

Uji Aktivitas Ekstrak *Mitragyna speciosa* Terenkapsulasi terhadap Tikus yang diinduksi Pakan Tinggi Lemak: Studi *In silico* dan *In vivo*

Activities Analysis of Encapsulated *Mitragyna speciosa* Extracts in High-Fat Diet-Induced Rats Model: *In vivo* and *In silico* Studies

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ABSTRACT

Background: UCP-1 deficiency can be addressed through the activation of thermogenesis using various herbs, including *Mitragyna speciosa* (*M. speciosa*). **Purpose:** This study aims to evaluate the efficacy of *M. speciosa* extracts, encapsulated in nanoparticles, in stimulating the activity of UCP-1. **Methods:** The research utilized an *in silico* approach to test the docking of Mitragynine and epinephrine ligands against $\beta 1$ and $\beta 3$ adrenergic receptors. The nanoparticles were prepared using chitosan, alginate, and polyethylene glycol (PEG). An *in vivo* study was performed utilizing Wistar rats subjected to a high-fat diet (HFD) and assigned to six groups: Normal, HFD, HFD + MG50, HFD + Npm5, HFD + Npm10, and HFD + Npm20. The induction period for all groups was set at four weeks. **Results:** The docking tests yielded binding scores of -189.55 for Mitragynine against the $\beta 1$ adrenergic receptor and -179.76 for the $\beta 3$ receptor. The interactions between Mitragynine and the adrenergic receptors involved amino residues similar to those found in the native ligand epinephrine, specifically Asn1006A, Ser1007A, and Asn1022A for $\beta 1$, and Asn332R for $\beta 3$. Nanoparticles at doses of 5, 10, and 20 mg/kg BW demonstrated significant differences ($p < 0.05$) in body temperature and UCP-1 levels. Notably, the 10 and 20 mg/kg BW dosages did not lead to weight gain in the rats ($p > 0.05$). **Conclusion:** Mitragynine shows potential as an agonist for $\beta 1$ and $\beta 3$ adrenergic receptors. Furthermore, the nanoparticles at 5, 10, and 20 mg/kg BW were effective in stimulating UCP-1 activity and elevating body temperature in rats subjected to a high-fat diet.

Keywords: HFD, *In silico*, *Mitragyna*, Nanoparticle, UCP-1.

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INTRODUCTION

Obesity has been recognized as a major global health issue since 2016, currently affecting over 650 million adults worldwide (WHO, 2021). Projections indicate that this number will continue to rise until 2030, with an estimated increase of 17.5%, potentially

reaching around 1 billion individuals affected globally (Federation, 2022). This surge in obesity prevalence is expected to coincide with a rise in hyperlipidemia cases. Notably, the Asian region reports the highest incidence of hyperlipidemia in the world, contributing

to roughly 100,000 deaths annually, including those in Indonesia (Lee et al., 2021).

The most effective approach to preventing hyperlipidemia is to curb the accumulation of body fat, particularly in cases of obesity. Changes in the lipid metabolism process that lead to hyperlipidemia can arise from hormonal imbalances and disruptions in appetite regulation. This, in turn, promotes increased fat storage in adipocytes (Maradonna & Carnevali, 2018). Additionally, another study highlights that hyperlipidemia may result from a deficiency in the thermogenesis-regulating protein known as uncoupling protein-1 (UCP-1). A lack of UCP-1 impairs the thermogenesis process, leading to reduced heat production for lipolysis and calorie expenditure (Kataoka et al., 2020).

Enhancing the function of UCP-1 can support adaptive thermogenesis, enabling the achievement of ideal body weight through effective and efficient methods (Rosenbaum & Leibel, 2013). Adaptive thermogenesis involves a variety of signaling processes occurring in Brown Adipose Tissue (BAT) (Z. Zhang et al., 2021). G protein-coupled receptors (GPCRs) play a crucial role in regulating thermogenesis in BAT. Upon activation by catecholamines like norepinephrine, β -adrenergic receptors, a subset of GPCRs, stimulate adenylyl cyclase via G_s proteins. This stimulation increases cyclic AMP (cAMP) levels, activating protein kinase A (PKA), which in turn enhances UCP-1 expression and activity, promoting thermogenesis (Fain & Garcia Sainz, 1983; Sveidahl Johansen et al., 2022).

