Molecular Docking Study of Active Compounds in Amaranthus tricolor Leaves as High Mobility Group Box 1 (HMGB1) Inhibitor in Breast Cancer

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ABSTRACT. Breast cancer shows the proliferation of malignant epithelial cells that limit the ducts and lobes of the breast. If this process is not controlled, it will cause lumps that can then spread to other parts of the body and cause death. High-mobility group box protein 1 (HMGB1) has been reported to play roles in promoting cell survival of breast cancer cells. The inhibition of HMGB1 could be a reasonable target for the treatment of breast cancer. Amaranthus tricolor has been found could reduce the viability of breast cancer cells. In this study, we aim to predict the ability of the active compounds in Amaranthus tricolor leaves to inhibit the HMGB1 through molecular docking study. The molecular docking was conducted by using the PyRx software. This study shows that the four active compounds in Amaranthus tricolor leaves, namely isorhamnetin, rutin, myricetin, and quercetin, have the smallest bond energy, indicating that the four compounds are the most stable and have the highest potency as HMGB1 inhibitor.

Keywords: Amaranthus tricolor, HMGB1, Flavonoid, Breast Cancer

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer among females and the main cause of cancer death. Breast cancer occurs almost entirely in women, but men can also develop breast cancer [1]. Breast cancer is defined as an uncontrolled growth of cells in the milk-producing glands or ducts that drain milk, causing lumps. If not treated immediately, it can metastasize throughout the body, causing death. In 2018, about 2.1 million newborn women were diagnosed with breast cancer, and 626,679 women with breast cancer died [2]. The incidence of breast cancer cases continues to increase annually by 3.1%, which continues every year [3].

High-mobility group box protein 1 (HMGB1) is a ubiquitous nuclear protein that contains DNA binding domains in the cell nucleus and is secreted into the extracellular environment to respond to the different stimuli, either through passive or active pathway [4]. HMGB1 levels in the sera and tissues of breast cancer patients were significantly higher than the healthy individuals or the patient with benign breast disease [5]. HMGB1 could promote the growth of breast cancer cells in vitro [6]. Thus, the inhibition of HMGB1 could become the target of breast cancer therapy.

Glycyrrhizin and quercetin are examples of HMGB1-targeting therapeutic agents. They directly bind to HMGB1 or inhibit phosphoinositide 3-kinases (PI3Ks) and increase the effectiveness of anticancer agents in several different tumor models. HMGB1’s primary role in the nucleus, HMGB1 could be a plausible target for cancers caused by genetic or hereditary factors such as breast cancer characterized by genomic instability [7].

Amaranthus belongs to the family Amaranthaceae, and 17 of 70 species of this family are used as edible leaves. Amaranthus leaves contain proteins with essential amino acids, vitamins, and minerals. Other than that, Amaranthus tricolor leaves contain antioxidant leaf pigments and flavonoids, such as rutin, isorquercetin, myricetin, quercetin, apigenin, and kaempferol [8]. Flavonoid components such as quercetin as dietary substances might provide the potential of alternative or complementary medicine in breast cancer [9]. Research conducted by Kanbarkar et al. showed that both ethanolic extract and aqueous extract of Amaranthus tricolor's whole plant could reduce the viability of MCF-7 and MDA-MB-231 breast cancer cell lines [10].

In silico study is the preliminary study in the discovery and development in drug design to predict a drug or compound's ability to target its therapy. The advantage of using the method is it could reduce the research expenses because it helps select potential molecules or compounds, thereby reducing the failure rate in research and its expense. Molecular docking is a type of in silico study to determine how a compound bonds with the target's active site. The output is in the form of bond energy, free energy, or qualitative numeric measures, or it can also be used to determine the orientation of the compound and conformational geometry [11]. Based on the facts above, we aim to predict the ability of the active compounds in
Amaranthus tricolor leaves as HMGB1 inhibitor

METHODS

Compounds and protein preparation

The active compounds contained in *Amaranthus tricolor* leaves are based on the results of previous studies [12]. Glycyrrhizin and doxorubicin were used as the inhibitor references. The 3D structure of the compound was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format, which was then converted into PDB format with the Openbabel application. The 3D HMGB1 protein structure was obtained from the Uniprot database (https://www.uniprot.org/) with the code PDB P09429. Furthermore, the protein structure validation was carried out with a Ramachandran plot through the site (http://molprobity.biochem.duke.edu/).

