

## IN SILICO STUDY OF ACTIVE COMPOUNDS OF RAMANIA LEAF EXTRACTS (*Bouea macrophylla* Griffith) ON PROTEIN KINASE C- $\beta$

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### Abstract

Background: Traumatic Dental Injury (TDI) causes injury to the teeth and tissues around the oral cavity. A total of 17.2% incidence of TDI in children and adolescents under the age of 18 years, in permanent teeth TDI as much as 15.2%. TDI will cause an inflammatory process that will increase the immune response. PKC- $\beta$  is a conventional type of PKC isoform. Prolonged PKC- $\beta$  activation is associated with impaired NF- $\kappa$ B activation by TNF- $\alpha$ . PKC- $\beta$  has been shown to reduce the activity of NF- $\kappa$ B. PKC has a clear role in several cellular functions and diseases, making PKC isoenzymes a very promising target for drug development. TDI can be treated with herbal medicine therapy, one of the plants that can be used as medicinal plants is ramania (*Bouea macrophylla* Griffith). Objective : To analyze the binding of the active compound of BMG leaf extract to the expression of PKC- $\beta$  as an anti-inflammatory with the in silico method. Methods: This research is computationally based with the in silico method. This research uses molecular docking analysis of the protein X-Ray diffraction results from the Protein Data Bank (PDB) ID web. The Docking process is carried out using Autodock Vina which is integrated in the PyRx application and visualized using the Biovia Discovery Studio application. Conclusion : The methyl ricinoleate compound in BMG is the most potent inhibitor of Protein Kinase C- $\beta$  and has the potential as an anti-inflammatory of the 10 BMG leaf extract compounds studied

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## 1. INTRODUCTION

*Traumatic Dental Injury* (TDI) is a risk factor in an individual's life (Khan & Jindal, 2022). TDI causes injury to the teeth and tissues around the oral cavity (Lam, 2016). The WHO classification of TDI consists of trauma to the periodontal tissues, pulp and hard tissues of the teeth, supporting bones, to the gingiva and oral mucosa with seven types of tooth fracture, six types of luxation, eight types of damage to the supporting bone and three types of damage to the oral mucosa or gingiva (Zaleckiene et al., 2014). The prevalence of TDI differs between geographic areas. According to the meta-analysis of Azami-Aghdash *et al* (2015) children and adolescents aged 18 years and under have a TDI prevalence of 17.2%. Petti *et al* (2015) stated that the prevalence of TDI was 15.2% in permanent teeth (Majewski et al., 2013).

TDI will cause an inflammatory process that will increase the immune response. Central inflammatory mediator in the form of *Nuclear factor kappa B* (NF- $\kappa$ B) will be activated and deregulated in various inflammatory diseases. NF- $\kappa$ B can be activated by activated Protein Kinase C (PKC) to stimulate pro-inflammatory genes to release inflammatory mediators, such as *Interleukin 1 Beta* (IL-1 $\beta$ ) and *tumor necrosis factor alpha* (TNF- $\alpha$ ) (Izzi et al., 2012; Shita, 2015). PKC- $\beta$  is a conventional type of PKC isoform. Prolonged PKC- $\beta$  activation is associated with impaired NF- $\kappa$ B activation by TNF- $\alpha$  (Alleboina et al., 2020). PKC- $\beta$  has been shown to reduce the activity of NF- $\kappa$ B. PKC has a clear role in several cellular functions and diseases, making PKC isoenzymes a very promising target for drug development (Leppänen et al., 2014; Liu et al., 2017; Robertson et al., 2007).

Prompt and appropriate treatment of TDI can result in long-term survival of the tooth (Majewski et al., 2013). TDI can be treated with herbal medicine therapy. Herbal therapy is a drug solution with ingredients that are relatively easy to obtain and have harmless side effects. One of the plants that can be efficacious as medicine is BMG which comes from Kalimantan. BMG has leaves with secondary metabolites such as flavonoids, phenols, tannins, saponins, terpenoids, steroids (Rahman et al., 2017; Rizkia et al., 2021; Sukalingam, 2018).

