Pharmacogn J. 2023; 15(1): 106-111
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Alkaloids from *Pandanus amaryllifolius* Roxb Leaf as Promising Candidates for Antidyslipidemic Agents: An *in silico* study

Martohap Parotua Lumbanraja, Kusnandar Anggadiredja*, Hubbi Nashrullah Muhammad, Neng Fisheri Kurniati

ABSTRACT

Introduction: The plant *Pandanus amaryllifolius* Roxb (pandan), has been shown to have antidyslipidemic potency. This study explored the potential of several alkaloids from pandan leaf as antidyslipidemia as well as their safety profile *in silico*. **Methods**: Analyses were carried out by studying the binding affinity of the alkaloids to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, peroxisome proliferator activator receptor (PPAR) alpha and Niemann Pick C1 Like 1 (NPC1L1). The structures of the alkaloids were downloaded from the Pubchem database and optimized using the ChemDraw Professional 16.0 to obtain 3D structures in protein data bank (PDB) format. The *in silico* testing was based on the interactions of the alkaloids with the HMG-CoA reductase (PDB ID 1HW9), PPAR alpha (PDB ID 6LX4) and NPC1L1 (PDB ID 7DFZ) proteins, downloaded from the Research Collaboratory for Structural Bioinformatics (RSCB) PDB website (http://www.rcsb.org/pdb). The preparation of protein structures was performed using the Discovery studio 2021 client and Gromacs applications, while optimization of the 3D structure of the alkaloids was carried out with the ChemDraw professional 16.0. Finally, validation was completed using AutoDock application. The safety profile was assessed by pkCSM online tool. **Results**: The respective root mean square deviation (RMSD) values of the 1HW9, 6LX4 and 7DFZ proteins were 1.677, 0.918 and 1.706, respectively. The alkaloids pandanusine B, pandamarilactonine A, pandamarilactonine B had respective values of binding energy for HMG-CoA of -5.52, -5.51 and -5.46 kcal/mol. The binding energy of pandamarilactonine B, pandamarilactonine A and pandanamine for PPAR alpha were -9.14, -9.10 and -8.48 kcal/mol, respectively, with the corresponding energy for t NPC1L1 of -9.63, -9.71 and -8.54 kcal/mol. The toxicity tests indicated that the alkaloids were safe, pandamarilactonines had the highest LD_{50} (2.736 mol/kg). **Conclusion**: The studied pandan alkaloids have potential antidyslipidemic activity by interacting with HMG-CoA reductase, PPAR alpha, and NPC1L1, with good safety profile.

Key words: *Pandanus amaryllifolius*, Pandan, Alkaloids, Dyslipidemia, *In Silico*.

Martohap Parotua Lumbanraja, Kusnandar Anggadiredja*, Hubbi Nashrullah Muhammad, Neng Fisheri Kurniati

Department of Pharmacology and Clinical Pharmacy, School of Pharmacy Institut Teknologi Bandung, Jl. Ganesa 10 Bandung 40132, INDONESIA.

Correspondence

Kusnandar Anggadiredja

Department of Pharmacology and Clinical Pharmacy, School of Pharmacy Institut Teknologi Bandung, Jl. Ganesa 10 Bandung 40132, INDONESIA.

E-mail: kunay.kusnandar@gmail.com

History

- Submission Date: 30-11-2022;
- Review completed: 16-01-2023;
- Accepted Date: 24-01-2023.

DOI : 10.5530/pj.2023.15.14

Article Available online

http://www.phcogj.com/v15/i6

Copyright

© 2023 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Dyslipidemia is one of leading factors of ischemic heart disease, which claims more than 4 million deaths yearly.[1] Treatment of dyslipidemia using synthetic drugs has limitations, especially due to the side effects, which include those of muscular, metabolic, and neurological.[2] Herbal medicine has long been used as an alternative for the prevention and treatment of diseases.[3] *Pandanus amaryllifolius* Roxb, pandan for short, is a plant that is distributed throughout Africa and the tropical areas of Pacific regions. The plant is widely used especially for their aroma and natural green coloring.[4]

