Berkala **PENELITIAN HAYATI**

ISSN: 08526834 | E-ISSN:2337-389X
Volume 30 | No. 1 | **April** | 2024

Original Article

Virtual antivenom prediction of tamarind compounds as inhibitor against King Cobra (*Ophiophagus hannah)* phospholipase A_2

Najma Zahira[1], Nia Kurnianingsih[2], Nia Kurniawan[1*]

[1]Department of Biology, Faculty of Mathematics and Natural Science, University of Brawijaya, Malang, Indonesia
[2]Department of Physiology, Faculty of Medicine, University of Brawijaya, Malang, Indonesia

Abstract

Ophiophagus hannah has significant medical important among snakebite cases. The enzyme Phospholipase A_2 (PLA_2) is crucial for venom impacts in life-threatening symptoms. However, specific antivenom against this species remains underexplored. This study aimed to analyze the potential of secondary metabolites from tamarind as PLA_2 protein inhibitor specifically from *Ophiophagus hannah* venom using in-silico approaches. The sequences and three-dimensional structure of the PLA_2 protein from *O. hannah* were physicochemicaly predicted using ProtParam. Virtual screening, drug-likeness, pharmacokinetic, and toxicity profiles of metabolite compound from Tamarind was performed using SwissADME. All ligands were downloaded from PubChem database. Molecular docking was carried out using PyRx and visualized using Discovery Studio 2016 software. All tamarind's compounds are safe for long term administration because they displayed minimum risk for hepatotoxicity and AMES. The best interaction of secondary metabolites with PLA_2 is owned by quercetin. Future in-vitro and in-vivo studies are needed to evaluate the use of secondary metabolite compounds as specific alternatives of antivenoms.

Keywords: king cobra, molecular docking, PLA_2, secondary metabolite, tamarind
Received: March 19, 2023 Revised: April 30, 2024 Accepted: April 30, 2024

Introduction

Snakebite is a neglected tropical disease according to the World Health Organization (WHO) in 2009. Snakebite cases are increasing rapidly in Indonesia. This is in accordance with the tropical climate in Indonesia (Gutierrez et al., 2013). Reported snakebite cases increased by 54% from 2017-2019. However, this number does not represent authentic data as many snakebite cases go unreported from community health centers and other small regional hospitals (Adiwinata & Nelwan, 2015). Despite the increasing number of snakebite cases, there is still a lack of attention from the pharmaceutical industry, government, and concern for public health. Snakebite cases are still not included in global health priority programs (William et al.,2019).

Out of all the snakes, the most venomous species is the King Cobra (*Ophiophagus hannah*) of the Elapidae family. The distribution of *O. hannah* in Indonesia covers three islands namely Java, Sumatra and Kalimantan. This species is classified in the first category of medical importance. This indicates that this venomous snake is widespread and causes high morbidity, disability or mortality (Tan et al., 2019; IUCN, 2012). Based on the snake venom category, *O. hannah* venom proteins are enriched in enzymes including phospholipase A_2 (PLA_2). This enzyme is the most abundant enzyme found in the venom of *Ophiophagus hannah* from Indonesia and Malaysia (Danpaiboon et al., 2014). PLA_2 acts on presynaptic and postsynaptic neurons causing neurotoxicity and muscle weakness. The protease effect of PLA_2 can damage local tissues, accompanied by hemorrhage, necrosis, and risk of edema. The PLA_2 enzyme in snake venom can induce local myonecrosis and systemic myotoxicity. Severe neurotoxic paralysis and cases of death have been reported in *Ophiophagus hannah* snake venom bites (Gutierrez & Rucavado, 2000). Previous reports showed that inhibition of Crotalus durissus terrificus PLA_2 by quercetin reduced myotoxic effects and platelet aggregation by about 40% and 55% (Cotrim et al., 2011). PLA_2 inhibitor drugs such as varespladib are used as first-line treatment of choice to increase the survival time of snakebite victims as shown in an in vivo study, *Vipera berus* pecked mice treated with varespladib could survive for 24 hours after injection of a lethal dose of V. Berus (Sales et al., 2017).

Antivenom is proposed as an effective treatment for snake bites. The development of antivenom is unique compared to other drugs as it is restricted based on the species and geographical distribution of the snake, which venom is used as an immunogen in the production of antivenom (Tan et al., 2016). Currently, there is only one type of polyvalent antivenom for the Java Cobra (*Naja sputatrix*), Welang Snake (*Bungarus fasciatus*), and Malayan Pit Viper (*Calloselasma rhodostoma*) commercially produced in Indonesia. This raises the limitation of the current polyvalent antivenom which may not be effective against other species (Gomez-Betancur et al., 2019). Multiple side effects of anti-snake venom serum, such as increased blood pressure, rash, and body pain should be considered (Liagat et al., 2022).

