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Profiling dan Analisis In Silico ekstrak Carica papaya terhadap Regulasi Nafsu makan

Profiling and In Silico Study of Carica papaya Extract on Appetite Regulation

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Abstrak

Carica papaya merupakan salah satu tanaman yang diketahui berkhasiat meningkatkan nafsu makan. Larutan daun papaya 15% dapat meningkatkan nafsu makan, namun pada pemberian larutan 20% terjadi penurunan berat badan. Daun Carica papaya mengandung papain, carpaine dan berbagai senyawa yang lain. Mekanisme yang mempengaruhi regulasi nafsu makan melibatkan ghrelin, leptin, ARC, NPY dan AgRP. Penelitian ini bertujuan mengetahui profil senyawa yang terdapat pada ekstrak daun papaya serta pengaruhnya terhadap regulasi nafsu makan yang di analisis secara insilico. Metode penentuan profiling senyawa ekstrak daun papaya dengan menggunakan UPLC/MS dan dianalisis dengan menggunakan watermaslinx, analisis in silico menggunakan metode molekuler docking menggunakan PatchDock. Hasil penelitian ini menunjukkan bahwa senyawa yang paling banyak terdapat pada ekstrak daun papaya (Carica papaya) adalah carpaine dengan konsentrasi sebesar 50,33 %. Hasil analisis in silico menunjukkan bahwa carpaine diprediksi dapat mempengaruhi baik dalam menurunkan nafsu makan maupun meningkatkan nafsu makan karena carpaine mempunyai ikatan yang stabil dengan ghrelin tetapi juga mempunyai energi ikatan yang kecil dengan leptin. Perlu dilakukan penelitian secara in vivo untuk mengetahui dosis carpaine yang tepat dalam menurunkan atau menaikkan nafsu makan

***Kata Kunci**: Carpaine, Leptin, ghrelin, molecular docking, nafsu makan*

Abstract

Carica papaya is one of the plants known to increase appetite. 15% papaya leaf solution can increase appetite, but if given in 20% solution there is a weight loss effect. Papaya leaves contain papain, carpaine and various other compounds. The mechanisms that affect appetite regulation involve ghrelin, leptin, ARC, IL-1β and NPY. This study aims to determine the compound profile contained in papaya leaf extract and its effect on appetite regulation that has been analyzed with in silico methods. The method of determining the profiling of papaya leaf extract compounds using UPLC / MS and analyzed using watermaslinx, in silico analysis used molecular docking methods using PatchDock. The results of this study are the most compounds found in papaya leaf extract (Carica papaya) is carpaine with a concentration of 50.33%. The results of in silico analysis show that carpaine is predicted to have an effect on both reducing appetite and increasing appetite because carpaine has a stable bond with ghrelin but also has a small binding energy with leptin. In vivo research needs to be carried out to find out the right dose of carpaine to reduce or increase appetite.

***Keywords**: Carpaine; leptin, ghrelin, molecular docking, appetite*

Introduction

Toddlerhood is a golden age or critical period, because during that time the growth and development of a human being is very rapid, especially brain development (Sakti, 2020). Nutritional deficiencies greatly affect children's brain development. One of the nutrition-related diseases that occur in toddlers is waste. Wasting occurs due to weight loss, or failure to gain weight due to acute lack of energy and protein intake (Harding et al., 2018). Deaths caused by waste accounted for 13% of all deaths in the world in 2015. The prevalence of wasting in children under five, ranges from 13% - 31% (WHO/UNICEF/WorldBank, 2021). Based on the Indonesian Health profile in 2021 (Ministry of Health Republic of Indonesia, 2022) under-fives who have a percentage of very underweight and underweight are 1.2 and 6.1. South Kalimantan is one of the provinces that has a percentage of very underweight and underweight above the Indonesian average, which is 1.8 and 8.6.

