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Molecular and Cellular Biomedical Sciences, Vol.7 No.1, March 2023, p.38-46
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i1.295

RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2023; 7(1): 38-46
DOI: 10.21705/mcbs.v7i1.295

Molecular Docking of *Citrus amblycarpa* Active Compounds against FTO, Leptin, and Resistin Protein

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Background: *Citrus amblycarpa* has been known to have various pharmacological activities, such as antioxidants, anticancer, antitumor, hepatoprotective, anti-inflammatory, antidiabetic, antiviral, antibacterial, and antifungal. Hesperidin, naringin, quercetin, rutin, gamma (γ)-aminobutyric acid (GABA), neoeriocitrin, and poncirin from *C. amblycarpa* were the major constituents that potentially act on some obesity proteins, such as fat mass and obesity-associated (FTO) protein, leptin, and resistin, the emerging targets in the treatment of obesity. This study aimed to investigate the interaction between major active compounds of *C. amblycarpa* with FTO, leptin and resistin.

Materials and methods: The ligands of the docking study were seven major chemical compounds found in peel of *C. amblycarpa*, *i.e.*, hesperidin, naringin, quercetin, rutin, GABA, neoeriocitrin, and poncirin. FTO, leptin and resistin structure were taken from Protein Data Bank, while the *C. amblycarpa* compounds were prepared using Open Babel integrated into PyRx 8.0. Molecular docking simulation was performed using Autodock Vina integrated into PyRx 8.0. Virtual prediction and visualization of protein–ligand complexes were analyzed and visualized using Discovery Studio.

Results: All major compounds of *C. amblycarpa* peel used in this study did not have hepatotoxicity and AMES toxicity. Hesperidin had the lowest binding affinity score when interacted with FTO, leptin and resistin compared to other compounds. Moreover, GABA had the highest binding affinity score compared to other compounds.

Conclusion: Hesperidin may be a candidate obesity protein antagonist and may have potential as a treatment for obesity.

Keywords: *Citrus amblycarpa, molecular docking, FTO, leptin, obesity, resistin*

Introduction

Obesity is an alteration in several conditions that are characterized by the occurrence of excess fat in adipose tissue.[1] Data from Basic Health Research (*Riset Kesehatan Dasar*) 2018 shows that the prevalence of obesity in South Kalimantan in men and women aged >18 years are 12.72% and 26.64%.[2] Obesity is indicated by the body

Submission: July 5, 2022
Last Revision: August 7, 2022
Accepted for Publication: August 9, 2022

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mass index (BMI)> 25.0 kg/m^2 accompanied by abdominal circumference >90 cm for men and >80 cm for women.[3] If obesity is not treated properly, obesity will cause serious health impacts. Obesity increases the risk of chronic diseases, such as cardiovascular diseases, stroke, diabetes mellitus, muscle and bone disorders (osteoarthritis).[4,5]

There are several proteins that can be used as obesity markers, such as fat mass and obesity-associated (FTO), leptin, and resistin. FTO is an important protein that regulates the development and function of adipose tissue, which adjusts the size and composition of the body.[6] One of the FTO genetic variants is rs993960, which is known to have a strong relationship with the level of obesity.[7] Leptin regulates homeostasis of energy, which is obtained from food intake and related to the body mass. Besides, it plays a role in reproductive system, fetal growth, proinflammatory immune responses, angiogenesis and lipolysis.[8,9] Resistin is the main protein expressed in adipose tissue that modulates molecular pathways of metabolism, inflammatory, and autoimmune diseases. This hormone is highly expressed in patients with obesity, diabetes and cardiovascular diseases.[10] Based on its function, these biomarkers could be targeted by obesity therapy.

In *Citrus amblycarpa* peel, there are several active compounds that can act as anti-obesity. *Citrus* peel extract has been reported to inhibit the accumulation of lipids, suggesting its use as an obesity therapy agent.[11] Peel of *Citrus* contains several main phenolic compounds in the form of flavanones (hesperidin, neohesperidin, narirutin, and naringin) and polycocothoxlation flavones (nobiletin, sinensetin, and tangeretin).[12,13] Several studies have shown that hesperidin and naringin in *Citrus* may be used to treat obesity.

