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In Silico Identification of Bajakah Root (*Spatholobus littoralis Hassk*) Alkaloid Compounds to Stimulate Lipolysis Through Inhibition of Phosphodiesterase-4

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ABSTRACT

Modulating the nucleotide cycle signaling of phosphodiesterases (PDEs) can facilitate thermogenesis. When it comes to obesity therapy, utilizing herbs as part of non-pharmacological treatment methods is highly recommended due to the lower risks involved compared to pharmacological methods. Bajakah root, a typical plant found in West Kalimantan, also known by its Latin name, *Spatholobus littoralis Hassk* (*S. littoralis Hassk*), is currently being extensively researched. Isorhynchophylline and rhynchophylline are common alkaloids found in *S. littoralis Hassk*. The objective of this study was to explore the potential of these two alkaloids through an *in silico* approach. Chem3D Pro software was used to prepare ligands with energy conformations for accurate docking results via AutoDock Vina software. The visualization was carried out in Biovia Discovery Studio. The lowest binding energy values were obtained for the PDE4C isoform, with values of -9.14, -7.61, and -5.61 kcal/mol, respectively. The interaction between isorhynchophylline and PDE4C had the lowest binding energy. *In silico* studies suggest that two alkaloid components, isorhynchophylline and rhynchophylline, found in *S. littoralis Hassk* have the potential to increase lipolysis activity.

Keywords: Bajakah; Isorphynchophylline; Lipolysis; Phosphodiesterase; Rhynchophylline

1. Introduction

Obesity is still a global health problem faced by every country. The cases of obesity that continue to increase are those with comorbid factors [1]. Developing countries such as Indonesia, Sri Lanka, and Sudan are

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the main territories where obesity cases are rapidly increasing [2, 3]. Various complications due to obesity that cause death in individuals include type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), hyperlipidemia, chronic kidney failure (CKD), cancer, and sleep disorders [3]. The World Health Organization (WHO) classifies obesity based on body mass index (BMI) with group divisions, namely for adults having a BMI > 30. In contrast, one is classified as overweight if their BMI is > 25. Meanwhile, in children aged between 5-19, the WHO classifies obesity as having a BMI > 2 standard deviations above the median and are said to be overweight if the BMI value is > 1 standard deviation above the median WHO growth reference value [4].

There are various approaches to addressing obesity, which include non-pharmacological, pharmacological, and surgical therapy [5, 6]. Non-pharmacological methods involve physical activity and dietary modifications involving calorie restriction and nutritional selection [5].

Currently, Semaglutide and Tirzepatide are the most commonly used pharmacological treatments for obesity [7]. However, pharmacological therapy and surgery are not entirely comfortable and safe for individuals struggling with obesity. Moreover, both methods carry the risk of side effects that may lead to other health complications.

Pharmacological agents such as Semaglutide and Tirzepatide are commonly utilized in the treatment of obesity. However, these medications may cause gastrointestinal system disorders such as nausea, vomiting, and constipation. Semaglutide operates by inhibiting glucagon secretion through the activation of the Glucose-like Peptide (GLP) hormone receptor [8], while Tirzepatide reduces food intake and increases calorie burning in the body [9].

For individuals struggling with obesity, non-pharmacological approaches are often recommended due to their relative safety compared to other methods. Non pharmacological strategies typically involve two models: calorie limitation and calorie burning [10, 11]. Calorie restriction can be achieved through dietary modifications such as low-carbohydrate, low-fat, and hypo-caloric diets. Meanwhile, calorie burning can be achieved through physical exercise such as walking, jogging, running, and swimming, or possibly through traditional therapies such as acupuncture, electrotherapy, and herbal products [12]. The process of burning calories within the body involves a mechanism known as thermogenesis, which generates heat.

The concept of thermogenesis can be classified into two categories: shivering and non-shivering. Shivering thermogenesis is a result of muscle contractions during physical exercise for a duration of 90-120 minutes at an intensity equivalent to 2.5-3.5 times the resting metabolic rate (xRMR) [13].

On the other hand, non-shivering thermogenesis is a calorie-burning process that involves the activation of adrenergic receptors (subtypes β1 and β3) in adipose tissue. When adrenergic receptors are stimulated, an intracellular signaling process is initiated, which triggers the activation of Protein Kinase A (PKA).