The prevention of hyperlipidemia can be effectively addressed through the use of herbal remedies as an alternative therapeutic approach, potentially offering a reduced risk of adverse effects compared to conventional pharmaceutical treatments (Chien et al., 2016). Numerous plants have been researched for their ability to enhance the

thermogenesis mechanism, including *Kaempferia parviflora*, *Aframomum melegueta*, and *Moringa oleifera* (Payab et al., 2019; Syamsunarno et al., 2021). Nevertheless, despite the existence of herbal candidates known to promote thermogenesis, there is still a significant need for further exploratory studies on plants found in Indonesia, particularly those that are indigenous and abundant in the West Kalimantan region.

Mitragyna speciosa (*M. speciosa*), commonly referred to as Kratom, is a widely accessible plant, particularly prevalent within communities in the West Kalimantan region (Novindriani et al., 2021). *M. speciosa* contains over 50 alkaloids, with Mitragynine being one of the most notable (Flores-Bocanegra et al., 2020). Previous studies utilizing in vitro experiments and molecular docking have demonstrated that Mitragynine can interact with and stimulate adrenergic receptors, specifically types $\alpha 1b$, $\alpha 1d$, $\alpha 2a$, and $\alpha 2b$ (Obeng et al., 2020). The stimulation of $\alpha 1$ -adrenergic receptors has been linked to the upregulation of thermogenic genes, initiating the thermogenesis process in adipocytes (Borst et al., 1994; Rahbani et al., 2022). However, in addition to stimulating adrenergic receptors, Mitragynine also exhibits a strong interaction with μ -opioid receptors (Obeng et al., 2020). The stimulation of μ -opioid receptors can lead to various health concerns, including respiratory depression, sedation, hallucinations, nausea, urinary retention, and constipation (Stein, 2013). Consequently, the use of *M. speciosa* for hyperlipidemia prevention necessitates a modification to prevent the activation of μ -opioid receptors by Mitragynine. A nanoparticle drug delivery system could serve as a viable solution for delivering Mitragynine in a targeted manner to adrenergic receptors.

The primary material commonly utilized for encapsulating drug substances in nanoparticles is

chitosan polymer, derived from the deacetylation of chitin (Wathoni et al., 2021). Chitosan is recognized for its stability, simplicity, biocompatibility, and ease of degradation (Ylitalo et al., 2002). It contains three reactive groups: amine, primary hydroxyl, and secondary hydroxyl (Raftery et al., 2013). Chitosan is soluble in water and exhibits a positive charge in environments with a low pH (less than 6.5). In contrast, it loses its charge and becomes insoluble in water when the pH exceeds 6.5 (Zargar et al., 2015). Chitosan's positive charge facilitates its interaction with negatively charged molecules, including phospholipids, glycosaminoglycans, and proteoglycans (Raftery et al., 2013; Wulandari, 2019). Additionally, incorporating polyethylene glycol (PEG) into the encapsulation material can enhance the affinity of the nanoparticles for targeted delivery to adrenergic receptors (Barthe et al., 2020; Qiao et al., 2017a). PEG-containing nanoparticles serve as a platform for ligand conjugation, thereby enhancing receptor expression on the surface of cell (Shi et al., 2021).

This study utilized *in silico* prediction methods to assess the potential of mitragynine, encapsulated within *M. speciosa* extract nanoparticles, in stimulating β -adrenergic receptors, comparing its efficacy to that of the native ligand, epinephrine. The *in silico* findings were further validated through *in vivo* experiments, which evaluated UCP-1 activity, a key regulator of thermogenesis.