The Factors that cause wasting are insufficient nutritional intake, lack of appetite, infection (Karlsson et al., 2022; (Mulu et al., 2020). Giving appetite enhancing supplements is one way to address waste. One of the plants that could increase appetite is papaya (*Carica papaya*). (Fajria & Rika, 2013; Olii et al., 2020; Mulyaningsih et al., 2022; Ramdhan et al., 2015).

A Carica papaya leaf water solution concentration 15% could increase the body weight of rats but giving a 20% of carica papaya causes a decrease in body weight (Ardiansyah, 2016). The difference in concentration causes differences in the activity of papaya leaves, this is influenced by the content of nutritious substances in papaya leaves and regulation of appetite. Carica papaya contains carpaine, dihydrocarpaine, flavonols, papaine, tannin, and nicotine. The main alkaloid compound in papaya is carpaine (Rahayu et al., 2019).

Appetite regulation is influenced by the Leptin and Ghrelin hormones. Ghrelin is a hormone produced in the stomach (Diz-Chaves, 2011). Appetite regulation also involves several neuropeptides such as Agouti related Peptide (AgRP). AgRP works to stimulate food intake and cause weight gain. Neuropeptide Y (NPY) works to stimulate appetite. NPY is expressed in the same neurons as AgRP, but NPY is expressed in other regions of the hypothalamus and brain (Sohn, 2015; Inui et al., 2004; Essner et al., 2017). Leptin suppresses appetite and regulate energy expenditure (Espinoza-García et al., 2022). Until now, there has been no analysis of the compound content of *Carica papaya* leaf extract on appetite-enhancing activity and the mechanism of action of Carica papaya leaf extract on appetite within silico analysis.

Methods

Tools and Materials

Carica papaya leaves were collected from Martapura, Banjar Regency, South Kalimantan, 99% technical grade methanol for extraction, methanol (hypergrade for UPHPLC), acetonitrile (hypergrade for UPLC), and water injection 0.05% for UPLC, rotary evaporator (Eyela), Mass Spectrometer Xevo G2-S QToF (Waters, USA), LC system using ACQUITY UPLC H-Class system (Waters, USA), LC column used ACQUITY UPLC BEH C18 (1.8 µm 2.1 x 50 mm; Waters, USA)

Extraction of Carica papaya Leaves

Young Carica papaya leaves (light green color) were washed thoroughly and oven-dried at 45^{o}C for 2 days. The dried leaves were then pulverized using a blender and sieved. The fine powder obtained was then soaked with 99% methanol in a closed container for 3 x 2 days. The liquid extract was evaporated using a rotary evaporator until a thick extract was obtained, then the liquid of papaya leaves evaporated on a waterbath until a thick extract was obtained. The extract was ready for further tests (Putri et al., 2022)

Phytochemical Screening

The *Carica papaya* extract carried out phytochemical screening of flavonoids with alkaline and lead acetate reagent tests, alkaloids with Dragendorf and Mayer reagents, tannins with gelatin test, phenols with iron (III) chloride test, saponins with foam method, anthraquinones with anthraquinone test, steroids with Liebermann Buchard's test, terpenoids with Salkowski's test. (Isnaini et al., 2021)

Profiling of metabolite compounds in Carica papaya extracts

Compound profiling analysis was carried out at the National Police Criminal Investigation Unit, Forensic Laboratory Center Bogor. The viscous extract of *Carica papaya* was prepared by Water Oasis Sample Extraction with Solid Phase Extraction (SPE). UPLC analysis was performed at 50°C (column) and 25°C (room). LC analysis used mobile phases of water + 5 mM ammonium formate (A) and acetonitrile + 0.05% formic acid (B), with a flow rate of 0.2 mL/min (step gradient) running for 23 min and an injection volume of 5 µL (initially filtered through a 0.2 µm syringe filter). Mass spectrometry (MS) analysis was performed using electrospray ionization (ESI) in positive mode with a mass range of 50-1200 m/z and source and desolvation, at 100^{o}C and 350°C, respectively. In addition, conical and desolvation gas flow rates of 0 L/h and 793 L/h were used respectively, while the collision energy was varied between 4 and 60 eV. Masslynx software version 4.1 was used for data acquisition and analysis as well as instrument control (Ismed et al., 2022).