The results of previous studies have suggested that pandan extracts were potential to be utilized in the management of dyslipidemia. Thoi *et al.*[5] demonstrated that the aqueous leaf extract of pandan decreased total cholesterol and triglyceride levels in mice induced by Triton WR-1339. In addition, the water extract of pandan was shown to ameliorate metabolic syndrome, including improvement of triglyceride and LDL levels, in a rat model induced by fructose administration.[6]

Several secondary metabolites expected to have roles in lipid metabolism have been isolated from pandan. Studies by Takayama *et al.*[7,8] have revealed the alkaloids pandamarilactonine A and pandamarilactonin B. It turned out that the two were derived from yet another alkaloid known as pandanamine.[9] Recently, the family of alkaloids pandanusines have also been unveiled.[10] These alkaloids have not been subject to thorough studies for their potential activities in lipid metabolism. Upon this consideration, therefore, the current study was carried out to probe this potency by using *in silico* method. The study was focused on the possible interaction of the alkaloids with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, Peroxisome Proliferator Activator Receptor (PPAR) alpha, and the Niemann Pick C1 Like 1 receptor (NPC1L1). They are three endogenous macromolecules which are essential for the transport and metabolism of lipid that are also the target of the currently used antidyslipidemic drugs. We also explore the safety profile of the alkaloids.

MATERIALS AND METHODS

Macromolecule and ligand interaction

The enzyme and receptor proteins were downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank with respective PDB ID of 1HW9, 6LX4, and 7DFZ for HMG-CoA reductase, PPAR alpha, and NPC1L1. The protein structures for visualization were prepared with the application Discovery Studio 2021 Client, while optimizations of the 3D structure of pandan alkaloids were carried out using the application ChemDraw Professional 16.0.

Cite this article: Lumbanraja MP, Anggadiredja K, Muhammad HN, Kurniati NF. Alkaloids from *Pandanus amaryllifolius* Roxb Leaf as Promising Candidates for Antidyslipidemic Agents: An *in silico* study. Pharmacogn J. 2023;15(1): 106-111.

Validation of docking method

The validation of the docking method was carried out to determine the molecular docking parameters. The validation was done using Autodock software. The validity was tested by re-docking the respective standard ligands for 1HW9, 6LX4, and 7DFZ, which were simvastatin, fenofibrate, and ezetimibe.

Toxicity prediction

Toxicity prediction of the test alkaloids in pandan was carried out using pkCSM online tool (http://biosig.unimelb.edu.au/pkcsm/prediction). The alkaloids were presented in SMILES format prior to pkSCM processing.

RESULTS

The 3D structure visualization of the HMG-CoA reductase (PDB ID 1HW9), PPAR alpha (PDB ID 6LX4) and NPC1L1 (PDB ID 7DFZ), based on the Discovery Studio software, is presented in Figure 1.

Validation of docking method

The grid box size for 1HW9 was 40x40x50 with the (x, y, z) value = (87.395, 69.765, 13.166). Meanwhile, that for 6LX4 grid box was 34x40x40 with the (x, y, z) value = (-0.416, -7.548, 15.717). The 7DFZ had grid box size of 34x40x40 with the (x, y, z) value = (172.778, 175.245, 184.277). The docking method is considered valid if the resulting root mean square deviation (RMSD) is less than or equal to 2.00. In the present study the *in silico* validation of the 1HW9, 6LX4 and 7DFZ proteins showed respective RMSD values of 1.677, 0.918 and 1.706. The validation results obtained indicate that the shifts in the binding position of the ligands were not far from the position before validation. These further confirmed the validity of the grid box parameters.

