



## **Prediction of Compounds from 96% Ethanol Extract of *Marsilea crenata* Presl. Leaves in Increasing Estrogen Receptor- $\alpha$ Activation**

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### **Abstract**

Women who experience menopause will experience estrogen deficiency which will have an impact on their health; one of which will increase the risk of neurodegenerative. Phytoestrogen compounds in *Marsilea crenata* are able to provide activity after binding to their receptors or through ER-dependent pathways. The research was conducted in silico with the molecular docking method using the ER $\alpha$  (1A52) receptor. In silico analysis was carried out on the metabolite profiling compound of the 96% ethanol extract of *M. crenata* leaves from the previous study. Sample preparation was carried out using the Biovia Discovery Studio 2021 application to separate macromolecules and native ligands and prepared to get a 3D structure using ChemDraw Ultra 12.0. then analyzed its pharmacokinetics and pharmacodynamics with the SwissADME webtool. Furthermore, the geometry of the compound was optimized using Avogadro 1.0.1 and molecular docking of the compound to the 1A52 receptor was carried out using Autodock vina (PyRx 0.8). The interaction visualization stage was carried out with Biovia Discover Studio 2021 and a toxicity test was carried out using the ProTox II online tool. The results of the in-silico study showed that there were 6 compounds that met the pharmacokinetic and pharmacodynamic criteria, toxicity, and had similar pharmacophore distances and amino acid binding with native 17 $\beta$ -estradiol, a 1A52 agonist with anti-neuroinflammatory effect. So, 96% ethanol extract of *M. crenata* leaves are predicted to have potential as an inhibitor of PD progression with an anti-neuroinflammatory mechanism.

**Keywords:** *Marsilea crenata* Presl.; Parkinson's Disease; neuroinflammation; in silico; 1A52

### **Introduction**

Women will experience menopause which is a transitional phase from productive to non-productive (Koeryaman and Ermiami, 2018). This period marked by the occurrence of estrogen deficiency or decreased levels of the hormone estrogen, where there is a deactivation process of

estradiol into estrone and estriol (Cui *et al.*, 2013). The impact can be in the form of osteoporosis, neurodegenerative, and others (Ma'arif *et al.*, 2019; Aditama *et al.*, 2020).

Neurodegenerative is a condition of the central nervous system that experiences loss of structure and function which is a sign of disease or disorder. The cause is an inflammatory state that attacks the central nervous system called neuroinflammation (Chen *et al.*, 2016). Parkinson's is a neurodegenerative disease that occurs when a person enters the age of 65 to 70 years, characterized by the presence of Lewy bodies with the main content of  $\alpha$ -synuclein (Balestrino and Schapira, 2020; Suharti, 2020).

Phytoestrogens are one of the alternative therapies to treat estrogen deficiency. This compound works as a support when the amount of estrogen in the body decreases by binding its activity to empty estrogen receptors. Phytoestrogens in the cytoplasm produce hormone receptor complexes that are active through their binding to estrogen receptors (Kargozar, 2017). Previous research has shown that phytoestrogen compounds in *Marsilea crenata* (clover) are able to provide activity after binding to their receptors or through ER-dependent pathways. This pathway can provide activity by binding to Estrogen Receptor (ER $\alpha$ ) (Ma'arif, 2020).

Based on this explanation, further research is needed on the 96% ethanol extract of *M. crenata* leaves obtained from the metabolite profiling results with UPLC-QToF-MS/MS (Ma'arif, 2020). The research was conducted *in silico* with the molecular docking method using the 1A52 receptor. *In silico* is a research method with computer simulation using certain programs or applications in designing or finding drugs (Suharna, 2012).

## Materials and Methods

The materials used consisted of 43 compounds resulting from metabolite profiling of 96% ethanol extract of *M. crenata* leaves using the UPLC-QToF-MS/MS method obtained from previous studies (Ma'arif, 2020), and the ER $\alpha$  receptor with ID 1A52 downloaded from [www.rcsb.org](http://www.rcsb.org) containing 17 $\beta$ -estradiol.