Research by Nguyen *et al*, (2020) stated that BMG leaves have several active compounds that function as antioxidants and anti-inflammatory, including *2-Methyl-cis-7,8-epoxynonadecane*; *Caryophyllene*; *Hexadecanoic acid, ethyl ester*; *Humulene*; *Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-*; *Phytol*; *Retinol, acetate*; *Squalene*; Vitamin E;  *$\gamma$ -himachalene* (Nguyen et al., 2020). The reaction between the active compounds of BMG must be known, one of which is the *in silico* method.

The *in silico* test can describe experiments performed with the help of a computer. One of the *in silico* processes is molecular docking which is the key to molecular biology and drug design methods using numerical aids. Docking can be used to perform virtual screening of large compound databases, classify results and propose structural hypotheses about how ligands inhibit targets, which is very important in primary optimization (Aziz et al., 2016; Dona et al., 2019; Setiawan dan Istyastono, 2015). Based on the description above, an *in silico* study with molecular docking technique can be carried out to determine the potential of BMG extract as an anti-inflammatory based on PKC- $\beta$  interactions, determine the test compound binding and interactions that can be formed, and predict the orientation and binding affinity of the active

compound from the BMG extract (Ngurah et al., 2021).

## 2. EXPERIMENTAL SECTION

### 2.1 Ingredient

Three-dimensional structure of protein kinase-c  $\beta$ II (2I0E) and comparison protein phospholipase C (7NXE) downloaded from the Protein Data Bank website (<http://www.rcsb.org/pdb/>) using the .pdb format. Three-dimensional structure of the active compound of BMG leaf extract, namely: *2-Methyl-cis-7,8-epoxynonadecane (Pristanal)* (CID 537313); *Caryophyllene* (CID 5281515); *Hexadecanoic acid, ethyl ester (Palmitic acid)* (CID 21859); *Humulene* (CID 5281520); *Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans- (Methyl ricinoleate)* (CID 22821244); *Phytol* (CID 5280435); *Retinol, acetate* (CID 638034); *Squalene* (CID 638072); *Vitamin E* (CID 14985);  *$\gamma$ -himachalene* (CID 577062) dan *Ruboxistaurin mesylate* (CID 153999) which were downloaded from the PubChem website (<http://www.pubchem.org/>) using SDF. format.



Figure 1. BMG plant leaves (Taufiqurrahman I)

### 2.2 Tool

Laptop that has Microsoft Windows 10 operating system. Protein Data Bank (PDB) Website ID (<https://www.rcsb.org>) to download protein data. Pubchem website (<https://pubchem.ncbi.nlm.nih.gov/>) to download the active compound. The PyRx app includes Vina's AutoDock for validation and docking. Biovia Discovery Studio application to remove docked ligands and other impurities. pkCSM website (<http://biosig.unimelb.edu.au/pkcsm/>) and molinspiration (<https://www.molinspiration.com/cgi-bin/properties>) to predict the pharmacokinetic properties of the active compound of BMG leaf extract and the comparative ligand compound.

## 3. Research methods

### 3.1 Protein and ligand preparation

Protein Kinase c- $\beta$ II (PKC- $\beta$ II) and phospholipase C (PLC) were cleaned of water molecules and residues using the Biovia Discovery Studio application. The downloaded ligands were minimized using the PyRx application on the open babel menu.

### 3.2 Validation

The validation process for the docking method was carried out by re-docking PKC- $\beta$ II with its natural ligand, C27 H28 N4 O2 (CID 11963533). The results are said to be valid if the RMSD (Root Mean Square Deviation) value < 2. The results of the PKC- $\beta$  protein validation process

obtained an RMSD value of 0 which means the docking parameter is valid and can be used to dock a compound.

### 3.3 Docking and visualization

The docking process is carried out using the PyRx application version 0.8 using the AutoDock vina menu. The docking results are then visualized using the Biovia Discovery Studio version 21 application.

