PAPER • **OPEN ACCESS**

Iron (Fe) Phytoremediation by *Hydrilla verticillata* and Its Antibacterial Activity Against *Staphylococcus aureus*: An In-vitro and In-silico Study

To cite this article: A G Fasya *et al* 2025 *IOP Conf. Ser.: Earth Environ. Sci.* **1439** 012011

Iron (Fe) Phytoremediation by *Hydrilla verticillata* and Its Antibacterial Activity Against *Staphylococcus aureus*: An In-vitro and In-silico Study

A G Fasya[1*], F A Aminata[1], M Fouzy[1], Z N Azizah[1], R Mahmudah[1], D K Kadarani[1], S Amalia[1], and D S Megawati[2]

[1] Department of Chemistry, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia
[2] Department of Pharmacy, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia

[*]E-mail: fasya.organik@kim.uin-malang.ac.id

Abstract. Iron (Fe) is essential for nutrition but excessive accumulation can be harmful to humans and the environment. Additionally, unhealthy aquatic environments can lead to bacterial diseases, such as those caused by *Staphylococcus aureus*. This study explores the potential of *Hydrilla verticillata*, an aquatic plant often deemed a nuisance, for iron (Fe) phytoremediation and antibacterial applications. The research assesses the impact of various iron concentrations on the remediation efficiency of *Hydrilla verticillata*, measuring parameters such as iron absorption percentage, Bioconcentration Factor (BCF), and Translocation Factor (TF). It also evaluates the plant's antibacterial activity against *Staphylococcus aureus* using in-vitro and in-silico methods. The results indicate that *Hydrilla verticillata* effectively remediates iron, with the highest absorption rate at 98.99% at a concentration of 30 ppm. The BCF for leaves and stems were 560.999 at 10 ppm and 161.197 at 30 ppm, respectively, while the TF was 6.087 at 15 ppm. The antibacterial activity was 8.313 mm at 10 ppm, and molecular docking revealed that the compound phytol has a binding affinity of -6,0 kcal/mol. This research highlights *Hydrilla verticillata*'s dual role in environmental remediation and antibacterial activity.

Keywords: Antibacterial, *Hydrilla verticillata*, Iron (Fe), Phytoremediation

1. Background

Hydrilla verticillata is an aquatic plant that often considered a weed that distrupts aquatic ecosystems. However, *Hydrilla verticillata* are known to have several secondary metabolites such as alkaloids, flavonoids, saponins, triterpenoids, and steroids [1-3]. Beside of that, *Hydrilla verticillata* can be utilized as a phytoremediation agent, and can also be utilized as an antibacterial agent. *Hydrilla verticillata* contains secondary metabolits that have proven antibacterial activity and thiol groups (SH), which allow the *Hydrilla verticillata* plant to absorb metals [4]. Currently, there are many environmental problems due to human activities, which of one is the environment being polluted by the heavy metal iron. Several studies show that iron metal pollution in the environment is above the predetermined threshold 0.3 ppm [5]. To overcome the environment

polluted by heavy metals, this can be done by applying an environmentally friendly concept using live plants, namely phytoremediation of the *Hydrilla verticillata* plant. Through the metal absorption process, the absorbed metal can enhance secondary metabolite production during the elicitation process.

An unhealthy environment can also cause disease which can be caused by bacteria, one of which is the *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a gram-positive bacterium that does not form spores. This bacterium commonly grows in pairs. It has a size of 0.8 - 1.0 micron. *Staphylococcus aureus* is a bacterium that causes disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis [6]. In addition to in-vitro assessments of antibacterial activity, in-silico methods can also be employed to evaluate the potential of compounds [7]. *Hydrilla verticillata* is known to have potential as an antioxidant [8-9] and may also exhibit anti-cancer properties. This underscores its broad potential for therapeutic applications beyond just iron remediation and antibacterial activity. Based on the background, a study will be conducted on the potential of *Hydrilla verticillata* as a phytoremediation agent in remediating iron metal and its antibacterial activity against *Staphylococcus aureus* with in-vitro and in-silico method.