Medicinal plants are growing along with the trend of research based on their potential as cures for diseases. Lower costs and fewer side effects are becoming superior to existing synthetic drugs (Liagat et al., 2022). Plant extracts rich in pharmacologically active

*Corresponding Author:
Nia Kurniawan
Department of Biology, Faculty of Mathematics and Natural Science, University of Brawijaya, Jl. Veteran 10-100, 65145, Malang, Indonesia
Phone: +62 813-3666-0007 Fax: -
E-mail: wawan@ub.ac.id

http://dx.doi.org/10.23869/bphjbr.30.1.20244
Published by © PBI East Java. Open Access www.berkalahayati.org

compounds that can interact with venom will have the potential to inhibit venom activation (Ushanandini et al., 2006). Herbal antivenoms can be prepared by making ethanolic, methanolic, or aqueous extracts of herbal plants. Tamarind (*Tamarindus indica* L.) is a versatile tree that almost every part of it has some advantages. Antioxidant, anti-inflammatory, antimicrobial, and potential antivenom activities have been explored from several parts of this plant in previous studies (Caluwe et al., 2010). Tamarind has several secondary metabolites that are classified into phenolic compounds, flavonoids, benzophenones, gallates, and galotanins. The compound content in tamarind seeds includes more than 50 phytochemicals that have different benefits. Compounds that are quite widely used in research are quercetin, catechin, epicatechin, procyanidin B2, β-amyrin, and so on (Sudjaroen et al., 2005). Examples of biochemical activity of β-amyrin compounds are able to be anti-inflammatory, analgesic, antidiabetic to antivenom. Research related to antivenom from secondary metabolites has been conducted in vivo against PLA_2 from *Daboia rusellii*. The results obtained showed a bond with amino acid residues (Kumar et al., 2015).

New drug discovery strategies are now being developed through Computer Aided Drug Design (CADD) systems that utilize computational resources, algorithms, and 3D visualization to assist in the early stages of drug discovery, namely target identification and target selection. Drug discovery is the process of identifying a compound that has potential therapeutic properties in the treatment of disease (Sliwoski et al., 2013). The early stages of drug discovery involve identification of candidate compounds, synthesis of the compound, characterization of pharmacokinetic and pharmacological properties, validation of the mechanism of action, optimization of the chemical structure of the compound, and various screening and testing related to the efficacy of the candidate drug compound (Deore et al., 2019). Subsequently, candidate compounds that show potential as new drug candidates will be further explored in preclinical and clinical studies (Deore et al., 2019; Sivaramakrishnan et al., 2016).

Based on the description above, PLA_2 inhibitor candidates using secondary metabolites are important to do as an initial step in drug development with a bioinformatics approach. While the therapeutic potential of tamarind metabolites and the toxic effects of King Cobra venom PLA_2 are well-documented individually, their interaction remains relatively unexplored. Understanding the interplay between tamarind secondary metabolites and PLA_2 King Cobra protein could unveil novel therapeutic strategies or aid in mitigating venom-induced toxicity.

Methods

Receptor preparation

The 3D protein structure of PLA_2 from *Ophiophagus hannah* was downloaded in PDB format from the Protein Data Bank database (www.rcsb.org) with PDB ID: 1GP7 containing Ca^{2+} as native ligand. The preparation was performed using Discovery Studio 2016 to remove water molecules and ligands.

Prediction of receptor physicochemical properties

PLA_2 sequences were submitted to the ProtParam tool on the ExPASy web server (https://web.expasy.org/protparam/) to predict several parameters, including molecular weight, isoelectric point, hydrophilicity, stability, and half-life.

Ligand preparation of tamarind secondary metabolites

Virtual screening was performed on compounds contained in tamarind (*T. indica* L) using SwissADME (http://www.swissadme.ch/) to predict ADME characteristics and identify compounds with potential as snake venom inhibitors based on drug similarity properties. The aim of this analysis is to estimate the pharmacokinetic profile associated with new drug candidates (Bitencourt-Ferreira et al., 2019; Patil et al., 2010). The 3D structures of the selected secondary metabolite compounds consisted of apigenin (CID: 5280443), β-amyrin (CID: 73145), catechin (CID: 9064), epicatechin (CID: 72276), eriodictyol (CID: 440735), luteolin (CID: 5280445), naringenin (CID: 932), orientin (CID: 5281675), procyanidin B2 (CID: 122738), and quercetin (CID: 5280343) in sdf format. The compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as a control, 3bI (CID: 135457934), belongs to the δ2-isoxazolines derivatives. This compound has been tested in vitro as a PLA_2 inhibitor in the snake genus *Daboia* (Sivaramakrishnan et al., 2016).23 Three amino acid residues (His48, Tyr73, and Asp99) are conserved in terms of their structure among snake venom phospholipases A_2 ($svPLA_2$s) (Holland et al., 1990), including *Daboia* (Kholilah et al., 2021) and *O. hannah* (Kini, 2003; Zhang et al., 2002). δ2-isoxazoline derivatives are ideal hydrophobic active side binders for enzymes with butyl substituents. From in vivo studies, only 3bI and 3bIV had strong inhibitory effects against $SvPLA_2$. 3bI showed the most excellent binding properties compared to other simulated derivatives as well as experimental results (Sivaramakrishnan et al., 2016; Kholilah et al., 2021). Then, all compound structures were energy minimized using PyRx 0.8 software.