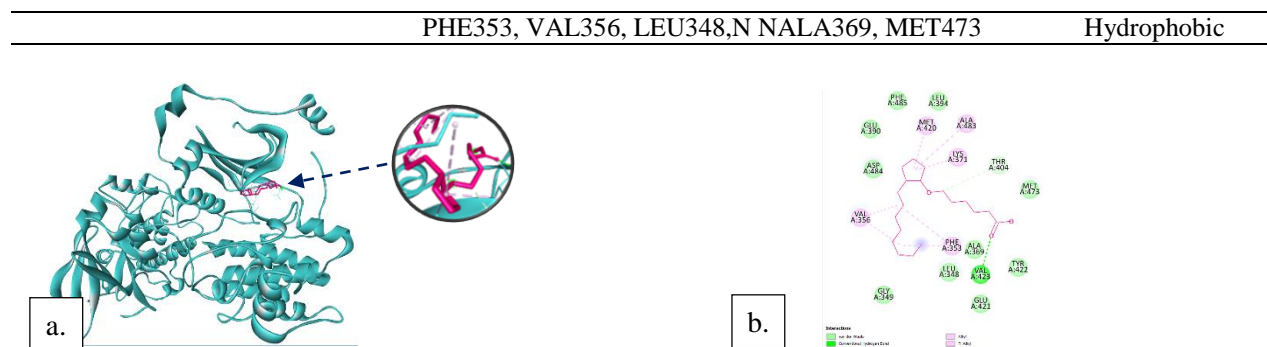
### 3.4 Prediction of drug-likeness, pharmacokinetics, and toxicity

*Drug-likeness* carried out based on Lipinski's rule of 5 to determine the similarity of the drug with the active compound. Pharmacokinetics and toxicity predictions were made through the pkCSM website (<http://biosig.unimelb.edu.au/pkcsm/>) and the molinspiration website (<https://www.molinspiration.com/cgi-bin/properties>).

## 4. RESULTS AND DISCUSSION

**Table 1.** Results of docking PKC- $\beta$  with the active compounds of BMG leaf extract and Ruboxistaurin

Ligand	Binding Affinity	Amino Residue	Category
<i>Pristanal</i>	-5,8	THR404	Hydrogen
		*PHE353, *LEU348, *VAL356, *VAL356, *ALA369, *ALA369, LYS371, MET420, VAL423, *MET473, ALA483, ALA483, *LEU348, TYR422	Hydrophobic
<i>Caryophyllene</i>	-7,6	*PHE353, *VAL356, MET420, ALA483	Hydrophobic
<i>Palmitic acid</i>	-5,9	PHE485, GLU390	Hydrogen
		*VAL356, *VAL356, *ALA369, *ALA369, VAL423, MET420, *LEU348, *PHE353, *PHE353	Hydrophobic
<i>Humulene</i>	-7,5	*VAL356, ALA483	Hydrophobic
<i>Methyl ricinoleate</i>	-6,3	VAL356, THR404	Hydrogen
		*VAL356, *VAL356, LYS371, MET420, ALA483, *PHE353, *PHE353	Hydrophobic
<i>Phytol</i>	-5,8	*VAL356, *VAL356, *ALA369, *ALA369, LYS371, ALA483, LYS371, MET420	Hydrophobic
		TYR422, TYR422	Hydrogen
<i>Retinol, acetate</i>	-7,8	*LEU348, *VAL356, *VAL356, *ALA369, *ALA369, VAL423, *MET473, *PHE353*	Hydrophobic
		*PHE353, *LEU348, *VAL356, *ALA369, MET420, ALA483, ALA483, *LEU348, TYR422	Hydrophobic
<i>Squalene</i>	-7	*PHE353, *VAL356, *VAL356, *VAL356, *ALA369, LYS371, MET420, ALA483, LYS371, *LEU348	Hydrophobic
<i><math>\gamma</math>-himachalene</i>	-8,1	*PHE353, *VAL356, *VAL356, *ALA369, *PHE353	Hydrophobic
<i>Ruboxistaurin</i>	-9,1	LEU348	Hydrogen



**Figure 2.** a. Result 3D structure docking of PKC- $\beta$  with Methyl ricinoleate. b. 2D structure of PKC- $\beta$  docking with Methyl ricinoleate.