The first step is preparation to separate native ligand macromolecules with the application of Biovia Discovery Studio 2021. The metabolite profiling compound from the 96% ethanol extract of *M. crenata* leaves was drawn 2-dimensional structure using ChemDraw Ultra 12.0. The compounds resulting from the metabolite profiling were geometrically optimized by the MMFF94 method using Avogadro 1.0.1. Internal validation is the initial stage of the molecular docking process on the receptor and native ligand using Autodock vina (PyRx 0.8) to get the root mean square deviation (RMSD) value. An RMSD result that is less than 2Å indicates the application is right to use. Molecular docking of each compound with 1A52 receptor with Autodock vina (PyRx 0.8). Results were visualized with Biovia Discover Studio 2021 to see bound amino acids and pharmacophore distances.

In the next step, the agonist compound is converted into a simplified molecular-input line-entry system (SMILES) in ChemDraw Ultra 12.0. This form is so that the compound can be analyzed for its physicochemical properties in a format that is in accordance with the IUPAC name (Sliwoski, 2014). The format is entered in the SwissADME webtool (<http://www.swissadme.ch>) and clicked to run to find out the value of molecular weight, log P, HBA (Hydrogen Bond Acceptor), HBD (Hydrogen Bond Donor), "Yes" or "No" statements in fulfilling Lipinski's rule of five, and topological polar surface area (TPSA). And the last step is toxicity analysis using SMILES format to predict compound toxicity in the form of LD50 (Lethal Dose 50) value and toxicity class based on globally harmonized system (GHS) using ProTox II online tool ([https://tox.charite.de/protox\\_II/](https://tox.charite.de/protox_II/)).

## **Results and Discussion**

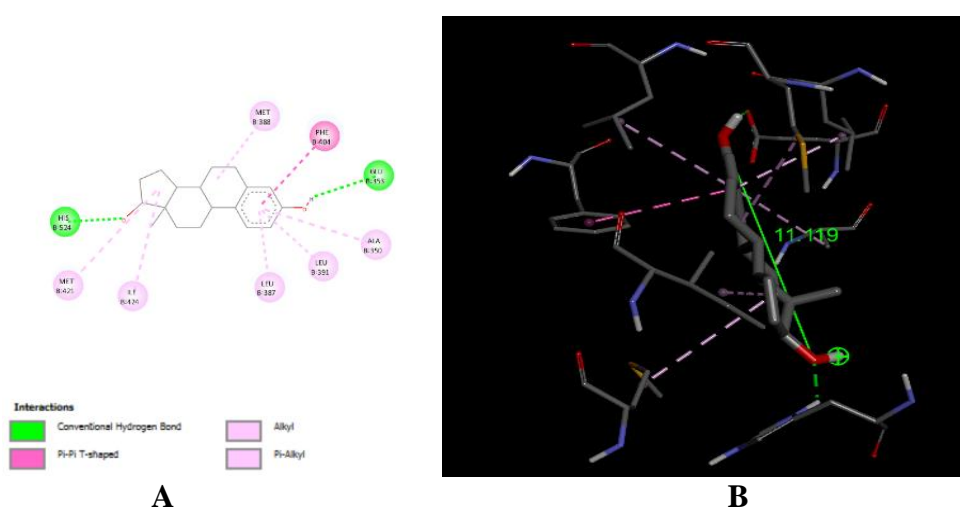
Phytoestrogens are an alternative treatment for postmenopausal women who have estrogen deficiency. This compound can replace the role of the hormone estrogen with an ER-dependent mechanism (Yang *et al.*, 2012). This pathway illustrates that estrogen will provide activity after penetrating the plasma membrane and providing it with an estrogen receptor in the form of ER $\alpha$  and activating it (Cui *et al.*, 2013).

The molecular docking process begins with method validation using Autodock Vina (PyRx 0.8) between the receptor and native ligand and obtained RMSD of 1,476. These results show that the molecular docking process that gives results is close to the experimental results, because the RMSD value is less than 2Å (Santoyo *et al.*, 2013). Compounds resulted from metabolite profiling with UPLC-QToF-MS/MS and the 1A52 receptor were re-tethered with Autodock Vina (PyRx 0.8). Visualization of molecular docking results with Biovia Discovery Studio 2021 to determine the type of bond, amino acids formed, and pharmacophore distance to be compared with native ligands (Mirza *et al.*, 2021). The results of molecular docking of agonist compounds with 1A52 can be observed in **Table 1**.