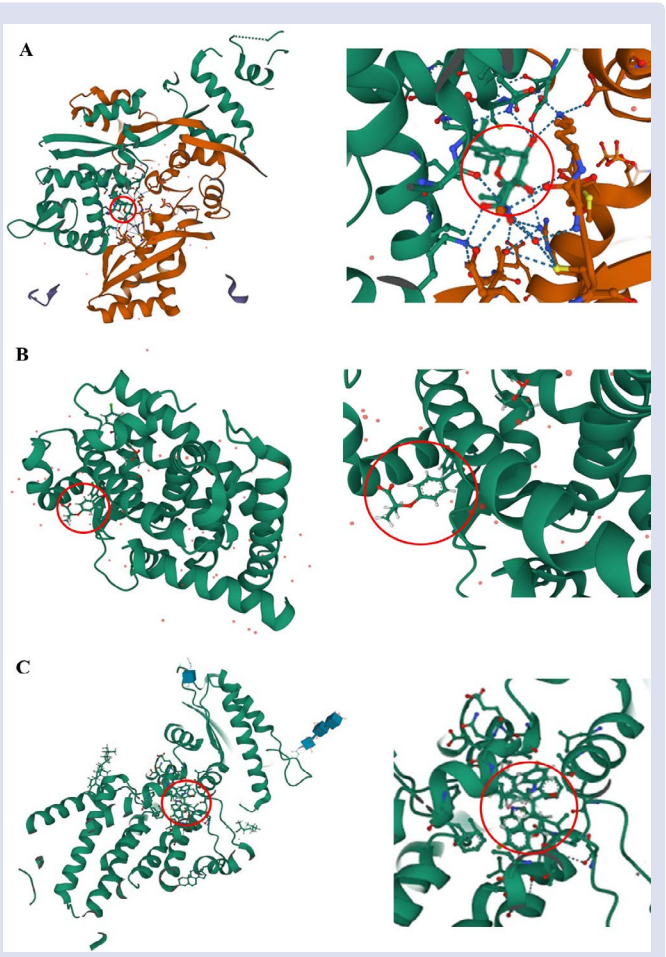

Figure 1: Visualization of the 3D structure of the HMG-CoA reductase (A), PPAR alpha (B), and NPC1L1 (C) proteins. Shown in red circles are respective binding sites of the standard ligands: Simvastatin, Fenofibrate, and Ezetimibe.

Table 1: Binding properties of the pandan leaf alkaloids to 1 HW9, 6 LX4 and 7 DFZ.

Protein (PDB ID)	Alkaloid/ Standard ligand	Binding Affinity (kcal/mol)	Inhibition constant (μM)
1HW9	Pandanusine B	-5.52	89.32
	Pandamarilactonine-A	-5.51	91.71
	Pandamarilactonine-B	-5.46	99.33
	Simvastatin	-3.21	442
6LX4	Pandamarilactonine B	-9.14	0.199
	Pandamarilactonine A	-9.10	0.215
	Pandanamine	-8.48	0.603
	Fenofibrate	-8.25	1.26
7DFZ	Pandamarilactonine A	-9.71	0.076
	Pandamarilactonine B	-9.63	0.087
	Pandanamine	-8.54	0.548
	Ezetimibe	-9.36	0.136

Molecular docking

Results of molecular docking carried out on the test alkaloids (pandanamine, pandanusine B, pandamarilactonine A, and pandamarilactonine B) for HMG Co-A reductase, PPAR alpha and NPC1L1 is presented in Table 1.

The respective binding energies to HMG Co-A reductase for pandanusine B, pandamarilactonine A, pandamarilactonine B were -5.52, -5.51 and -5.46 kcal/mol, more negative compared to the standard ligand simvastatin. For PPAR alpha, pandamarilactonine B, pandamarilactonine A and pandanamine had free energies of -9.14, -9.10 and -8.48 kcal/mol, more negative than fenofibrate as the standard ligand. In regard to NPC1L1, the binding energies for pandamarilactonine A, pandamarilactonine B and pandanamine were -9.71, -9.63 and -8.54 kcal/mol, respectively, which were comparable to the standard ligand ezetimibe. Data in Table 1 further indicates that the aforementioned alkaloid of pandan, with the exception of pandanamine against 7DFZ, had lower inhibition constant compared to the standard ligands, suggesting more potent activity against the protein.

Analyses of the interaction of the pandan alkaloids to the HMG-CoA reductase PPAR alpha and NPC1L1 was performed. The interactions were in the form of hydrogen, hydrophobic, and electrostatic bonds, as well as the binding of amino acid residues on the active site of the receptor. The data further showed the interaction distance and the effectiveness of the interactions (Table 2-4).

As Table 2 presents, the interaction of simvastatin with HMG CoA reductase involved 2 hydrogen bonds (Glu 559 and Gly 276), while that of pandanusine B took one hydrogen bond (Gly 560). In each interaction of pandamarilactonine A and B with HMG CoA reductase, two hydrogen bonds (Ser 565 and Thr 566) were demonstrated. Presented in Figure 2 is the interaction profile between pandanusine B, as the alkaloid with the highest level of interaction, with 1HW9 protein.