Drug likensness analysis

After obtaining each ligand and protein structure, a potential drug prediction analysis is then carried out by referring to the five Lipinski rules (http://www.scbio-iitd.res.in/software/drugdesign/lipinski.jsp). The result must meet at least two of the five Lipinski’s rules [13].

Molecular docking study

Compounds that meet Lipinski’s rules are docking with HMGB1 protein with the PyRx software, and then we get binding affinity. A negative binding affinity indicates a bond between with the molecular docking study, the ligand and the protein. The more negative the binding affinity value is predicted, the stronger the bond between the ligand and the protein [14].

Protein-ligan interactions

Compounds that showed a more negative binding affinity were analyzed for the interaction between ligands and HMGB1 protein by applying the Ligplot. The Ligplot application is used to determine the hydrophobic interactions and hydrogen bonds between ligands and proteins. The inhibition potential of HMGB1 can be seen from the presence of bonds between compounds on the active side of the amino acid HMGB1.

Molecular visualization

Furthermore, molecular visualization was carried out with the Pymol application to determine the ligand and protein bonds [15].

RESULTS AND DISCUSSIONS

Protein validation

The structure of the HMGB1 protein that had been obtained was then validated for the Ramachandran plot. The results obtained by the number of residues in the favored region are 92.9% (Figure 1). A good value for the number of residues is 90-98%, so it can be concluded that the HMGB1 protein model obtained is good enough and can be used for further analysis [16].

![Figure 1](https://example.com/figure1.png)

Figure 1. Result of MolProbity Ramachandran analysis (a) General case, (b) Isoleucine and valine, (c) Pre-proline, (d) Glycine, (e) Trans proline, and (f) Cis proline.
The potency of chemical compounds of leaf *Amaranthus tricolor* as a drug candidate

The chemical compounds were predicted as drug-like molecule with the five Lipinski rules. The rule explains that compounds with high probability as drug-like molecules must follow a less than two rules [17]. The rules consist of molecular weight \( \leq 500 \) Dalton, hydrogen bond acceptors \( \leq 10 \), hydrogen bond donors \( \leq 5 \), high lipophilicity \( \leq 5 \), and molar refractivity between 40-130 [13]. The prediction showing all chemical compounds in *Amaranthus tricolor* is drug-like molecules (Table 1). These compounds can enter the next analysis steps to determine the binding energy of the target protein.

**Table 1.** The result of drug-like molecule prediction from the active compounds in *Amaranthus tricolor* leaves

| Compound              | MW   | HBD | HBA | LOGP   | MR   |
|-----------------------|------|-----|-----|--------|------|
| Ellagic acid          | 302.000 | 4   | 8   | 1.241  | 68.454 |
| Beta-caroten          | 536.000 | 0   | 0   | 12.606 | 181.392 |
| Isorhamnetin          | 316.000 | 4   | 7   | 2.314  | 78.938 |
| Rutin                 | 610.000 | 10  | 16  | -1.879 | 137.495 |
| Myricetin             | 318.000 | 6   | 8   | 1.716  | 75.715 |
| Quercetin             | 302.000 | 5   | 7   | 2.011  | 74.050 |
| Apigenin              | 270.000 | 3   | 5   | 2.420  | 70.814 |
| Kaempferol            | 286.000 | 4   | 6   | 2.305  | 72.386 |
| Hyperoside            | 464.000 | 8   | 12  | -0.731 | 106.274 |
| Isoquercetin          | 464.000 | 8   | 12  | -0.731 | 106.273 |
| Chlorogenic acid      | 354.000 | 6   | 9   | -0.645 | 82.519 |
| Sinapic acid          | 224.000 | 2   | 5   | 1.507  | 57.881 |
| Caffeic acid          | 180.000 | 3   | 4   | 1.196  | 46.441 |
| Gallic acid           | 170.000 | 4   | 5   | 0.502  | 38.396 |
| Ferulic acid          | 194.000 | 2   | 4   | 1.499  | 51.329 |
| Hydroxycinnamic acid  | 164.000 | 2   | 3   | 1.490  | 44.777 |
| M-coumaric acid       | 164.000 | 2   | 3   | 1.490  | 44.777 |
| Trans-cinnamic acid   | 148.000 | 1   | 2   | 1.784  | 43.112 |
| Syringic acid         | 198.000 | 2   | 5   | 1.108  | 48.170 |
| Phytol                | 296.000 | 1   | 1   | 6.364  | 95.562 |
| Vanilic acid          | 168.000 | 2   | 4   | 1.099  | 41.618 |
| P-hydroxybenzoic acid | 138.000 | 2   | 3   | 1.090  | 35.066 |
| Salicylic acid        | 138.000 | 2   | 3   | 1.090  | 35.066 |
| Palmitic acid         | 256.000 | 1   | 2   | 5.552  | 77.948 |
| Ascorbic acid         | 176.000 | 4   | 6   | -1.407 | 35.256 |
| Oleic acid            | 282.000 | 1   | 2   | 6.109  | 87.088 |

