goal was blind docking. A value of 0 means that the RMSD value is neglected, so it can be assumed that the ligand is flexible while the protein remains rigid or rigid.

3. **Result and discussion**
In this study, the extraction process was carried out by maceration, while the separation process between the extract and the solvent was carried out using a rotary vacuum evaporator. The purpose of using a rotary vacuum evaporator is to avoid damage to thermolabile compounds (easily damaged by heating). The solvent used is 70% ethanol. The use of ethanol as a solvent is based on the ability to bind polar to semipolar compounds. The yield produced in the manufacture of pineapple weevil ethanol extract was 79.269%. The results of the extract were then tested for antibacterial activity using the disk diffusion method based on Kirby beur. The diameter of the inhibition zone formed can be seen in Table 1.
Table 1. The antibacterial test result of *S. aureus*, *Shigella*, and *S. typhi*

| Treatment          | Inhibition Zone (mm) | *S. aureus* | *Shigella* | *S. typhi* |
|-------------------|----------------------|-------------|------------|------------|
| Negative control  | 6                    | 6           | 6          |            |
| Positive control  | 14.5                 | 10.3        | 8.45       |            |
| Extract 80%       | 10.35                | 7.56        | 8.1        |            |
| Extract 90%       | 12.9                 | 9.05        | 9          |            |
| Extract 100%      | 15.5                 | 10          | 9.95       |            |

Based on the results of the antibacterial activity test in Table 1, it can be seen that the highest activity was found in the ethanolic extract of pineapple weevil with a concentration of 100%. According to Elganjar [10], the antibacterial power is classified into three, namely strong if it produces inhibition zone diameter more than 8 mm, moderate activity if it produces an inhibition zone diameter of 7-8 mm, and weak activity if it has less inhibition zone diameter than 7 mm. Based on this, it can be concluded that the pineapple weevil ethanol extract has strong antibacterial power at a concentration of 90%. This ability is also the same as the inhibitory activity in the positive control treatment using Chloramphenicol. To determine the content of compounds in the ethanol extract of pineapple weeds that act as antibacterial, LC-MS analysis was carried out.

The analysis of the LC-MS results in Figure 1 shows that there are 58 chromatogram peaks. This means that there are 58 compounds identified in the 70% ethanol extract of pineapple weevil. There are several advantages of analyzing compound content using LC-MS, including being able to analyze thermolabile, semipolar to polar compounds, high sensitivity, selective, able to determine the type of compound based on its molecular weight, able to analyze compounds qualitatively and quantitatively [11]. The results of the classification of compounds can be seen in Table 2.