In silico Analysis

1. Scores

The score obtained represents the geometric shape complementarity score of the docking solutions. It is a quantitative measure used to assess how well the shapes of the ligand (a small molecule) and the receptor (a protein) molecules fit together in the complex formed during docking. The score considers factors such as surface contours, protrusions, and concavities of the interacting molecules. A higher score indicates better shape complementarity between the ligand and receptor, suggesting a more favorable interaction and a higher likelihood of forming a stable complex. The solutions generated by PatchDock are sorted based on this score, with the top-ranked solutions considered to have the best shape complementarity and are more likely to represent biologically relevant binding modes (Duhovny et al., 2002).

2. Area

Area parameters refers to the approximate interface area of the complex formed by the docked ligand and receptor molecules. It provides a quantitative measure of the surface area in contact between the two molecules in their docked conformation. The interface area is calculated by identifying the regions where the

ligand and receptor come into close proximity and have significant intermolecular interactions. It represents the extent of molecular contact and interaction between the two molecules within the complex. A larger interface area generally indicates a more extensive and intimate interaction between the ligand and receptor. It suggests a greater number of amino acid residues or functional groups from the receptor and atoms from the ligand being involved in specific binding interactions such as hydrogen bonding, Van der Waals interactions, or electrostatic interactions (Schneidman-Duhovny et al., 2005)

3. ACE

ACE (Atomic Contact Energy) is a parameter used in PatchDock that stands for Atomic Contact Energy. It is a measure of the desolvation free energy required for transferring atoms from water to the interior of the protein in the docked complex. The estimation of ACE involves determining effective atomic contact energies for different atom types, which are resolved based on the clustering of their properties in the 20 common amino acids. The method considers various factors, including the reference state (random crystal structure) to remove compositional bias and a scaling factor to make the contact energies comparable with experimentally measured energies. By calculating ACE, it becomes possible to evaluate the free energies associated with transferring sidechains from the protein interior to water. Comparisons with experimental data have indicated that the magnitude of protein-to-water transfer free energies for hydrophobic sidechains is larger than that of n-octanol-to-water transfer free energies. This finding is consistent with previous observations and experiments on protein unfolding and mutant substitutions. Additionally, ACE can be used to calculate and compare binding free energies of protease-inhibitor complexes. This involves creating a full free energy function by incorporating direct electrostatic interactions, an appropriate entropic component, and the solvation free energy term. The calculated free energies have shown good agreement with observed values, suggesting that ACE can provide an accurate and efficiently computable solvation component of free energy (Zhang et al., 1997)

4. Transformation

Transformation indeed refers to the 3D transformation parameters required to position the ligand molecule in the docked conformation relative to the receptor molecule. The transformation consists of three rotational angles and three translational parameters, collectively known as the 6-degree-of-freedom (6-DOF) transformation. The rotational angles define the rotation of the ligand molecule around the X, Y, and Z axes in three-dimensional space. These rotations determine the orientation of the ligand relative to the receptor. Each rotational angle represents the amount of rotation around a specific axis. The translational parameters represent the displacement of the ligand molecule in three dimensions - X, Y, and Z. They specify the movement of the ligand along each axis, allowing it to be positioned at a specific location within the receptor's binding site. By adjusting the rotational angles and translational parameters of the transformation, PatchDock explores different orientations and positions of the ligand with respect to the receptor. It searches for the optimal combination of these parameters that maximizes the shape complementarity and interaction between the ligand and receptor molecules (Yunta, 2016).

