Pharmacogn J. 2021; 13(6)Suppl: 1639-1647
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Molecular Study of *Acalypha indica* to Leptin, Alpha Glucosidase, and its Antihyperglycemic Effect on Alpha Glucosidase

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ABSTRACT

Introduction: The purpose of this study is to find potential inhibitors of leptin as a proinflammatory adipokine and alpha glucosidase as an enzyme that mediate hyperglycaemia; to alter the chronic complications of obesity from herbal *Acalypha indica* (Ai). This study was conducted using in silico molecular docking to evaluate the Ai compounds interaction with leptin and alpha glucosidase. The *in vitro* assay to alpha glucosidase was done to explore antihyperglycemic effect of Ai, as hyperglycaemia is the key process of chronic complication of obesity. **Material and Methods:** Protein target were leptin and alpha glucosidase; compounds from Ai plant were repundusinic, mauritanin, hesperetin, acaindinin, and glucogalin in pdb format. Molecular docking using autodock vinna. *In vitro* assay of Ai antihyperglycemic activity was done to alpha glucosidase and was define as IC50 level. **Result:** The results from the docking analysis demonstrated that compounds from Ai roots contain antihyperglycemic-antiobesity activity which acted by inhibiting leptin and alpha glucosidase receptors. Repundusininc and mauritanin compounds contain hydrogen bond with the greatest leptin enhancer activity on Ser9, Thr35, Glu8, Ser9, Thr25, Gln111, Lys211, Leu7 for repundisinic and Glu8, Thr25, Gly112 and Leu7 for mauritanin. Hesperetin, acaindinin and glucogallin were the most identical compounds with similar affinity binding value to alpha glucosidase. *Ai roots* was already proven as anti-hyperglycemic-antiobesity which was further confirmed by *in vitro* assay to alpha glucosidase (IC50 19,429 µg/ml.). **Conclusion**: The results demonstrated that Ai have anti hyperglycaemic-antiobesity effects and was found to be potentially as antihyperglycemic by *in vitro* assay to alpha glucosidase.

Key words: Acalypha indica, Antiobesity, Leptin, Alpha glucosidase.

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History

- Submission Date: 04-08-2021;
- Review completed: 25-08-2021;
- Accepted Date: 02-09-2021.

DOI : 10.5530/pj.2021.13.211

Article Available online

http://www.phcogj.com/v13/i6

INTRODUCTION

Obesity is defined as abnormal or excessive fat accumulation that presents a risk to health.[1,2] Nowadays, obesity has become a global health problem.[3-5] Availability of highly processed foods, which are very easy to handle or do not require any handling and—most importantly—are very cheap, contributes greatly to the continuous increase in the incidence of obesity.[1] Obesity is associated with impaired glucose tolerance or type 2 diabetes mellitus. The underlying mechanism is due to insulin resistance which results in further complications of obesity.[6]

Leptin is the product of the obesity (Ob) gene and currently considered as a satiety hormone.[7] Leptin is released into the circulating blood; transported to the brain and bound to its receptors in the hypothalamus, where it causes repression of genes encoding neuropeptide Y (NPY) and induction of genes encoding proopiomelanocortin (POMC) and corticoliberin (CRH).[7,8] This results in decreased appetite and reduced food intake with subsequent body fat reduction and increased energy expenditure, which finally leads to body mass decrease.[7] Leptin has been shown to enhance insulin sensitivity in peripheral tissues and increase glucose uptake and oxidation in skeletal muscles.[9] In leptin resistant over-weight individuals, the permeability of the blood brain barrier (BBB) to leptin is decreased in high-fat diet-induced obesity despite the increase in plasma leptin levels. This impaired transport of leptin across the BBB is one of the causes of leptin resistance. Insufficiency of leptin signaling in the hypothalamus (induced by hyperleptinemia in obese subjects), causes hyperglycemia and hyperinsulinemia, which lead to diabetes mellitus.[10]

Alpha-glucosidases are enzymes in the digestive tract that hydrolyze carbohydrates into glucose.[11] In particular, mammalian α-glucosidase in the mucosal brush border of the small intestine catalyzes the end step digestion of starch and disaccharides that are abundant in the human diet causing an increase of blood glucose. Inhibitors of alpha-glucosidases delay the breakdown of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion. Thus, inhibition of glycosidases has a significant effect on polysaccharide metabolism, glycoprotein processing, and cellular interaction. This mechanism widens opportunities for the discovery and development of new therapeutic agents against diabetes and obesity.[6]

Considering the complexity of chronic complications that could be happen in obesity patient, it seems probable that it will be necessary to apply combined treatment, with pathways directed at various types of cells in various stages of the disease process. *Acalypha indica L* (Ai) has a promising potential to be developed as an herb to treat obesity. Ai comes from the Euphorbiaceae family and taxonomically belongs to the genus Acalypha which is the fourth largest genus in the Euphorbiaceae family.[12] This plant can grow to 1.5-2.5m and has empirically been used to treat diabetes and hyperlipidaemia. Most of the international manuscripts on Ai were published from the Indian region because this plant

Cite this article: Hakim RW, Fadilah F, Tarigan THE, Jusman SWA, Purwaningsih EH. Molecular Study of *Acalypha indica* to Leptin, Alpha Glucosidase, and its Antihyperglycemic Effect on Alpha Glucosidase. Pharmacogn J. 2021;13(6)Suppl: 1639-1647.

has a close relationship with Ayurvedic medicinal practices done by the older Indian generation. Meanwhile, in other countries, this plant is used for medical purposes but is still limited.[12] Various studies have been conducted to determine the effect of Ai administration on diseases associated with metabolic syndrome. However, the effect of Ai on inflammatory adipokines such as leptin and alpha glucosidase as enzymes of obesity-related metabolic syndrome has not been studied.