2. Material and Methods

2.1 Material

Material used in this research are *Hydrilla verticillata* as a sample, $FeSO_4.7H_2O$ (E-Merck), HNO_3 65% (E-Merck), H_2O_2 p.a (Smartlab), FG Ethanol, aquademin, aquadest, Nutrient Agar, Nutrient Broth, *Staphylococcus aureus* bacteria, DMSO 100%, sterile disc (Himedia), antibiotic disc chloramphenicol (Oxoid). While on in-silico, the material used are 3D structure of 7 ethanolic extract compound on *Hydrilla verticillata* that are phytol, isophytol, 2,6,6-Trimethyl-bicyclo [3.1.1] heptane, cis, linoleic acid, 6,10,14-Trimethylpentadecan-2-one, ethyl palmitate, and 9,12,15-Octadecatrienoic acid ethyl ester [10]. Receptor from *Staphylococcus aureus* is Penicillin Binding Protein PBP2a with PDB ID 1MWT.

2.2 Sample Preparation

Hydrilla verticillata samples were collected from Lake Ranu Grati in Pasuruan. The sampling took place at the lake's surface, with an estimated depth of around 2 meters from the surface to the bottom. Wet samples, approximately 5 kg, were placed in plastic bags. The samples were then cleaned of any adhering dirt and acclimatized for 14 days with a sample weight to water ratio of 1:50.

The stock solution for phytoremediation and elicitation was prepared by dissolving 4.975 grams into 1000 mL of demineralized water. Working solutions were made from the 1000 ppm stock solution by diluting it to 0 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm, and 30 ppm. The acclimatized *Hydrilla verticillata* samples were treated with the prepared working solution for 7 days with a sample weight to solution ratio of 1:30.

After the treatment, 50 grams of *Hydrilla verticillata* were separated between leaves and stems then powdered for destruction. Approximately 200 grams of *Hydrilla verticillata* were dried to obtain *Hydrilla verticillata* powder as a sample for antibacterial activity test.

2.3 Destruction Using Hotplate Method

Destruction was carried out using the open destruction method with a hotplate. For the destruction of biomass, 0.5 grams of each leaf and stem sample were added to 6 mL of 65% HNO_3 and 2 mL of H_2O_2 p.a. For water destruction, 10 mL of the water sample was added to 2.5 mL of 65% HNO_3 and 0.75 mL of H_2O_2 p.a.

2.4 Atomic Absorption Spectroscopy (AAS)

Fe^{2+} analysis was performed using an AAS (Atomic Absorption Spectrometer) Varian Spectra AA 240, with a wavelength of 248.3 nm. The acetylene flow was set at a rate of 2 L/min, and the air flow was set at a rate of 13.50 L/min. The slit width and current strength were set to 0.5 nm and 5 mA, respectively. Absorbance was measured in the biomass (leaves and stems) and in the water samples at concentrations of 0, 10, 15, 20, 25, and 30 ppm.

2.5 Ultrasonic Assisted Extraction (UAE)

Extraction using sonication was performed by weighing 5 grams of *Hydrilla verticillata* powder and adding 50 mL of food-grade ethanol. Sonication was carried out for 40 minutes. The samples obtained from sonication were then concentrated using a rotary evaporator.

2.6 Antibacterial Activity

Antibacterial activity was carried out by dissolving 5 mg of crude *Hydrilla verticillata* extract in each concentration (0, 10, 15, 20, 25, 30 ppm) into 5 mL of DMSO to make a 1000 ppm solution. Then, 0.1 mL of *Staphylococcus aureus* inoculum was added to the antibacterial solution, and a small amount of NA media was poured in and allowed to solidify. Sterile discs were then placed into each concentration for 30 minutes. The sterile discs were placed on the solidified agar media with 5 replicates and antibiotic discs along with negative controls on separate plates. The plates were incubated for 24 hours, and the inhibition zones were measured.

2.7 Molecular Docking

The Penicillin Binding Protein PBP2a receptor from Staphylococcus aureus (1MWT) was prepared by removing contaminants and the native ligand using Biovia Discovery Studio. The receptor, once purified, was then refined by adding missing amino acids using Modeller. The target ligand was then prepared in PyRx Open Babel with minimized energy. After preparation, the target ligand and receptor were docked using AutoDock Vina with an exhaustiveness of 200. The results obtained were in the form of binding affinity.

