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RESEARCH PAPER

Identification of Active Compound from *Mitragyna speciosa* Leave as Antiinflammation Agent: *In Silico* Study.

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Abstract: The study aims to identify the most responsible compound for the antiinflammation activity from *Mitragyna speciosa* leaves. Seventeen compounds previously reported to have been isolated from the leave were virtually screened against human 5-lipoxygenase protein and analyzed according to their binding energies. The native ligand used was arachidonic acid, and mitragynine was found to be the strongest binding compound (Pubchem ID: 3034396). In addition, ADMET profiling shows that mitragynine was not violating Lipinski's rule of five and was not toxic.

Keywords: *Mitragyna speciose*, antiinflammation, virtual screening, ADMET

INTRODUCTION

Kratom (*Mitragyna speciosa*) was reported to have several health benefits, i.e., as antiinflammation, antioxidant, sedative, antiobesity, analgesics, and anti-breast cancer [1]. In more detail, the leaf of kratom was found to have 17 compounds dominated by alkaloids [2]. However, there has yet to be a clear explanation about the compound most responsible for those activities.

Antiinflammation activity from a compound could be investigated by its capability to inhibit the lipoxygenase (LOX) protein. The LOX produces hydroxyeicosatetraenoic acids, which induce an inflammatory response [3]. Several studies about LOX inhibition using the *in silico* method were conducted by previous researchers, including the eugenol [4], mycophenolic acid derivatives [5], and *Melissa officinalis* subsp. *officinalis* essential oil [6].

Virtual screening as one of the *in silico* methods has been used for various purposes; one of them was to investigate the potent compound as an inhibitor [7], [8] and another one was to identify the most responsible compound for a particular pharmacological activity [9], [10].

Besides the compound's activity in inhibiting the targeted protein, the adsorption, distribution, metabolism, excretion, and toxicity (ADMET) profile was another important aspect to ensure the compound could reach the target. Some web servers could predict the ADME profile, i.e., SwissADME [11], while the toxicities of the compound could be calculated by the ProTox-II server [12].

In this study, we investigate the most responsible compound from kratom leaves to its antiinflammation activity using docking-based virtual screening followed by ADMET profiling.

MATERIALS AND METHODS

The targeted protein was downloaded from the database (https://www.rcsb.org) with the PDB ID of 3V99 [13]. The kratom's compounds (ligands) were downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov) with the CID of 65080, 72276, 94160, 120678, 441975, 3000341, 3034396, 3037629, 5742590, 9930064, 10475115, 10948612, 11726520, 15560576, 44301524, 44568160, and 102183193. The protein and the ligands were then prepared using the DockPrep feature in Chimera 1.16 [14].

The virtual screening process was done based on the native ligand (arachidonic acid) position in the LOX protein using Autodock Vina [15] implemented in PyRX 0.7 package [16]. The grid box was assigned 20 Å in the x, y, and z axis in each, while centered in x=17.1028, y=-77.7762, and z=-34.8646. The exhaustiveness level was adjusted to 20. The ligand with the most similar interaction with the native ligand was selected for the next process.

The ADMET profiling was done using SwissADME and ProTox-II server. The analysis and visualization of protein-ligand interactions were conducted using Discovery Studio Visualizer 2021 Client [17].

RESULTS AND DISCUSSION

The first step in docking-based virtual screening was protocol validation. It was sentenced that the docking protocol has to reproduce the native ligand similarly to the crystallized position. The reproducibility was measured by the RMSD value, in which the threshold was 2 Å [18]. Our result shows the RMSD value between the co-crystallized native ligand and after it was docked of 1.53 Å (Figure 1). This number of RMSD shows that the docking protocol used was valid.
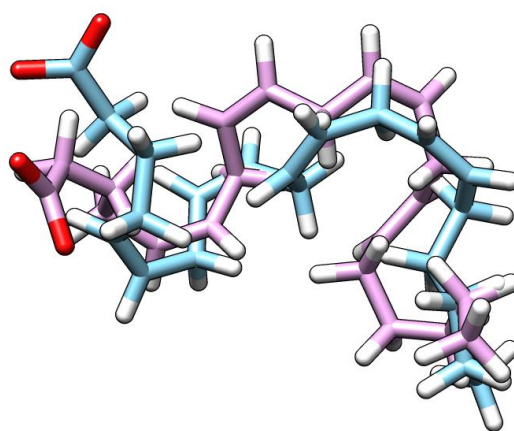