The purpose of this study is to explore the molecular docking of *Acalypha indic*a active compounds to leptin and alpha glucosidase which has a role to overcome chronic complications of obesity such as hyperlipidemia, cardiovascular abnormalities, and atherosclerosis. The molecular docking study is assessed by binding energy values. Binding energy values are the sum of intermolecular energies (kcal/mol), comprising hydrogen bond energy, Van Der Waals energy, desolvation energy, and electrostatic energy. Binding energy is described as the energy released due to the interaction between a ligand and its receptor. The ligand with the less binding energy had a greater possibility of interacting with a protein target. The level of the compound affinity to the molecular target is represented by the pKi value. The ligand with greater pKi value has more possibility to interact with a protein target.

Furthermore, *in vitro* assay of *Acalypha indica* roots extract to alpha glucosidase were done in this study to assess its antihyperglycemic effect, reminding hyperglycemia is the main condition in obesity related to metabolic syndrome which often causes chronic complications such as heart diseases and stroke.

MATERIAL AND METHOD

Computational Materials

All computational studies to perform molecular docking were done using MacBook Air with High Sierra 10.13.3, 1.8GHz Intel Core i5 processor, 8 GB RAM, 1600 MHz DDR3. Marvin Sketch was used to draw ligand structures in 2 and 3 dimensions. AutoDockVina was used for docking process, while AutoDockTools was used to analyze the docking result and create 3D visualization of the ligand-receptor complex. LigPlus was used for the analysis of the amino acid residues of 2D ligand-receptor complex.

Receptor Preparation

We downloaded the receptor from www.rcsb.org in the .pdb file format and performed separation between the receptor chain and ligands. The water was removed by deleting water in Autodock software and hydrogen atoms (polar only) was added and non polar atoms were merged. Finally, gesteiger load was added to the recipe and the file was saved as .pdbqt.

Preparation of ligands

The native PDB ligand was isolated from the receptor ligand complex (specifically the ligand from the PDB receptor ligand complex). Images of 50 compounds were download from Chemspide in .pdb file format using Marvin Sketch.

Validate the docking method

We performed docking on the original PDB ligands by optimizing the grid box size and grid center position. The optimum grid box and grid center were selected by looking at the docking results.

Docking and analysis of docking results

A folder containing proteins and ligands was created in the .pdbqt format and the docking configuration (protein file name, ligand file name, grid box coordinates, grid box size, output file name, and the number of models produced) was set. AutodockVina was used to run the docking through the terminal. The docking file output (.pdbqt) was separated by the best docking model with vina split and docking receptor-ligand complexes were analyzed and visualized through Ligplus.

Extraction and Phytochemical Test

Acalypha indica L dry-powder underwent maceration process with 96% ethanol, ethyl acetate, and n-hexane for 3x24 hours. Extract and solvent was separated with a rotary evaporator and stored in oven until it become solvent free. The phytochemical analysis of saponin, flavonoid, tannin, glycoside, triterpenoid, steroid, and alkaloid were carried out using chemical reactions.

In vitro Assay to Alpha- Glucosidase

Alpha-glucosidase assay of extracts was carried out according to literature with slight modification.[13] In a 96-well plate, reaction mixture containing 120µl phosphate buffer (0,1 M, pH= 6.9), 20µl alpha-glucosidase (0,5U/ml), 20µl p-nitrophenyl-α-D-Glucopyranoside (PNPG) (5mM), and 10µl of varying concentrations of extracts was pre-incubated at 37°C for 15 min. The reaction was stopped by adding 80µl sodium carbonate (0.2M). The yellow color produced was read at 405nm using microplate Reader. Each experiment was performed in triplicates, along with appropriate blanks. Acarbose at various concentrations (0.1-10 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The results were expressed as percentage inhibition, which were calculated as:

$$\text{Inhibition (\%)} = A_{\text{Negative control}} - A_{\text{Test}}/A_{\text{Negative control}} \text{ X } 100,$$

where, A is absorbance. The result is also expressed as IC_{50} value.

RESULT

The Ai compounds were assessed through the energy variation and the formation of the ligand-receptor structure, given by the binding constant and the Gibbs free energy (ΔG) values. Ligand-protein binding was evaluated by their binding affinity, binding structure, binding energy, and residue component.

