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Evaluation of *in silico* antidiabetic activity of phytocompounds of wild bitter gourd on DPP4 target

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ABSTRACT
Wild bitter gourd (Momordica charantia var. abbreviata Ser.) shows diverse pharmacological effects, especially containing many phytocompounds with potential antidiabetic activity. Forty-one bioactive compounds were screened for antidiabetic activity through the inhibition of DPP4 (dipeptidyl peptidase 4) by molecular docking with AutoDock Vina software. Momordicoside T (-10.1 Kcal/mol), Momordicoside B (-9.9 Kcal/mol), Momordicoside F1 (-9.8 Kcal/mol), and Momordicoside I (-9.7 Kcal/mol) showed the strongest interaction with the DPP4 target compared to co-crystallized ligand and reference drug Vildagliptin (-6.9 Kcal/mol). These Momordicosides formed many strong hydrogen bonds at the DPP4 active site. In particular, Momordicoside T established the most hydrogen bonds with bond lengths ranging from 1.92-2.94 Å. The structure linked with sugar moieties increased the hydrogen bond formation and binding affinity of these phytocompounds with DPP4. Therefore, these compounds could be potential molecules to develop new antidiabetic drugs on DPP4.

Keywords: *wild bitter gourd, antidiabetic, in silico, molecular docking, DPP4*

1. INTRODUCTION

Wild bitter gourd (*Momordica charantia* var. abbreviata Ser.) belongs to the Cucurbitaceae family and is a climbing plant that grows in tropical and subtropical regions. According to Oriental medicine, wild bitter gourd (WBG) has a cold property, a bitter taste, and no toxicity. Therefore, WBG exhibits detoxifying, anti-inflammatory, heat-clearing, phlegm-reducing, and cough-reducing effects. Various biological activities of WBG have been reported such as hypoglycemic, antibacterial, antiviral, antitumor, immunomodulatory, anti-oxidant, anthelmintic, antipyretic, anticoagulant, liver protection, and anti-inflammatory [1]. Around the world, oral tablets from WBG fruit extract have been produced for weight loss and blood sugar stabilization.

Many studies have documented the hypo-glycemic effect of bitter melon extracts and some active ingredients through *in vitro* tests [2-4]. Besides, DPP4 (dipeptidyl peptidase 4) is a common enzyme expressed on the surface of most cell types with the function of inactivating many types of biologically active peptides such as glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1. Therefore, inhibition of DPP4 may affect glucose regulation through multiple effects [5, 6]. Medicines in the DPP4 inhibitor class include sitagliptin, saxagliptin, linagliptin, and alogliptin. However, research data on the mechanism of action of the active ingredients of WBG on DPP4 are also limited. Therefore, this *in silico* study aimed to identify active ingredients with strong or potential effects on the DPP4 target for antidiabetic activity of WBG.

2. METHOD

2.1. Ligand preparation

The structures of the active ingredients of WBG were downloaded from the PubChem database of NLM (National Library of Medicine). The structure of new ligands was drawn in ChemBioDraw Ultra

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19. The energy of these ligands was minimized using ChemBio3D Ultra 19 software [7, 8].

2.2. Protein preparation

The protein molecule of dipeptidyl peptidase 4 (DPP4, PDB ID: 6B1E) was retrieved from the protein data bank (rcsb.org) (Figure 1). All the water molecules were removed from the DPP4 target. Then, DDP4 was added to only polar hydrogen and Kollman charges. The grid box for docking simulations was set by AutoDock tools (Table 1) [9, 10].