Phytochemical screening also proves the presence of glycoside content in *C. amblycarpa* ethanol extract. It is suspected that glycosides found in *C. amblycarpa* plants are flavonoid glycosides. Flavonoid glycosides that are found in large quantities in *Citrus* plants are neohesperidine, naringin, neoeriocitrin, and poncirin, which has a role in bitter taste of *Citrus*. In addition, aqueous extract from *C. amblycarpa* has been reported to contain phenolic, quercetin, rutin, and gamma (γ)-aminobutyric acid (GABA), which has been reported to inhibit angiotensin converting enzyme.[14]

Flavonoid glycosides have a tendency to form bonds with plasma protein, hence the higher concentration of these compounds, the longer it can last in blood plasma. This is one of the desired pharmacokinetic properties of a drug. Flavonoid glycosides in *Citrus* have various pharmacological activities such as antioxidants, anticancer, antitumor, hepatoprotective, antiinflamation, antidiabetic, antiviral, antibacterial, and antifungal.[15]

Currently, *in silico* studies using molecular docking is a popular technique for designing new drug. This technique is based on the prediction of the best bond pairs between ligand and macromolecules, consisting of several ligand conformations that are formed in target proteins.[16] Molecular docking is one of several solutions that help to uncover the mechanism of action of active compounds as obesity protein inhibitor agents. Therefore, this study aimed to investigate the interaction between major active compounds of *C. amblycarpa* with markers of obesity (FTO, leptin and resistin) to find novel anti-obesity candidates.

Materials and methods

Ligand and Protein Sampling

Three dimensional (3D) structure of FTOT (3LFM), leptin (1AX8) and resistin (1RH7) were taken from the Protein Data Bank (https://www.rcsb.org/). Structure of *C. amblycarpa* bioactive compounds and reference ligand (Orlistat; CID 3034010) were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Protein structures were downloaded in .pdb format, while ligands were downloaded in SDF format.

Protein and Ligand Preparation

Seven major chemical compounds found in peel of *C. amblycarpa*, *i.e.*, hesperidin (CID 10621), naringin (CID 442428), quercetin (CID 5280343), GABA (CID 119), Rutin (CID 5280805), neoeriocitrin (CID 114627), and poncirin (CID 442456) were used in this study. Obesity proteins were prepared using Discovery Studio version 16 (Dassault Systèmes BIOVIA, Vélizy-Villacoublay, France, 2020) to remove previously attached ligands, while ligands were prepared using Open Babel integrated into PyRx 8.0 (SourceForge, San Diego, CA) to minimize their energy and convert them to.pdb format.[17]

Docking and Visualization of Protein–Ligand Complex

Molecular docking simulation was performed using Autodock Vina integrated into PyRx 8.0. Virtual prediction analysis and visualization of protein–ligand complexes were performed using Discovery Studio. The interaction site was analyzed based on the ligand–residue interaction and structural conformation.

Molecular and Cellular Biomedical Sciences, Vol.7 No.1, March 2023, p.38-46
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i1.295

Drug-likeness, Pharmacokinetic, and Safety Predictions

Drug-likeness of *C. amblycarpa* bioactive compounds was evaluated on swissadme.ch. website. Pharmacokinetic and safety predictions were performed on http://biosig.unimelb.edu.au/pkcsm/. Pharmacokinetic parameters assessed in this study included absorption, distribution, metabolism, elimination, and toxicity (ADMET).

Results

Table 1 showed that the major compounds of *C. amblycarpa* did not have hepatotoxicity. Meanwhile, Orlistat had hepatotoxicity but did not have AMES toxicity. The molecular docking results of *C. amblycarpa* active compounds against FTO, leptin and resistin were presented in Table 2, while two dimensional (2D) interactions were shown in Supplementary Data. Similar interactions with binding site residue indicated that the compounds might exhibit inhibitory activity toward the obesity proteins.