METHODS

Materials

A total of 1 kg of *M. speciosa* powder was sourced from a West Borneo Kratom vendor (Pontianak, Indonesia; <https://westborneokratom.com>). The botanical identification and authentication were performed by Dr. Nelly Wahyuni at the Central Laboratory of Tanjungpura University, Pontianak, Indonesia. The

plant material was identified based on morphological characteristics and further confirmed through thin-layer chromatography (TLC) and UV-Vis spectrophotometry. A voucher specimen was deposited under the accession number 041/UN22.17/TA.00.001/SA/2022. Nanoparticle encapsulation was carried out using sodium alginate and tripolyphosphate as the crosslinking agent, both obtained from Wako Pure Chemical Industries (Tokyo, Japan). Additionally, PEG 6000 was acquired from Sigma-Aldrich (Missouri, USA), and potassium bromide (KBr) was purchased from PT Merck (West Java, Indonesia) (Wathoni et al., 2019).

Methods

In Silico Study

Mitragynine (PubChem ID: 3034396) was docked to the β 1-adrenergic receptor (PDB ID: 7BU6) and the β 3-adrenergic receptor (PDB ID: 7DH5) for comparison with epinephrine (PubChem ID: 5816). Docking simulations were conducted using the HDock Server (<http://hdock.phys.hust.edu.cn/>). Prior to docking, both mitragynine and epinephrine ligands underwent energy minimization using Chem3D Pro 12 to ensure stability during the simulation (Khoirul Rista Abidin et al., 2024).

A more negative docking score, coupled with a confidence score > 0.7, indicates a stronger binding affinity between the ligand and the receptor (Abd El-Aziz et al., 2023). In contrast, a confidence score between 0.5 and 0.7 suggests a probable binding interaction between the ligand and the receptor (H. Li et al., 2022). The interaction profiling was conducted using Biovia Discovery Studio 2021 and the Protein-Ligand Interaction Profiler (PLIP) (<https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>). The analysis focused on evaluating hydrogen bond interactions and bond distances, expressed in angstroms (Å), between amino acid residues and the ligands mitragynine and epinephrine.

***M. speciosa* Extract Preparation**

Extraction Process

The extraction method employed was adapted from the study by Cheaha et al., with several modifications (Cheaha et al., 2017). The process began with the preparation of *M. speciosa* extract using the maceration method. A total of 1 kg of *M. speciosa* powder was mixed with 1 L of 98% methanol (MeOH) in a sealed container and left to macerate under continuous stirring at room temperature (25°C) for 72 hours to maximize the extraction of bioactive compounds. The extraction was performed in triplicate trials to ensure reproducibility.

Following maceration, the mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract from the plant residue. The filtrate was then concentrated using a rotary evaporator at 40°C under reduced pressure to remove excess solvent. The resulting semi-solid extract was further dried in a vacuum oven at 50°C until a stable, dried extract was obtained. The final extract was stored at –20°C in an airtight container until further analysis.

Mitragynine Percentage Analysis

The quantification of Mitragynine in the *M. speciosa* extract was performed using High-Performance Liquid Chromatography (HPLC) analysis. To determine the levels of Mitragynine, an optimization approach utilizing an Ultra Violet (UV) λ_{max} detector was employed. The process included creating a standard curve (linearity) with concentration levels set at 200, 100, 50, 25, 12.5, and 6.25 $\mu\text{g/mL}$. The Mitragynine content in the extract is reported in mg/g. Furthermore, the Limit of Detection (LoD) and Limit of Quantification (LoQ) values were determined.

A total of 1000 mg of the extract was dissolved in 25 mL of methanol (MeOH), with the dissolution process performed using a sonicator for 20 minutes. Next, 500 μL of the resulting solution was

aliquoted and diluted to 1000 μL with MeOH. For the HPLC analysis, 20 μL of the prepared solution was injected at 30°C using an Agilent 1220 Infinity LC HPLC system (Waldbronn, Germany). The HPLC parameters were as follows: a Waters C18 column (150 x 4.6 mm) with a mobile phase composed of A (water + 0.1% formic acid) and B (acetonitrile + 0.1% formic acid), using the following gradient: 0.0–2.0 min at 30% B; 2.0–8.0 min from 30% to 35% B; 8.0–8.1 min from 35% to 100%; 8.1–11.0 min held at 100%. The flow rate was maintained at 1 mL/min, and UV detection was performed at a wavelength of λ_{max} 254 nm.