Example: 0.1 -0.2 -0.3 1.2 -1.5 1.8

0.1 -0.2 -0.3 1.2 -1.5 1.8, represents the 6-degree-of-freedom (6-DOF) transformation parameters for the ligand molecule in the docked conformation relative to the receptor molecule. Let's break down the result:

- Rotational angles:
    - Rotation around the X-axis: 0.1
    - Rotation around the Y-axis: -0.2
    - Rotation around the Z-axis: -0.3

These values specify the amount of rotation, in degrees, around each of the three axes: X, Y, and Z. They determine the orientation of the ligand molecule relative to the receptor.

- Translational parameters:
    - Displacement along the X-axis: 1.2
    - Displacement along the Y-axis: -1.5
    - Displacement along the Z-axis: 1.8

These values represent the amount of displacement, in units of distance (e.g., Angstroms), along each of the three axes: X, Y, and Z. They indicate how the ligand molecule is shifted in three-dimensional space relative to the receptor.

Together, these six parameters (three rotational angles and three translational parameters) define the transformation required to accurately position the ligand molecule in the docked conformation with respect to the receptor molecule.

5. Conclusion

Based on the information provided, the ACE (Atomic Contact Energy) is a parameter that represents the desolvation free energies required to transfer atoms from water to the interior of the protein. In the context of PatchDock results, a more negative ACE value indicates a more favorable interaction between the ligand and receptor molecules. Therefore, in PatchDock results, a more negative ACE value can be considered as a favorable outcome. It suggests stronger interactions and a better fit between the ligand and receptor in terms of atomic desolvation energies. Other factors such as the overall score, interface area, and transformation parameters also contribute to the quality of the docking prediction. In summary, while a more negative ACE value in PatchDock results can indicate a more favorable interaction, it is essential to consider multiple parameters and evaluate the overall quality and reliability of the docking solutions. ACE used when assessing the specific energetic contributions and desolvation effects in the docking complex. while Score is often considered for shape complementarity between the ligand and receptor (Lauritano et al., 2023).

The scientific method employed in this research project involved a series of systematic steps. Firstly, ligands and proteins relevant to the research question were identified by conducting thorough searches on databases such as RCSB.org and PubChem. The search criteria included specific structural features, functional properties, and biological relevance to the research focus. Next, the ligand structures were modified by inserting metal ions, such as calcium (Ca), iron (Fe), and zinc (Zn), using the BIOVIA Discovery Studio Visualizer. This software tool facilitated precise placement of the metal ions within the ligand molecules, ensuring accurate representation of the desired metal-ligand interactions. Subsequently, water molecules and inherent ligands were removed from the protein structure using PyMOL. This step aimed to isolate the protein molecule and eliminate

any potential interference or bias caused by solvent molecules or pre-existing ligands. By removing these components, the focus was solely on the interaction between the prepared ligands and the protein receptor. After these preparations, the ion-inserted ligands, non-inserted ligands, and their respective receptor proteins were prepared for docking simulations using PatchDock.

PatchDock operates on the principle of geometric matching, employing a scoring function to predict binding conformations between a ligand and receptor protein through rigid body docking simulations. This powerful software tool utilized algorithms and scoring functions to generate potential binding conformations between the ligands and receptors. The simulations considered the three-dimensional structures and molecular interactions, providing insights into the most favorable binding modes and potential affinity between the ligands and the receptor. By aligning complementary surface patches, the algorithm identifies favorable interactions and assigns scores based on shape complementarity, electrostatics, and desolvation energy. Finally, the docking results were visualized and analyzed using the BIOVIA Discovery Studio Visualizer. This software facilitated the examination and interpretation of the docking poses and interactions. The visual representation of the docked complexes offered valuable insights into the spatial arrangement of the ligands within the receptor's binding site, as well as the nature of the interactions, such as hydrogen bonding, hydrophobic contacts, or metal coordination.

Ethical Clearance

This study has received ethical approval from the Ethics Committe of the Faculty of Medicine Lambung Mangkurat University with the number 149/KEPK-FK ULM/EC/VII/2023

Results.

Phytochemical Screening

The results of phytochemical screening of *Carica papaya* extract can be seen in table 1.