The interaction of fenofibrate with PPAR alpha did not involve hydrogen bonds (Table 3), while the respective interactions of pandamarilactonine A and B had one hydrogen bond (Thr 283), and there was also one hydrogen bond involved in the interaction of pandanamine (Ser 323). Depicted in Figure 3 is the interaction

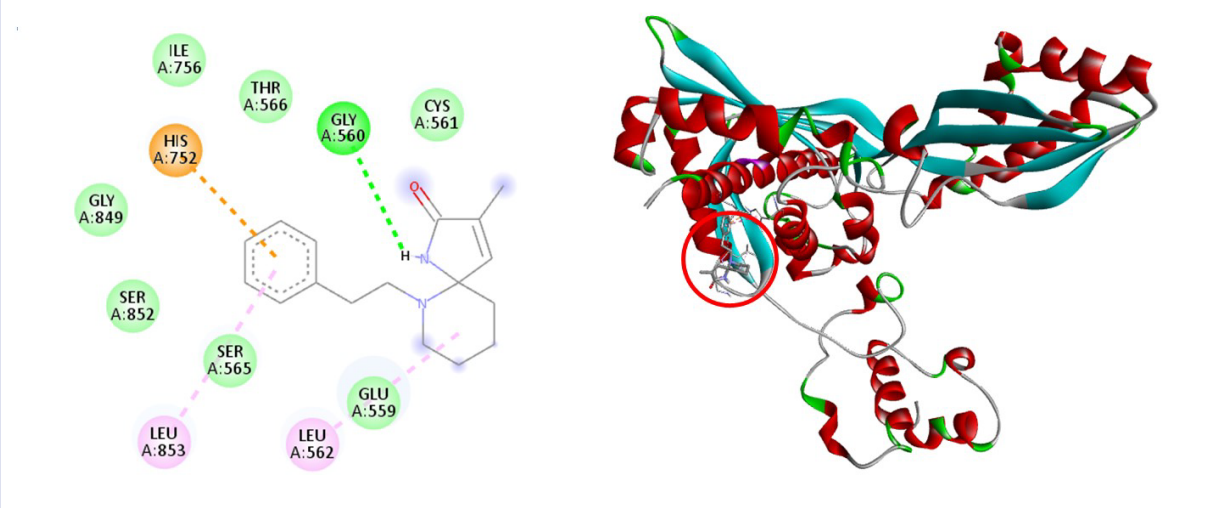

Figure 2: The profile of interaction of pandanusine B with HMGCoA reductase (PDB ID 1HW9). Red circle: binding site of the alkaloid.