We found that hesperidin had the lowest binding affinity score when interacted with FTO (-8.5 kcal/mol), leptin (-8.2 kcal/mol) and resistin (-8.1 kcal/mol) compared to other compounds. Low binding affinity score indicated that the interaction between ligand and target protein was stable. Hesperidin interacted with TRP452, ASN235, GLU373, and THR328 residues in FTO by forming hydrogen bonds. The interaction of hesperidin-leptin consisted mainly of hydrogen bonds, which formed in GLU81, ARG84, THR66, SER102, ALA101, and ALA59 residues. Hesperidin-resistin interaction also consisted mainly of hydrogen bonds, which formed in GLU20, LYS22, GLU20, LYS22, and LYS13 residues. Amino acid residue in obesity proteins that interacted with *C. amblycarpa* active compounds were presented in Table 3.

Naringin also had the lowest binding affinity score when interacted with resistin (-8.1 kcal/mol). SER17, GLU20, and LYS22 residues in resistin were interacted with naringin by forming hydrogen bonds. Naringin interaction with leptin and FTO had binding affinity scores of -7.2 and -8.0 kcal/mol, respectively. Hydrogen bonds were formed in SER120, TYR119, LEU39, and SER127 residues in the naringin-leptin complex. Meanwhile, hydrogen bonds were formed in TYR106, ARG322, ASN110, SER229, and GLN306 residues in the naringin-FTO complex.

The binding affinity of other glycosides, such as rutin, quercetin, neoeriocitrin and poncirin was slightly different compared to other compounds, ranged from -6.8 to -8.3 kcal/mol. However, both TRP42 and TYR39 residues in FTO were interacted with poncirin and Orlistat. Ligand interaction in GLN19 residue of resistin was not only found in Orlistat-resistin complex, but also found in interactions of resistin with glycosides. In addition, amino acid residues in leptin that interacted with either Orlistat or *C. amblycarpa* active compounds were not similar each other. Interestingly, GABA had the highest binding affinity score in all obesity proteins.

Discussion

According to previous *in vivo* studies, the main compounds of *Citrus i.e.*, hesperidin and naringin have many physiological effects, such as antioxidant, anticancer, and anti-inflammatory activities. These compounds also help to reduce cholesterol level.[18] To confirm the results of the *in vivo* studies, present molecular docking study were conducted to predict the interaction between FTO, leptin and resistin and several potential anti-obesity compounds from *C. amblycarpa*.

The results of the present study showed that hesperidin had the lowest binding affinity among all *C. amblycarpa* active compounds, suggesting that there was a strong interaction between hesperidin and the obesity proteins used in this study (FTO, leptin and resistin). Meanwhile, GABA had the weakest interaction with FTO, leptin and resistin, since it had the highest binding affinity score. Binding energy analysis is conducted to determine the spontaneity of a reaction and the stability of the macromolecule-ligand interaction. Stable interaction between target protein and ligand is indicated by low binding energies. Binding energy is proportional to the ability of a compound to form strong chemical bonds with its target. The more negative the binding energy value, the higher the tendency of the ligand to interact with its target.[19] GABA has been reported to have a small effect on inhibition of obesity protein. This can be attributed to the γ-aminobutyric acid (GABA) type B receptor (GABAB-R) which causes a proinflammatory effect. Previous study revealed that GABAB-R signaling in subcutaneous inguinal adipose tissue (IAT) might affect inflammatory responses as well as adipose tissue macrophages (ATM) infiltration.[20] However, the specific mechanism has not been fully understood.

In obesity, there is an increase in FTO expression, leptin resistance and an increase in resistin level. Bioactive compounds could act as inhibitors for expression of obesity

Table 1. ADMET analysis of *C. amblycarpa* active compounds and Orlistat.