![Figure 1. LC-MS chromatogram of ethanolic steam extract of pineapple](image-url)
| No. | Compound                  | Composition (%) | Molecule Formula | Molecule Weight (Mr) | Compound Class       |
|-----|---------------------------|-----------------|------------------|----------------------|----------------------|
| 1.  | Acetaldehyde              | 0.55277         | C₂H₃O           | 44.053               | Aldehyde             |
| 2.  | Acetic acid               | 2.98975         | C₂H₄O₂          | 60.052               | Carboxylic Acid      |
| 3.  | Cinnamic acid             | 2.72999         | C₆H₈O₃          | 148.161              |                      |
| 4.  | Oxalic acid               | 1.13118         | C₂H₂O₄          | 90.034               |                      |
| 5.  | Malic acid                | 2.72999         | C₆H₁₀O₅         | 134.087              |                      |
| 6.  | Citric acid               | 3.40812         | C₆H₈O₇          | 192.123              |                      |
| 7.  | Ethyl formate             | 0.89755         | C₁₈H₂₀O₂        | 74.079               |                      |
| 8.  | Ethyl acetate             | 1.31698         | C₂H₄O₂          | 88.106               |                      |
| 9.  | Isobutyl formate          | 0.93914         | C₆H₁₀O₂         | 102.133              |                      |
| 10. | Butyl formate             | 1.85235         | C₁₂H₂₂O₃        | 102.133              |                      |
| 11. | Ethyl propanoate          | 1.51383         | C₆H₁₀O₂         | 102.133              |                      |
| 12. | 2-methylpropyl formate    | 0.76967         | C₆H₁₀O₂         | 102.133              | Ester                |
| 13. | Methyl butanoate          | 1.70966         | C₁₂H₁₀O₂        | 102.133              |                      |
| 14. | Methyl 2-methyl propanoate| 1.19487         | C₁₂H₁₂O₂        | 102.133              |                      |
| 15. | Propyl acetate            | 0.70737         | C₆H₁₀O₂         | 102.133              |                      |
| 16. | Isobutyl acetate          | 0.93883         | C₁₂H₁₂O₂        | 116.16               |                      |
| 17. | Ethyl butanoate           | 1.24563         | C₁₂H₁₀O₂        | 116.16               |                      |
| 18. | Ethyl isobutanoate        | 1.33628         | C₁₄H₁₄O₂        | 116.16               |                      |
| 19. | Ethyl 2 hydroxypropanoate | 1.09249         | C₁₂H₁₀O₃        | 118.132              |                      |
| 20. | Methyl 3-hydroxybutanoate | 1.96638         | C₁₄H₁₆O₃        | 118.132              |                      |
| 21. | Ethyl isovalerate         | 1.19487         | C₁₄H₈O₂         | 130.187              |                      |
| 22. | Methyl hexanoate          | 0.82302         | C₁₄H₈O₂         | 130.187              |                      |
| 23. | Dimethyl malonate         | 1.70972         | C₆H₈O₄          | 132.115              |                      |
| 24. | Isopropyl isobutyrate     | 1.41118         | C₆H₄O₂          | 130.187              | Ester                |
| 25. | Methyl 3 hydroxyhexanoate | 1.06756         | C₁₄H₈O₃         | 146.186              |                      |
| 26. | Ethyl 3 propanoate        | 0.82113         | C₁₂H₁₄O₂S       | 148.0558             |                      |
| 27. | Fructose                  | 5.64827         | C₆H₁₂O₆         | 180.156              | Polysaccharide       |
| 28. | Glucose                   | 6.56002         | C₆H₁₂O₆         | 180.156              | (carbohydrate)       |
| 29. | Sucrose                   | 4.24424         | C₁₂H₂₂O₁₁       | 342.297              |                      |
| 30. | Pectin                    | 2.40431         | C₁₀H₂₀O₇        | 194.139              |                      |
| 31. | Ethyl hydroxyhexanoate    | 0.95163         | C₈H₁₆O₃         | 160.213              |                      |
| 32. | Ethyl hexanoate           | 0.93911         | C₁₂H₁₆O₂        | 144.214              | Fatty Acid           |
| 33. | Ethyl octanoate           | 0.93911         | C₁₀H₂₀O₂        | 172.146              |                      |
| 34. | Pentyl hexanoate          | 1.32883         | C₁₁H₂₂O₃        | 186.295              |                      |
| 35. | Thiamine                  | 0.56591         | C₁₂H₁₇N₂O₄⁺     | 265.545              | Vitamin B1           |
| 36. | Niacin                    | 0.70594         | C₁₂H₂₀N₂O       | 123.111              | Vitamin B3           |
| 37. | Riboflavin                | 1.06358         | C₁₂H₂₀N₂O₆      | 376.369              | Vitamin B12          |
| 38. | Ascorbic acid             | 3.70526         | C₆H₈O₆          | 176.124              | Vitamin C            |
Based on the data from the LC-MS analysis in Table 2, it can be concluded that several compounds that have antibacterial activity include acetic acid, cinnamic acid, oxalic acid, malic acid, thiamine, niacin, riboflavin, p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, quercetin, ananasic acid, subaphyllin, beta carotene, lutein, cyanidin, subaphyllin, methoxyflavone, beta-sitosterol, campesterol, and hydroxysitosterol. The components of these compounds can be divided into groups of carboxylic acid compounds, vitamin B1, vitamin B12, vitamin C, vitamin B3, phenolics, flavonoids, steroids, alkaloids, carotenoids, anthocyanins, sterols, and serotonin.

### 3.1. Toxicity and Lipinski test

The toxicity of the compounds in the ethanolic extract of pineapple hump was analyzed in silico. This test was conducted to predict the level of toxicity of the compounds contained in the pineapple hump for the human body. This test uses the Cramer rules parameter. These parameters predict toxicity based on the molecular structure when taken orally. The results of the toxicity test with the Toxtree application can be seen in Table 3. In the table, it can be seen that the compounds from pineapple weevil have different levels of toxicity.

Class III compounds include terrestribisamide, cyanin, campesterol, hydroxysitosterol, ascorbic acid, riboflavin, quercetin, and -sitosterol. Class III category means that the compound has a low safety risk or allows a significant risk of toxicity. Compounds that fall into the intermediate category or Class II are oxalic acid and -carotene compounds. These compounds have a moderate risk of toxicity. Compounds classified as Class I have a low risk of toxicity, including Subaphylline, 5-Hydroxytryptamine, cinnamic acid, acetic acid, malic acid, citric acid, caffeic acid, sinapic acid, ferulic acid, thiamin, nicotinic acid, p-coumaric acid, and ananasic acid [12].
Compounds belonging to Class II and III have toxic effects, and there is a potential risk to health that is quite large for humans if given more than the exposure threshold of 0.15 g/day. Compounds that are in Class I have low toxicity so that these compounds do not pose risks such as genetic damage or cancer [12]. The physicochemical properties of a compound were analyzed using the Lipinski role. The Lipinski test is carried out to determine the physicochemical properties of a ligand when it crosses cell membranes in the body, as for the conditions that must be met by a compound based on Lipinski's rules. The results of the physicochemical analysis of the compounds with the Lipinski role can be seen in Table 3 below.