3. Results and Discussion

3.1 Sample Preparation

Sampling was conducted using the cluster sampling method. *Hydrilla verticillata* samples were collected from all parts of the plant that were on the water surface. Acclimatization was carried out for 14 days to allow the *Hydrilla verticillata* plants to adapt and survive in the new environmental conditions. The acclimatized *Hydrilla verticillata* can be observed in Figure 1.

Figure 1. *Hydrilla verticillata* after acclimatization shows a greener and fresher appearance, with new branches growing on the stems, elongation of the stems, and the development of new roots.

3.2 Percentage Absorbed Iron Metal

The results of the test on the percentage of iron absorption at concentrations of 10, 15, 20, 25, and 30 ppm were 93.656 %, 95.315 %, 96.415 %, 97.297 %, and 98.990 %, respectively. The highest iron absorption was observed at a concentration of 30 ppm, with a value of 98.990 %. This indicates that *Hydrilla verticillata* can effectively remediate iron contamination with an average absorption rate of over 90 %.

3.3 Iron Content in Hydrilla verticillata Biomass

The results show that the concentration of absorbed iron is higher in the leaves than in the stems. This is due to the larger surface area of the leaves and the fact that the stems are more covered by leaves, allowing the leaves to have more direct contact with the metal. The results are shown in **Table 1.**

Table 1. Result of Iron Content in *Hydrilla verticillata* Biomass

Concentration (ppm)	Biomass Concentration of Iron Metal (mg/Kg BK)			
	Leaf	Notation	Stem	Notation
10	5352.692	A	831.306	A
15	6663.037	A	1122.142	A
20	8431.639	A	2084.694	B
25	11376.290	AB	3057.471	C
30	20635.210	C	7827.284	D

The iron concentration in the leaves rose from 5352.692 mg/kg at 10 ppm to 20635.210 mg/kg at 30 ppm, indicating that the leaves absorbed more iron as the solution's concentration increased. Similarly, the iron concentration in the stems increased from 831.306 mg/kg at 10 ppm to 7827.284 mg/kg at 30 ppm. Although the stems had lower values than the leaves, the increase was still considerable. Statistical analysis revealed significant differences in iron concentration at several levels for both leaves and stems. In the leaves, concentrations of 10, 15, and 20 ppm showed no notable differences (all marked as A), but differences emerged at 25 ppm (AB) and 30 ppm (C). For the stems, distinct notations appeared starting at 20 ppm, reflecting a significant increase in concentration from that point onward

3.4 Phytoremediation Parameter

3.4.1 Bioconcentration Factor (BCF)

The Bioconcentration Factor (BCF) is the process of chemical absorption by aquatic organisms from their surrounding environment. The BCF results show significantly high values. This is because *Hydrilla verticillata* is an aquatic plant where all parts of the plant are submerged in water and have direct contact with iron. The BCF results are presented in Figure 2. From the Bioconcentration Factor (BCF) data in Figure 2, it is evident that the leaves of *Hydrilla verticillata* accumulate more iron compared to the stems across all concentrations. In addition, the high BCF in the leaves of *Hydrilla verticillata* is caused by the direct contact of the metals in the water with the leaves, resulting in a higher BCF in the leaves compared to the stems. At a 10 ppm concentration, the BCF for the leaves reaches 560.999, while the stems only accumulate 97.879. This trend continues at 15 ppm, where the leaf BCF is 534.340, and the stem BCF drops to 88.907. At 20 ppm, the leaf BCF slightly decreases to 466.865, while the stem BCF increases to 114.929. At 25 ppm, the leaf BCF rises again to 507.554, and the stem BCF continues to increase, reaching 135.610. At the highest concentration of 30 ppm, the leaf BCF decreases to 425.072, with the stem BCF increasing to 161.197. Overall, the leaves consistently show higher BCF values, highlighting their dominant role in iron accumulation. With leaf BCF values consistently above 100, *Hydrilla verticillata* is classified as an effective hyperaccumulator plant for absorbing iron from aquatic environments