Compounds	Physicochemical Properties	Water Solubility	Druglikeness	Pharmacokinetics
Hesperidin (ID : 10621)	Formula: $C_{28}H_{34}O_{15}$ Weight: 610.56 g/mol Num. heavy atoms: 43 Num. arom. heavy atoms: 12 Fraction Csp3: 0.54 Num. rotatable bonds: 7 Num. H-bond acceptors: 15 Num. H-bond donors: 8 Molar refractivity: 141.41 TPSA: 234.29 Å²	Log *S* (ESOL): -3.28 Class: Soluble Log *S* (Ali): -4.33 Class: Moderately soluble Log *S* (SILICOS-IT): -0.58 Class: Soluble	Lipinski: No Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD_{50}: 2.506 mol/kg Hepatotoxicity: No AMES toxicity: No
Naringin (ID : 442428)	Formula: $C_{27}H_{32}O_{14}$ Weight: 580.53 g/mol Num. heavy atoms: 41 Num. arom. heavy atoms: 12 Fraction Csp3: 0.52 Num. rotatable bonds: 6 Num. H-bond acceptors: 14 Num. H-bond donors: 8 Molar Refractivity: 134.91 TPSA: 225.06 Å²	Log *S* (ESOL): -2.98 Class: Soluble Log *S* (Ali): -3.82 Class: Soluble Log *S* (SILICOS-IT): -0.49 Class: Soluble	Lipinski: No Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD_{50}: 2.495 mol/kg Hepatotoxicity: No AMES toxicity: No
Quercetin (ID: 5280343)	Formula: $C_{15}H_{10}O_7$ Weight: 302.24 g/mol Num. heavy atoms: 22 Num. arom. heavy atoms: 16 Fraction Csp3: 0.00 Num. rotatable bonds: 1 Num. H-bond acceptors: 7 Num. H-bond donors: 5 Molar Refractivity: 78.03 TPSA: 131.36 Å²	Log *S* (ESOL): -3.16 Class: soluble Log *S* (Ali): -3.91 Class: Soluble Log *S* (SILICOS-IT): -3.24 Class: Soluble	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability: 0.55	Predicted LD_{50}: 2.471 mol/kg Hepatotoxicity: No AMES toxicity: No
GABA (ID: 119)	Formula: $C_4H_9NO_2$ Weight: 103.12 g/mol Num. heavy atoms: 7 Num. arom. heavy atoms: 0 Fraction Csp3: 0.75 Num. rotatable bonds: 3 Num. H-bond acceptors: 3 Num. H-bond donors: 2 Molar Refractivity: 25.82 TPSA 63.32 Å²	Log *S* (ESOL): -1.72 Class: Highly soluble Log *S* (Ali): -2.41 Class: Highly soluble Log *S* (SILICOS-IT): -0.04 Class: Soluble	Lipinski: Yes Ghose: No Veber: Yes Egan: Yes Muegge: No Bioavailability: 0.55	Predicted LD_{50}: 1.642 mol/kg Hepatotoxicity: No AMES toxicity: No
Rutin (ID: 5280805)	Formula: $C_{27}H_{30}O_{16}$ Weight: 610.52 g/mol Num. heavy atoms: 43 Num. arom. heavy atoms: 16 Fraction Csp3: 0.44 Num. rotatable bonds: 6 Num. H-bond acceptors: 16 Num. H-bond donors: 10 Molar Refractivity: 141.38 TPSA 269.43 Å²	Log *S* (ESOL): -3.30 Class: Soluble Log *S* (Ali): -4.87 Class: Moderately soluble Log *S* (SILICOS-IT): -0.29 Class: Soluble	Lipinski: No Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD_{50}: 2.491 mol/kg Hepatotoxicity: No AMES toxicity: No

Molecular and Cellular Biomedical Sciences, Vol.7 No.1, March 2023, p.38-46
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i1.295

Table 1. ADMET analysis of *C. amblycarpa* active compounds and Orlistat (cont.).