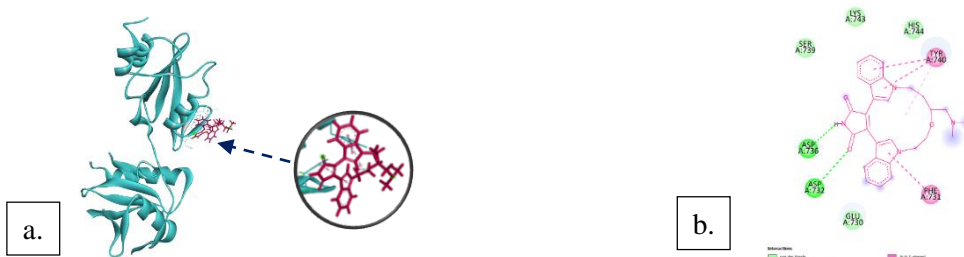
**Figure 3.** 1a. 3D structure of PKC- $\beta$  docking with Ruboxistaurin. b. 2D structure of PKC- $\beta$  docking with Ruboxistaurin.

**Table 2.** The results of PLC-PKC- $\beta$  docking with the active compounds of BMG leaf extract and Ruboxistaurin

Ligand	Binding Affinity	Amino Residue	Category
<i>Pristanal</i>	-3,5	ARG609	Hydrogen
		ARG609, PHE591	Hydrophobic
<i>Caryophyllene</i>	-5,3	ARG609, PH591	Hydrophobic
<i>Palmitic acid</i>	-3,9	ARG586, ARG586, THR590, THR596, SER588	Hydrogen
		ARG609, PHE591, HIS607	Hydrophobic
<i>Humulene</i>	-5,5	ARG609, PHE591	Hydrophobic
<i>Methyl ricinoleate</i>	-4,5	GLU730, GLU730	Hydrogen
		LYS743, *PHE731, *TYR740, HIS744	Hydrophobic
<i>Phytol</i>	-4,9	ARG5860, THR596, THR590	Hydrogen
		CYS608, CYS608, ARG609, ARG609, LEU623, LEU644, LEU623, LEU644, PHE591	Hydrophobic
<i>Retinol, acetate</i>	-5,1	GLN659	Hydrogen
		LYS549, PRO658, ALA662, ALA662	Hydrophobic
<i>Squalene</i>	-4,7	LYS743, *PHE731, *PHE731, *TYR740, *TYR740, *TYR740, *TYR740,	Hydrophobic
		ARG562, THR590	Hydrogen
Vitamin E	-5,8	ARG562	Electrostatic
		CYS608, CYS608, ARG609, ARG609, LEU623, PHE591	Hydrophobic
<i><math>\gamma</math>-himachalene</i>	-5,5	ALA760, LYS763, ILE764, TYR754	Hydrophobic
<i>Ruboxistaurin</i>	-7,2	ASP732, ASP736, H-O	Hydrogen
		TYR740, TYR740, PHE731, TYR740	Hydrophobic



**Figure 4. a. Result 3D structure docking PLC with results of docking PKC- $\beta$  with *Methyl ricinoleate*. b. 2D structure of PLC docking results with PKC- $\beta$  docking results with *Methyl ricinoleate*.**



**Figure 5. a. Result 3D structure PLC docking with PKC- $\beta$  docking results with *Ruboxistaurin*. b. 2D structure of PLC docking results with PKC- $\beta$  docking results with *Ruboxistaurin*.**

*Binding affinity* is a measure of a compound's ability to bind to a receptor. The smaller the binding affinity value, the stronger and more stable the bond, and vice versa (Pantsar & Poso, 2018; Supriyanto et al., 2020).