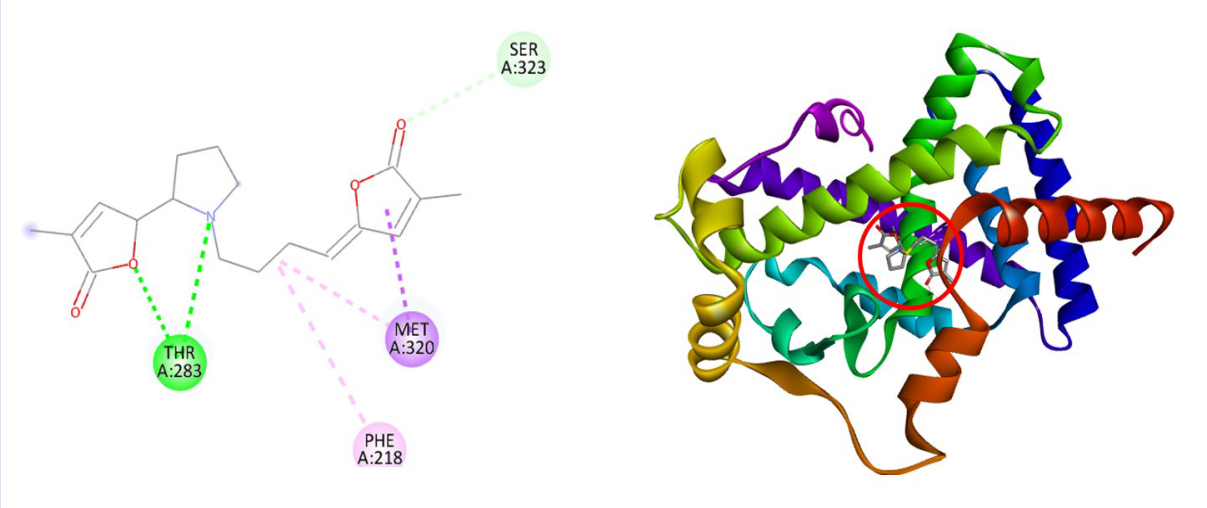

Figure 3: The profile of interaction of pandamarilactonine B with PPAR alpha (PDB ID 6LX4). Red circle: binding site of the alkaloid.

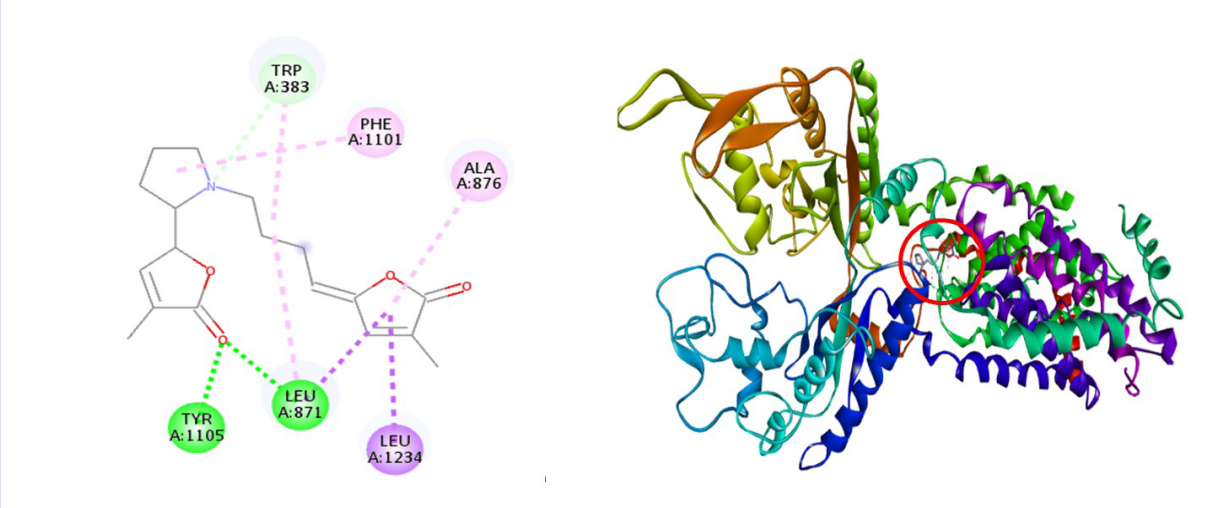

Figure 4: The profile of interaction of pandamarilactonine A with NPC1L1 (PDB ID 7DFZ). Red circle: binding site of the alkaloid.

Table 2: The characteristics of interaction of the pandan leaf alkaloids and HMG Co-A reductase.

Ligand	Binding Affinity (kcal/mol)	Amino acid residues with hydrogen bond	Distance of hydrogen bond (Å)	Hydrophobic amino acid residue
Simvastatin (standard ligand)	-3.21	Glu 559 Gly 560	2.21 2.76	Leu 562, His 752, Asn 755, Gly 849, Ser 852, Ser 565, Arg 568 & Ala 856.
Pandanusine B	-5.52	Gly 560	2.32	His 752, Leu 562, Leu 853, Ser 565, Ser 852, Gly 849, Ile 756, THR 566, Cys 561 & Glu 559.
Pandamarilactonine B	-5.51	Ser 565 Thr 566	2.76 2.72	His 752, Leu 853, Leu 562, Gly 849, Ser 852, Glu 559, Gly 560, Cys 561 & Asn 755
Pandamarilactonine A	-5.46	Thr 566 Ser 565	2.81 2.96	Leu 853, Leu 562, His 752, Gly 849, Ser 852, Glu 559, Gly 560, Cys 561 & Asn 755

Table 3: The characteristics of interaction of the pandan leaf alkaloids and PPAR alpha.