PKA facilitates lipolysis reactions that release free fatty acids (FFA), which then enter the mitochondria to activate uncoupling protein 1 (UCP1). UCP1 facilitates the subsequent process in the mitochondria until lipid oxidation reactions and thermogenesis occur [14].

Another study explains that the activation of thermogenesis can also be facilitated through modulation of phosphodiesterase (PDE) nucleotide cycle signaling. PDEs typically work by suppressing the thermogenesis reaction. Thus, inhibition of PDEs will stimulate cAMP-dependence to produce signaling to increase UCP1 gene expression [15]. Research by Jang et al. (2020) explained that Theobromine, a natural compound derived from chocolate, can inhibit PDE activity. Theobromine was shown to inhibit the activity of PDE4D, a PDE

subtype found in adipose tissue, through β3 adrenergic receptor signaling in the C57BL/6 mouse model induced by high-fat diet (HFD) [16]. Forsythin, an alkaloid from the Chinese plant *Forsythia suspensa*, can also lead to the inhibition of PDE4 [17].

The use of herbs as a non-pharmacological treatment for obesity prevention is based on the resultant stimulation of lipolysis; it is highly recommended because the risks tend to be smaller than pharmacological methods. Thus, exploring the most efficient types of herbs for obesity therapy must continue. Indonesia, especially West Kalimantan, is home to many unique plants whose potential in obesity therapy should be explored. One such plant in West Kalimantan, currently being widely researched, is Bajakah, also known by its Latin name, *Spatholobus littoralis Hassk* (*S. littoralis Hassk*).

The alkaloid content often found in *S. littoralis Hassk* includes isorhynchophylline and rhynchophylline. These two alkaloids have been studied for their ability to effectively reduce hyperglycemia problems in diabetic mouse models [18]. Isorhynchophylline and rhynchophylline are also effective in improving endothelial dysfunction in blood vessels, helping reduce cardiovascular problems, and functioning as anti-hypertension compounds [19]. The potential of isorhynchophylline and rhynchophylline from *S. littoralis Hassk* for obesity problems has not been widely studied. Especially lacking are studies relating to the ability of isorhynchophylline and rhynchophylline to inhibit PDE activity. This study aims to investigate the potential of *S. littoralis Hassk* through an *in silico* approach by considering its potential benefits for the prevention of diseases associated with lipid profile disorders due to obesity.

2. Materials and Methods

2.1. Hardware spesifications

For the docking process, this study used a computer with specifications: Intel (R) Core (TM) i5-5300U CPU @ 2.30 GHz, 2301 Mhz, 2 Core(s), 4 Logical Processors, 8 GB RAM, and Hewlett Bios system. Packard M71 Ver 01.24.

2.2 Receptors and ligands preparation

AutoDock Vina software was utilized for this research, with visualization carried out through Biovia Discovery Studio. To ensure accurate results during the docking process, ligands were prepared with energy conformations using Chem3D Pro software [20].

PubChem website provided the necessary ligands: theobromine, rhynchophylline, and isorphynchophylline. Receptors were obtained from the UniProt website with codes O89084, Q3UEI1, Q9QXI7, and Q01063. The Swiss Model at https://swissmodel.expasy.org/ was utilized to build the protein for target docking against the ligand, and the receptor was modified by removing any extraneous ligands, water molecules, and proteins [16]. The modelling results were validated through the saves webserver site at https://saves.mbi.ucla.edu/.

2.3 Docking process

The docking process was carried out using AutoDock Software, with both the ligand and receptor files placed in a Grid Box with dimensions of 70 x 40 x 40 centered on the ligand. To perform the Genetic Algorithm (GA) Runs, 100 repetitions were utilized. The file to be docked was saved in .gpf format, with docking parameters and Lamarckian GA added, and then saved in .dpf format [21].

Based on the docking results, the ligand and receptor demonstrated the lowest binding energy value, indicating the best interaction value. Bond energy was measured in kcal/mol, and hydrogen and hydrophobic interactions were also taken into account when analyzing the docking results [20].

3. Results and Discussion

3.1 Receptors and ligands preparation

An analysis was conducted on receptors sourced from UniProt, resulting in similarity

values spanning from 0.57 to 0.61. The GMQE score, derived from the four receptors, ranges from 0.60 to 0.72. With a sequence identity of over 85%, the quality factor value obtained was greater than 90. As shown in Table 1, the sequence length ranged from 643 to 844.