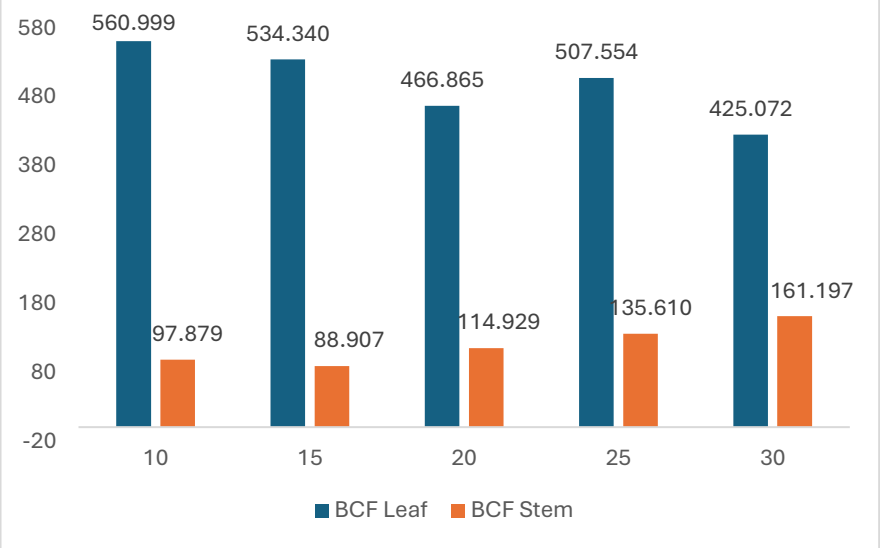

Figure 2. The BCF results show that the leaf section accumulates higher values than the stem section, with the highest leaf value at 10 ppm concentration being 560.999 and the stem section at 30 ppm concentration being 161.197. A BCF > 100 is categorized as an excellent hyperaccumulator plant.

3.4.2 Translocation Factor (TF)

The determination of the Translocation Factor (TF) aims to identify the movement of metals from one part of the plant to another and to categorize a plant as a potential phytoremediation agent. The TF results are presented in Figure 3.

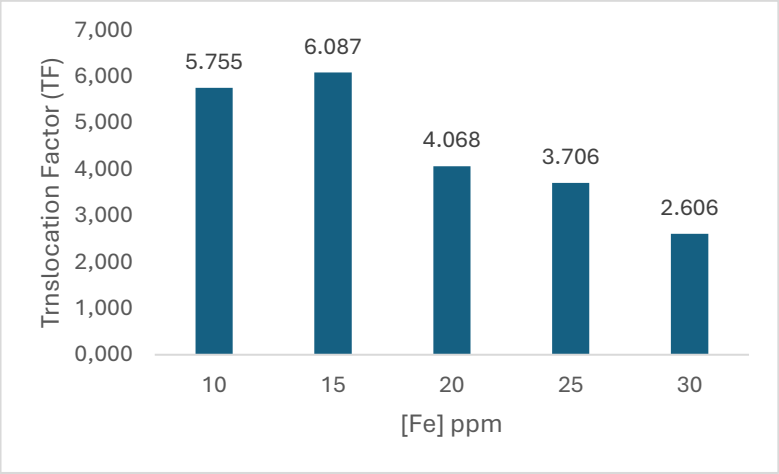

Figure 3. All concentrations showed TF>1, indicating a phytoextraction mechanism, which suggests that iron is efficiently translocated from the stem to the leaves. The plant is capable of accumulating iron at all concentrations, with the highest TF value observed at the 15 ppm concentration.

From Figure 3, it can be observed that all tested iron (Fe) concentrations yield Translocation Factor (TF) values greater than 1. This indicates that *Hydrilla verticillata* has the capability to efficiently translocate iron from the stem to the leaves of the plant. The highest TF value is recorded at the 15 ppm concentration, measuring 6.0874, which signifies that at this concentration, the efficiency of transporting iron to the leaves is very high. At 10 ppm, the TF value is 5.7550, also reflecting good translocation capability. However, as the concentration increases, the TF values decrease at 20 ppm (4.0676), 25 ppm (3.7057), and 30 ppm (2.6059). Despite this decline at higher concentrations, all TF values remain above 1, indicating that the plant consistently maintains a strong ability to translocate iron. Overall, these results suggest that *Hydrilla verticillata* is a promising candidate for phytoremediation, owing to its ability to accumulate and translocate heavy metals like iron from the lower parts of the plant to the upper parts.