Ligand	Binding Affinity (kcal/mol)	Amino acid residues with hydrogen bond	Distance of hydrogen bond (Å)	Hydrophobic amino acid residue
Fenofibrate (standard bawaan)	-8.05	-	-	Cys 276, Lys 257, Ile 272, Cys 275, Val 332 & Ala 333
Pandamarilactonine B	-9.14	Thr 283	1.88	Phe 218, Met 320 & Ser 323
Pandamarilactonine A	-9.10	Thr 283	1.69	Met 220, Met 320, Ser 323 & Phe 218
Pandanamine	-8.48	Ser 323	2.45	Phe 218, Leu 321, Met 320, Ile 317 & Met 220

Table 4: The characteristics of interaction of the pandan leaf alkaloids and NPC1L1.

Ligand	Binding Affinity (kcal/mol)	Amino acid residues with hydrogen bond	Distance of hydrogen bond (Å)	Hydrophobic amino acid residue
Ezetimibe (standard ligand)	-9.36	Gln 873 Tyr 1105 Asp 378	2.23 2.31 1.81	Leu 871, Ile 625, Val 380, Leu 382, Leu 1234, Val 1166, Val 380 & Trp 383
Pandamarilactonine A	-9.71	Tyr 1105 Leu 871 Tyr 1102 Leu 382	1.74 2.97 2.92 2.57	Trp 383, Val 380 & Phe 1101
Pandamarilactonine B	-9.63	Tyr 1105 Leu 871	1.72 2.95	Leu 1234, Ala 876, Phe 1101 & Trp 383
Pandanamine	-8.54	Leu 382 Leu 871 Tyr 1105	2.50 3.00 1.40	Trp 383 & Val 380

Table 5: Prediction of toxicity profile of the pandan leaf alkaloids.

Alkaloid/standard ligand	Ames Toxicity	Hepato-totoxicity	Skin sensiti-zation	LD 50 (mol/kg)	LOAEL (mg/kg/d)
Pandanusine B	No	Yes	No	2.134	1.014
Pandamarilactonine A	Yes	No	No	2.736	1.044
Pandamarilactonine B	Yes	No	No	2.736	1.044
Pandanamine	No	Yes	No	2.523	1.188
Simvastatin	No	No	No	2.075	0.264
Fenofibrate	No	No	No	2.572	1.451
Ezetimibe	No	Yes	No	2.127	2.164

between pandamarilactonine B, as the alkaloid with the highest level of interaction, with 6LX4.

Data presented in Table 3 shows that the interaction of ezetimibe with NPC1L1 was partly established by 3 hydrogen bonds (Gln 873, Tyr 1105 & Asp 378), while that of pandamarilactonine A was constituted by 4 hydrogen bonds (Tyr 1105, Leu 871, Tyr 1102 and Leu 382). Pandamarilactonine B had 2 hydrogen bonds (Tyr 1105 and Leu 871) for the interaction, and pandanamine has three (Leu 382, Leu 871 & Tyr 1105). Outlined in Figure 4 is the interaction between pandamarilactonine A, as the alkaloid with the highest level of interaction, with 7DFZ.

Toxicity prediction

The data on prediction of toxicity of the pandan leaf alkaloids is presented in Table 5.

As the results show, the alkaloids pandamarilactone A and B had the highest values of LD_{50}, indicating good safety profiles. In addition, the two substances also lacked of hepatotoxicity and skin sensitizing potencies.

DISCUSSION

Pandan has been indicated as one of the plants expected to have the potency for use in dyslipidemia management. More than 170

chemical constituents have been identified in plants belonging to pandanus species.[11] The species *Pandanus amaryllifolius* contains flavonoids, alkaloids, benzenoid, steroids, lignans, and triterpenoids. Pandanusines A, B, pandalizine C, D, E, norpandamarilactonine C, D3, pandamarilactonine-A, pandamarilactonine-B4 are among the alkaloids. Meanwhile, phenolic and flavonoid contents cover catechin, epicatechin, naringin and ferulic acid. In addition, essential oils are also present, including 2-acetyl-1-pyrroline, dodecane, 2-hexenal, nonanal, pentadecanal, phytol, and norisoprenoid.[10,12,13] Using molecular docking, to the best of our knowledge, this is the first study to explore the potential of representative alkaloids in pandan leaf for their antidyslipidemic activity as well as their safety profile.