This study showed that docking PKC- $\beta$  with 10 active compounds of BMG leaf extract and a comparison ligand (*Ruboxistaurin*) resulted in a binding affinity that varied from -5.8 kcal/mol to -9.1 kcal/mol. It is known that the strongest interaction is in the PKC- $\beta$ -*Ruboxistaurin* bond (comparison ligand) with a value of -9.1 kcal/mol, while the strongest bond of BMG leaf extract is -himachalene bond with a value of -8.1 kcal/mol. The weakest bonds were PKC- $\beta$ -*Pristanal* and PKC- $\beta$ -*Phytol* bonds with a value of -5.8 kcal/mol.

The results of protein to protein docking found that the highest binding affinity value was in the comparison ligand binding, namely PLC-(PKC- $\beta$ -*Ruboxistaurin*) with a value of -7.2 kcal/mol. Whereas the lowest value was found in the PLC-(PKC- $\beta$ -*Pristanal*) bond with a value of -3.5 kcal/mol. It is known that the 10 active compounds of BMG leaf extract that have been tested for protein to protein have less stable bonds compared to their comparison ligands (*Ruboxistaurin*) because it has a higher binding affinity value.

Hydrogen bonding is a bond to consider when searching for active sites, especially since hydrogen bonding is the most important contributor to the structural stability of a protein. This is due to the fact that proteins are made of NH and OH groups that can donate hydrogen bonds and other groups that will accept them. Thus, hydrogen bonding helps in the specificity of the protein-ligand interaction stabilizing the ligand in the binding pocket (Ami Fini Faqiha et al., 2022; Dhorajiwala et al., 2019).

The results of docking PKC- $\beta$  with 10 active compounds of BMG leaf extract and the comparison ligand (*Ruboxistaurin*) which has the most hydrogen bonds are owned by three bonds, namely the PKC- $\beta$ -*Palmitic acid* bond (at residues PHE485 and GLU390), PKC- $\beta$ - *Methyl ricinoleate* (at residues VAL423 and THR404), and PKC- $\beta$ -*Retinol Acetate* (at residues TYR422 at a distance of 2.85162 and 3.03661Å). In the results of protein-to-protein docking, the most hydrogen bonds were in the PLC-(PKC- $\beta$ -*Palmitic acid*) bond as many as 5 hydrogen bonds at residues ARG586 (3.17751Å and 3.04531Å), THR590, THR596, and SER588. The ligand-receptor bond will be more stable the more the number of hydrogen bonds, because hydrogen bonds affect the stability of the structure of a protein (Rena et al., 2022). The smaller the hydrogen bond distance, the stronger the bond will be and not easily separated (Pratama & Nashihah, 2021). The results of visual docking of PKC- $\beta$  bonds with 10 active compounds of BMG leaf extract seen the same amino acid residue interactions as the comparison ligand (*Ruboxistaurin*). The similarity of interactions with the comparison ligand allows for the same biological activity as the comparison ligand (Prasetiawati et al., 2021). The amino residue bonds that have the most similarities with the comparison ligand are found in the PKC- $\beta$ -*Palmitic acid* (at PHE485 and GLU390) residues, PKC- $\beta$ -*Methyl ricinoleate* (at the VAL423 and THR404) residues, and PKC- $\beta$ -*Retinol Acetate* (at residues VAL423 and THR404). at residue TYR422 at a distance of 2.85162 and 3.03661).