***M. speciosa* Extract Nanoparticles Preparation**

Nanoparticles Formulation

Nanoparticles from *M. speciosa* extract were synthesized using the ionic gelation method, which is based on electrostatic interactions between two distinct ions under controlled conditions. The outcome of this process is the transformation of at least one of the molecules into a polymer. The active compound, such as Mitragynine, is subsequently encapsulated by a polymer chain, forming a protective capsule (Pedroso-Santana & Fleitas-Salazar, 2020).

The extract of *M. speciosa* is encapsulated into nanoparticles at a dosage of 20 mg. Each portion of the *M. speciosa* extract is dissolved in 20 mL of 95% ethanol. This selected dosage is based on the studies conducted by Wathoni et al. and Ramanathan et al. to evaluate the effects of Mitragynine alkaloids (Ramanathan et al., 2015; Wathoni et al., 2021). The next step involves dissolving the extract in a chitosan solution mixed with 1% acetic acid, maintaining a ratio of 200 mg per 200 mL. Subsequently, TPP is added at a ratio of 40 mg per 40 mL while stirring with a magnetic stirrer. All solution mixtures were subsequently subjected to ultrasonication for 30 minutes to reduce the droplet suspension size.

In the coating process, various suspensions are introduced into several polymeric formulations.

The specific variants being utilized include F1 (MG-Ch/Al5), F2 (MG-Ch/Peg3/Al7), F3 (MG-Ch/Peg7/Al9), and F4 (MG-Ch/Peg12), which differ in their sodium alginate content, while F2, F3, and F4 contain varying amounts of PEG (Wathoni et al., 2021). A complete overview of the formulation design is provided in Table 1. The final step entails transforming the suspension into nanoparticle powder through Spray Pyrolysis, performed at an ESP reactor temperature of 100°C, an SP reactor temperature of 80°C, and a flow rate pressure of 1 atm. After the nanoparticle powder was collected, its weight was measured.

Table 1. Formula Designs of *M. speciosa* Extract Nanoparticles

Materials Composition	Formulas Design			
	F1	F2	F3	F4
<i>M. speciosa</i> (mg)	20	20	20	20
chitosan (mg)	200	200	200	200
TPP (mg)	28	28	28	28
PEG6000 (mg)	0	30	75	120
Sodium Alginate (mg)	50	70	90	0

Particles Size Analysis (PSA)

The expected parameters for this study include nanoparticle sizes ranging from 10 to 100 nm, using chitosan, alginate, and PEG as encapsulating materials. A 5 mg sample of powdered *M. speciosa* extract nanoparticles was dissolved in 30 mL of distilled water and sonicated for 30 minutes. Particle size analysis (PSA) was conducted using the monodisperse Dynamic Light Scattering (DLS) technique with a Zetasizer (Horiba Scientific, Kyoto, Japan) at 25°C, with a scattering angle of 90°. The PSA results are presented in nanometers (nm) along with the Poly Dispersity Index (PDI) values. Smaller nanoparticle sizes are indicative of enhanced stability and uniformity (Oksal et al., 2020).

Scanning Electron Morphology (SEM) Analysis

Morphological analysis of *M. speciosa* extract nanoparticles was performed using the SEM method. The nanoparticles in powder form were mounted on stubs and coated with platinum for 30 seconds at 10 mA. Observations were conducted at a magnification of 10,000 and an accelerating voltage of 15 kV. The obtained data were processed using the ImageJ software (Madison, WI, USA) and Origin software (Northampton, MA, USA) to assess the particle distribution and size (Wathoni et al., 2021).

Fourier-Transform Infrared Spectrophotometry (FTIR) Analysis

The interaction between *M. speciosa* extract and the nanoparticle components was analyzed using FTIR. A total of 2 mg of nanoparticle powder was homogenized with 200 mg of KBr to form a fine mixture. The blended sample was then compressed into a transparent pellet using a hydraulic press under a vacuum at a pressure of 60 kN for 15 minutes to ensure proper pellet formation.

Subsequently, FTIR spectra were recorded in the mid-infrared region (4000–400 cm⁻¹) to detect characteristic functional groups and potential molecular interactions between the extract and the nanoparticle matrix. The obtained spectra were compared to those of pure *M. speciosa* extract, blank nanoparticles, and encapsulated nanoparticles to identify any shifts or new peaks indicative of encapsulation.