### Table 3. Toxicity and Lipinski rules

| Ligands            | Toxicity | Mass (Da) | Hydrogen bond donor | Hydrogen bond acceptor | Log P | Molar Refractivity |
|--------------------|----------|-----------|---------------------|------------------------|-------|-------------------|
| Terrestribisamide  | III      | 440       | 4                   | 8                      | 2.85  | 122.63            |
| Subaphylline       | I        | 264       | 4                   | 5                      | 1.27  | 74.99             |
| Cyanin             | III      | 611       | 11                  | 16                     | -2.33 | 139.18            |
| Campesterol        | III      | 400       | 1                   | 1                      | 7.63  | 123.59            |
| Hydroxysitosterol  | III      | 430       | 2                   | 2                      | 6.99  | 129.60            |
| 5-Hydroxytryptamine| I        | 176       | 4                   | 2                      | 1.37  | 52.72             |
| Cinnamic acid      | I        | 148       | 1                   | 2                      | 1.78  | 43.11             |
| Oxalic acid        | II       | 90        | 2                   | 4                      | -0.84 | 15.27             |
| Acetic acid        | I        | 60        | 1                   | 2                      | 0.09  | 13.30             |
| Malic acid         | I        | 134       | 3                   | 5                      | -1.09 | 25.89             |
| Citric acid        | I        | 192       | 4                   | 7                      | -1.24 | 37.09             |
| Ascorbic acid      | III      | 176       | 4                   | 6                      | -1.40 | 35.25             |
| Riboflavin         | III      | 376       | 5                   | 9                      | -0.9  | 94.61             |
| Caffeic acid       | I        | 180       | 3                   | 4                      | 1.19  | 46.44             |
| Sinapic acid       | I        | 224       | 2                   | 5                      | 1.51  | 57.88             |
| Ferulic acid       | I        | 194       | 2                   | 4                      | 1.49  | 51.32             |
| Thiamin            | I        | 265       | 3                   | 4                      | 0.61  | 70.32             |
| Nicotinic acid     | I        | 123       | 1                   | 3                      | 0.78  | 31.19             |
| β-carotene         | II       | 536       | 0                   | 0                      | 12.61 | 181.39            |
| Quercetin          | III      | 302       | 5                   | 7                      | 2.01  | 74.05             |
| β-sitosterol       | III      | 414       | 1                   | 1                      | 8.02  | 128.22            |
| p-Coumaric acid    | I        | 164       | 2                   | 3                      | 1.49  | 44.78             |
| Ananasic acid      | I        | 488       | 4                   | 5                      | 5.18  | 135.46            |

Lipinski test is a test to determine the physicochemical properties of a ligand when it crosses cell membranes in the body and to determine the hydrophilic or hydrophobic character of a compound that passes through the cell membrane by passive diffusion. The Lipinski test has five rules that must be met, namely the molecular weight of the compound < 500 Da, the donor number of hydrogen bonds < 5, the acceptor number of hydrogen bonds < 10, the molar refractivity ranging from 40-130 and the Log P value < 5 [13,14].

Lipinski's rule is a parameter that indicates the oral bioavailability of a compound. The first rule is that a molecular weight of more than 500 Da cannot diffuse through the cell membrane, the number of hydrogen donors is not more than 5 and a maximum hydrogen bond acceptor of 10 describes the higher the hydrogen bonding capacity, the higher the energy required for the absorption process to occur. The log P value indicates the solubility coefficient in water or fat is not more than 5. The greater the log P value, the more hydrophobic the molecule [15].
Molecules that are too hydrophobic tend to have a high level of toxicity because they will be retained longer in the lipid bilayer and distributed more widely in the body so that the selectivity of binding to the target enzyme is reduced. A log P value that is too negative is also not good because the molecule cannot pass through the lipid bilayer membrane [15].

The results in Table 3 above show that the compounds that meet Lipinski’s rules are 5-hydroxytryptamine, subaphylline, cinnamic acid, caffeic acid, ferulic acid, and sinapic acid compounds. The characteristics of ligands that meet the requirements of Lipinski’s rule show that they can easily penetrate cell membranes and enter the circulation of the human body, and can be used as oral drugs.

### 3.2. Molecular docking

Parameters seen in molecular docking research include binding affinity, amino acid residues, and amino acids connected by hydrogen bonds to receptor proteins. Phytochemical compounds are considered agonists if they have bonding interactions at the two binding sites [16]. It can be seen in Table 4 the following.