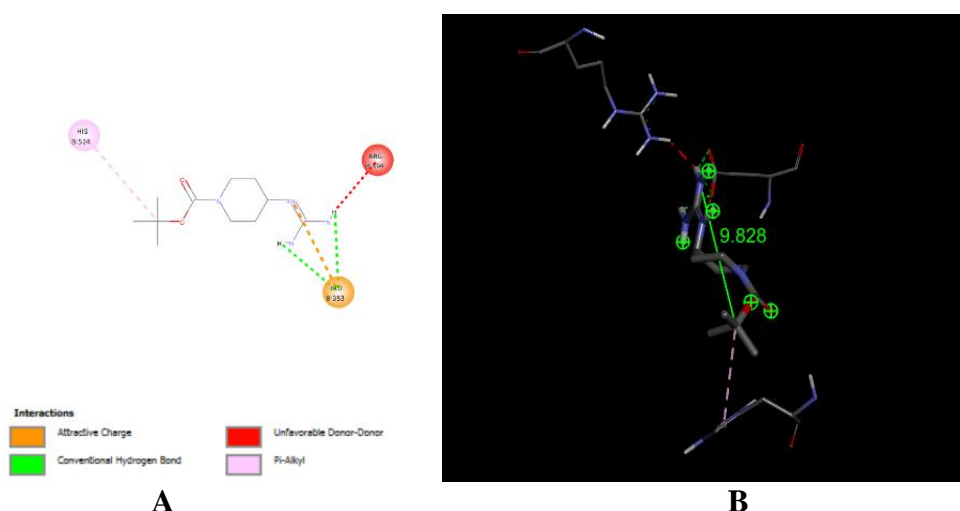
**Tabel 1.** 1A52 agonist compounds from 96% ethanol extract of *M. crenata* leaves along with pharmacokinetic and pharmacodynamic analysis

No.	Compound	Binding Affinity (kcal/mol)	Amino Acids (Types of Bonds)	Pharmacophore Distance (Å)	Lipinski's parameters			Lipinski role of live	TPSA (Å <sup>2</sup> )	LD <sub>50</sub> class	
					BM < 500 g/mol	HBA ≤ 10	HBD ≤ 5				Log P ≤ 5
<b>Native Ligand</b>											
1.	17β-estradiol	-10,7	Glu353 (Hidrogen) His524 (Hidrogen)	11,119	272,38	2	2	3,40	Yes	40,46	IV
<b>96% ethanol extract of <i>Marsilea crenata</i> leaves</b>											
1.	2-Methyl-2-propanyl 4-carbamimidamido-1-piperidinecarboxylate hydrochloride	-6,2	Glu353 (Hidrogen) His524 (Hidrogen)	9,828	278,78 g/mol	3	3	0,63	Yes	91,44	IV
2.	2,2'-(Tridecylimino) diethanol	-5,2	Glu353 (Hidrogen) His524 (Hidrogen)	10,310	287,48 g/mol	3	2	4,02	Yes	43,70	IV
3.	2-Deoxy-2-(((2-methyl-2-propanyl)oxy)carbonyl)amino)-D-glucopyranose	-6,8	Glu353 (Hidrogen) His524 (Hidrogen)	8,678	279,29 g/mol	7	5	-0,77	Yes	128,48	V
4.	4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo-1,3-dihydronaphtho[2,3-c]furan-5-yl hexopyranoside	12,4	Glu353 (Hidrogen) His524 (Hidrogen)	11,605	498,44 g/mol	1	5	1,06	Yes	164,37	V
5.	6-O-(N-(((2-Methyl-2-propanyl)oxy)carbonyl)-D-leucyl)-α-D-allopyranose	-4,4	Glu353 (Hidrogen) His524 (Hidrogen)	9,760	393,43 g/mol	9	5	0,02	Yes	154,78	IV
6.	N2-((2S)-1-[(3aR,6a'S)-1'-(Cyclopropylcarbonyl)-2'-oxohexahydro-4'H-spiro[cyclobutane-1,3'-pyrrolo[3,2-b]pyrrol]-4'-yl]-3-methyl-1-oxo-2-butanyl)carbamoyl)-N,N-dimethyl-L-valinamide	14	Glu353 (Hidrogen) His524 (Hidrogen)	10,606	504,64 g/mol	6	2	0,76	Yes	133,12	IV