Docking and visualization

Docking simulations were performed on the prepared PLA_2 receptors (without native ligands) with 10 tamarind (*T. indica* L.) secondary metabolites and controls using PyRx 0.8 software. Docking simulations were performed with a grid of X: 35.0012 Å, Y: 49.5501 Å, and Z: 44.4462 Å equipped with 3bI and applied to other ligands both with and without the presence of Ca^{2+} because the reaction of PLA_2 is Ca^{2+} dependent (Sivaramakrishnan et al., 2016). All ligands were paired with PLA_2 protein in turn and their binding affinity was observed (Holland et al., 1990). The docking results were visualized using Discovery Studio 2016 software to observe the type of binding and ligand-receptor

interaction. Analysis was done by observing the significance of the energy changes that occurred.

Results

Physicochemical properties PLA_2

The PLA_2 protein sequence is composed of 124 amino acids with H (His) as the N-terminal point. Based on ProtParam analysis, the predicted physicochemical characteristics of PLA_2 show a low molecular weight (13733.42 Da), an isoelectric point (pI) of 5.40, and hydrophilic properties due to low Grand Average of Hydropathicity (GRAVY) values (Table 1).

Table 1. Physicochemical properties of PLA_2 based on ProtParam analysis

Parameter	Skor
Amino acid	124
Molecular weight	13733.42
Isoelectric point	5.40
Positively charged residue	12
Negatively charged residue	9
Formula	$C_{587}H_{886}N_{162}O_{188}S_{16}$
Number of atom	1839
Extinction coefficient	19785 18910
Half life	3.5 hours (mammalian reticulocyte, in vitro) 10 minutes (ragi, in vivo) > 10 hours (*E. coli,* in vivo)
Instability index	30.54
Aliphatic index	57.50
GRAVY	-0.256

Table 2. Interaction of PLA_2 with secondary metabolites of tamarind and 3bI

Receptor	Compound (ligand)	Binding affinity (PLA_2) (kcal/mol)	Binding affinity (PLA_2 + Ca^{2+}) (kcal/mol)	Hydrogen bond (Å)		Hydrophobic bond (Å)	
PLA_2 (PDB ID: 1GP7)	Ca^{2+} (Cofactor)	-7,3					
	3bI (control)	-6,6	-6,7	**His48** Gly30	3.148 2.814	Trp19 Leu2 Ala23 Phe5	3.778 5.234 4.012 4.913
	Apigenin	-8,1	-8,1			Leu2 Leu2 Ala23	3.902 5.163 4.649
	β-amyrin	-6,9	-7,9	Ala31	2.536	Lys71 Ile64	5.136 5.321
	Catechin	-8	-8	Tyr22	2.522	Trp19 Phe5 Leu2	4.231 5.003 5.102
	Epicatechin	-8,2	-8,2	Leu2 Tyr28 Gly30	1.748 2.152 2.473	Trp19 **His48** Cys45 Leu2	5.387 4.722 4.735 4.134
	Eriodictyol	-8,3	-8,3	**Asp49** Gly30	2.924 3.029	Trp19 **His48** Cys45 Leu2	5.479 4.674 4.804 4.002
	Luteolin	-8,1	-8,4	Gly30	2.962	Tyr69 Phe5 Leu2 Ala23	5.394 4.877 5.189 4.611
	Naringenin	-8,1	-8,1	Leu2 Gly30	2.994 2.898	Trp19 **His48** Cys45 Leu2	5.500 4.712 4.849 3.979
	Orientin	-8,6	-8,3	Asp89 Thr12 Gly26 Cys29 Ser34 Ser62 Ala60 Thr12	2.386 2.469 2.535 1.661 3.046 2.827 3.410 3.512	Thr120 Ile64	3.492 3.696
	Procyanidin B2	-8,4	-8,6	**Asp49** Gly30	2.516 3.278	Asp24 Trp19 Ala23 Asp24 Leu2 Ala23 Cys29 Cys45 Ala23	3.916 5.052 4.678 4.962 4.507 5.258 4.999 4.861
	Quercetin	-7,9	-8	Leu2 Tyr22 Tyr28 Gly30 Ala23	2.332 2.852 1.995 3.276 3.256	Trp19 Phe5 **His48** Leu2	5.447 5.955 5.529 4.181