Note: MW: Molecular Weight (D); HBD: Hydrogen Bond Donors; HBA: Hydrogen Bond Acceptors; LOGP: High lipophilicity; MR: Molar refractivity.
Potential analysis of the active compounds in Amaranthus tricolor leaves as HMGB1 inhibitors

Based on the data above, this study purposed is to examine the potency of the active compounds in Amaranthus tricolor as HMGB1 inhibitor with in silico study, compared to control drugs that are known to inhibit HMGB1 protein. The active compounds that potentially inhibit HMGB1 protein are compounds with free binding energy that is almost equal or less than control. The control drugs used were doxorubicin and glycyrrhizin. Ligand-protein interactions are shown based on the type of chemical bond formed and the binding site. The results of the potential analysis of the active compounds of Amaranthus tricolor leaves on HMGB1 protein inhibition can be seen in Table 2.

Table 2 shows that the ellagic acid, beta-carotene, isorhamnetin, rutin, myricetin, and quercetin have the free bond energy that are almost closer to the control. It can be concluded that these active compounds could potentially inhibit HMGB1 protein. The binding affinity of drug control to HMGB1 protein was -7.1.

Table 2. Analysis of the inhibitory potential of the active compounds in Amaranthus tricolor leaves with the HMGB1

| Compound     | CID     | Binding Affinity | Compound     | CID     | Binding Affinity |
|--------------|---------|------------------|--------------|---------|------------------|
| Ellagic acid | 5281855 | -6.9             | Gallic acid  | 370     | -4.8             |
| Beta-carotene| 5280489 | -6.9             | Ferulic acid | 445858  | -4.8             |
| Isorhamnetin | 5281654 | -6.6             | Hydroxycinnamic acid | 637542 | -4.7 |
| Rutin        | 5280805 | -6.4             | M-coumaric acid | 637541 | -4.6 |
| Myricetin    | 5281672 | -6.3             | Trans-cinnamic acid | 444539 | -4.5 |
| Quercetin    | 5280343 | -6.2             | Syringic acid | 10742   | -4.4             |
| Apigenin     | 5280443 | -6.2             | Phytol       | 5280435 | -4.3             |
| Kaempferol   | 5280863 | -6.1             | Vanilic acid | 8468    | -4.3             |
| Hyperoside   | 5281643 | -6.1             | P-hydroxybenzoic acid | 135 | -4.3 |
| Isoquercetin | 5280804 | -5.9             | Salicylic acid | 338    | -4.2             |
| Chlorogenic acid | 1794427 | -5.8             | Palmitic acid | 985    | -4.0             |
| Sinapic acid | 637775  | -4.9             | Ascorbic acid | 54670067 | -4.0 |
| Caffeic acid | 689043  | -4.9             | Asam Oleat  | 445639  | -3.9             |

Analysis of interaction between ligand of the active compounds in Amaranthus tricolor leaves and HMGB1

The interaction between ligands and HMGB1 protein can be analyzed by the strength of the bonds formed. A strong bond with HMGB1 protein is indicated by the formation of hydrogen bonds and hydrophobic interactions, which are the same as the interaction value of control compounds. Analysis of the bond interaction between the active compounds in Amaranthus tricolor and HMGB1 protein is shown in Table 3 and Figure 2.

The hydrogen bond interaction of the control compound glycyrrhizin with the active site of the HMGB1 protein is the amino acids Thr80, Glu77, Arg73, Arg13, Gly14, and for hydrophobic interactions with the amino acids Tyr81, Tyr74, Lys10, Pro12. The hydrogen bonding for doxorubicin control compounds on amino acids...
His30, and hydrophobic interactions on amino acids Lys47, Phe44, His34, Lys33, Phe22, Cys26.