Based on the result of docking with leptin (Table 1), repandusinic bound to leptin with the least binding energy −7,3495 kcal/mol and pKi 10,237. Repandusinic bound to leptin with 8 hydrogen bindings which involved binding sites of Ser9, Thr35, Glu8, Ser9, Thr25, Gln111, and Leu7 (Table 1). Another significant compound binding to Leptin is mauritanin with a binding energy of -5,6617 kcal/mol and pKi 7,886. Mauritanin had hydrogen bound to leptin in sites Glu8, Thr25, Gly112 and Leu7. In visual observation through the docking simulation (Figures 1 and 2), the two observed ligands (repandusinic and mauritanin) made similar conformation to Leptin structure on the active site.

On the other hand, the study of alpha glucosidase molecular docking to Ai (Table 2) determined that hesperetin has the least binding energy of −7,2135 kcal/mol and pKi 11,2135. Hesperetin bound to alpha glucosidase with 7 hydrogen bindings which involved binding sites of His34, Asp303, Asp633, Phe30, His34, His691, and Glu29 (Table 2). Another significant compound binding to alpha glucosidase is acaindinin with a binding energy of -6, 7586 kcal/mol and pKi 10,667. Acaindinin had 10 hydrogen binding sites which involved binding sites of Tyr746, Tyr 747, Asn765, His825, Glu858, Lys742, His825, Arg880, and Lys387. Glucogalin bound to alpha glucosidase with a binding energy of -5, 1502 kcal/mol and pKi 9,823. Gly764, His825, Tyr893, Lys742, Asn765, His825, Arg880, Tyr893, and Thr738 were hydrogen binding sites of glucogalin to alpha glucosidase. In visual observation through the docking simulation (Figure 3, 4 and 5), the three observed ligands (hesperetin, acaindin, and glucogalin) made similar conformations to alpha glucosidase molecule on the active site.

Table 1: Binding energy and residue components of each ligand with leptin.

No	Molecules	ΔG (kcal/mol)	KI	H don and H acc
1	Repandusinic acid B	-7.3495	10, 237	Ser9, Thr35, Glu8, Ser9, Thr25, Gln111, Lys211, and Leu7
2	Tannin	-6.9753	8,927	Thr25, Asp633, Phe30, His34
3	Chebulagic	-5.7741	7,925	Phe30, Gly112
4	Mauritanin	-5.6617	7,886	Glu8, Thr25, Gly112 and Leu7
5	Clitorin	-5.4841	7,561	Glu8, Ser9
6	Acalyphamide	-5.1322	6.925	Asp633,
7	Tri-O-Metylellagic	-5.0676	6.820	Pro206
8	Acetonylgreanin	-5.0603	6.729	Thr35
9	Geranin	-4.9229	5.736	Gly112
10	Acalyphin amide	-4.7762	5.509	

ΔG (mean binding energy); pKi (binding affinity), H don (Hydrogen donor); H acc (Hydrogen Acceptor)

Table 2: Binding energy and residue components of each ligand with alpha glucosidase.

No	Molecules	ΔG (kcal/mol)	pKI	H don and H acc
1	Hesperetin	-7.2135	11,673	His34, Asp303, Asp633, Phe30, His34, His691, and Glu29
2	Chrsysin	-7, 0256	10,902	His691, and Glu29, Phe30
3	Acaindinin	-6, 7586	10,331	Asn765, His825, Lys742, His825, Arg880
4	Tannin	-6, 3464	10,667	Tyr746, Tyr 747, Asn765, His825, Glu858, Lys742, His825, Arg880, and Lys387
5	Chebulagic acid	-5,9039	10,030	Arg880, Tyr893, Thr738
6	Geranin	-5,8510	9,922	Gly764, His825, Tyr893, Lys742, Asn765
7	Glucogallin	-5.1502	9,823	Gly764, His825, Tyr893, Lys742, Asn765, His825, Arg880, Tyr893, Thr738
8	Acetonylgreanii	-4,9814	8,739	His825
9	Mauritanin	-4.9628	8,654	Tyr893
10	Kaempferol	-4, 9597	7,938	Arg880

ΔG (mean binding energy); pKi (binding affinity), H don (Hydrogen donor); H acc (Hydrogen Acceptor)

Table 3: Inhibition percentage and IC50 value of acalypha and acarbose towards α-glucosidase.

Sample	Cons (mg/ml)	%Inhibition			Mean Inh	IC50 (mg/ml)			Mean IC50 (mg/ml)
		1	2	3		1	2	3	
Ai roots ethanol extract	12,5	47,684	49,380	48,076	48,380 ± 0,888	19,683	18,947	19,655	**19,429**
	25	50,554	50,424	51,468	50,815±0,569				
	50	59,556	58,121	57,860	58,513±0,913				
	100	62,557	62,296	62,035	62,296±0,261				
	200	65,427	64,644	64,123	64,731±0,656				
	400	66,601	69,602	69,472	68,558±1,696				
	800	82,909	81,213	79,909	81,344±1,504				
	1600	84,083	84,083	84,214	84,127C0,076				
Acarbose (standard)	0,156	-1500,274	-1666,301	-1786,301	-1650,958±143,629	1,461	1,502	1,581	**1,515**
	0,3125	-1211,781	-1271,781	-1298,082	-1260,547±44,233				
	0,625	-929,863	-993,973	-1127,123	-1016,986±100,623				
	1,25	-651,233	-567,397	-671,781	-630,137±55,297				
	2,5	-522,192	-436,712	-519,726	- 492,877±48,655				
	5	-411,233	-384,110	-359,452	-384,931±25,9				
	10	-357,808	-274,795	-356,164	-329,589±47,460				

This result was supported by the *in vitro* assay of Ai extract to alpha glucosidase. The IC_{50} of Ai extract to alpha glucosidase was 19,429 mg/ml compared to IC_{50} of Acarbose, an established alpha glucosidase inhibitor, was 1,515 mg/ml (Table 3). This activity is probably due to the content of polyphenol compounds in Ai, like flavonoid compounds. The results of the phytochemical screening of Ai are as shown in Table 4; flavonoid, tannin and triterpenoid were positive in Ai roots of ethanol extract.