Compounds	Physicochemical Properties	Water Solubility	Druglikeness	Pharmacokinetics
Neoeriocitrin (ID: 114627)	Formula: $C_{27}H_{32}O_{15}$ Weight: 596.53 g/mol Num. heavy atoms: 42 Num. arom. heavy atoms: 12 Fraction Csp3: 0.52 Num. rotatable bonds: 6 Num. H-bond acceptors: 15 Num. H-bond donors: 9 Molar Refractivity: 136.94 TPSA 245.29 Å²	Log *S* (ESOL): -2.85 Class: Soluble Log *S* (Ali): -3.87 Class: Soluble Log *S* (SILICOS-IT): -0.10 Class: Soluble	Lipinski: No Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD_{50}: 2.487 mol/kg Hepatotoxicity: No AMES toxicity: No
Poncirin (ID: 442456)	Formula: $C_{28}H_{34}O_{14}$ Weight: 594.56 g/mol Num. heavy atoms: 42 Num. arom. heavy atoms: 12 Fraction Csp3: 0.54 Num. rotatable bonds: 7 Num. H-bond acceptors: 14 Num. H-bond donors: 7 Molar Refractivity: 139.38 TPSA 214.06 Å²	Log *S* (ESOL): -3.21 Class: Soluble Log *S* (Ali): -3.93 Class: Soluble Log *S* (SILICOS-IT): -1.18 Class: Soluble	Lipinski: No Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.17	Predicted LD_{50}: 2.545 mol/kg Hepatotoxicity: No AMES toxicity: No
Orlistat (ID: 3034010)	Formula: $C_{29}H_{53}NO_5$ Weight: 495.73 g/mol Num. heavy atoms: 35 Num. arom. heavy atoms: 0 Fraction Csp3: 0.90 Num. rotatable bonds: 24 Num. H-bond acceptors: 5 Num. H-bond donors: 1 Molar Refractivity: 145.36 TPSA 81.70 Å²	Log *S* (ESOL): -7.60 Class: Poorly soluble Log *S* (Ali): -11.60 Class: Insoluble Log *S* (SILICOS-IT): -7.97 Class: Poorly soluble	Lipinski: Yes Ghose: No Veber: No Egan: No Muegge: No Bioavailability: 0.55	Predicted LD_{50}: 1.972 mol/kg Hepatotoxicity: Yes AMES toxicity: No

proteins. An *in vivo* study shows that hesperidin significantly reduced leptin level in serum and tissue, as well as some inflammatory markers, such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) compared to Orlistat.[21] Hesperidin could stimulate the release of cholecystokinin (CCK) and other hormones in secretin tumor cell line-1 (STC-1) cells, hence suppressing appetite. Administration of high doses of hesperidin regulates the expression of adenosine 5'-monophosphate (AMP), which activates mRNA protein kinase, hence affecting insulin signaling pathways and activating peroxisome proliferator-activated receptor (PPAR)-α expression. As an anti-obesity candidate, hesperidin regulates genes, cytokines, adipokines, and the like in lipid metabolism to reduce fat accumulation.[15]

Naringin is a flavonoid compound contained in the peel of *C. amblycarpa* which also has anti-obesity properties. Naringin reduces LDL cholesterol and triglycerides in animals and humans.[22] Naringin plays a role in regulating metabolism, such as fatty acids and cholesterol metabolism by utilizing glucose-regulating enzymes. Previous study shows that 100 mg/kg/day naringin improves glucose intolerance and reduces plasma lipid concentrations in rats with diet-induced obesity.[23] Flavonoid compounds, such as naringin and hesperidin has been reported to reduce the concentration of triacylglycerol by 30%. In addition, naringin and hesperidin has been reproted to increase plasma antioxidant activity and significantly inhibit the increase in plasma lipids. Therefore, hesperidin and naringin in *C. amblycarpa* peel may have a potential to reduce plasma lipids, and play an important role as antioxidants.[22]

Orlistat inhibits fat absorption from triglyceride hydrolysis by inhibiting lipase[24], which reduces and

Table 2. Molecular docking results of *C. amblycarpa* active compounds and Orlistat against obesity proteins.