Figure 1. Superimposition of co-crystallized native ligand (purple) and the docked native ligand (blue)

The validated protocol was then used to virtually screen the 17 compounds from kratom leaves against the LOX protein. The binding energy from the 17 compounds is shown in Figure 2. It was seen that the compound quinovic acid (CID: 120678) shows the strongest binding energy to LOX. However, this compound did not interact with LOX similarly to the native ligand. The docking analysis does not only focus on the binding energy but also on the interaction between the ligand and the main residue of the targeted protein [19], [20]. The same phenomenon occurred to the other compounds, and resulting the selected one was mitragynine (CID: 3034396) since this compound shows the same hydrogen bond interaction compared to the

native ligand. The hydrogen bond has occurred from the O atom from the ligand to the H atom from the amino acid residue. The main literature on the LOX protein used in this study mentioned that arachidonic acid interacts with the protein through hydrogen bonding to the GLN557 [13]. The same interaction was shown by the mitragynine, as depicted in Figure 3. This result concluded mitragynine was the most responsible for the antiinflammation activity from kratom leaves. Hydrogen bonding has become the most studied interaction in protein-ligand due to this interaction being the strongest among the non-covalent interaction [21].
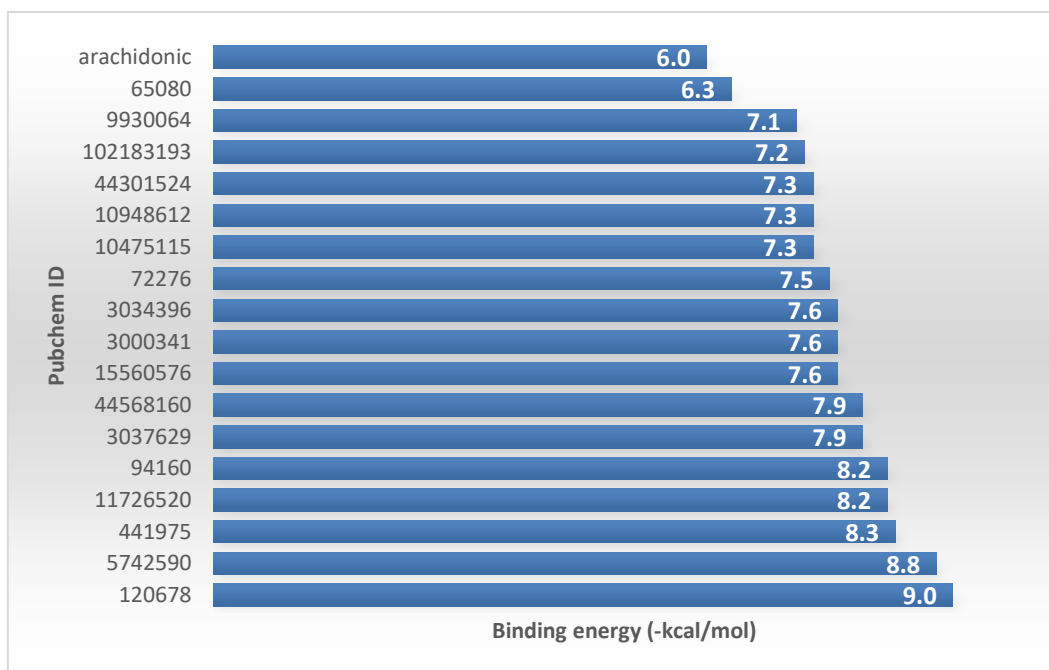
Figure 2. Binding energy from the native ligand (arachidonic acid) and the compounds from kratom leaves
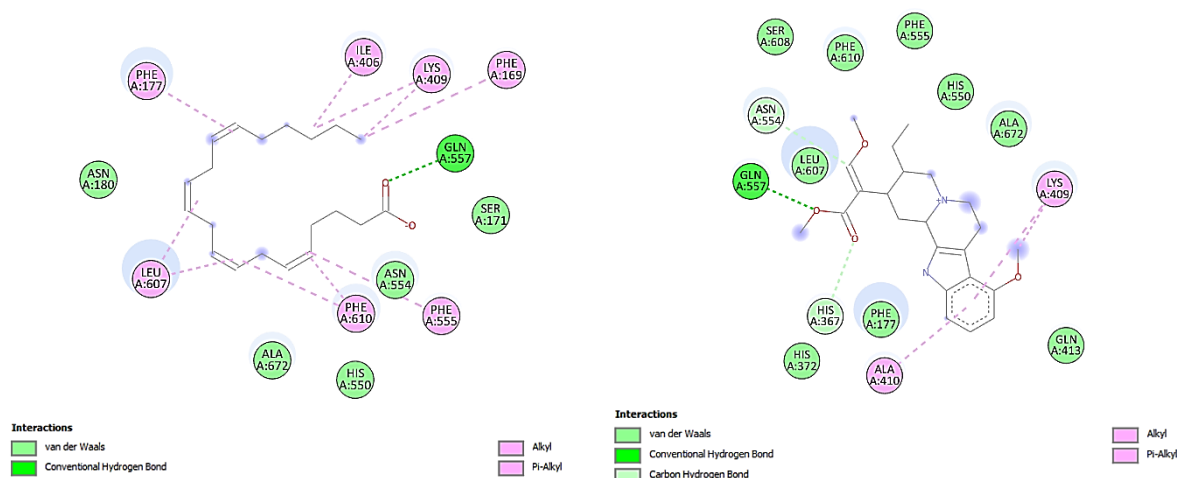