DISCUSSION

Molecular Study of Ai to Leptin

Leptin, the adipocyte hormone, is well known for its role in the regulation of food intake and energy expenditure. Since the discovery of leptin, leptin is acknowledged for its therapeutic potential to obesity and diabetes.

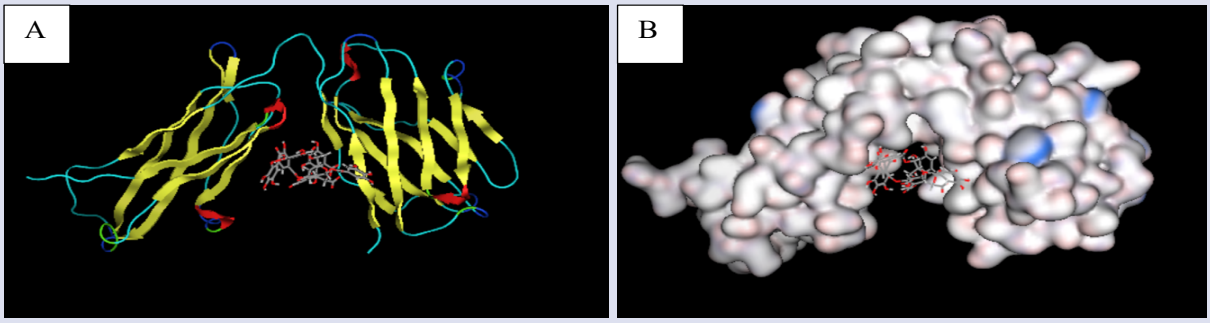

Figure 1. A. Repandusinic. B. Model binding of repandusinic acid and leptin.

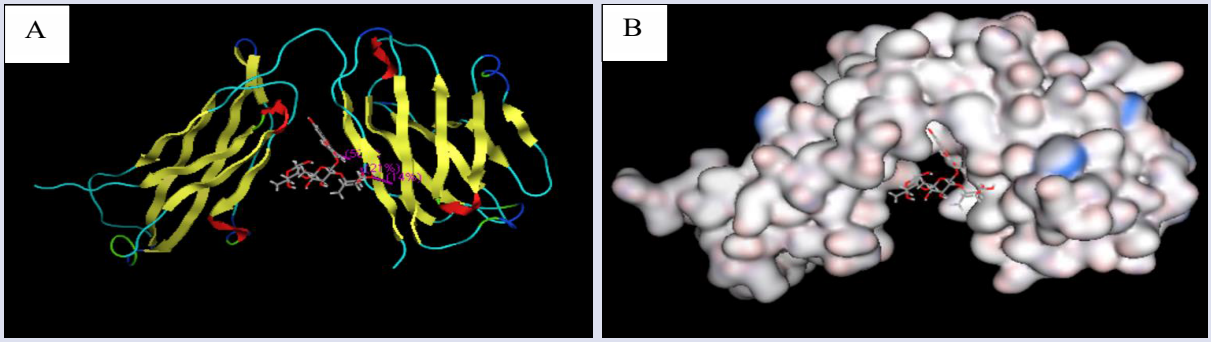

Figure 2. A. Mauritanin. B. Model binding of mauritanin and leptin.