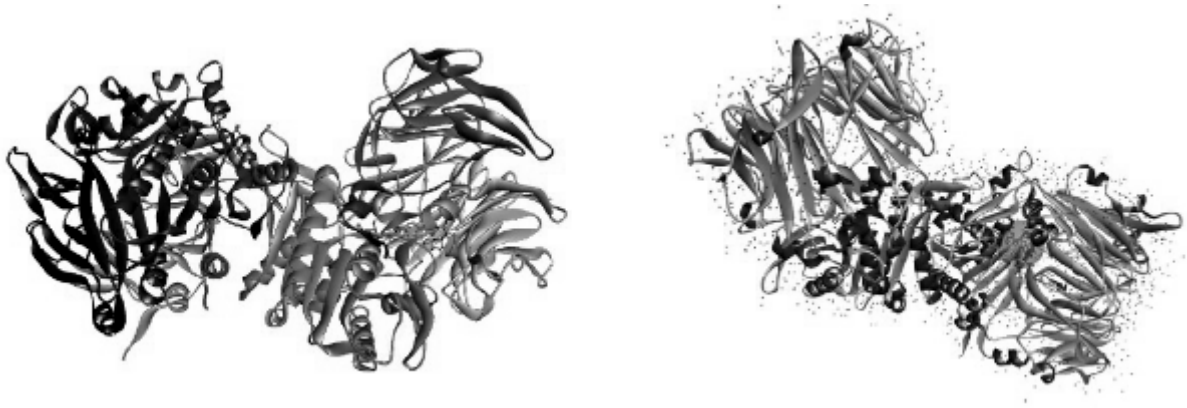


Figure 1. Structure and active site of DPP4 receptor

Table 1. Grid box parameters for DPP4 target

Target	Size			Center		
	x	y	z	x	y	z
DPP4	40	40	40	38.8930	50.9776	36.6248

DPP4 - dipeptidyl peptidase 4, exhaustiveness = 8, num modes = 10, energy range = 4

2.3. Molecular docking

All the minimizations were performed by AutoDock Vina docking simulation protocol with AMBER force field and the partial charges were automatically calculated. The search algorithm of AutoDock Vina is a Monte-Carlo iterated search combined with the BFGS17 gradient-based optimizer, which comprises iterations of sampling, scoring, and optimization. AutoDock Vina actually uses a united-atom scoring function (one that involves only the heavy atoms) that combines knowledge-based and empiric scoring function features as well as supports the AutoDock4.2 scoring function [11]. Besides, AutoDock Vina was compiled and run under Windows 10.0 Professional operating system. The pictorial representation of the interaction between the ligands and the target protein was performed by BIOVIA Discovery Studio 2021 software [10, 11].

3. RESULTS AND DISCUSSION

The phytocompounds of WBG were screened and assembled through published *in vitro* and *in vivo* studies [2-4]. The results showed that 41 bioactive compounds of WBG with hypoglycemic potentials were identified for the interaction study on the DPP4 target compared to the antidiabetic drug and co-crystallized ligand for optimal structure determination (Table 2). The docking results of the receptor-ligand interaction are shown in Table 2 and 3 and Figure 2-4.

Table 2. The binding affinity of ligands with DPP4 target

Entry	Phytocompound	Affinity (Kcal/mol)		Entry	Phytocompound	Affinity (Kcal/mol)
1	**Momordicoside T**	**-10.1**		22	Karaviloside X	-8.7
2	**Momordicoside B**	**-9.9**		23	Momordicoside A	-8.7
3	**Momordicoside F1**	**-9.8**		24	Momordicoside R*	-8.7
4	**Momordicoside I**	**-9.7**		25	MC2	-8.7
5	Kuguaglycoside G	-9.5		26	25-O-methylkaraviagenin D	-8.7

Entry	Phytocompound	Affinity (Kcal/mol)		Entry	Phytocompound	Affinity (Kcal/mol)
6	Momordicilin	-9.4		27	Momordicoside F2	-8.6
7	Momordicoside Q	-9.4		28	Momordicoside L	-8.6
8	Karaviloside XI	-9.3		29	Karaviloside XI*	-8.6
9	Momordicoside D	-9.3		30	MC3	-8.6
10	Momordicoside S	-9.3		31	Momordicoside K	-8.5
11	Momordicin	-9.2		32	Momordicoside G	-8.3
12	Momordicoside E	-9.2		33	Charantoside IX	-8.3
13	Stigmasterol glucoside	-9.1		34	Momordicine I	-8.2
14	Momorcharaside B	-9.0		35	Momordicoside U	-8.2
15	β-Sitosterol-D-glucoside	-8.9		36	Karaviloside II	-8.2
16	Momordicoside C	-8.9		37	Momordicoside S*	-8.1
17	β-Sitosterol-β-D-glucoside	-8.8		38	Stigmasterol	-7.9
18	(-)-Momordenol	-8.8		39	β-Sitosterol	-7.9
19	Momordicoside U*	-8.8		40	Momordicoside R	-7.9
20	MC1	-8.8		41	Momordenol	-7.2
21	Momordicin II	-8.7		42	**Vildagliptin**	**-6.9**