Table 1. Results of protein building using Swiss Model.

Uniprot ID	GMQE	Similarity	Seq Identity (%)	Quality Factor (%)	Seq. Length	PDB ID
O89084	0,65	0,57	86,89	94,81	844	PDE4A
Q9QXI7	0,6	0,60	97,10	96,24	643	PDE4B
Q3UEI1	0,72	0,61	100,00	95,71	686	PDE4C
Q01063	0,7	0,61	100,00	96,47	747	PDE4D

Note: GMQE (Global Model Quality Estimate); Seq (Sequence); PDB (Protein Data Bank).

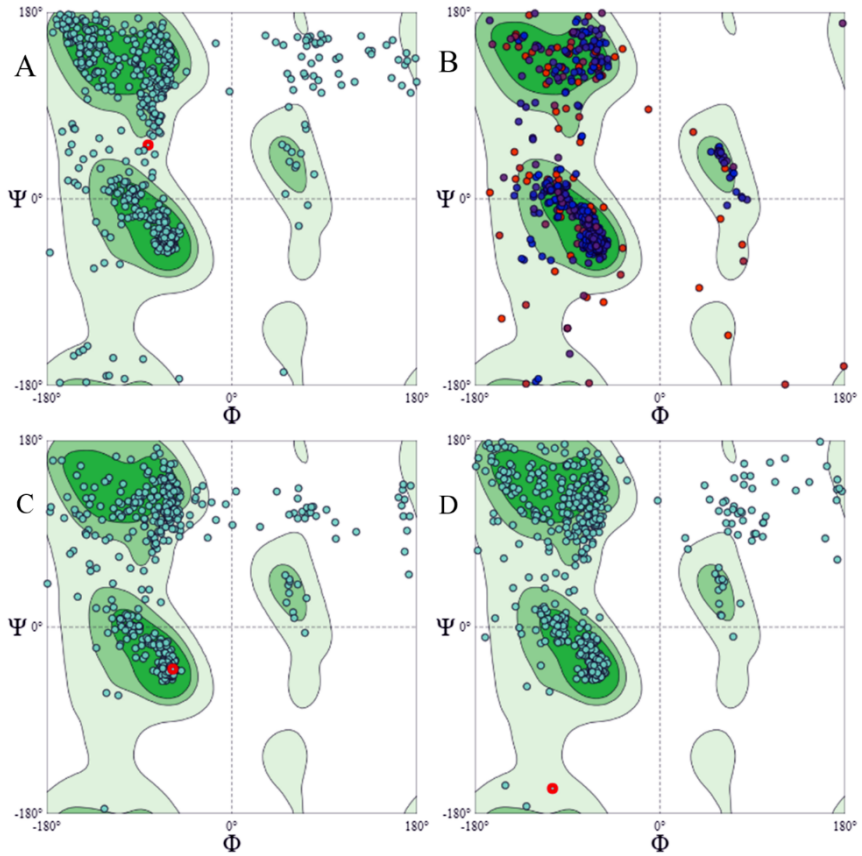

Fig. 1. Ramachandran plot (A) Phosphodiesterase-4A (PDE4A) (B) Phosphodiesterase-4B (PDE4B) (C) Phosphodiesterase-4C (PDE4C) (D) Phosphodiesterase-4 D (PDE4D).

Validation results were obtained using a Swiss-PDB viewer, which generated Ramachandran plot results along with corresponding colors. Fig. 1(A) showed 78.6% of the deposit in the most favored area, 14.9% in the allowed area, and 3.5% in the disallowed area. In Fig. 1(B), 88.2% of residues were in the most favored area, 10.0% in the allowed area, and 0.6% in the disallowed area.

Fig. 1(C) displayed 76.8% of the residues in the most favored area, 17.7% in the allowed area, and 1.0% in the disallowed area. Finally, Fig. 1(D) showed 78.9% of the residues in the most favored area, 15.3% in the allowed area, and 2.3% in the disallowed area. These results were validated through a saved web server.

According to the four PDEs, the distribution of diagram data is adjusted to colors that indicate differences in area status. Both dark green and light green colors represent permitted areas, with the dark green area indicating no steric clash in the tripeptide model [22].