Docking analysis

Tamarind secondary metabolites formed different types of bonds with PLA_2 receptors such as hydrogen, electrostatic, hydrophobic, and unfavorable donor-donor bonds. Each bond had its own conformational complex characteristics. Table 2 showed the binding affinity of the control ligand (3bI) with PLA_2 protein was -6.6 and -6.7 kcal/mol with and without Ca^{2+}. The more negative binding affinities indicated the stability of the 3bI-PLA_2 interaction either with or without Ca^{2+} as a cofactor. Similar interactions were formed for several tamarind metabolite compounds namely β-amyrin, luteolin, procyanidin B2, and quercetin. The interaction between β-amyrin as ligand showed the highest binding affinity change from -6.9 kcal/mol to -7.9 kcal/mol in the presence of Ca^{2+}. Stable binding affinities were shown by the compounds apigenin, catechin, epicatechin, eriodictyol, and naringenin, which were -8.1; -8; -8.2; -8.3; and -8.1 kcal/mol. In contrast, the presence of Ca^{2+} increased the positivity of the binding affinity which was assumed to be a reduction in conformational stability.

3bI as a control (δ2-isoxazolines derivative) formed a hydrophobic interaction at Gly53 residue responsible for the anti-coagulant response. This compound bound to the active site of PLA_2 receptor at Asp49 residue. Interaction with His48 through hydrophobic bonding was exhibited by the metabolites epicatechin, eriodictyol, naringenin, and quercetin. Meanwhile, the interaction with Asp49 through hydrogen bonding was shown by the metabolites eriodictyol and procyanidin B2 (Figure 1).

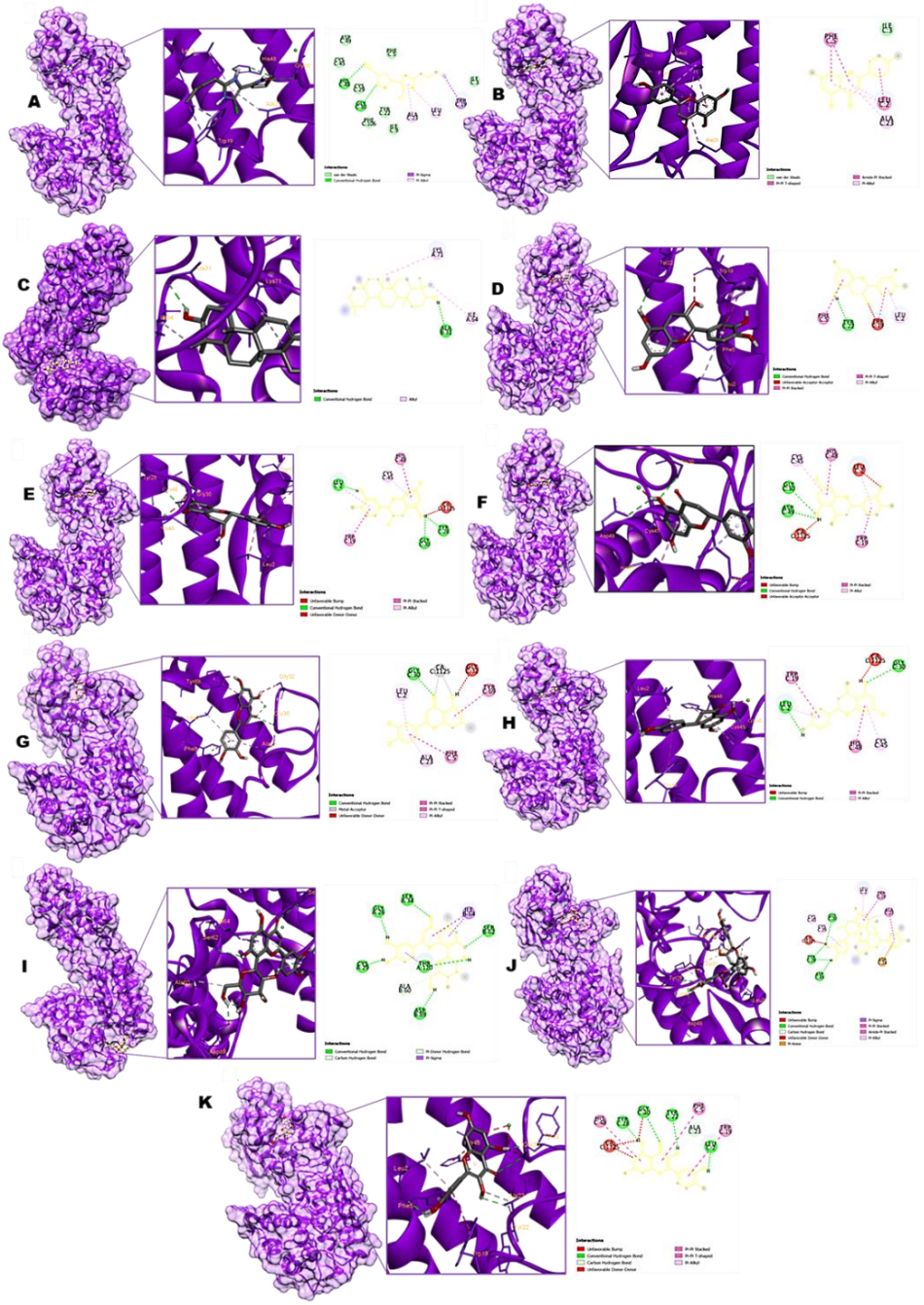

Figure 1. Docking of PLA_2 with secondary metabolites and control. Description: (A) 3bI (control); (B) Apigenin; (C) β-amyrin; (D) Catechin; (E) Epicatechin; (F) Eriodictyol; (G) Luteolin; (H) Naringenin; (I) Orientin; (J) Procyanidin B2; (K) Quercetin