Table 1. The Results of Phytochemical Screening of *Carica papaya* Extract

Compounds Identification	Testing	Result	Description
Flavonoid	Reagen Alkaline Reagent Test	+	The result was a greenish yellow and a greenish yellow faded after it been added dilute acid
	Lead Acetate Test	+	The result was a yellowish green precipitate (cloudy)
Alkaloid	Dragendroff Test	+	The result was a brownish red color
	Mayer Test	+	The result was a greenish yellow precipitate
Tannin	Gelatine Test	+	The result was a greenish yellow (cloudy) precipitate
Phenol	Iron (III) Chloride Test	+	The result was a yellow-brown precipitate (cloudy)
Saponin	Foam Method	-	The result was only a small of foam appears and lasts less than 1 minute after shaking
Anthraquinone	Anthraquinone Test	+	The result was a white precipitate
Steroid	Libermann Burchard's Test	+	The result was a green ring
Terpenoid	Salkowski's Test	+	The result was a dark green color

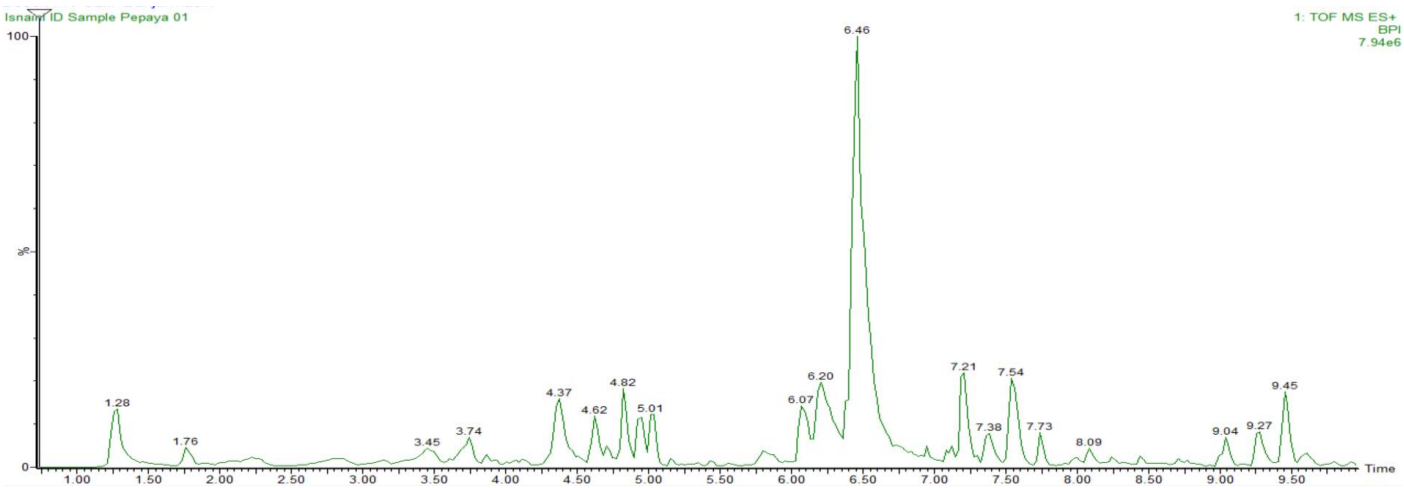

Figure 1. Compound Profiling from UPLCS MS Based on RT

Table 2. Analysis of Area

No	RT	Area	Length (%)
1	1.28	96.300	4,46
2	1.79	18.585	0,86
3	2.2	30.247	1,40
4	2.81	25.797	1,19
5	3.45	52.645	2,44
6	3.74	52.374	2,43
7	4.37	107.716	4,99
8	4.92	243.731	11,29
9	5.43	1.747	0,08
10	5.85	15.500	0,72
11	6.46	1.086.561	50,33
12	7.21	106.180	4,92
13	7.54	120.351	5,57
14	8.09	25.546	1,18
15	8.44	4.402	0,20
16	8.73	5.120	0,24
17	9.04	24.983	1,16
18	9.45	140.909	6,53
19	9.92	226	0,01