The sequence identity value derived from the four PDEs is over 85%, indicating good modeling results. In addition to the sequence identity value, the authors also identified disallowed areas, with PDE4B, PDE4C, and PDE4D receptors having less than 2.5% disallowed area, while the PDE4A receptor has 3.5% disallowed area. It is worth noting that the tolerance limit for disallowed areas used in previous research is less than 2.5% [23, 24]. Hence, good receptor modeling is crucial for obtaining favorable results in the ligand docking process.

3.2 Docking process

Table 2 displays comprehensive information on the docking process between isorphynchophylline, rhynchophylline, and theobromine with the PDE receptor. The lowest binding energy values were obtained for the PDE4C isoform, with values of -9.14, -7.61, and -5.61 kcal/mol, respectively.

On the other hand, the PDE4A receptor isoform had the highest binding energy values, with values of 583.97, 295.42, and 12.51 kcal/mol, respectively. The hydrogen and hydrophobic interaction values for the PDE4B isoforms were found to be similar overall. The docking results revealed a Root Mean Square Deviation (RMSD) value > 5 for all ligands and receptors.

Table 2. Binding energy, hydogen, and hydophobic interactions between ligand and receptors docking results.

Ligands	Receptor's ID	Binding Energy (kcal/mol)	Hydrogen Interactions	Hydrophobic Interactions	RMSD Values
Isorphynchophylline	PDE4A	583,97	Arg570A; Ala529A	Cys575A; Trp640A; Leu539A	6,84
Rhynchophylline		295,42	Not detected	Val433A; Leu435A; Phe444A	6,72
Theobromine		12,51	Not detected	Leu578A; Met532A; His419A; His459A	9,03
Isorphynchophylline	PDE4B	-4,87	**Thr564A**	**Met568A; Phe571A; Met504A; Met588A; Ile567A; Phe603A**	5,47
Rhynchophylline		-5,43	**Thr564A**	**Met568A; Phe571A; Met504A; Met588A; Ile567A; Phe603A**	6,35
Theobromine		-3,8	**Thr564A**	**Met568A; Phe571A; Met504A; Met588A; Ile567A; Phe603A**	7,19
Isorphynchophylline	PDE4C	-9,14	His389A; **Asp547A**; Tyr388A; His393A; His429A; Asp430A; Glu459A	**Ile565A**; Met502A; His433A	7,66
Rhynchophylline		-7,61	**Asp547A**; Tyr388A; Leu548A	Met502A; **Ile565A**; Tyr558A	6,87
Theobromine		-5,61	**Asp547A**; Gln598A	Phe601A; Phe569A; **Ile565A**; Leu548A; Tyr388A; Pro551A	8,51
Isorphynchophylline	PDE4D	-6,18	**Asp507A**; Asp523A; Gln552A	Ile548A; **Leu520A**; **Lys516A**; **Ala511A**	7,98
Rhynchophylline		-6,24	**Asp507A**; Asp523A; Gln552A	Leu510A; **Leu520A**	8,59
Theobromine		-3,5	Leu510A; Gln552A; **Asp507A**	**Leu520A**; **Lys516A**; **Ala511A**	6,02

Note: kcal/mol (kilocalories per mol); RMSD (Root Mean Square Deviation)

3.2.1 Docking of ligands and PDE4A

The docking analysis between the isorphynchophylline ligand and the PDE4A receptor resulted in a weak binding energy value of 583.97 kcal/mol. The RMSD value was also more than 2, higher than the recommended minimum tolerance limit of an RSMD value ≤ 2 [25]. Similar weak binding energy values were also observed for rhynchophylline and theobromine ligands. Therefore, the result of theobromine docking in this study differs from Jang et al.'s findings [16]. All three ligands, isorphynchophylline, rhynchophylline, and theobromine, showed negative binding energy values to the PDE4B, PDE4C, and PDE4D receptors. Isorphynchophylline and rhynchophylline had hydrogen and hydrophobic interactions in the PDE4B receptor, similar to those of theobromine.