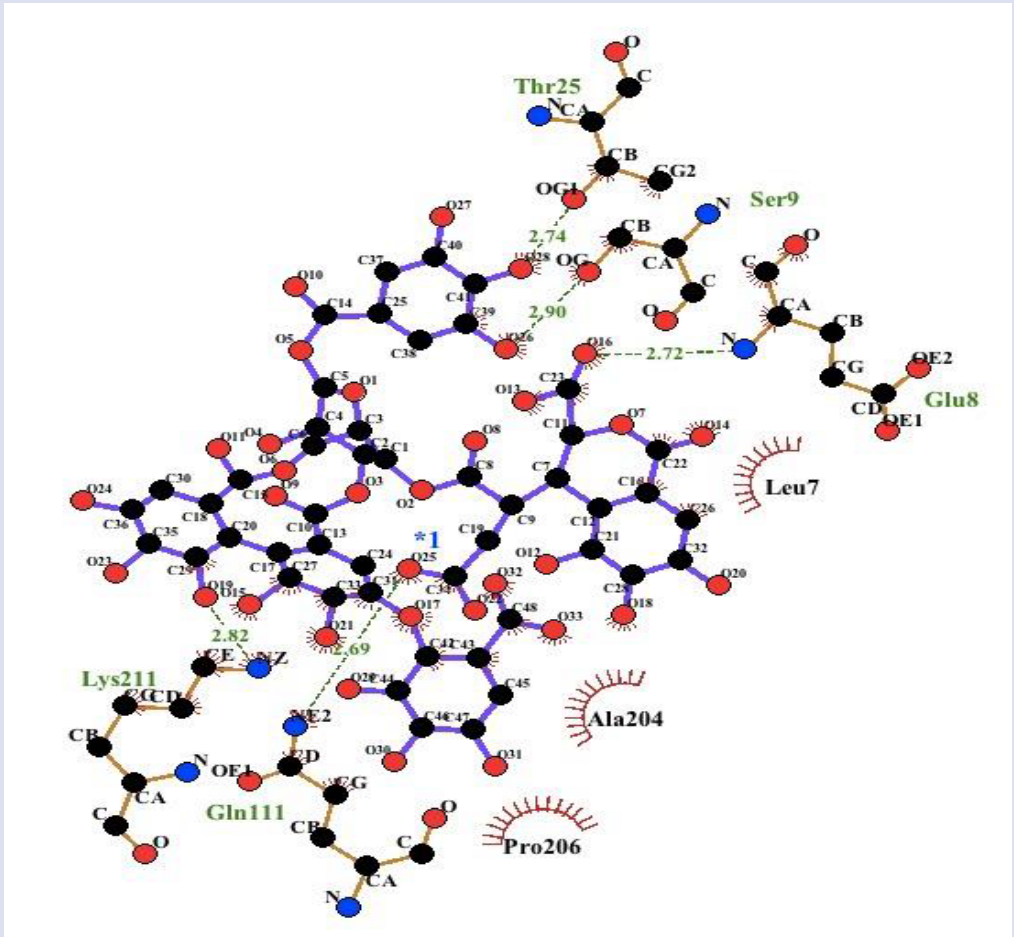

Figure 3. 2D Interaction between repandusinic and leptin.

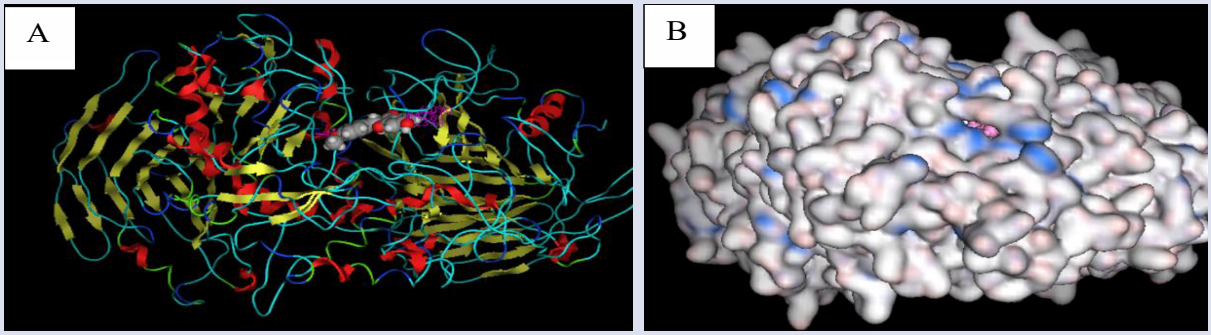

Figure 4. A. Hesperetin. B. Model binding of hesperetin and alpha glucosidase.

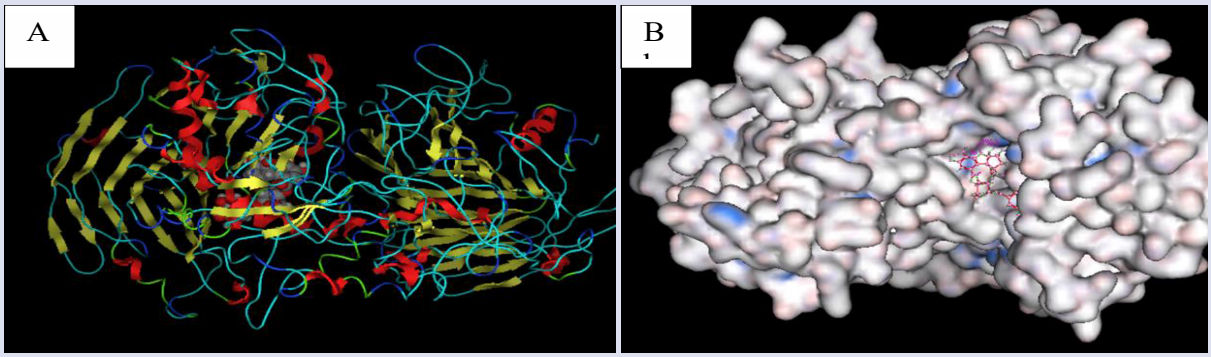

Figure 5. A. Acaindinin. B. Model binding of acaindinin and alpha glucosidase.