Parameters			Protein		
			FTO	Leptin	Resistin
Autogrid	Center (Å)	X	21.7625	57.9802	10.3221
		Y	-12.4296	-31.1269	32.8082
		Z	-28.4808	4.5009	70.8399
	Dimensions (Å)	X	73.9912	31.9733	46.7603
		Y	57.0589	38.7809	57.0854
		Z	66.9995	44.3399	145.2301
Binding affinity (kcal/mol)	Hesperidin		-8.5	-8.2	-8.1
	Naringin		-8.0	-7.2	-8.1
	Quercetin		-7.8	-7.0	-7.1
	GABA		-3.9	-3.6	-3.6
	Rutin		-8.3	-6.8	-7.9
	Neocitrin		-8.0	-7.3	-7.5
	Poncirin		-8.1	-7.0	-7.9
	Orlistat		-5.5	-4.4	-5.4

prevents obesity prevalence. Previous research revealed that Orlistat reduces the risk of cardiovascular disease in overweight and obese patients.[25] Docking results showed that binding affinity scores of Orlistat with obesity proteins were higher compared to 6 compounds from *C. amblycarpa* (hesperidin, naringin, quercetin, rutin, neoeriocitrin and poncirin). ADMET test results also showed that Orlistat had a hepatotoxicity effect compared to other active compounds (Table 1).

FTO is an essential protein in adipogenesis. Increased FTO expression in 3T3-L1 preadipocyte cells derived from mice has been reproted to increase adipogenesis process, followed by a decrease in N^6-methyladenosine (m^6A) levels. This preadipocyte differentiation process is mediated by PPARγ. FTO gene knock-down using small interfering ribonucleic acid (siRNA) in 3T3-L1 preadipocyte culture cells causes a decrease in preadipocyte differentiation, followed by a decrease in PPARγ and CCAAT/enhancer-binding protein α (C/EBPα) mRNA levels, which are regulators of adipogenesis.[26] FTO is a key protein in body mass composition. It also regulates the activity of dopaminergic circuitry. Therefore, if there is a malfunction in this activity, it will impact food intake rates, as evidenced by uncontrolled overeating and increased food responsiveness.[27-30] Inhibition of FTO expression will normalize dopamine activity in order to control food intake.

Leptin is mostly produced by fat cells. In obesity, numerous cellular processes attenuate leptin signaling. As a result, high expression of leptin is still unable to provide an energy adequacy response to the body. Leptin mRNA levels in adipose tissue and serum leptin are positively correlated with total body fat mass.[27] An increase in free leptin levels is correlated with a decrease in adiponectin levels. An increase in free leptin levels is correlated with a decrease in adiponectin levels, which cause macrophages accumulation by migrating to adipose tissue and will increase macrophage adhesion to vascular endothelial cells.[31,32] High levels of leptin in the blood indicates leptin resistance as an effect of reduced tissue sensitivity to leptin. Sulforaphane has been reported to stabilize leptin receptor through nuclear factor erythroid-derived 2-like 2 (NRF2)-dependent pathway, hence hyperleptinemia can be resolved. There will be a suppression of fatty acid synthesis, reduction of reactive oxygen species (ROS) accumulation and recovery of inflammation. This phenomenon describes a special transcriptional program that leads to obesity protection.[33]

Additionally, preventive action through resistin open control against obesity progression. In Hispanic subjects, higher resistin levels are associated with higher adiposity and lower insulin sensitivity.[34] Several experiments and clinical studies have shown that resistin is associated with activated insulin resistance via the toll-like receptor (TLR)-4 signaling pathway in obese patients.[35]

Molecular and Cellular Biomedical Sciences, Vol.7 No.1, March 2023, p.38-46 Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i1.295

Table 3. Results of molecular interaction analysis.