#### Table 4. Binding affinity query compound and interactions ligands and proteins

| Receptor | Ligand       | Binding affinity (Kcal/mol) | Interaction                                      |
|----------|--------------|----------------------------|-------------------------------------------------|
| 3SRW     | 5-hydroxytryptamine | -6.3                      | ILE15, PHE93, SER50, LYS46, THR47               |
|          | Caffeic acid  | -6.4                      | PHE93, ALA8, SER50, THR47                       |
|          | Cinnamic acid | -5.8                      | ILE15, GLN20                                   |
|          | Ferulic acid  | -6.4                      | SER50, ASN19, THR47, GLN96                     |
|          | Sinapic acid  | -6.6                      | THR122, ALA8, LEU6                             |
|          | Subaphyllin   | -6.9                      | ASN19, PHE93                                   |
|          | Anansic acid  | -7.3                      | PHE93, Val32, LEU29, ILE51, LEU55, PR O56      |
|          | Ascorbic acid | -5.7                      | THR122, ASN19                                  |
|          | β-carotene    | -9.8                      | LEU21, ILE15, LYS46, LEU29, LYS33, PR O56, LEU55, VAL32, PHE93 |
|          | β-sitosterol  | -9.8                      | THR122, LEU55, LEU21, VAL32, LYS33, LEU29, ILE51, PHE93, ILE15 |
|          | Nicotinic acid| -5.1                      | THR47, THR122, GLN20, LEU21, ASN19             |
|          | p-coumaric acid| -6.1                     | THR122, ILE15, LEU21, Val7, ALA8               |
|          | Quercetin     | -9                        | ILE15, LEU21, ALA8, THR47, SER50, GLY16, THR122, ASN19, ASP121 |
|          | Thiamin       | -7.1                      | PHE99, LEU21, PHE93, GLY16, ILE15              |
|          | Chloramphenicol| -7.6                     | THR47, ALA8, THR122, GLN96, GLN20, VAL7, ALA8, ILE15, LEU21 |
| 5ZTJ     | 5-hydroxytryptamine | -5.4                      | HIS545, ILE631                                 |
|          | Caffeic acid  | -5.9                      | VAL735, SER734                                 |
|          | Cinnamic acid | -5.5                      | ASP579, GLY547                                 |
|          | Ferulic acid  | -5.6                      | HIS545, GLY547, GLU629                         |
|          | Sinapic acid  | -5.8                      | ARG838, ARG739, ARG580, THR632                 |
|          | Subaphyllin   | -5.8                      | ARG580, ARG838, LEU836                         |
|          | Anansic acid  | -8.4                      | ILE634, ARG580, ALA786, LEU735, VAL733, THR632, VAL787 |
|          | Ascorbic acid | -5.5                      | ARG580, LEU735, VAL733, SER734, LEU836        |
The interaction that occurs between the ligand and the receptor will result in the value of the binding energy (binding affinity) and the activity of the molecule [17]. The result of the docking process that produces bond energy is used as the main parameter to determine the stability of the bond between protein and ligand. The binding interaction between the ligand and the receptor will be at the lowest energy state. The lowest energy yield indicates the stability of the molecular position, so the lower the bond energy value, the more stable the interaction between the ligand and the receptor [18]. A small Gibbs free energy value indicates a stable conformation, while a large Gibbs free energy value indicates an unstable complex [19].

The molecular docking process has several conditions. The requirement for molecular docking is that the desired protein structure must be present. The structure of the protein to be used is determined using biophysical techniques. This biophysical technique is x-ray crystallography or NMR spectroscopy. The existence of this protein structure and ligand database has a function in the input of the docking program.

Compounds that have the potential as antibacterial are compounds that have hydrogen bonds, and the binding affinity value is less than 10. Score from binding affinity are indicators of the binding ability of active compounds to the target protein. Free energy is the enthalpy change needed to break certain bonds in 1 mole of gas inhibitor molecules. Active compound is predicted to potentially have inhibitory properties and strong interaction if it has the same chemical interaction position on the target protein with the control [20].

Compounds that have the potential as antibacterial are compounds that have hydrogen bonds, and the binding affinity value is less than 10. Namely subaphyllin, 5-hydroxytryptamine, cinnamic acid, caffeic acid, ferulic acid, and sinapic acid. These compounds are agonists. Phytochemical compounds are considered agonists if they have bonding interactions at both sites of the bond.

### 4. Conclusion

Compounds that have the potential as antibacterial are compounds that have hydrogen bonds, and the binding affinity value is less than 10. Namely subaphyllin, 5-hydroxytryptamine, cinnamic acid, caffeic acid, ferulic acid, and sinapic acid. These compounds are agonists.

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