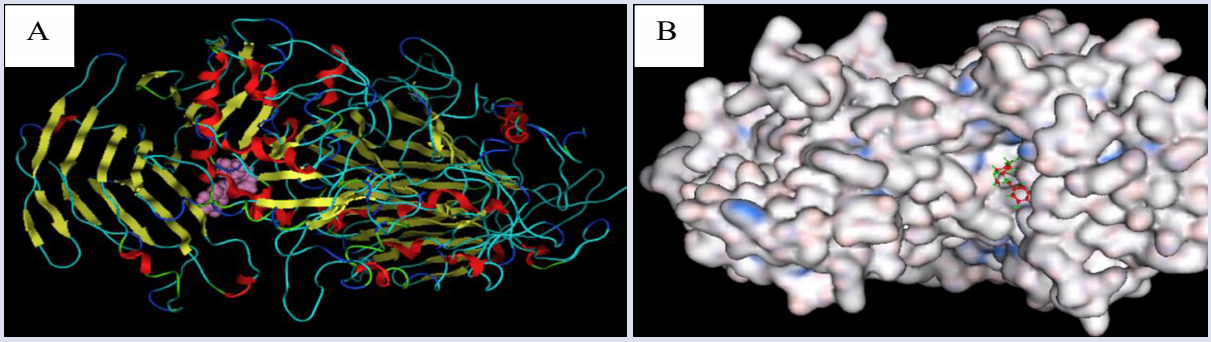

Figure 6. A. Glucogalin. B. Model binding of glucogallin and alpha glucosidase.

Table 4: Phytochemical screening result of Acalypha indica extract.

	Metabolite	Ethanol Extract	Ethyl Acetate Extract	n-Hexane Extract
1	Saponin	-	-	-
2	Flavonoid	+	+	-
3	Tannin	+	+	-
4	Glycoside	-	-	-
5	Triterpenoid	+	+	+
6	Steroid	-	-	-
7	Alkaloid	-	+	+

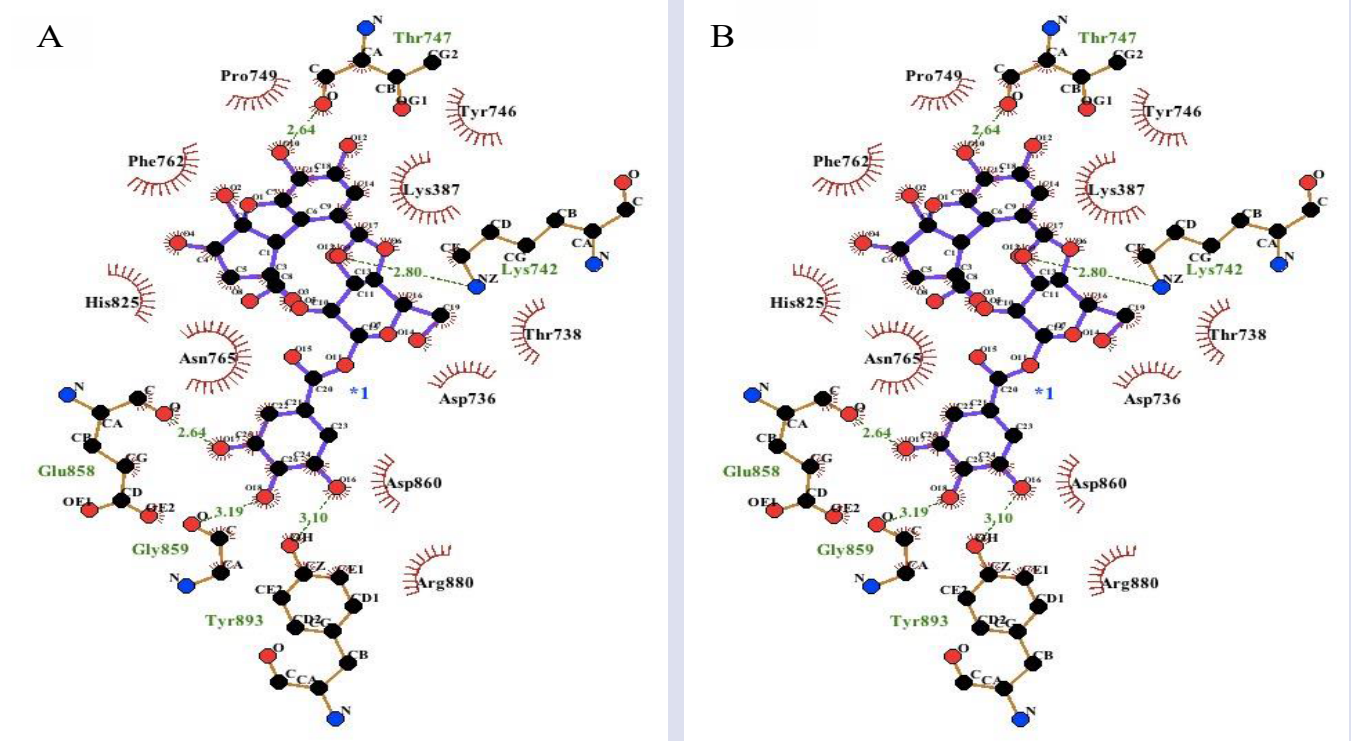

Figure 7. 2D Interaction between: A. acaindinin and B. glucogallin with alpha glucosidase.