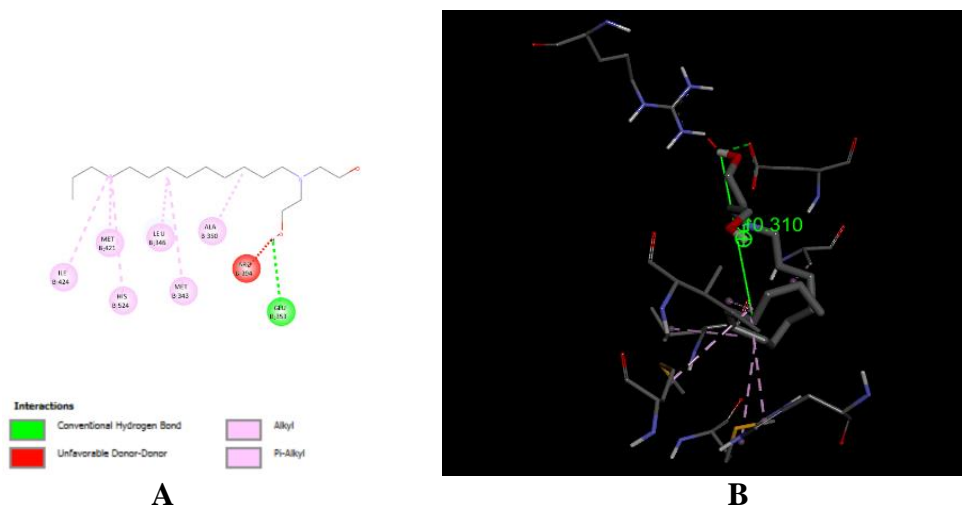
Native ligands are agonists in activating ER $\alpha$  by binding to amino acids in the form of Glu353 and His524, each of which is in the form of hydrogen bonds. Amino acids bound by a hydroxyl group (OH) will produce estrogenic activity (Prabowo and Santoso, 2018). The resulted pharmacophore distance is 11.119. A compound can be said to be an agonist if it binds to the same two amino acids as the native ligand, in the form of Glu353 and His524 which can be seen in **Figure 1**. The similarity of the amino acids bound by the compound and the pharmacophore distance that is close to the yield of the native ligand gives a similarity in the resulted interactions (Ekins *et al.*, 2007; Suhud *et al.*, 2015; Muchtaridi *et al.*, 2018). The smaller the binding affinity or the negative value, the stronger the bond formed and the stable interaction (Arwansyah *et al.*, 2014). Visualization of the molecular docking results of agonist compounds using the Biovia Discovery Studio 2021 application is shown in **Figure 2 - Figure 5**.