Table 3. Analysis of the bond interaction between the active compound *Amaranthus tricolor* and HMGB1 protein

| Ligan          | Interaction                                      |
|---------------|--------------------------------------------------|
| Ellagic acid  | Hydrogen bond: Lys10, **Gly14**                  |
|               | Hydrophobic bond: **Pro12**, Arg13, **Tyr74**, Arg73, Glu77 |
| Beta-caroten  | Hydrogen bond: -                                 |
|               | Hydrophobic bond: Arg73, **Pro12**, Lys10, Tyr19, Lys53, Trp52, Ala20, Gly14, Glu77 |
| Isorhamnetin  | Hydrogen bond: **Arg13**, **Gly14**, Glu77       |
|               | Hydrophobic bond: Asp8, **Lys10**, **Tyr81**, **Pro12** |
| Rutin         | Hydrogen bond: **Gly14**, Arg73, Lys10           |
|               | Hydrophobic bond: **Pro12**, **Tyr74**, Glu77, Lys11, Tyr01 |
| Myricetin     | Hydrogen bond: **Gly14**, Glu77                  |
|               | Hydrophobic bond: **Lys10**, Lys11, Asp8, **Pro12**, Arg73 |
| Quercetin     | Hydrogen bond: **Gly14**, Asp8, Glu77            |
|               | Hydrophobic bond: Arg73, **Pro12**, Lys11, **Lys10** |
| Inhibitor reference 1/ | Hydrogen bond: **Thr80**, Glu77, Arg73, Arg13, **Gly14** |
| Glycyrrhizin  | Hydrophobic bond: **Tyr81**, **Tyr74**, **Lys10**, **Pro12** |
| Inhibitor reference 2/ | Hydrogen bond: **His30** |
| Doxorubicin   | Hydrophobic bond: **Lys47**, Phe44, **His34**, Lys33, **Phe22**, Cys26, Cys48, Arg51 |

Notes: The bold and underlined letter indicated the same bonds as the control compound.

Figure 2. Molecular docking simulation results of HMGB1 the active compound *Amaranthus tricolor* (a) structure of the active compound *Amaranthus tricolor* (b) overview (c) analysis of the bond interaction
The hydrogen bonds formed by theisorhamnetin compound have three similarities with the amino acid binder with the control compound, then rutin compounds, myricetin, and quercetin have two things in common, and ellagic acid compounds have 1 in common. Meanwhile, theisorhamnetin compound’s hydrophobic interaction has three similarities with the control compound, and for the ellagic acid, beta carotene, routine, myricetin and quercetin compounds they have two similarities. The active compound is predicted to have the potency to inhibit HMGB1 if it has the same interaction ability compared to the control compound amino acid. The results obtained show that the compoundsisorhamnetin, routine, myricetin, and quercetin have almost the same ability as the control in inhibiting HMGB1.

High-mobility group box 1 (HMGB1) is a multifunctional factor involved in various biologically diverse processes, including DNA repair, recombination, replication, transcription, differentiation, development, and extracellular signaling. HMGB1 has an intricate role in carcinogenesis because it has pro and anti-tumorigenic bioactivity. Once released, extracellular HMGB1 engages HMGB1 signaling with RAGE leading to chemotaxis and migration, proliferation, and differentiation of immune and cancer cells and upregulation of cell surface receptors and autophagy. Downregulation of HMGB1 inhibits proliferation, migration, cell invasion, induced apoptosis, and cell cycle cessation of some cancers, including breast cancer. HMGB1 inhibition suppresses tumorigenesis by interacting with p53, p73, retinoblastoma (RB) protein, Rel / NF-κB family [18]. So that HMGB1 inhibition can reduce the viability of breast cancer cells.

CONCLUSION

Of the several active compounds contained in Amaranthus tricolor leaves, based on the in silico molecular docking test that we conducted, the compoundsisorhamnetin, rutin, myricetin, and quercetin have the best prediction as HMGB1 inhibitor. They have the smallest bond energy, indicating that the four compounds are the most stable and have the highest activation to inhibit HMGB1. The inhibition of HMGB1 thus could inhibit the proliferation, migration, and invasion of breast cancer cells. As a result, we recommend that in vitro is needed to prove our finding in this study.

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