Pharmacokinetic and toxicological analysis of secondary metabolites

Lipinski's rule of five rules was fulfilled by eight secondary metabolites of tamarind except procyanidin B2 and orientin. Thus, these compounds had potential as drugs compared to 3bI as a control (Table 3). All metabolites had moderate to good solubility range in water, except β-amyrin. All candidates were well absorbed in the gastrointestinal tract, except β-amyrin, orientin, and procyanidin B2. Distribution to the blood-brain barrier of all compounds was not available except 3bI. Based on toxicity tests, all secondary metabolites used were found not to be toxic except 3bI as a control. However, according to the hepatoxicity test, all secondary metabolites including 3bI as a control were not categorized as hepatoxic (Table 4).

Table 3. ADME and drug similarity profiles of tamarind (*T. indica* L.) secondary metabolites and 3bI

Compound	MW (g/ mol)	Lipophilicity (Log P)	Water solubility	Absorbtion (GI Abs)	Distribution (Blood-Brain Barrier)	Metabolism	Bioavailabliity	Drug-likeness Lipinski's rule of 5 (violation)
Apigenin	270.24	2.58	Moderatelysoluble	High	No	• CYP1A2 inhibitor • CYP2D6 inhibitor • CYP3A4 inhibitor	0.55	Yes (0)
β-amyrin	426.73	8.17	Poorly soluble	Low	No	-	0.55	Yes (1)
Catechin	290.27	1.55	Soluble	High	No	-	0.55	Yes (0)
Epicatec-hin	290.27	1.55	Soluble	High	No	-	0.55	Yes (0)
Eriodictyol	288.25	2.22	Soluble	High	No	• CYP3A4 inhibitor	0.55	Yes (0)
Luteolin	286.24	2.28	Moderately soluble	High	No	• CYP1A2 inhibitor • CYP2D6 inhibitor • CYP3A4 inhibitor	0.55	Yes (0)
Naringenin	272.25	2.52	Soluble	High	No	• CYP1A2 inhibitor • CYP3A4 inhibitor	0.55	Yes (0)
Orientin	448.38	-0.53	Soluble	Low	No	-	0.17	No (2)
Procya-nidin B2	578.53	2.35	Moderately soluble	Low	No	• CYP3A4 inhibitor	0.17	No (3)
Quercetin	302.24	1.99	Soluble	High	No	• CYP1A2 inhibitor • CYP2D6 inhibitor • CYP3A4 inhibitor	0.55	Yes (0)
3bI	252.70	2.04	Soluble	High	Yes	• CYP1A2 inhibitor • CYP2C19 inhibitor	0.55	Yes (0)

Table 4. Toxicity profile of tamarind (*T. indica* L.) secondary metabolites and 3bI

Ligand	AMES toxicity	Maximum dosage (manusia) (log mg/kg/day)	Hepatotoxicity	Oral Rat Acute Toxicity (LD50) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_body weight/day)
Apigenin	**No**	0.328	**No**	2.45	2.298
β-amyrin	**No**	-0.56	**No**	2.478	0.873
Catechin	**No**	0.438	**No**	2.428	2.5
Epicatechin	**No**	0.438	**No**	2.428	2.5
Eriodictyol	**No**	0.014	**No**	2.03	2.475
Luteolin	**No**	0.499	**No**	2.455	2.409
Naringenin	**No**	-0.176	**No**	1.791	1.944
Orientin	**No**	0.567	**No**	2.572	4.386
Procyanidin B2	**No**	0.438	**No**	2.482	4.349
Quercetin	**No**	0.499	**No**	2.471	2.612
3bI	Yes	-0.105	**No**	3.021	0.843

Discussion

Physiochemical profile of phospholipase A_2

Phospholipase A_2 functions to catalyze fatty acids on membrane glycerophospholipids. The catalysis process can induce hemotoxicity, neurotoxicity, cytotoxicity, nephrotoxicity, hemorrhage, and edema. Previous studies found that neutralization of PLA_2 envenomation can be achieved by inhibiting PLA_2 activity. These days, a number of well-studied inhibitors for PLA_2 are derived from antibodies and plant compounds of both synthetic and natural origin (Sudjaroen et al., 2005; Kumar et al., 2015; Sliwoski et al., 2013).