Based on the aforementioned findings on validation experiments, one might suggest that the amino acids interacted with the alkaloids of interest in this study might also be involved in the binding of the standard ligands (simvastatin, fenofibrate, and ezetimibe) and act as constituents of the active site of the target macromolecules HMG-CoA reductase, PPAR alpha, and NPC1L1.

HMG-CoA reductase is essential in the biosynthesis of cholesterol, and the statins are drugs which are effective in lowering blood cholesterol through the inhibition of HMG-CoA reductase. It has been shown that some catalytically pertinent residues were disordered around the area where the complex of the enzyme-statin occurred, in the site adjacent to the C-terminus of the enzyme. The undisturbed residues would, thus, deter the protein-ligand binding.[14] As we found in our result (Table 5), the alkaloid pandanusin B shared the same one hydrogen bond (Gly 560) as simvastatin. The hydrogen bonds have been indicated to reduce the barrier of free energy in enzyme reactions.[15] The more negative binding energy shown by pandanusine B compared to simvastatin might be related to more disturbances exerted by this alkaloid on amino acid residues around the C-terminus[14,16] although it shared some common hydrophobic amino acid residues with simvastatin.

With PPAR alpha, our results showed that pandamarilactonine B had more negative binding energy compared to the reference ligand fenofibrate. The binding site for fenofibrate has been shown to be the arm I of PPAR alpha.[17] However, the 3D structure of a PPAR alpha ligand seems to dictate the fitment of the ligand in the binding site. Thus, pemafibrate which has a Y-structure (with the presence of unique features aside from the common structure of carboxylic group), had improved fit compared to the more linear fenofibrate.[18] Pandamarilactonine B (Figure 3) seemed to also have a unique feature at the binding site where it interacted with Phe 218, Met 320 and Ser 323 moieties of PPAR alpha.

In the NPC1L1 protein, the site bound to ezetimibe was reported to form a continuous tunnel, formed by the rotating N-terminal domain, that ease the movement of cholesterol towards the plasma membrane. Ezetimibe acted by stopping up the tunnel and not as a competitor for cholesterol binding.[19] Data from our study, as presented in Figure 4, shows that pandamarilactonine A shared a common hydrogen bond with ezetimibe (Tyr 1105). The smaller number of hydrophobic amino acid residues might be associated with the higher level of interaction between the alkaloid and NPC1L1 protein. As demonstrated by Huang *et al.*[19] ezetimibe interaction with the protein rotated the N-terminal domain to a degree that enabled the movement of cholesterol. Indeed, amino acid hydrophobicity has been associated with the parametric flexibility.[20]

In light of toxicity study, although caution should be taken when concluding the safety profile due to positive result of Ames toxicity test, this result should also be carefully interpreted since it might have high sensitivity and low specificity that leads to the opposite consequence.[21] *In vivo*/*ex vivo* toxicity studies should be performed to further explore this issue.

CONCLUSION

Overall, the results of our present work show that the alkaloids of pandan leaf are promising candidates for antidyslipidemic agents, acting through the inhibition of HMG-CoA reductase, activating PPAR alpha, and interfering with cholesterol transport *via* NPC1L1.

ACKNOWLEDGMENT

This work was funded by the Doctoral Dissertation Program Scheme, Grant no. 329/IT1.B07.1/SPP-LPPM/V/2022.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interests in this work.