** - Aglycone; MC1 - 3-hydroxycucurbita-5,24-dien-19-al-7,23-di-O-β-glucopyranoside; MC2 -3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al; MC3 - (19R,23E)-5β-19-epoxy-19,25-dimethoxycucurbita-6,23-dien-3β-ol*

Phytocompounds of WBG showed strong interactions with DPP4 with an affinity between -7.2 and -10.1 Kcal/mol. In particular, all bioactive compounds showed better affinity than the reference drug Vildagliptin (-6.9 Kcal/mol). Momordicoside T (-10.1 Kcal/mol), Momordicoside B (-9.9 Kcal/mol), Momordicoside F1 (-9.8 Kcal/mol), and Momordicoside I (-9.7 Kcal/mol) showed the strongest interaction with the DPP4 target compared to the co-crystallized ligand and reference drug Vildagliptin. The structures of these compounds are shown in Figure 3. These potential compounds are glycosides with structures attached to sugar moieties, especially β-D-glucose (Figure 2). This may be the reason for increased interaction at the active site of DPP4.

Figure 2. Interaction sites of potential ligands on DPP4

Table 3. Molecular docking results of potential phytocompounds and reference drug

Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond types	Amino acid
Momordicoside T	**-10.1**	2.59	Hydrogen bond	ARG356
		2.56	Hydrogen bond	TYR547
		2.34	Hydrogen bond	LYS554
		2.94	Hydrogen bond	SER630

Ligand	Affinity (Kcal/mol)	Distance (Å)	Bond types	Amino acid
		2.42	Hydrogen bond	TYR662
		2.88	Hydrogen bond	HIS740
		1.92	Hydrogen bond	GLU206
		2.73	Hydrogen bond	TYR662
		2.26	Hydrogen bond	ARG358
		2.32	Hydrogen bond	ARG358
		2.07	Hydrogen bond	ILE405
		2.68	Hydrogen bond	ILE405
Momordicoside B	**-9.9**	2.34	Hydrogen bond	ARG356
		1.94	Hydrogen bond	PHE357
		2.30	Hydrogen bond	ARG358
		2.73	Hydrogen bond	TYR662
		2.31	Hydrogen bond	VAL303
		2.34	Hydrogen bond	GLU206
		2.58	Hydrogen bond	GLU205
		3.36	Hydrogen bond	GLU361
Momordicoside F1	**-9.8**	3.03	Hydrogen bond	LYS554
		2.73	Hydrogen bond	ARG560
		2.68	Hydrogen bond	SER577
		3.79	Hydrophobic (π-σ)	TRP629
Momordicoside I	**-9.7**	2.98	Hydrogen bond	LYS122
		3.09	Hydrogen bond	ARG560
		2.77	Hydrogen bond	ARG560
		2.56	Hydrogen bond	SER577
		3.00	Hydrogen bond	GLN553
		3.80	Hydrophobic (π-σ)	TRP629
Vildagliptin	**-6.9**	2.19	Hydrogen bond	LYS122
		3.75	Hydrogen bond	GLN123
		3.60	Hydrogen bond	ASP737
		3.46	Hydrogen bond	ASP739
		5.10	Hydrophobic (Alkyl)	VAL252
		4.70	Hydrophobic (π-Alkyl)	TRP124
		4.09	Hydrophobic (π-Alkyl)	PHE240

Hydrophobic interaction (π-σ, π-π stacked, amide-π stacked, alkyl, π-alkyl)

Four phytocompounds formed 3-12 strong hydrogen bonds with DPP4. Momordicoside T established the most hydrogen bonds with 12 strong hydrogen bonds with bond lengths ranging from 1.92-2.94 Å at ARG356, TYR547, LYS554, SER630, TYR662, HIS740, GLU206, ARG358, and ILE405 amino acids. The structure linked with 4 sugar moieties increased the hydrogen bonding of Momordicoside T. Meanwhile, Momordicoside B formed eight strong hydrogen bonds with lengths in the range of 1.94-3.36 Å at ARG356, PHE357, ARG358, TYR662, VAL303, GLU206, GLU205, and GLU361 amino acids. However, Momordicoside T and Momordicoside B did not show hydrophobic interactions with DPP4. Besides, Momordicoside F1 formed three strong hydrogen bonds (2.68-3.03 Å) and showed a hydrophobic (π-σ) interaction with TRP629 amino acid. Momordicoside I formed five strong hydrogen bonds (2.56-3.09 Å) and showed a hydrophobic (π-σ) interaction with TRP629 amino acid similar to Momordicoside F1. Therefore, these four compounds need to continue studying *in vitro* biological activity on DPP4 to confirm the *in silico* research results.