**Table 3.** Lipinski's Rule of Five test on the active compound of BMG leaf extract and the comparative ligand *Ruboxistaurin*

<i>Ligand</i>	MW (g/mol)	Log P	HBA	HBD	TPSA Å	nviolations
<i>Pristanal</i>	296.539	6.8911	1	0	12.53	1
<i>Caryophyllene</i>	204.357	4.7252	0	0	0	0
<i>Palmitic acid</i>	284.484	6.1884	1	1	37.30	1
<i>Humulene</i>	204.357	5.0354	0	0	0	1
<i>Methyl ricinoleate</i>	312.494	5.5673	2	1	46.53	1
<i>Phytol</i>	296.539	6.3641	1	1	20.23	1
<i>Retinol, acetate</i>	328.496	6.0811	2	0	26.30	1
<i>Squalene</i>	410.73	10.605	0	0	0	1
<b>Vitamin E</b>	430.717	8.84026	2	1	29.46	1
<i><math>\gamma</math>-himachalene</i>	204.357	4.7252	0	0	0	0
<i>Ruboxistaurin</i>	468.557	3.5137	6	1	72,27	0

MW : Molecular Weight (<500); HBA : Hydrogen Bond Acceptors (<10); HBD : Hydrogen Bond Donor (< 5); LogP : partition coefficient 5; TPSA : Topological Polar Surface Area; Nviolations : number of violations of lipinski's rules

**Table 4.** Predictive test of pharmacokinetic properties and toxicity of active compounds of BMG leaf extract and *Ruboxistaurin*

<b>ligand</b>	<b>HI A (%)</b>	<b>Ca co- 2 (10<sup>-6</sup> cm/ s)</b>	<b>VD ss (Lo g L/k g)</b>	<b>CYP2 D6 Inhib itor</b>	<b>Total Cleara nce</b>	<b>AME S Toxi city</b>	<b>Hep ato toxic ity</b>	<b>LD50 (mol/ kg)</b>	<b>LOA EL (log mg/k g _bw/d ay)</b>
<i>Pristanal</i>	91.8 08	1.2 26	0.4 06	No	1.631	No	No	1.40	0.991
<i>Caryophyl- lene</i>	94.8 45	1.4 23	0.6 52	No	1.088	No	No	1.61	1.416
<i>Palmitic acid</i>	91.4 62	1.5 72	- 0.5 78	No	1.678	No	No	1.42	3.313
<i>Humulen- e</i>	94.6 82	1.4 21	0.5 05	No	1.282	No	No	1.76	1.336
<i>Methyl- ricinoleat- e</i>	91.9 56	1.5 7	- 0.5 09	No	1.615	No	No	1.57	2.701
<i>Phytol</i>	90.7 1	1.5 15	0.4 68	No	1.686	No	No	1.60	1.043
<i>Retinol, acetate</i>	94.3 3	1.1 88	0.4 08	No	1.503	No	Yes	1.67	2.276
<i>Squalene</i>	90.3 41	1.2 16	0.4 11	No	1.791	No	No	1.84	0.946
<b>Vitamin E</b>	89.7 82	1.3 45	0.7 09	No	0.794	No	No	2.07	1.987
<i>γ- himachal- ene</i>	94.5 56	1.4 18	0.6 48	No	1.093	No	No	1.68	1.346
<i>Ruboxist- aurin</i>	94.2 91	0.9 18	0.5 84	No	0.719	Yes	Yes	2.381	1.576



#### 4.1 Pharmacokinetics

Human Intestinal Absorption (HIA) and permeability of Caco-2 cells were studied to predict drug absorption (Prasetiawati et al., 2021). The results of the pharmacokinetic prediction test showed that the 10 active compounds of BMG leaf extract and *Ruboxistaurin* tested had a percentage above 89%. The absorption is said to be good if the absorption value is >80%, while the absorption is bad if the absorption value is <30% (Maslikah et al., 2019). Caco-2 was used to predict the gastrointestinal permeability of drugs. Based on the nature of the human small intestine, this model expresses enterocytes, transporters, cytochrome P450 enzymes and microvilli. The active compounds of leaf extract BMG and *Ruboxistaurin* have high Caco-2 permeability because they have log Papp values above  $0,9^{-6}$  cm/s (Yeni & Rachmania, 2022; Zackria et al., 2022).