However, the RMSD value obtained was not less than 2. Therefore, it can be concluded that isorphynchophylline and rhynchophylline have the potential to inhibit PDE4B receptor activity. The PDE4B receptor isoform is expressed in all adipose tissue [26]. Inhibiting PDE4B signaling in all adipose tissue can lead to massive lipolysis activity. According to Fig. 2A, the residues Arg570A and Ala529A engage in hydrogen bonding with isorphynchophylline, whereas Cys575A, Trp640A, and Leu539A form hydrophobic interactions. The bond energy between isorphynchophylline and PDE4A is relatively weak, at 583.97 kcal/mol. In Fig. 2B, although no hydrogen bonds were detected, Val433A, Leu435A, and Phe444A formed hydrophobic interactions. Similarly, the bond energy was weak at 295.42 kcal/mol. In Fig. 2C, there were no hydrogen bonds found, but hydrophobic interactions were formed by Leu578A, Met532A, His419A, and His459A.

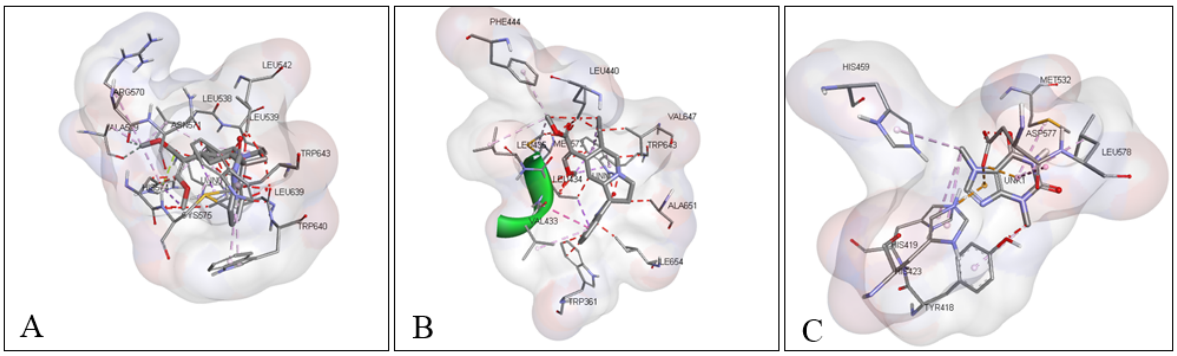

Fig. 1. Docking results of 3D interactions between (A) Isorphynchophylline and PDE4A (B) Rhynchophylline and PDE4A (C) Theobromine and PDE4A.

The docking test between the isorphynchophylline ligand and the PDE4A receptor resulted in a weak binding energy value of 583.97 kcal/mol. The RMSD value was also found to be more than 2, which is higher than the recommended minimum tolerance limit of an RSMD value ≤ 2 [25]. Similar weak binding energy values were observed for rhynchophylline and theobromine ligands as well.

However, the results of this study were different from the study of Jang et al. [16] who found a negative binding energy value between the theobromine ligand and the PDE4A receptor.

3.2.2 Docking of ligands and PDE4B

In Fig. 3(A), the docking analysis indicates that the isorphynchophylline ligand interacts with the PDE4B receptor via a hydrogen bond with the Thr564A residue. Additionally, hydrophobic bonds are formed with other residues, including Met568A,

Phe571A, Met504A, Met588A, Ile567A, and Phe603A.

Notably, the residue depicted in Fig. 3(A) is the same as that observed with the rhynchophylline ligand in Fig. 3(B) and theobromine in the ligand in Fig. 3(C). All three ligands, isorphynchophylline, rhynchophylline, and theobromine, showed negative binding energy values to the PDE4B, PDE4C, and PDE4D receptors. Isorphynchophylline and rhynchophylline had hydrogen and hydrophobic interactions in the PDE4B receptor, similar to those of theobromine.

However, the RMSD value obtained was not less than 2. Therefore, it can be concluded that isorphynchophylline and rhynchophylline have the potential to inhibit PDE4B receptor activity. The PDE4B receptor isoform is expressed in all adipose tissue [26]. Inhibiting PDE4B signaling in all adipose tissue can lead to massive lipolysis activity.