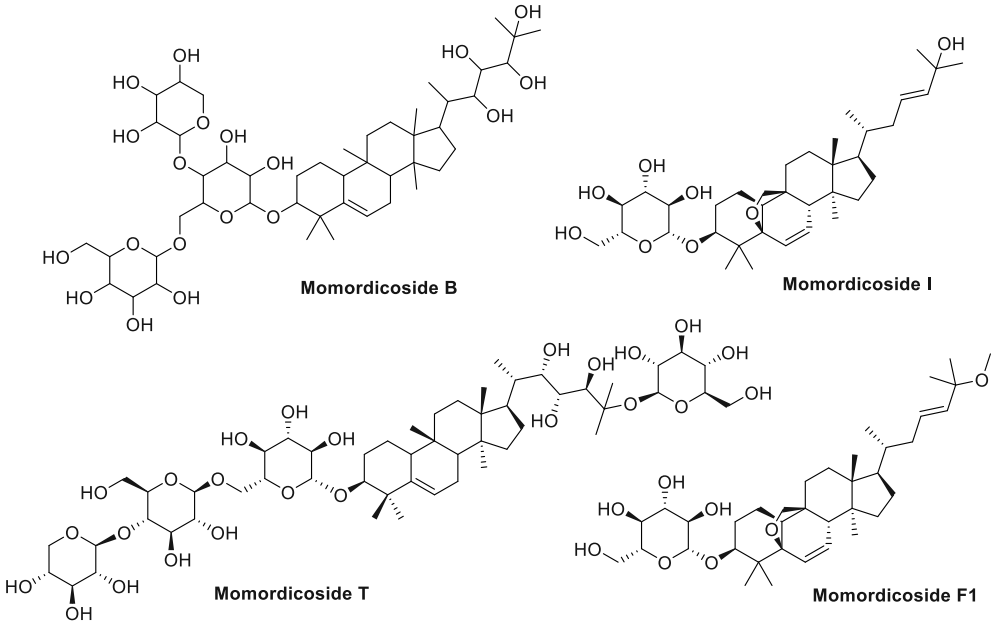
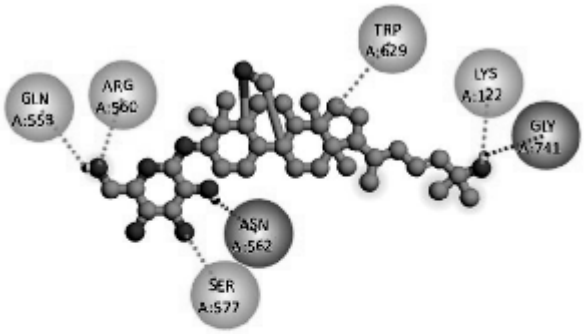
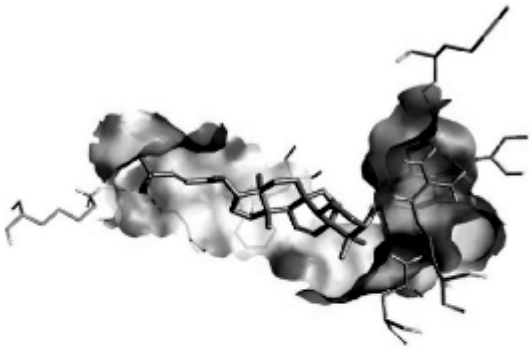