3.5 Ultrasonic Assisted Extraction (UAE)

Sonication was performed with a sample to ethanol ratio of 1:10, using 5 grams of *Hydrilla verticillata* with 50 mL of food-grade ethanol. Food-grade ethanol was used to minimize the use of hazardous chemicals by applying the concept of green chemistry. Sonication was conducted for 40 minutes at 40°C. The resulting extract was then concentrated using a rotary evaporator to obtain a crude extract. The results of the extract can be seen in Table 2.

Table 2. Result of Iron Content in *Hydrilla verticillata* Biomass

Concentration	Crude Extract (g)
0 ppm	0.229
10 ppm	0.168
15 ppm	0.163
20 ppm	0.243
25 ppm	0.317
30 ppm	0.178

3.6 Antibacterial Activity

Antibacterial activity was assessed using the Kirby-Bauer method, also known as the disk diffusion method. This technique involves the use of paper discs impregnated with different antimicrobial agents. The results indicated that the best antibacterial activity was observed at a concentration of 10 ppm. (Figure 4, Table 3).

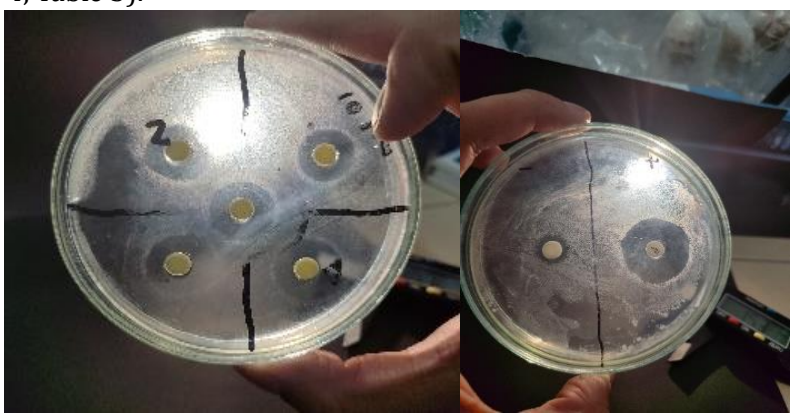

Figure 4. Best Antibacterial Acitivity of *Hydrilla verticillata* on 10 ppm

Table 3. Inhibition Zone of *Hydrilla verticillata*

Concentration	Inhibition Zone (mm)	Category*
0 ppm	4.000^{ac}	Weak
10 ppm	8.313^{b}	Average
15 ppm	5.713^{c}	Average
20 ppm	7.813^{b}	Average
25 ppm	3.138^{a}	Weak
30 ppm	5.225^{c}	Average

* Category of Inhibition Zone

weak	= < 5 mm	strong	= 10-20 mm
average	= 5-10 mm	very strong	= > 2 mm

The table presents the results of inhibition zone testing from *Hydrilla verticillata* extract at different concentrations against microorganisms. At a concentration of 0 ppm, the inhibition zone produced is 4.000 mm, categorized as weak (Weak). The highest inhibition zone occurs at 10 ppm, measuring 8.313 mm, followed by 20 ppm with an inhibition zone of 7.813 mm; both are classified as average (Average). For concentrations of 15 ppm and 30 ppm, the inhibition zones are 5.713 mm and 5.225 mm respectively, which are also categorized as average. Meanwhile, at 25 ppm, the inhibition zone drops to 3.138 mm, classified as weak. Overall, the inhibition zones show varying antimicrobial activity, with the highest effectiveness seen at 10 ppm and 20 ppm.

3.7 Molecular Docking

Molecular docking was conducted to assess the binding affinity between the ligand and the molecule. The results of the molecular docking indicated that the compound with potential antibacterial activity in *Hydrilla verticillata* is Phytol, with a binding affinity of -6,0 kcal/mol. The interaction between ligand and receptor are shown on Figure 5, Table 4.