Molecular docking

Ca^{2+} ions act as cofactors to catalyze the inflammatory reaction caused by PLA_2. The binding of PLA_2 with Ca^{2+} causes bleeding (hemorrhagic) conditions through the release of lysophospholipids and fatty acids (Mahmud et al., 2020; Sakthivel et al., 2013). Secondary metabolite interactions can also bind to the active sites His48 and Asp49, which stimulate inflammatory reactions in *O. hannah* snake venom (Kholilah et al., 2021; Mahmud et al., 2020). The amino acids His48 and Asp49 play important roles in the enzymatic activity of $sPLA_2$. His48 is involved in the initial step of the enzymatic mechanism, while Asp49 is essential for binding the Ca^{2+} cofactor (Verheij et al., 1980). This indicates that some of the secondary metabolites of tamarind have the potential as enzymatic inhibitors that inhibit the enzymatic reaction resulting in chronic inflammation in snakebite victims. In addition, the development and administration of drugs targeting different venom families are mandatory to propose a comprehensive treatment to preserve the life of snakebite victims (Sales et al., 2017).

Based on the interaction on active site residues, binding affinity, and drug similarity, procyanidin B2 metabolite compounds are not recommended for the oral drug route. The best interaction of secondary metabolites with PLA_2 along with Ca^{2+} was shown by quercetin (Figure 2) in accordance with previous studies (Cotrim et al., 2011).

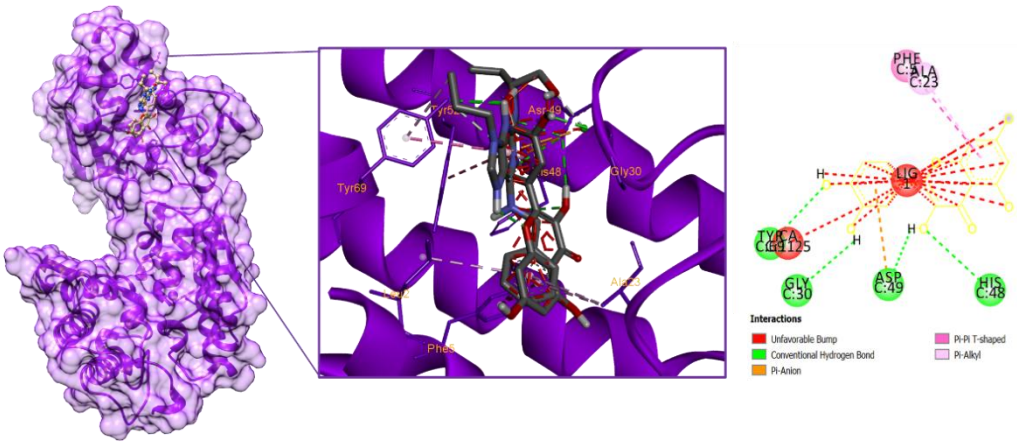

Figure 2. Docking PLA_2+Ca^{2+} with quercetin

Pharmacokinetic and toxicological analysis of secondary metabolites

According to Lipinski's rule of five, a drug compound should have a molecular mass <500 g/mol, hydrogen bond donor <5, octane-water partition coefficient (log P) <5, and hydrogen bond acceptor <10 (Lipinski et al., 2012). These characteristics indicate a chemical compound that acts as an orally active compound on the desired target (Doak et al., 2014).

Tamarind metabolite compounds were characterized in terms of lipophilicity pharmacokinetic profile, water solubility, and absorption rate in the gastrointestinal tract. The β-amyrin compound had the highest log P value and was therefore considered likely to diffuse across the lipid bilayer component, resulting in widespread distribution throughout the body. Conversely, negative log P values indicate a lower ability to diffuse across lipid bilayer membranes (Lipinski et al., 2012; Doak et al., 2014).

All metabolites have a moderate to good solubility range in water, except β-amyrin. All candidates are well absorbed in the gastrointestinal tract, except β-amyrin, orientin, and procyanidin B2. Distribution to the blood brain barrier of all compounds was not available except 3bI. Several secondary metabolites of tamarind are suspected to act as inhibitors of cytochrome P450 enzymes, such as apigenin, eriodictyol, luteolin, naringenin, procyanidin B2, and quercetin - including 3bI as a control (Tsantili-Kakoulidou & Demopoulos, 2021). Inhibition of cytochrome P450 enzymes reduces drug metabolism in liver tissue thereby increasing the duration of metabolites in the body thereby increasing potential toxicity. Regarding the toxicity profile, all tamarind compounds are safe for long-term administration as they show minimum risk for hepatotoxicity and AMES. The AMES test indicates the potential of a compound to induce mutagenesis or be labeled as carcinogenic (Han et al., 2019). Drug-likeness assessment and pharmacokinetic evaluation will shed light on the feasibility of utilizing these compounds as potential therapeutics. Furthermore, toxicity prediction analyses will help in assessing the safety profiles of the

compounds for human consumption or therapeutic applications.