From the Ai molecular docking study, we understood that repandusinic acid and mauritanin are Ai compounds that have strong interactions with leptin. The strong binding between ligan and protein in molecular docking is described by lowest ΔG, highest pKI and many hydrogen bonds between them. Repandusinic acid has the lower **ΔG** (-7.3495kcal/ml), the stronger pKI (10, 237) and more hydrogen bonds (7 hydrogen bonds) compare to mauritanin (**ΔG** -5,6617 kcal/ml, pKI 7,886, and 5 hydrogens bonds). Either repandusinic acid and mauritanin has a perfect conformation site to inhibit leptin receptors. Repandusinic acid is the main alkaloid of *Phyllanthus amarus* medicinal plant. It was confirmed to have a wide range of biopotential for many disease complications, which includes radical scavenging activity.[14]

Phil Scherer *et al*. reported that in mice with diet-induced obesity, a partial reduction of circulating leptin either by genetic approaches or by a leptin neutralizing antibody restores sensitivity of the body to the remaining circulating leptin, resulting in weight loss and improvements in diabetes. Thus, he proposed a major conceptual shift to the treatment of obesity; namely, rather than treating obese individuals with exogenous leptin, the appropriate approach would be to induce a partial reduction in endogenous circulating leptin by treatment with leptin neutralizing antibody. This avenue might constitute a promising antiobesity and antidiabetic clinical therapy in the near future.[15] On the other hand, there is only limited research in herbal medicine which makes leptin reduction as a target for obesity therapy. Despite using leptin neutralizing antibody which has a very complicated procedure, herbal medicine is a promising therapy to improve obesity through leptin reduction. This molecular study showed us that Ai as herbal medicine can be developed as an antiobesity that works on leptin reduction mediated by repandusinic acid and mauritanin as Ai compounds.

Molecular Study of Ai to Alpha glucosidase

One strategy that has been developed to treat type-2 diabetes, which usually occurs in obesity complication, is inhibition of alpha-glucosidase activity using synthetic drugs. However, these inhibitors are usually associated with gastrointestinal side effects.[11] Therefore, the development of alpha glucosidase inhibitors from natural products offers an alternative option for the control of hyperglycemia. In recent years, various studies have been conducted to identify alpha-glucosidase inhibitors from plants and many candidates have transpired to be secondary metabolites including alkaloids, flavonoids, phenols, and terpenoids. Among these compounds, flavonoid is considered to be responsible for the inhibitory activity of alpha glucosidase because it has a strong antioxidant effect.[11]

Based on the results of the Ai molecular docking study; hesperetin, acaindinin and glucogalin are compounds that have strong bindings to alpha glucosidase on the active site. Among these three compounds, hesperitin has the lowest **ΔG** (-7.2135 kcal/ml) and the strongest pKI (11,673) than acaindinin and glucogalin. Acaindinin has the moderate **ΔG** (-6, 7586kcal/ml) and pKI (10,331) than hesperetin and glucogalin. Hesperetin had 7 hyrogen bonds compared to acaindinin with 5 hydrogen bonds and glucogalin 9 hydrogen bonds. This data showed us that glucogalin has the highest hydrogen bonds to alpha glucosidase, despite the fact that it has the highest **ΔG** (-5.1502 kcal/ml) and the lowest pKI (9,823) compared to hesperetin and acaindinin. In overall, these three compounds have the most suitable conformation site to inhibiting alpha glucosidase action compare to other compounds of Ai.

From the literature review we found that hesperetin, acaindinin and glucogalin are the member of phenolics or flavonoids compounds. Hesperetin is a natural compound belonging to the flavanone class of flavonoids. It is the aglycone of hesperidin (β-7-rutinoside of hesperetin), a predominant flavonoid component of citrus fruits. Although hesperidin exhibits a wide range of biological activities, its aglycone, hesperetin, has a greater bioactivity as a result of more efficient absorption from the intestine, than hesperidin.[16] Acaindinin is a phenolic compound of Ai, first isolated from Ai acetone extract in 1997 by Chinese researcher and it has only limited information.[17] On the other hand, glucogalin is chemical compound formed

from gallic acid and β-D-glucose. It is a major component of *Emblica officinalis* (gooseberry) fruits which have been used for thousands years as a traditional Indian Ayurvedic preparation for the treatment of diabetes in humans. Gooseberry extracts which contain glucogalin have been shown to be efficacious against the progression of cataract in a diabetic rat model.[18] These data gave a strong suggestion that Ai may have inhibition activity to alpha glucosidase which is mediated by its polyphenol/flavonoid compounds: hesperetin, acaindinin and glucogalin.