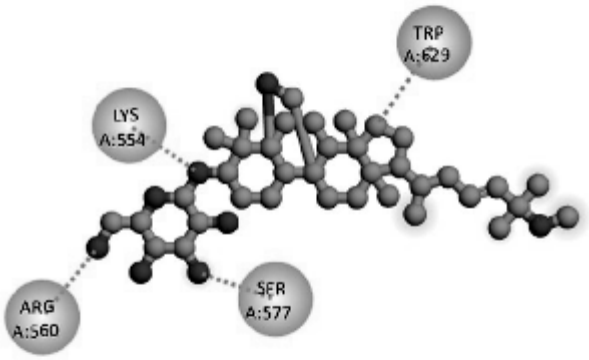
Figure 3. Structure of phytocompounds of WBG with potential *in silico* antidiabetic activity



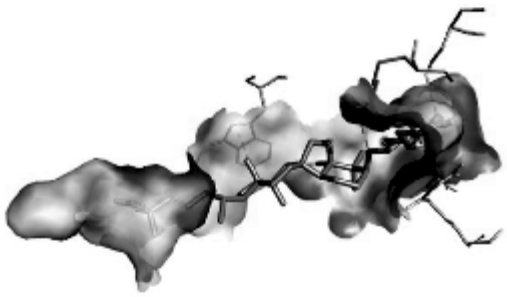
Momordicoside I – DPP4 (2D)



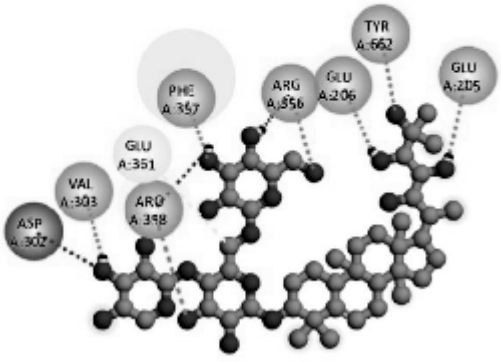
Momordicoside I – DPP4 (3D)



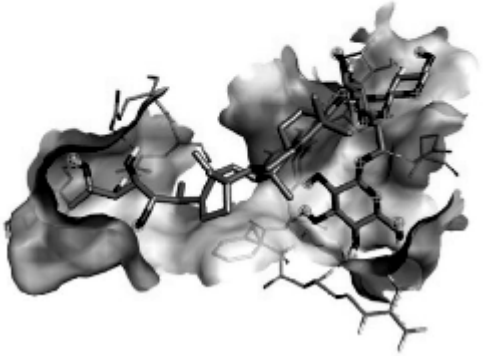
Momordicoside F1 – DPP4 (2D)



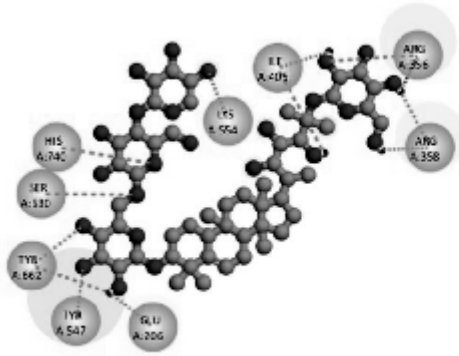
Momordicoside F1 – DPP4 (3D)



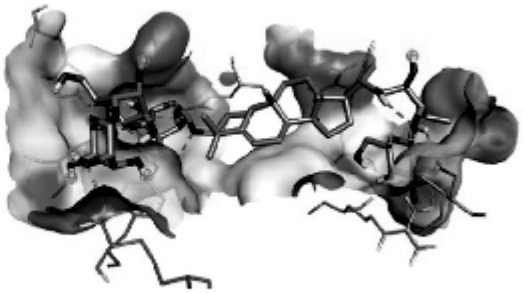
Momordicoside B – DPP4 (2D)



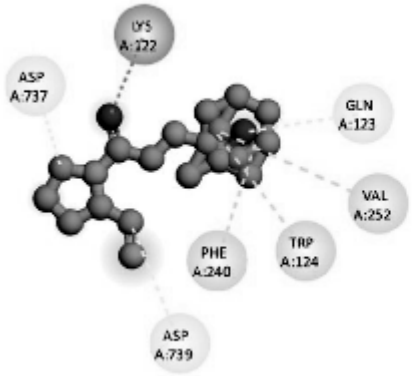
Momordicoside B – DPP4 (3D)