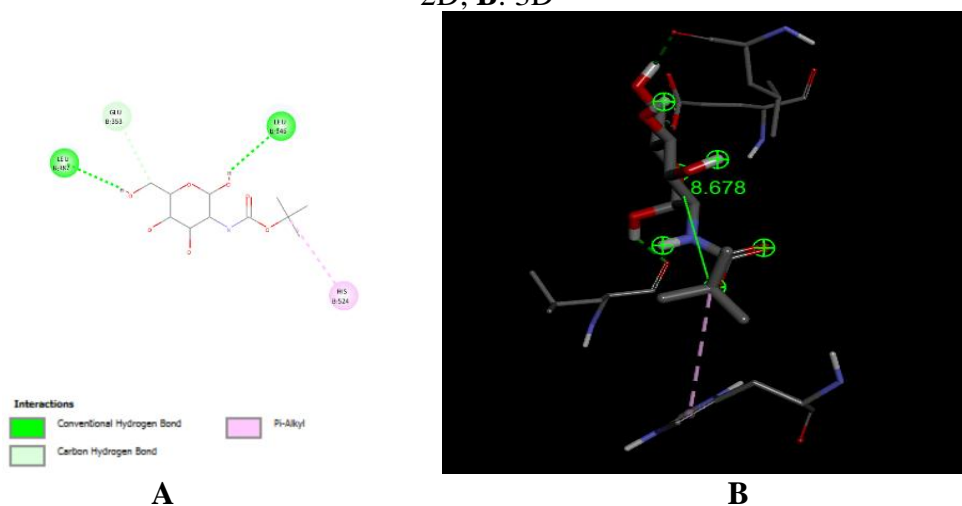
**Figure 1.** Visualization of native ligand with 1A52 receptor. **A:** 2D; **B:** 3D



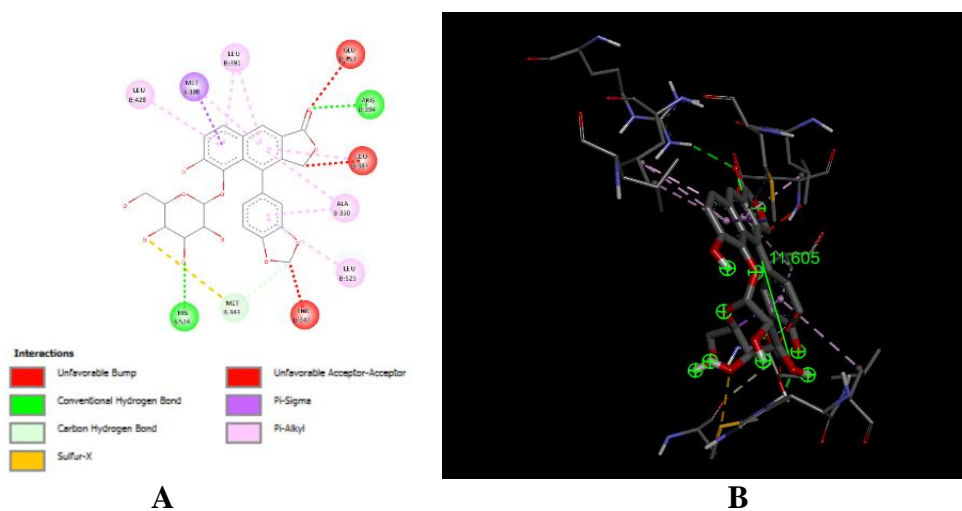
**Figure 2.** Visualization of 2-Methyl-2-propanyl 4-carbamimidamido-1-piperidinecarboxylate hydrochloride compounds with 1A52 receptor. **A:** 2D; **B:** 3D



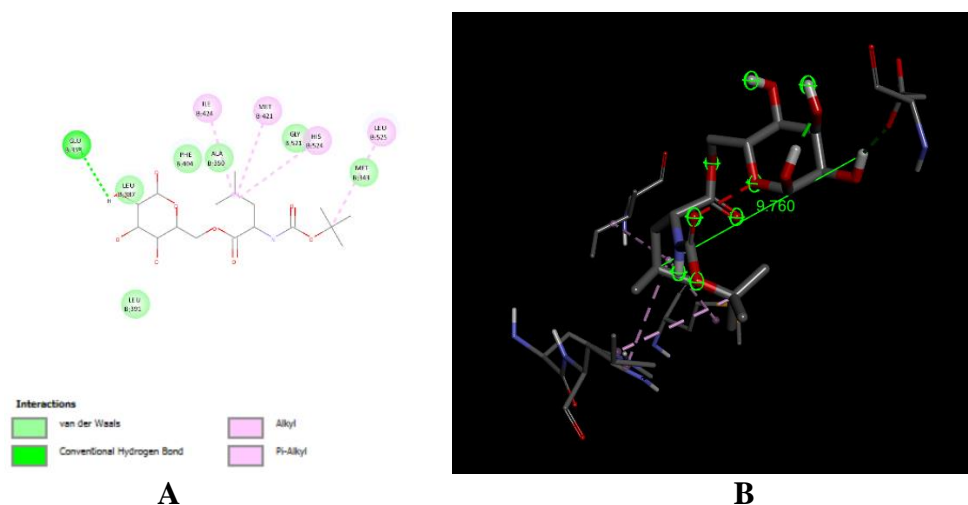
**Figure 3.** Visualization of 2,2'-(*Tridecylimino*) diethanol compounds with 1A52 receptor. **A:** 2D; **B:** 3D