To strengthen the molecular docking results of Ai to alpha glucosidase, we performed an *in vitro* alpha glucosidase assay to Ai. *In vitro* alpha glucosidase assay is a parameter that can predict the capability of extract to reduced intestinal glucose assimilation that can attenuate hyperglycaemia. The IC_{50} value of Ai to alpha glucosidase (19,429 mg/ml) showed that Ai can competitively inhibit the alpha-glucosidase enzyme. This result is in accordance with Saha *et al.* and Masih *et al.* which showed that Ai can decrease the sugar blood level in diabetic rats.[18,19] The result of Saha *et al.* exhibited a potent hypoglycemic effect of Ai in streptozotocin-induced diabetic rats given for 28 days. On the other hand, Masih *at el.* showed the same result of Ai as an antihyperglycaemia in alloxan-induced diabetic Wistar rats.[19]

Phytochemical Compound and α-Glucosidase Inhibitory Activity

Differences of compounds found among the extracts are caused by the polarity of the solvent and solubility of the samples.[20-22] Phytochemistry screening of Ai extract in this study showed that Ai contain flavonoid in ethanol and ethyl acetate extract, but not in n-Hexane extract, due to the flavonoid polarity to ethanol and ethyl acetate. Phytochemical test showed that triterpenoid was found in three extracts, flavonoid and tannin found in ethanol and ethyl acetate extracts. Both ethyl acetate and n-hexane contain alkaloid. These phytochemical compounds can be potentially used in diabetes treatment. Flavonoids were reported to inhibiting the alpha-glucosidase activity.[23-25] Several studies showed that triterpenes isolated from plants inhibit α-glucosidase and α-amylase.[26-28] These inhibition activities were also reported from tannin and alkaloid compounds.[29,30] These compounds found in the extract supported the inhibition activity of the extract. The IC_{50} of Ai ethanol extract to alpha glucosidase was 19,429 mg/ml compared to IC_{50} of Acarbose which was 1,515 mg/ml. The smaller the IC_{50}, the better inhibition activity of the extract towards α-Glucosidase. Hence the activity from the Ai extract was not as potent as acarbose. However, the Ai extract was still considered as an antidiabetic agent active category based on its IC_{50} value compared to the reference.[31]

Ai as Promising Herbal Medicinal to Improve Obesity

Based on this molecular docking study, we found that Ai can inhibit leptin and alpha glucosidase which play a role in obesity. This makes Ai a suitable herbal candidate to be developed as an antiobesity considering obesity is related to various pathways directed at various types of cells in various stages of the disease process. Further research should do to explore another pathways that Ai could improve in obesity condition.

CONCLUSSION

The molecular study of Ai to leptin and alpha glucosidase, complemented by *in vitro* alpha glucosidase assay, suggest that Ai has a strong potency to be developed as antihyperglycaemia-antiobesity. This suggestion requires further research *in vitro*, *in vivo*, and in clinical trials.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENT

This study was supported by Universitas Indonesia research grant under the scheme "International Indexed Publication Universitas Indonesia 2020" (PUTI UI 2020).

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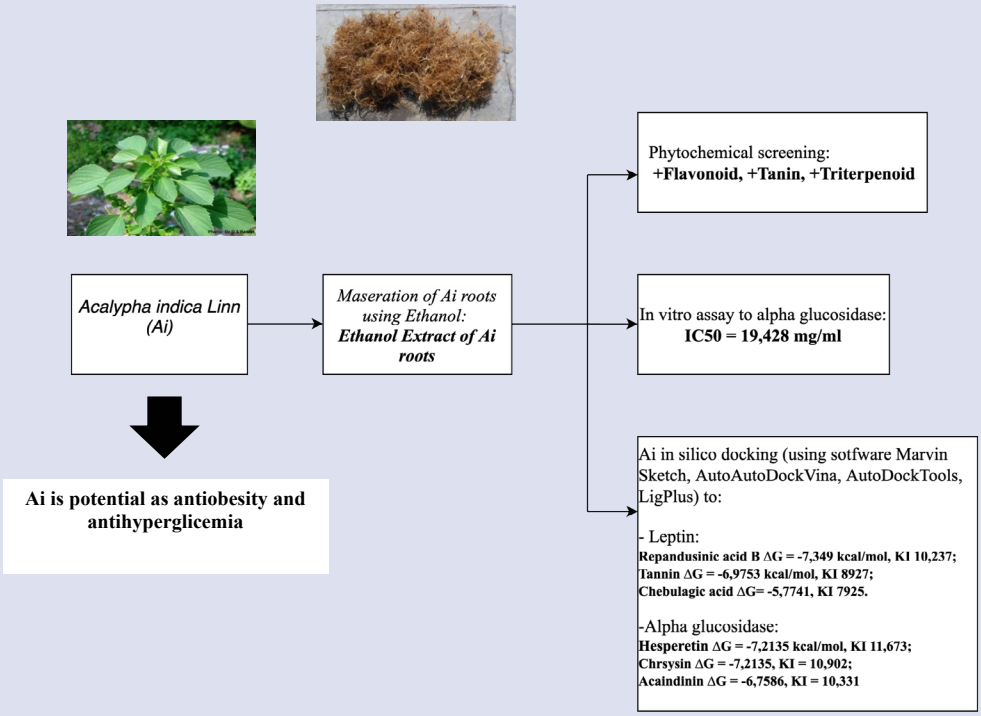
GRAPHICAL ABSTRACT



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Cite this article: Hakim RW, Fadilah F, Tarigan THE, Jusman SWA, Purwaningsih EH. Molecular Study of *Acalypha indica* to Leptin, Alpha Glucosidase, and its Antihyperglycemic Effect on Alpha Glucosidase. Pharmacogn J. 2021;13(6)Suppl: 1639-1647.