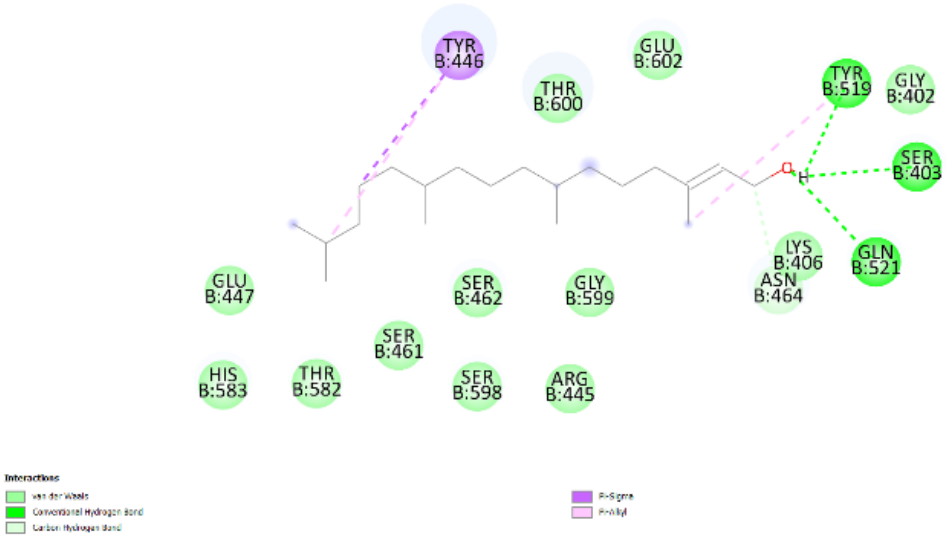

Figure 5. The best result of molecular docking compound is Phytol with the binding afinity -6.0 kcal/mol

Table 4. Result of Molecular Docking

Compound	Binding Affinity (kcal/mol)
PNM (Native Ligand)	-7.2
Chloramphenicol	-6.7
Phytol	-6.0
Isophytol	-5.5
2,6,6-Trimethyl-bicyclo [3.1.1] heptane, cis	-5.3
Linoleic Acid	-5.2
6,10,14-Trimethylpentadecan-2-one	-5.1
Ethyl Palmitate	-5.1
9,12,15-Octadecatrienoic acid ethyl ester	-5.1

Based on the **Table 4.** phytol is the compound with the highest potential for antibacterial activity due to its binding affinity of -6.0 kcal/mol, which is close to the binding affinity of chloramphenicol at -6.7 kcal/mol. Chloramphenicol is an antibiotic often used as a positive control in in-vitro research, and docking was performed to observe its binding affinity to *Staphylococcus aureus*, allowing a comparison with active compounds from *Hydrilla verticillata*. The active compound most similar to chloramphenicol is phytol, although its binding affinity is not as strong as chloramphenicol or PNM, which has a value of -7.2 kcal/mol as the native ligand. The phytol compound interacts with the receptor by forming hydrogen bonds, hydrogen-carbon bonds, Pi-Sigma, and Pi-Alkyl interactions. The bond between the phytol compound and the receptor can be seen in Figure 6.

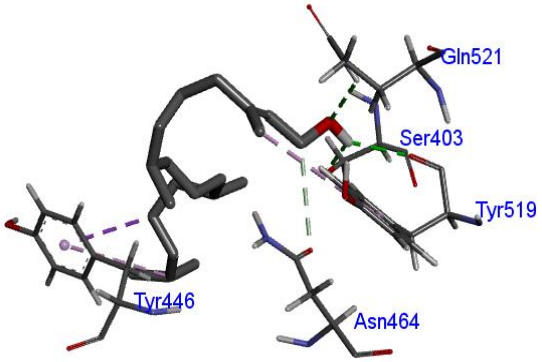

Figure 6. Interaction between phytol with receptor

The bonds formed between the phytol compound and the receptor were then compared with the native ligand and chloramphenicol, as shown in **Figure 7** and **Figure 8**.