Volume of distribution (VDSS) is the volume of drug dose needed to be distributed homogeneously at a balanced level in blood plasma, the higher the volume of distribution, it means that more compounds are distributed compared to blood plasma in the body (Purwanto et al., 2021; Wahyuningsih et al., 2022). Based on pkCSM predictions, the Log VD value <-0.15 means that the volume of distribution of the compound is low, and is said to be high if >0.45 (Firdausy et al., 2020; Kirtishanti et al., 2020). **Table 3.** The prediction results show that the VDss ranges from -0.578 L/kg to 0.709 L/kg, the compound *Methyl ricinoleate* and *Retinol*, acetate has a low VDss value.

Cytochrome P450 (CYP450) is an important detoxifying enzyme in the body found in the liver and is responsible for many drugs. Cytochrome P450 inhibitors can affect drug metabolism, so it is necessary to evaluate whether a compound can affect CYP450 which in this study is represented by the isoform of cytochrome P2D6 (CYP2D6) (Ekowati et al., 2018; Sugiharto et al., 2021). The active compound of ramania leaf extract and the comparison ligand tested in this study did not have inhibitory power against CYP450, so that the pharmacokinetic predictions were enzymatic and not hepatotoxic (Sugiharto et al., 2021; Utami et al., 2022).

Excretion prediction value *total clearance* can be performed using CLTOT (a combination of hepatic clearance and renal clearance) (Krihariyani et al., 2021). Result of prediction *total clearance* of the active compound of ramania leaf extract and the comparison ligand ranged from 0.719 log mL/minute/kg to 1.791 mL/minute/kg where the comparison ligand had a value of *total clearance* the smallest, so that *Ruboxistaurin* as a comparison ligand is the slowest to be excreted from the body.

## 4.2 Toxicology

Determining the toxicity present in a compound is done by using the Ames toxicity test, this test has been widely used to measure the mutagenic potential of a compound using bacteria. The mutagenicity of a compound that can act as a carcinogen shows a positive result (Purwanto et al., 2021). **Table 4.** Shows the results of all tested BMG leaf extract compounds that are neither mutagenic nor carcinogenic, except for Ruboxistaurin (comparison ligand). It was also known in the hepatotoxicity test that all BMG leaf extract compounds were not hepatotoxic, except for *retinol acetate* and *Ruboxistaurin* compounds which were known to be hepatotoxic.

Prediction of toxicity for drug-like molecules is an important parameter for calculating the total tolerability before being administered to animal and human models. The compounds tested using pkCSM predictions showed acute and chronic lethal doses as Oral Rate Acute Toxicity (LD50) and Oral Rate Chronic Toxicity (LOAEL) (**Table 4.**) (Bharadwaj et al., 2022; Simoben et al., 2020). LD50 of compound *Methyl ricinoleate* worth 1.57 mol/kg which is smaller than the comparison ligand *Ruboxistaurin* with value 2,381 moles/kg. In contrast to the results of nLOAEL value, where compound *Methyl ricinoleate* higher by value 2,701 log mg/kg\_bb/day, compared to *Ruboxistaurin* (comparison ligand) which is worth 1,576 log mg/kg\_bb/day.

The results of the Molecular docking study showed that 10 active compounds of BMG extract were potential candidates for anti-inflammatory drugs. Methyl ricinoleate compound is a compound that shows the best affinity compared to 9 other compounds. Methyl ricinoleate compounds work in anti-inflammatory processes by inhibiting the activity of PKC- $\beta$ , thereby inhibiting the activation of the NF- $\kappa$ B pathway which results in reduced pro-inflammatory mediators such as Nitric Oxide (NO), inducible Nitric Oxide Synthase (iNOS), Prostaglandin E2 (PGE2), cyclooxygenase-2 (COX-2), and cytokines, such as Tumor necrosis factor alpha (TNF- $\alpha$ ) (Da-Silva et al., 2019; Martin-Perez et al., 2019, 2021).

## COMPETING INTERESTS

The author has no competing interests to declare.

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