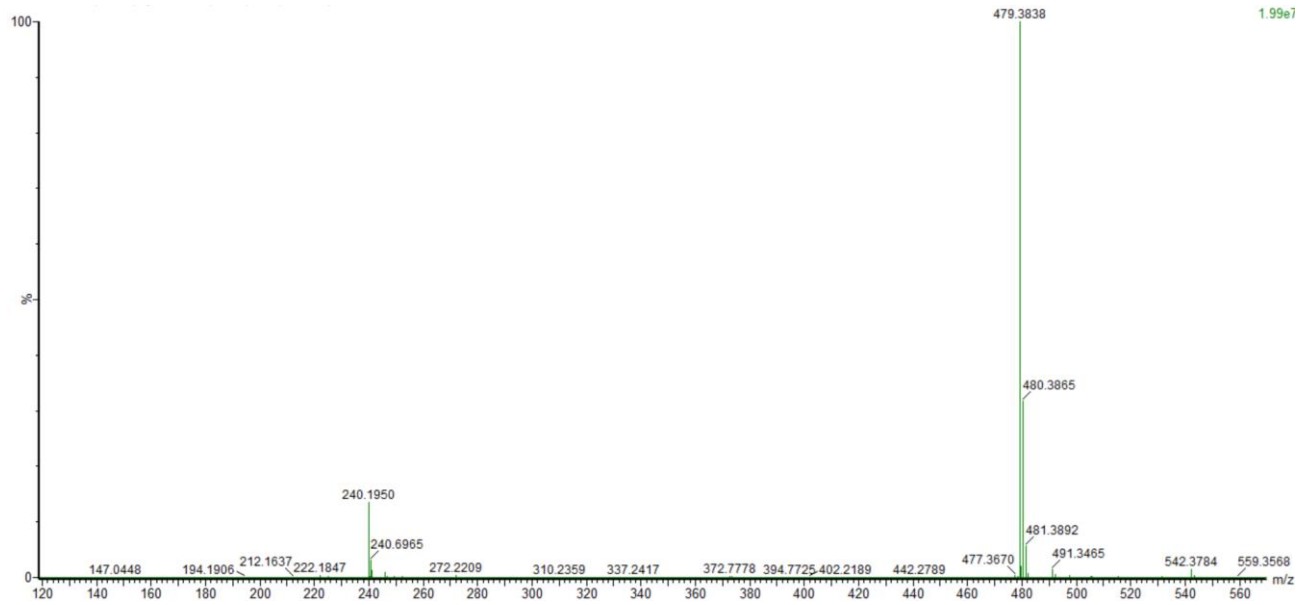

Figure 2. Element Spectrum Based on Molecular Weight (m/z)

Based on the results of analysis and area, it was known that the most abundant compound in the extract is carpaine with a concentration of 50.33% of all compounds in the extract with a molecular weight of 479.3838 m/z. The results of spectrum element analysis showed that the molecular formula of the compound was C28H51N2O4 (H+) with fit conf of 100%. Then analysis using chemspider that the compound that has C28H50N2O4 is carpaine with 46 references and 4 references on Pubmed. Furthermore, carpaine will be analyzed with in silico study to determine its ability to increase appetite by using ligands ghrelin, NPY (Neuropeptide Y), Leptin and ARC.

In Silico Analysis

The results of in silico analysis of carpaine with ghrelin, NPY (Neuropeptide Y), Leptin and ARC can be seen in table 3 below.