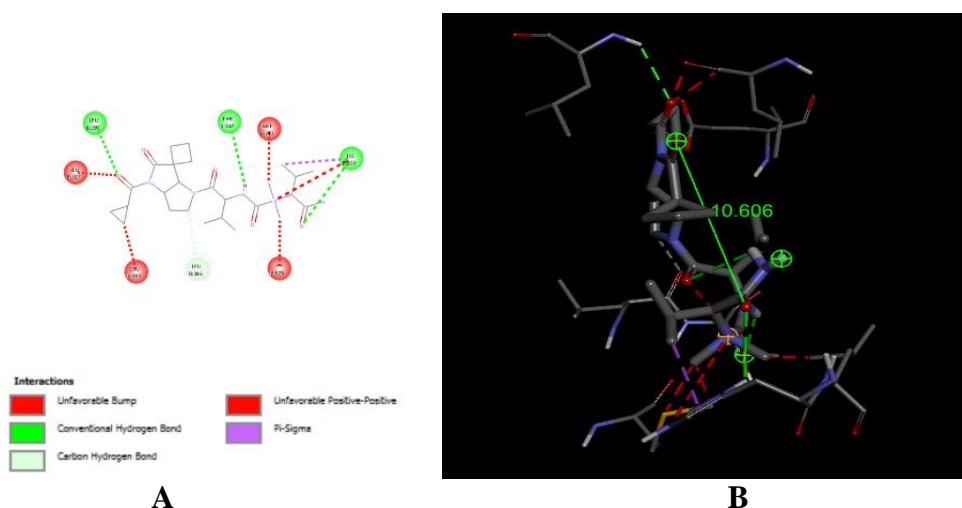
**Figure 4.** Visualization of 2-Deoxy-2-([(2-methyl-2-propanyl)oxy]carbonyl)amino)-D-glucoopyranose compounds with 1A52 receptor. **A:** 2D; **B:** 3D



**Figure 5.** Visualization of 4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo-1,3-dihydronaphtho[2,3-c]furan-5-yl hexopyranoside compounds with 1A52 receptor. **A:** 2D; **B:** 3D



**Figure 6.** Visualization of 6-O-(N-[(2-Methyl-2-propanyl)oxy]carbonyl)-D-leucyl)-α-D-allopyranose compounds with 1A52 receptor. **A:** 2D; **B:** 3D



**Figure 7.** Visualization of N2-((2S)-1-[(3a'R,6a'S)-1'-(Cyclopropylcarbonyl)-2'-oxohexahydro-4'H-spiro[cyclobutane-1,3'-pyrrolo[3,2-b]pyrrol]-4'-yl]-3-methyl-1-oxo-2-butanyl}carbamoyl)-N,N-dimethyl-L-valinamide compounds with 1A52 receptor. **A:** 2D; **B:** 3D

Screening of agonist compounds using the SwissADME application showed that there were 6 compounds that met Lipinski's rule of five requirements with a molecular weight of <500 g/mol, HBD<5, HBA<10, and log p<5, so that these compounds can be accepted by the body which can be observed in Table 1. The molecular weight of the compound can penetrate biological membranes in the range of less than 500 g/mol. The log P results obtained are less than 5 which indicates the ability of the compound to dissolve in membrane fluids. H-acceptor and H-donor show hydrogen bonding capacity where the greater the value, the higher the energy required for the absorption process with the results obtained HBA is less than 10 and HBD is less than 5 (Lipinski *et al.*, 1997). The TPSA value expresses the ability of the compound to penetrate the body's cell membranes (Chagas *et al.*, 2018).