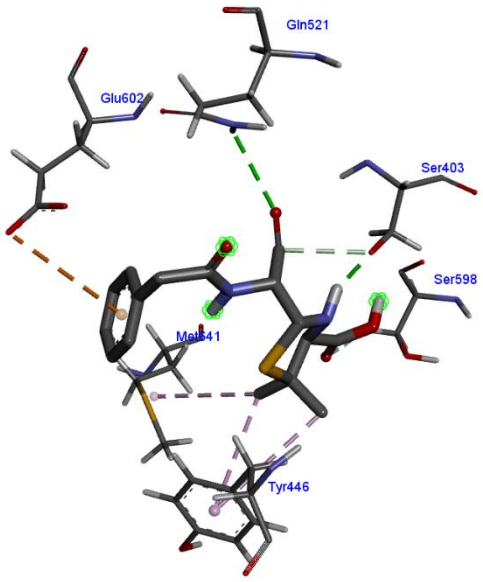

Figure 7. Interaction between PNM (native ligand) with receptor

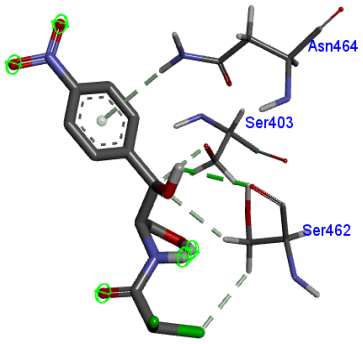

Figure 8. Interaction between chloramphenicol with receptor

In Figure 6, Figure 7, and Figure 8, it can be seen that the amino acid bonds formed are similar to each other, where the phytol compound binds to Tyr446, Asn464, Tyr519, Ser403, and Gln521, while the native ligand binds to Tyr446, Ser403, Gln521, Met641, Glu602, and Ser598, and chloramphenicol binds to Ser462, Ser403, and Asn464. This indicates a similarity in the binding of amino acids to the receptor, suggesting that the active compounds from *Hydrilla verticillata* possess antibacterial properties.

4. Conclusion

Conclusion of this research are that *Hydrilla verticillata* can remediate iron metal with the highest value in the absorption percentage namely at a concentration of 30 ppm of 98.99%, BCF of leaves and stems were 560.999 at a concentration of 10 ppm and 161.197 at a concentration of 30 ppm, TF was 6.087 at a concentration of 15 ppm, antibacterial activity was 8.313 mm at a concentration of 10 ppm, and in molecular docking, the compound phytol had a binding affinity of -6,0 kcal/mol. Thus, *Hydrilla verticillata* is a hyperaccumulator plant because the BCF value is >100 and the TF value is >1. *Hydrilla verticillata* exhibits moderate antibacterial activity, and the results of docking show that the active compounds present in *Hydrilla verticillata* also have potential as antibacterial agents.

5. Acknowledgment

We would like to express our deepest gratitude to the Ministry of Religious Affairs of the Republic of Indonesia and LP2M Universitas Islam Negeri (UIN) Maulana Malik Ibrahim Malang for their support through the Applied National Development Research Grant. Our appreciation also extends to the Faculty of Science and Technology at UIN Maulana Malik Ibrahim Malang for their support through the Competitive Student Research (RKM) program.

References

[1] Ginting, M., Marbun, N. R., Sinaga, M., Fitri, K., & Leny, L. 2022. *Majalah Farmasetika*, 8(1), 13
[2] Fasya, A. G., Amalia, S., Megawati, D. S., Kusuma, V. A., & Purwantoro, B. 2020. *IOP Conference Series: Earth and Environmental Science*, 456(1): 012009
[3] Fasya, A. G., Wati, V. S., Vania, V., Mahbubi, H. A., Fauzi, V. S. H., Jannah, M., Masrihanah, A., Kholidah, I., Fatimah, F., Amalia, S., & Megawati, D. S., 2023. *AIP Conference Proceedings*, 2818(1): 070002
[4] Dennis, K. K., Uppal, K., Liu, K. H., Ma, C., Liang, B., Go, Y. M., & Jones, D. P. 2019. *Database: the journal of biological databases and curation*, 2019
[5] Peraturan Pemerintah Republik Indonesia No. 22. 2021. *Penyelenggaraan Perlindungan Dan Pengelolaan Lingkungan Hidup*, *Jakarta*
[6] Del Giudice P. 2020. *Acta Derm Venereol*. 2020 Apr 20;100(9)
[7] Megawati, D. S., Ekowati, J., Siswodihardjo, S., & Fasya, A. G., 2023. *Journal of Medicinal and Pharmaceutical Chemistry Research*,5(11)**:** 1077-1088
[8] Fasya, A. G., Warsito, Masruri, Rollando & Megawati, D. S. 2024. *IOP Conference Series: Earth and Environmental Science*, 1312(1)**:** 012051
[9] Fasya, A. G., Warsito, Iftitah, E., D. & Rollando, 204. *AIP Conference Proceedings*, 3055(1): 040002
[10] Prabha, P. & Rajkumar, J. 2015. *Journal of Chemical and Phramaceutical Research, 2015,* 7(3):1809-1815