Table 3. In silico Analysis between Carpaine and Ghrelin, NPY, Leptin and ARC

Structure	Highest Score	Atomic Contact Energy (ACE) (KJ/mol)	Transformation	Area
NPY (5ZBH)	6002	-151.48	0.51 0.45 2.69; 17.47 20.94 -56.95	693.70
ARC (6TN7)	4542	-133.79	0.97 -0.42 -2.19; 6.79 -2.29 144.28	528.10
Ghrelin (7NA8)	6358	-121.24	-0.51 1.18 0.47; 124.76 134.88 94.15	761.50
Leptin (1AX8)	4650	-263.02	-2.30 -1.49 -0.27; 51.23 -22.67 -4.73	608.90
AgRP	3546	-53.40	0.45 0.35 -1.17 3.35 -1.21 -5.81	424.70

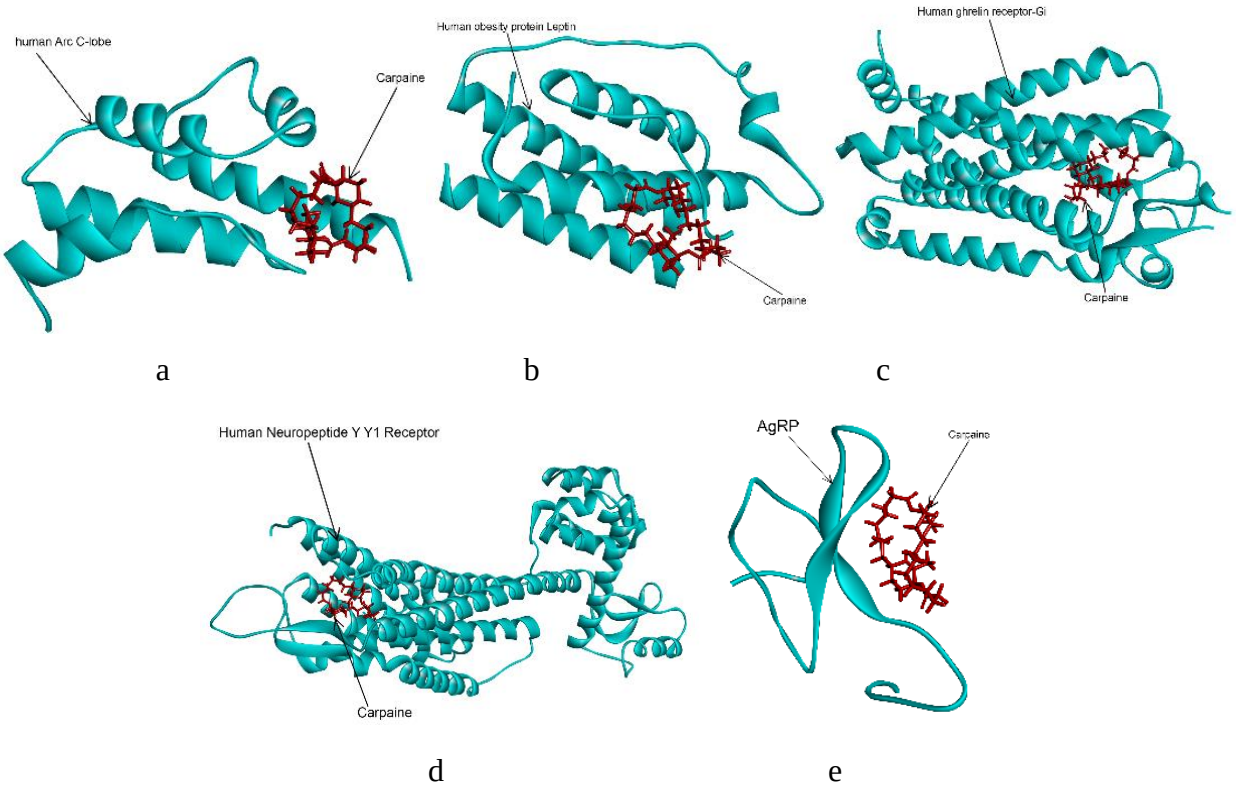

Figure 3. Molecular docking between carpaine and Ghrelin, NPY, Leptin, ARC and AgRP (a) carpaine – ARC, (b) Carpaine – Leptin, (c) Carpaine – Ghrelin, (d) Carpaine – NPY, € Carpaine – AgRP

