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 female 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 metastasizes 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, isoquercetin, 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://molprobtty.biochem.duke.edu/>).

Drug likeness 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.scfbio-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. 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 the 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 ≤ 500 Dalton, hydrogen bond acceptors ≤ 10 ,

hydrogen bond donors ≤ 5 , high lipophilicity ≤ 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	Lipinski Rule of Five				
	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-caroten	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,

Cys48, Arg51.

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/ Glycyrrhizin	Hydrogen bond: Thr80 , Glu77 , Arg73 , Arg13 , Gly14 Hydrophobic bond: Tyr81 , Tyr74 , Lys10 , Pro12
Inhibitor reference 2/ Doxorubicin	Hydrogen bond: His30 Hydrophobic bond: Lys47 , Phe44 , His34 , Lys33 , Phe22 , Cys26 , Cys48 , Arg51

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

A. HMGB1 with ellagic acid

B. HMGB1 with beta-caroten

C. HMGB1 with isorhamnetin

D. HMGB1 with rutin

E. HMGB1 with myricetin

F. HMGB1 with quercetin

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 the Isorhamnetin 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, the isorhamnetin 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 compounds isorhamnetin, 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- κ B 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 compounds isorhamnetin, 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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