Conclusion

The secondary metabolites of tamarind used and 3bI showed potential to be inhibitory drugs as they were less toxic and fulfilled Lipinski's rule of five, except procyanidin B2 and orientin. The best interaction of secondary metabolites with PLA_2 along with Ca^{2+} as cofactor is owned by quercetin. This study needs to be conducted further in-vivo and in-vitro to confirm the antivenom potential of tamarind secondary metabolites by inhibiting the PLA_2 enzymatic reaction.

Acknowledgement

Author acknowledges and appreciates the NK Research University of Brawijaya, as well as all members of Laboratorium Diversitas Fauna for their assistance during this research.

Disclosure of interest

The authors report no conflict of interest.

References

Adiwinata R., & Nelwan EJ. (2015). Snakebite in Indonesia. *Acta Medica Indonesia, 47*(4), 358-365.

Bitencourt-Ferreira G., Veit-Acosta M., and de Azevedo WF Jr. (2019). Hydrogen Bonds in Protein-Ligand Complexes. *Methods Mol Biol 2053*, 93-10.

Caluwe ED., Katerina H., & Patrick VD. (2010). *Tamarindus indica* L. – A review of traditional uses, phytochemistry and pharmacology. *Afrika Focus, 23*(1), 53-83.

Cotrim, C. A., de Oliveira, S. C., Diz Filho, E. B., Fonseca, F. V., Baldissera, L., Jr, Antunes, E., Ximenes, R. M., Monteiro, H. S., Rabello, M. M., Hernandes, M. Z., de Oliveira Toyama, D., & Toyama, M. H. (2011). Quercetin as an inhibitor of snake venom secretory phospholipase A2. *Chemico-biological interactions*, *189*(1-2), 9–16. https://doi.org/10.1016/j.cbi.2010.10.016

Danpaiboon, W., Reamtong, O., Sookrung, N., Seesuay, W., Sakolvaree, Y., Thanongsaksrikul, J., Dong-din-on, F., Srimanote, P., Thueng-in, K., & Chaicumpa, W. (2014). Ophiophagus hannah venom: proteome, components bound by Naja kaouthia antivenin and neutralization by N. kaouthia neurotoxin-specific human ScFv. *Toxins*, *6*(5), 1526–1558. https://doi.org/10.3390/toxins6051526

Deore A., Dhumane J., Wagh R., & Sonawane R. (2019). The Stages of Drug Discovery and Development Process. *Asian Journal of Pharmaceutical Research and Development, 7*(6), 62-67.

Doak BC., Over B., Giordanetto F., & Kihlberg J. (2014). Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates.*Chemistry & Biology, 21*(9), 1115-42.

Gómez-Betancur I., Gogineni V., Salazar-Ospina A., & León F., (2019). Perspective on the Therapeutics of Anti-Snake Venom. *Molecules, 24*(18), 3276.

Gutiérrez JM., & Rucavado A. (2000). Snake venom metalloproteinases: their role in the pathogenesis of local tissue damage. *Biochimie, 82*(9-10), 841-50.

Gutierréz JM., Warrel DA., Williams DJ., Jensen S., Brown N., Calvete JJ., &Harrison RA. (2013). The Need for Full Integration of Snakebite Envenoming within a Global Strategy to Combat The Neglected Tropical Diseases: The Way Forward. *PLoS Neglected Tropical Disease, 7*(6), e2162.

Han Y., Zhang J., Hu CQ., Zhang X., Ma B., & Zhang P. (2019). In silico ADME and Toxicity Prediction of Ceftazidime and Its Impurities. *Front Pharmacol, 24*(10), 434.

Holland, D. R., Clancy, L. L., Muchmore, S. W., Ryde, T. J., Einspahr, H. M., Finzel, B. C., Heinrikson, R. L., & Watenpaugh, K. D. (1990). The crystal structure of a lysine 49 phospholipase A2 from the venom of the cottonmouth snake at 2.0-A resolution. *The Journal of biological chemistry*, *265*(29), 17649–17656. https://doi.org/10.2210/pdb1ppa/pdb

IUCN. 2012. King cobra (*Ophiophagushannah*). www.iucnredlist.org. Accessed: September 2020.

KholilahT., Widodo N., & Nia K. (2021). In silico Study Reveals Potential Docking Sites of δ 2-isoxazolines derivates for Inhibiting Russell's Viper PLA_2 Toxin. *J.Trop.Life.Science, 11*, 45–51.

Kini RM. (2003). Excitement ahead: structure, function and mechanism of snake venom phospholipase A2 enzymes. *Toxicon, 42*(8), 827-40.

Kumar JR., Basavarajappa BS., Vishwanath BS., & Gowda TV. (2015). Biochemical and pharmacological characterization of three toxic phospholipase A_2s from *Daboia russelii* snake venom. Comparative Biochemistry and Physiology Part C. *Toxicology Pharmacology, 168*, 28-38.