The toxicity test was seen based on the LD50 value in finding a single dose that could kill 50% of the experimental animals when given a single administration of the compound, so that it could see the potential toxicity of the compounds in the 96% ethanol extract of *M. crenata* leaves which are toxic to the body (Nurmianti and Gusmawarni, 2020). The division of the level of



toxicity according to GHS is 6 classes. The six toxicity classes are class I ( $LD50 \leq 5$  mg/kg) fatal if swallowed, class II ( $5 < LD50 \leq 50$  mg/kg) fatal if swallowed, class III ( $50 < LD50 \leq 300$  mg/kg) toxic if swallowed, class IV ( $300 < LD50 \leq 2000$  mg/kg) harmful if swallowed, class V ( $2000 < LD50 \leq 5000$  mg/kg) harmful if swallowed, and class VI ( $LD50 > 5000$  mg/kg) non-toxic. The greater the LD50 value, the more secure the compound is for the body and vice versa. The results showed that there were 6 compounds in classes 4 and 5, so they had low toxicity (Supandi, *et al.*, 2018) which can be seen in **Table 2**.

The agonist compound that has the potential as a phytoestrogen so that it becomes an anti-neuroinflammatory agent by meeting Lipinski's rule of five parameters "Yes" and low toxicity consists of 6 compounds, 2-Methyl-2-propanyl 4-carbamimidamido-1-piperidinecarboxylate hydrochloride, 2,2'- (Tridecylimino) diethanol, 2-Deoxy-2-([(2-methyl-2-propanyl)oxy]carbonyl)amino)-D-glucopyranose, 4-(1,3-Benzodioxol-5-yl)-6-hydroxy-1-oxo-1,3-dihydronaphtho[2,3-c]furan-5-yl hexopyranoside, 6-O-(N-([(2-Methyl-2-propanyl)oxy]carbonyl)-D-leucyl)- $\alpha$ -D-allopyranose, and N2-([(2S)-1-[(3a'R,6a'S)-1'-(Cyclopropylcarbonyl)-2'-oxohexahydro-4'H-spiro[cyclobutane-1,3'-pyrrolo[3,2-b]pyrrol]-4'-yl]-3-methyl-1-oxo-2-butanyl} carbamoyl)-N,N-dimethyl-L-valinamide.

Inflammatory response will be shown in Parkinson's patients; estrogen can be an anti-inflammatory agent to reduce the severity of Parkinson's. Besides, estrogen can protect memory by inhibiting the activity of NF- $\kappa$ B and being an antidote to oxidative stress. This is because estradiol is useful for increasing the synthesis, release, and turnover of dopamine (Cherry *et al.*, 2014). So, the hormone provides neuroprotection by inhibiting protein aggregation, thereby slowing down synucleinopathies (Marota *et al.*, 2015). This similarity of activity was produced by the compound from the 96% ethanol extract of *M. crenata* leaves (Cherry *et al.*, 2014).

The compounds contained in *M. crenata* are the best choice in dealing with the problem of estrogen deficiency which leads to an inflammatory state in the central nervous system, by inhibiting the activity of microglia at M<sub>1</sub> polarity so that it becomes M<sub>2</sub> polarity to become an anti-neuroinflammatory agent. These compounds can be used as an alternative to anti-neuroinflammatory treatment which is one of the causes of Parkinson's disease (Cui *et al.*, 2013). *M. crenata* is a plant that has health benefits for the community as showed by molecular docking observations. The results show that the compounds contained in *M. crenata* have anti-neuroinflammatory activity that is safe for the body.

## Conclusion

The *in silico* activity of the metabolite profiling compound of 96% ethanol extract of *M. crenata* leaves showed that there were 6 compounds that had similar activity with 17 $\beta$ -estradiol which was seen from the similarity of the bound amino acids and the distance of the pharmacophore so that they became agonists of 1A52 which has the potential as an anti-neuroinflammatory. So, the 96% ethanol extract of *M. crenata* leaves was predicted to be a therapy for Parkinson's Disease with an anti-neuroinflammatory mechanism.

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