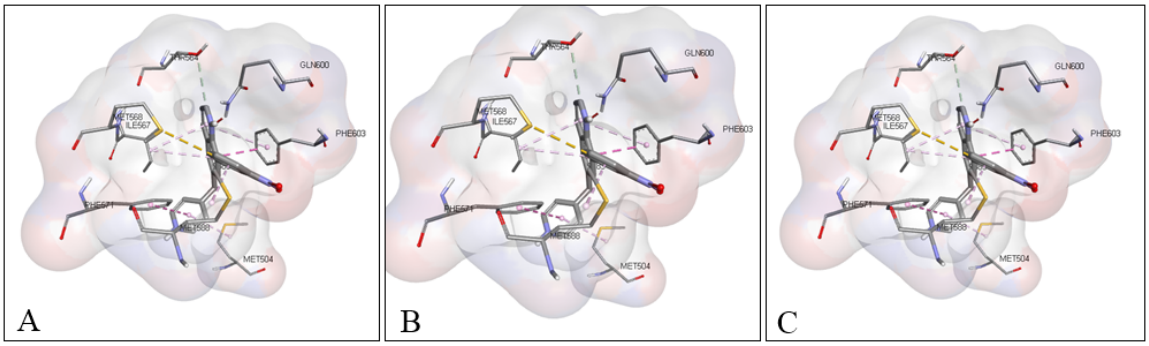

Fig. 2. Docking results of 3D interactions between (A) Isorphynchophylline and PDE4B (B) Rhynchophylline and PDE4B (C) Theobromine and PDE4B.

3.3.3 Docking of ligands and PDE4C

According to the docking results presented in Fig. 4(A), the interaction between the ligand isorphynchophylline and the PDE4C receptor involves several residues, including His389A, Asp547A, Tyr388A, His393A, His429A, Asp430A, and Glu459A. Additionally, residues Ile565A, Met502A, and His433A are also displayed.

Fig. 4(B) illustrates the residue interaction of the ligand rhynchophylline and the PDE4C receptor, which forms a hydrogen bond between Asp547A, Tyr388A, and Leu548A. The figure also highlights hydrophobic bonds formed by Met502A, Ile565A, and Tyr558A. Finally, Fig. 4(C) shows the hydrogen interactions between the Theobromine ligand and the PDE4C receptor, which involve Asp507A, Asp523A, and Gln552A. Moreover, residue Phe601A forms hydrophobic bonds with Phe569A, Ile565A, Leu548A, Tyr388A, and Pro551A.

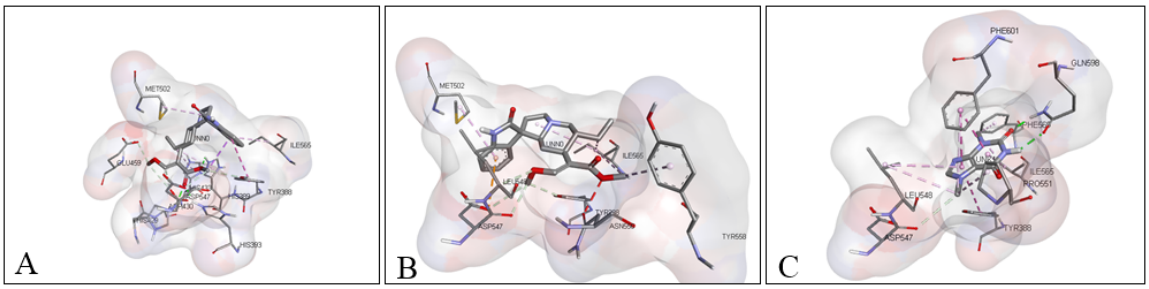

Fig. 3. Docking results of 3D interactions between (A) Isorphynchophylline and PDE4C (B) Rhynchophylline and PDE4C (C) Theobromine and PDE4C.

According to a recent study by Jang et al. [27], ligands isorphynchophylline and rhynchophylline were found to have hydrogen interactions with specific residues, such as Asp547A in PDE4C and PDE4D, and Ile565A (as shown in Table 2), which is similar to theobromine. Asparagine (Asp) is a negatively charged residue that can establish stable interactions with ligands and receptors. Although Asp residues typically only contribute minimally to protein interactions through salt bridges, the stabilization of interactions resulting from the charge of Asp can be stronger [28].

This stabilization occurs through Asp forming salt bridges, which has been found to produce an inhibitory effect on the target protein. Both *in silico* and *in vitro* studies have demonstrated that the formation of salt bridges by Asp leads to the inhibition of STAT3-PIM1 signaling in cancer cells through the interaction between curcumin and Asp155 [29].

Another study explains that the interaction with the Asp residue causes the inhibition of translation through a mutagenesis reaction. This process of inhibiting translation is supported by the discovery of interactions between Asp-189 and Lithium (Li+) or Rubidium (Rb+) [30].