Liaqat A., Tauqeer HM., Yusra HK., Aisha K., Sadia C., & Muhammad A. (2022). Anti-Snake Venom Properties of Medicinal Plants: A Comprehensive Systematic Review of Literature. *Brazilian Journal of Pharmaceutical Sciences, 58*, e191124.

Lipinski CA., Lombardo F., Dominy BW., &Feeney PJ. (2012). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews, 64*, 4–1.

Mahmud S., Parves MR., Riza YM., Sujon KM., Ray S., Tithi FA., Zaoti ZF., Alam S., & Absar N. (2020). Exploring the potent inhibitors and binding modes of phospholipase A_2 through in silico investigation. *Journal of Biomolecular Structure and Dynamics, 38*(14), 4221-4231.

Sakthivel G., Dey A., Nongalleima K., Murthy C., Rimal I., Surjit S., & Lokesh D. (2013). In Vitro and In Vivo Evaluation of Polyherbal Formulation against Russell's Viper and Cobra Venom and Screening of Bioactive Components by Docking Studies. *Evidence-Based Complementary and Alternative Medicine, 2013*, 1-12.

Sales TA., Marcussi S., da Cunha EFF., Kuca K., & Ramalho TC. (2017). Can Inhibitors of Snake Venom Phospholipases A_2 Lead to New Insights into Anti-Inflammatory Therapy in Humans? A Theoretical Study. *Toxins, (Basel) 9*(11), 341.

Sivaramakrishnan, V., Ilamathi, M., Ghosh, K. S., Sathish, S., Gowda, T. V., Vishwanath, B. S., Rangappa, K. S., & Dhananjaya, B. L. (2016). Virtual analysis of structurally diverse synthetic analogs as inhibitors of snake venom secretory phospholipase A2. *Journal of molecular recognition : JMR*, *29*(1), 22–32. https://doi.org/10.1002/jmr.2492

Sliwoski G., Kothiwale S., Meiler J., & Lowe EW Jr. (2013). Computational methods in drug discovery. *Pharmacologycal Review, 66*(1), 334-95.

Sudjaroen, Y., Haubner, R., Würtele, G., Hull, W. E., Erben, G., Spiegelhalder, B., Changbumrung, S., Bartsch, H., & Owen, R. W. (2005). Isolation and structure elucidation of phenolic antioxidants from Tamarind (Tamarindus indica L.) seeds and pericarp. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *43*(11), 1673–1682. https://doi.org/10.1016/j.fct.2005.05.013

Tan CH., Jia LL., Kae YT., & Nget HT. (2016). Assessing SABU (Serum Anti Bisa Ular), the sole Indonesian antivenom: A proteomic analysis and neutralization efficacy study. *Scientific Reports 6*, 37299.

Tan, K. Y., Ng, T. S., Bourges, A., Ismail, A. K., Maharani, T., Khomvilai, S., Sitprija, V., Tan, N. H., & Tan, C. H. (2020). Geographical variations in king cobra (*Ophiophagus hannah*) venom from Thailand, Malaysia, Indonesia and China: On venom lethality, antivenom immunoreactivity and in vivo neutralization. *Acta tropica*, *203*, 105311. https://doi.org/10.1016/j.actatropica.2019.105311

Tsantili-Kakoulidou A., & Demopoulos VJ. (2021). Drug-like Properties and Fraction Lipophilicity Index as a combined metric. *ADMET DMPK 9*(3),177-190.

Ushanandini, S., Nagaraju, S., Harish Kumar, K., Vedavathi, M., Machiah, D. K., Kemparaju, K., Vishwanath, B. S., Gowda, T. V., & Girish, K. S. (2006). The anti-snake venom properties of Tamarindus indica (leguminosae) seed extract. *Phytotherapy research : PTR*, *20*(10), 851–858. https://doi.org/10.1002/ptr.1951

Verheij HM., Volwerk JJ., Jansen EHJM., Puyk WC., Dijkstra BW., Drenth J., & De Haas GH. (1980). Methylation of histidine-48 in pancreatic phospholipase A_2. Role of histidine and calcium ion in the catalytic mechanism. *Biochemistry, 19*, 743–750.

Williams, D. J., Faiz, M. A., Abela-Ridder, B., Ainsworth, S., Bulfone, T. C., Nickerson, A. D., Habib, A. G., Junghanss, T., Fan, H. W., Turner, M., Harrison, R. A., & Warrell, D. A. (2019). Strategy for a globally coordinated response to a priority neglected tropical disease: Snakebite envenoming. *PLoS neglected tropical diseases*, *13*(2), e0007059. https://doi.org/10.1371/journal.pntd.0007059

Zhang HL., Xu SJ., Wang QY., Song SY., Shu YY., & Lin ZJ. (2002). Structure of a cardiotoxic phospholipase A(2) from *Ophiophagushannah* with the "pancreatic loop". *J StructBiol 138*(3), 207-15.