REFERENCES

1. Moini J, Miller C, Ahangari R, Samsam M. Global health complications of obesity. 2020, Elsevier, Chennai. ISBN: 978-0-12-819751-6.
2. Thompson PD, Panza G, Zaleski A, Taylor B. Statin-associated side effects. J Am Coll Cardiol. 2016;67(20):2395-410.
3. Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, *et al.* Herbal medicine for cardiovascular diseases: Efficacy, mechanisms, and safety. Front Pharmacol. 2020;11:422.
4. Azhar ANH, Amran NA, Yusup S, Mohd Yusoff MH. Ultrasonic extraction of 2-Acetyl-1-Pyrroline (2AP) from *Pandanus amaryllifolius Roxb.* using ethanol as solvent. Molecules. 2022;27(15):4906.
5. Thoi NDN, Hung TM, Trinh HN. Hypolipidemic effect of ethanolic extract from Pandanus amaryllifolius leaves on triton WR-1339-induced hyperlipidemia in mice/ Int J Pharmacol Res. 2018;8(12):131-6.
6. Reshidan NH, Abd Muid S, Mamikutty N. The effects of *Pandanus amaryllifolius* (Roxb.) leaf water extracts on fructose-induced metabolic syndrome rat model. BMC Complement Altern Med. 2019;19(1):232.
7. Takayama H, Ichikawa T, Kuwajima T, Kitajima M, Seki H, Aimi N, *et al.* Structure characterization, biomimetic total synthesis, and optical purity of two new pyrrolidine alkaloids, Pandamarilactonine-A and -B, isolated from *Pandanus amaryllifolius* Roxb. J Am Chem Soc. 2000;122(36):8635-39.
8. Takayama H, Ichikawa T, Kitajima M, Aimi N, Lopez D, Nonato MG. A new alkaloid, pandanamine; finding of an anticipated biogenetic intermediate in Pandanus amaryllifolius Roxb. Tetrahedron Lett. 2001;42(16):2995-96.
9. Salim AA, Garson MJ, Craik DJ. New Alkaloids from *Pandanus amaryllifolius*. J Nat Prod. 2004;67(1):54-7.
10. Cheng YB, Hu HC, Tsai YC, Chen SL, El-Shazly M, Nonato MG, Wu YC, Chang FR. Isolation and absolute configuration determination of alkaloids from *Pandanus amaryllifolius. Tetrahedron. 2017;73(1):3423-29.*
11. Son NT. Secondary Metabolites of Genus Pandanus: An Aspect of Phytochemistry. Mini-Rev Org Chem. 2019;16:689-710.
12. Gopalkrishnan B, Agashe S, Kumavat U. Pharmacognostical screening of flavouring leaves *Pandanus amaryllifolius* Rox. Int J Pharmacogn Phytochem Res. 2015;7(4):745-49.
13. Tsai YC, Yu ML, El-Shazly M, Beerhues L, Cheng YB, Chen LC, *et al.* Alkaloids from Pandanus amaryllifolius: Isolation and Their Plausible Biosynthetic Formation. J Nat Prod. 2015;78(10):2346-54.
14. Istvan ES. Structural mechanism for statin inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Am Heart J. 2002;144(6 Suppl):S27-32.
15. Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci Adv. 2016;2(3):e1501240.

16. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292(5519):1160-4.
17. Kamata S, Oyama T, Saito K, Honda A, Yamamoto Y, Suda K, *et al.* PPARα Ligand-Binding Domain Structures with Endogenous Fatty Acids and Fibrates. Science. 2020;23(11):101727.
18. Fruchart JC, Santos RD, Aguilar-Salinas C, Aikawa M, Al Rasadi K, Amarenco P, *et al.* The selective peroxisome proliferator-activated receptor alpha modulator (SPPARMα) paradigm: conceptual framework and therapeutic potential: A consensus statement from the International Atherosclerosis Society (IAS) and the Residual Risk Reduction Initiative (R3i) Foundation. Cardiovasc Diabetol. 2019;18(1):71.
19. Huang CS, Yu X, Fordstrom P, Choi K, Chung BC, Roh SH, *et al.* Cryo-EM structures of NPC1L1 reveal mechanisms of cholesterol transport and ezetimibe inhibition. Sci Adv. 2020;6(25):eabb1989.
20. Perunov N, England JL. Quantitative theory of hydrophobic effect as a driving force of protein structure. Protein Sci. 2014;23(4):387-99.
21. Hillebrecht A, Muster W, Brigo A, Kansy M, Weiser T, Singer T. Comparative evaluation of in silico systems for ames test mutagenicity prediction: scope and limitations. Chem Res Toxicol. 2011;24(6):843-54.

Cite this article: Lumbanraja MP, Anggadiredja K, Muhammad HN, Kurniati NF. Alkaloids from *Pandanus amaryllifolius* Roxb Leaf as Promising Candidates for Antidyslipidemic Agents: An *in silico* study. Pharmacogn J. 2023;15(1): 106-111.