3.3.4 Docking of ligands and PDE4D

The results of docking depicted in Fig.5(A) highlight the hydrogen interaction between the ligand isorphynchophylline and the PDE4D receptor's Asp507A residue, Asp523A, and Gln552A. Additionally, the residue group includes Ile548A, Leu520A, Kys516A, and Ala511A, all of which contribute to hydrophobic interactions. Fig. 5(B) reveals hydrogen interactions formed by Asp507A, Asp523A, and Gln552A, along with hydrophobic interactions by Leu510A and Leu520A. Finally, Fig. 5(C) displays the docking result between the theobromine ligand and PDE4D, where Leu510A, Gln552A, and Asp507A show hydrogen interactions. Additionally, Leu520A, Lys516A, and Ala511A play a crucial role in forming hydrophobic interactions.

Several compounds have been studied for their ability to inhibit PDE activity, including xanthine and its derivatives. Notably, 1,3-diethylxanthine and 1-pentyl-3-ethylxanthine have shown roughly 20% inhibition of PDE activity. Additionally, some xanthine derivatives like methylxanthine have been found to directly stimulate lipolysis activity [31]. From a physiological perspective, these findings suggest that isorphynchophylline and rhynchophylline, identified in *S. littoralis Hassk*, may have potential in inhibiting the activity of PDE4B, PDE4C, and PDE4D, leading to an increase in cAMP activity in adipocytes. This increase in activity can promote lipid oxidation or lipolysis, influencing lipid reserves in adipocytes. A previous study by Zhang *et al*. (2009) showed that a deficiency in PDE4B activity can reduce both adipocyte numbers and the activity of pro-inflammatory cytokines in white adipose tissue (WAT) caused by obesity [32]. Thus, based on the results of this study, the effect that will emerge in the *in vivo* study is the modulation of WAT in the form of an increase in adipocytes and a reduction in lipid droplet volume. Differences in lipid profile levels and cAMP activity can also be used to analyze lipolysis activity. Additionally, inhibiting PDE4 activity can reduce 2-deoxyglucose uptake into muscles, providing further insights into the potential benefits of *S. littoralis Hassk* in improving metabolic diseases. The potential for upregulating lipolysis through PDE inhibition using *S. littoralis Hassk* in this study still needs to be proven through *in vivo* studies that analyze the several parameters that have been mentioned.

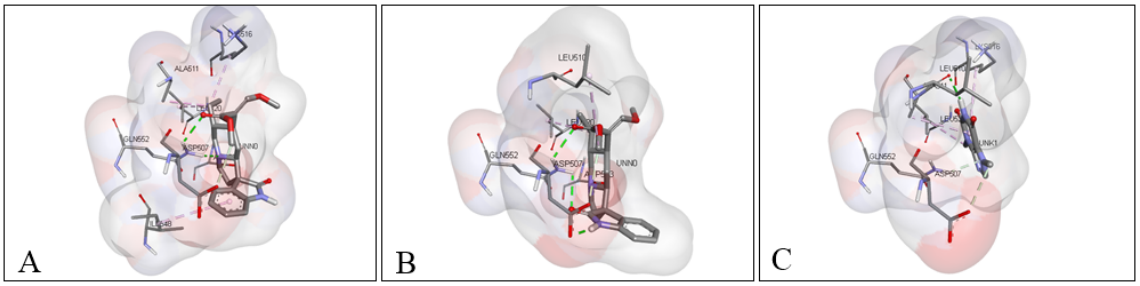

Fig. 4. Docking results of 3D interactions between (A) Isorphynchophylline and PDE4D (B) Rhynchophylline and PDE4D (C) Theobromine and PDE4D.

4. Conclusion

According to *in silico* studies, the alkaloid components isorhynchophylline and rhynchophylline, which are present in *S. littoralis Hassk*, have the ability to increase lipolysis activity by inhibiting PDEs. Interestingly, the interaction between isorhynchophylline and PDE4C appears to have the lowest binding energy, similar to theobromine's inhibition of PDE4B, PDE4C, and PDE4D activity. The results of this research can be used to develop health supplements that prevent diseases related to various complications, especially related to lipid profile disorders due to obesity, by utilizing the properties of the alkaloids contained in the *S. littoralis Hassk* plant.

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