Protein	Ligand	Chemical Interactions
FTO	Hesperidin	**Hydrogen bond:** TRP452, ASN235, GLU373, THR328 **Pi:** LEU448, GLU377, LYS107 **Unfavorable donor-donor:** ARG112
	Naringin	**Hydrogen bond:** TYR106, ARG322, ASN110, SER229, GLN306 **Pi:** TYR108, HIS231, LEU109
	Quercetin	**Hydrogen bond:** ARG96, TRP230, SER229, TYR106 **Pi:** ASP233, HIS231, LEU109, TYR108 **Unfavorable donor-donor:** GLU234, ARG322
	GABA	**Hydrogen bond:** SER240, LEU236 **Unfavorable donor-donor:** ALA303
	Rutin	**Hydrogen bond:** ARG112, THR328, LYS107 **Pi:** GLU369 **Unfavorable donor-donor:** ARG337, ASN372
	Neoeriocitrin	**Hydrogen bond:** ARG112, THR328, ARG337, LYS107, SER441 **Pi:** GLU377, GLU373
	Poncirin	**Hydrogen bond: TYR39**, ARG52, GLU53 **Pi: TRP42** **Alkyl:** LEU157
	Orlistat	**Hydrogen bond:** PRO47, GLN43 **Pi:** TRP42, TYR39 **Alkyl:** LEU51, ILE50, PRO33
Leptin	Hesperidin	**Hydrogen bond:** GLU81, ARG84, THR66, SER102, ALA101, ALA59 **Pi:** LEU107, GLN63
	Naringin	**Hydrogen bond:** SER120, TYR119, LEU39, SER127 **Pi:** ILE24, VAL123
	Quercetin	**Hydrogen bond:** SER127, ASN22 **Pi:** ILE24, VAL123, LEU126
	GABA	**Hydrogen bond:** SER52, PRO99, GLN56, SER102
	Rutin	**Hydrogen bond:** GLY103, ARG84, ALA59, GLN62, GLU81 **Pi:** GLN63 **Unfavorable acceptor-acceptor:** ALA101
	Neoeriocitrin	**Hydrogen bond:** GLN130, SER127, TYR119, SER120, SER70, ASN72 **Pi:** VAL123, ILE24
	Poncirin	**Hydrogen bond:** GLN130, LEU39, SER127, ILE24, ASN22 **Pi:** VAL123
	Orlistat	**Hydrogen bond:** GLY44, PRO47, GLN139 **van der Waals:** TRP138 **Pi:** PHE41 **Salt bridge:** ASP135
Resistin	Hesperidin	**Hydrogen bond:** GLU20, LYS22, GLU20, LYS22, LYS13 **Pi:** ILE12, LEU16, GLN19
	Naringin	**Hydrogen bond:** SER17, GLU20, LYS22 **Pi:** PRO21 **Unfavorable donor-donor:** GLN19
	Quercetin	**Pi:** LYS22, PRO21, MET81, LYS22 **Unfavorable donor-donor:** GLN19
	GABA	**Hydrogen bond:** GLN67, SER75, THR30 **Pi:** TRP73
	Rutin	**Hydrogen bond:** GLU20, GLN19 **Pi:** LYS22, LYS22, PRO21 **Unfavorable donor-donor:** GLN19

Table 3. Results of molecular interaction analysis (cont).

Protein	Ligand	Chemical Interactions
Resistin	Neoeriocitrin	**Hydrogen bond:** ARG59, SER75, THR30, SER28, ILE58, HIS65 **Pi:** TRP73, GLN67
	Poncirin	**Hydrogen bond:** LYS22, GLN19 **Pi:** ALA15, ILE12
	Orlistat	**Hydrogen bond:** GLN19 **Alkyl:** PRO21, LYS22

In the present study, compounds interacting with obesity proteins showed varied results in binding affinity and type of interaction, which may depict the inhibition of obesity proteins, culminating in the lipolysis of fats that may primarily lead to reverse obesity. This study may predict the forthcoming treatment strategies for obesity.

Conclusion

Hesperidin has the lowest binding affinity score when it interacts with FTO, leptin, and resistin, suggesting its potential as an inhibitor of the obesity protein and its future use as an anti-obesity agent. Analysis of the structure-activity relationship indicates that the ligand-receptor binding energy is the parameter that most influenced the activity of the compound. Further pre-clinical research of *C. amblycarpa* active compounds is needed to validate the results of this *in silico* study.

Authors Contribution

RP and JPU were involved in concepting and planning the research and performed the data acquisition. MAB and NDM performed the data analysis. JPU, MAB and NDM designed the figures and tables and aided in interpreting the results. RP and JPU took parts in giving critical revision of the manuscript. All authors drafted the manuscript and have read and approved the final manuscript.

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Molecular and Cellular Biomedical Sciences, Vol.7 No.1, March 2023, p.38-46
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i1.295

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