Figure 3. The interaction between arachidonic acid (left) and mitragynine (right) to LOX (PDB ID: 3V99)

The ADMET profile was predicted to ensure the compound would reach the target, as shown in Table 1. Based on the ADME prediction, mitragynine did not find a significant obstacle to reaching the target. This compound was predicted to be permeant through the brain-blood barrier. This result agreed that mitragynine from kratom could be used as an opioid and bind to μ-opioid and к-opioid receptors [22].

Fortunately, mitragynine does not violate Lipinski's rule of five, which has become the most common drugability rule in drug discovery.

This rule limits the compound to show some particular physicochemical properties: < 5 hydrogen bond donor, < 10 hydrogen bond acceptor, molecular weight < 500, and log P < 5 [23]. In addition, the synthetic accessibility value of this compound was found of 4.49, which indicates a medium level in terms of synthetic difficulty [24].

Table 1. The result of ADMET profiling

Parameter	Prediction	Range
GI absorption	High	Low-High
BBB permeant	Yes	Yes/No
CYP1A2 inhibitor	No	Yes/No
CYP2C19 inhibitor	No	Yes/No
CYP2C9 inhibitor	No	Yes/No
CYP2D6 inhibitor	Yes	Yes/No
CYP3A4 inhibit or	Yes	Yes/No
Lipinski #violations	0	0-4
Synthetic Accessibility	4.49	1 (easy) – 10 (hard)
Predicted LD_{50}	300	mg/kg
Predicted toxicity class	3	1 (very toxic) – 6 (Non-toxic)
Hepatotoxicity	Inactive	Active/Inactive
Carcinogenicity	Active	Active/Inactive
Immunotoxicity	Inactive	Active/Inactive
Mutagenicity	Inactive	Active/Inactive
Cytotoxicity	Inactive	Active/Inactive
Tox21-nuclear receptor signaling pathways	Inactive	Active/Inactive
Mitochondrial membrane potential	Inactive	Active/Inactive

In the toxicity prediction, most of the results show that mitragynine was not toxic. In exception, this compound was predicted to have the potential to be carcinogenic. The step to reduce carcinogenicity is to make derivatives of mitragynine, as done by Chakraborty [25] and Bhowmik [26].

CONCLUSION

The virtual screening result shows that mitragynine (CID: 3034396) was predicted as the most responsible compound for the antiinflammation activity from kratom (*Mitragyna speciosa*) leaves. The ADMET profiling also predicts that the compound has good drugability but may also have toxic and carcinogenic properties, so it must be modified before being administered to humans.

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