The data presented in the table represents the docking results of Carpaine with the structures of NPY, ARC, Ghrelin, and Leptin. The highest score indicates the quality of the docking interaction, while the Atomic Contact Energy (ACE) represents the stability of the complex formed between Carpaine and each structure. The highest docking score is Ghrelin with 6358 score and the lower docking score is AgRP with 3546 score. Ghrelin has a strong binding affinity and potential with Carpaine in a stable manner and the other side, AgRP have a weak binding affinity with carpaine. Leptin has a lower ACE value, so carpaine will interact with leptin more easily than others. Besides that, AgRP indicated the lowest docking scores and lowest ACE than another structure. This shows AgRP interaction between Carpaine and AgRP may be weaker or less favorable.

Discussion

The highest docking score is Ghrelin with 6358 score and the lower docking score is AgRP with 3546 score. Ghrelin has a strong binding affinity and potential with Carpaine in a stable manner and the other side, AgRP have a weak binding affinity with carpaine. A higher score indicates better shape complementarity between the ligand and receptor, suggesting a more favorable interaction and a higher likelihood of forming a stable complex (Duhovny et al., 2002).

Leptin has a lower ACE value, so carpaine will interact with leptin more easily than others. ACE is the energy needed to move from water to protein, where the bond occurs. The less energy required, the easier it is for a compound to bind to its receptor (Ahmad & Komari, 2022).

Based on the area, it is known that ghrelin has the largest area compared to leptin, NPY, ARC, AgRP with an area of 761.50. A larger interface area generally indicates a broader and tighter interaction between ligand and receptor. This indicates that more amino acid residues or functional groups of the receptor and atoms of the ligand are involved in specific binding interactions such as hydrogen bonds, Van der Waals interactions, or electrostatic interactions. This large area shows that carpaine has a close bond with ghrelin (Schneidman-Duhovny et al., 2005).

Carpaine is an alkaloid compound found in *Carica papaya*. Carpaine is found in all parts of papaya, both in leaves, seeds, and root (Bukhori et al., 2014). Carpaine exhibits a strong potential for interaction with Leptin and NPY. Appetite is regulated by a complex physiological system. Several hormones involved in regulation appetite including ghrelin, NPY, and leptin (Armani et al., 2023). Ghrelin and NPY can stimulate appetite and leptin make satiesty and the other side leptin can inhibit appetite.

Ghrelin stimulates food intake and the release of growth hormone. Ghrelin is known as the "hunger hormone" (Pradhan et al., 2013). Leptin, a hormone released by adipose tissue, plays a crucial role in appetite regulation, they can inhibit appetite (Al-Hussaniy et al., 2021). Leptin acts on the hypothalamus to decrease the production of neuropeptide Y (Armani et al., 2023). Carpaine's interaction with NPY could impact it signaling pathways, potentially influencing hunger and satiety mechanisms (Inui et al., 2004; Beck, 2006).

The results of in silico analysis show that the carpaine contained in Carica papaya leaf extract can interact with leptin and ghrelin, so that carpain has 2 different activities, namely increasing and decreasing appetite. This is in accordance with research from Ardiansyah (2016) that giving carica papaya at a concentration of 15% increased body weight but at a concentration of 20% reduced body weight in mice. Further research needs to be done to determine carpaine levels and their effect on body weight.

Conclusion

Based on the results of the study, it can be said that *Carica papaya* extract can be used for diet programs by reducing appetite.

Conflict of Interest

No potential competing interest was reported by the authors.

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Author contribution

II provided the research idea, conducted the research, analyzed the results of compound profiling, and drafted the manuscript, WW conducted the research, and conducted proof reading of the manuscript, DS conducted the research, RYR assisted in the implementation of the research, KKI assisted in the implementation of the research, AAP conducted the insilico analysis.

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