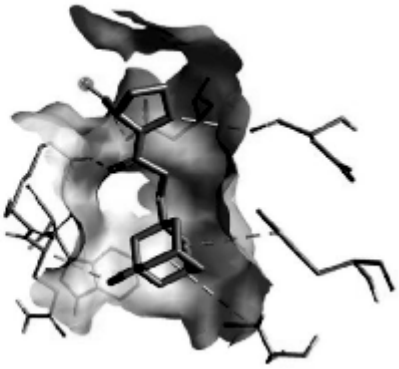
Momordicoside T – DPP4 (2D)



Momordicoside T – DPP4 (3D)



Vildagliptin – DPP4 (2D)



Vildagliptin – DPP4 (3D)

Figure 4. 2D and 3D representation of the interaction of potential phytocompounds and reference drug Vildagliptin with DPP4 target

Several *in silico* studies on DPP4 have been performed on the bioactive compounds of different plant species such as *Artocarpus champeden* and *Pisum sativum* to explore new potential antidiabetic compounds [12, 13]. Twelve phytocompounds from *A. champeden* have the potential as DPP4 inhibitors based on ΔG value and interaction conformation, of which seven phytocompounds showed ΔG values and inhibition constants close to the native ligand [12]. Besides, four peptides (IPYWTY, IPYWT, LPNYN, and LAFPGSS) from pea (*Pisum sativum* L.) showed good interaction ability mainly because these compounds formed hydrophobic interactions with the S1 pocket in DPP4 [13]. Moreover, eleven potential asymmetric bioactive natural products were screened from 224,205 NPs by molecular docking and predicted as potential DPP4 inhibitor candidates from natural sources [14]. In particular, to block the active site of GSK-3 protein three anti-diabetic compounds namely, Charantin, Momordenol, and Momordicilin were taken from *Momordica charantia* for docking study and calculation of binding energy [15]. However, the potential phytocompounds (Momordicosides) of WBG on DPP4 in this study showed differences with the potential compounds on GSK-3 due to different targets. Additionally, these potential phytocompounds from WBG also exhibited differences with bioactive compounds of *A. champeden* on DPP4 due to the different active ingredients of the two species.

4. CONCLUSION

Bioactive compounds of WBG showed stronger interactions than Vildagliptin on DPP4. In particular, Momordicoside T, Momordicoside B, Momordicoside F1, and Momordicoside I demonstrated potential *in silico* antidiabetic activity with strong binding affinity to DPP4 target. The structures of these compounds are all attached to sugar moieties and form many strong hydrogen bonds at the DPP4 active site.

ACKNOWLEDGMENTS

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Đánh giá hoạt tính chống đái đường *in silico* của các phytocompound trong khổ qua rừng trên mục tiêu DPP4

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TÓM TẮT

Khổ qua rừng (Momordica charantia var. abbreviata Ser.) có tác dụng dược lý đa dạng, đặc biệt chứa nhiều phytocompound có hoạt tính trị đái tháo đường tiềm năng. 41 hợp chất có hoạt tính sinh học đã được sàng lọc hoạt tính trị đái tháo đường thông qua ức chế DPP4 (dipeptidyl peptidase 4) bằng docking phân tử với phần mềm AutoDock Vina. Momordicosid T (-10.1 Kcal/mol), Momordicosid B (-9.9 Kcal/mol), Momordicosid F1 (-9.8 Kcal/mol) và Momordicosid I (-9.7 Kcal/mol) thể hiện tương tác mạnh nhất với mục tiêu DPP4 so với phối tử đồng kết tinh và thuốc đối chiếu Vildagliptin (-6.9 Kcal/mol). Các Momordicosid này đã hình thành nhiều liên kết hydrogen mạnh tại vị trí tác động của DPP4. Trong đó, Momordicosid T tạo được nhiều liên kết hydrogen nhất với độ dài liên kết dao động từ 1.92-2.94 Å. Cấu trúc liên kết với các nhóm đường làm tăng sự hình thành liên kết hydrogen và ái lực liên kết của các phytocompound này với mục tiêu DPP4. Do đó, các hợp chất này có thể là phân tử tiềm năng để phát triển các loại thuốc điều trị đái tháo đường mới trên DPP4.

Từ khóa: *khổ qua rừng, chống đái tháo đường, in silico, docking phân tử, DPP4.*

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