In Vivo Study

Animal Models

The treatment of rats was approved by the Animal Ethics Committee of Padjadjaran University, as confirmed by certificate number 309/UN6KEP/EC/2023. The male Wistar rats used in this study were sourced from the Bogor Agricultural Institute (IPB) laboratory. Each rat weighed between 200 and 300 g and was 13 weeks old, all in good

health. The rats were assigned to six groups, with seven rats in each group, designated as treatment subjects.

Prior to the experiment, the rats underwent a 21-day adaptation period, during which they were housed in cages measuring 51 x 36 x 29 cm. A maximum of five rats were kept per cage, resulting in a total of 12 cages. The cages were furnished with rice husks as bedding and covered with iron wire mesh to ensure security. To maintain hygiene, the rice husks were replaced every three days, and a 12-hour dark-light cycle was observed. The environmental conditions were regulated to maintain a temperature of 22-25°C and a relative humidity of 55 ± 10%. During the adaptation phase, the rats were provided with standard BR-1 feed and had unrestricted access to distilled water to support normal metabolic processes.

Experimental Protocol

In this study, the division of rats also involved categorizing groups based on their diets—those receiving a normal diet and those on a high-fat diet. The group of normal rats was provided with standard feed, whereas the group on a high-fat diet received a specialized high-fat feed for a duration of 4 weeks, which contained 12.9% carbohydrates, 16.3% protein, and 70.8% fat (Noor et al., 2023). Both the standard feed and the high-fat diet (HFD) were procured from PT. Surya Sains Indonesia in Bandung, Indonesia. *M. speciosa* extract nanoparticle therapy was administered orally once daily.

The dosage for the conventional *Mitragyna speciosa* extract was established at 50 mg/kg body weight (BW), based on previous studies that reported a substantial reduction in food intake and significant changes in body weight in rats (Kumarnsit et al., 2006). For the nanoparticle dosage, this study employed a modified induction dose, as outlined in the research by Suryani et al., with doses

of 5, 10, and 20 mg/kg body weight (BW) selected (Suryani et al., 2022). The dose selection considered both the effective formulation of nanoparticles and safety concerns to mitigate toxicity risks associated with doses up to 64 mg/kg BW, as indicated in previous findings (Marianti et al., 2024). Detailed grouping and treatment protocols for the rat subjects are outlined in Table 2.

Table 2. Animals Group Model

Groups	Induction Models	Rats Groups (n)
Normal	No induction	7
HFD	HFD induction	7
HFD+MG50	HFD+ 50 mg/kg BW <i>M. speciosa</i> conventional extract	7
HFD+Npm5	HFD+ 5 mg/kg BW <i>M. speciosa</i> loaded in nanoparticles	7
HFD+Npm10	HFD+10 mg/kg BW <i>M. speciosa</i> loaded in nanoparticles	7
HFD+Npm20	HFD+20 mg/kg BW <i>M. speciosa</i> loaded in nanoparticles	7

Description: HFD (*High-fat diet*), BW (Body Weight)

Body Weight Analysis

The body weight measurements were conducted at the start and final of the study, with results expressed in grams (g).

Rat’s Body Temperature Analysis

Body temperature measurements were performed using a thermometer in the rectum of rats (Meyer et al., 2017). The measurement of the rat’s body temperature was carried out 15 minutes after the treatment of extract therapy. The results of the temperature examination were analyzed between pre- and post-research. In addition, body temperature analysis was also compared between groups at the end of the study.

Histological Analysis

At the final of the study, all rats were weighed prior to euthanasia. The rats were euthanized through the administration of ketamine at 75-100 mg/kg BW

and xylazine at 5-10 mg/kg BW. Organ samples taken for histological analysis were in the iBAT section. The method for making tissue preparations to analyse the appearance of adipose tissue was adopted from the study of Oksal et al., 2020 with modifications (Oksal et al., 2020).

The adipose tissue to be used was soaked in 10% formalin. The sample was then dehydrated (50%, 70%, 80%, 90%, and 100% alcohol, each process 1.5 hours with three repetitions). The following process was impregnation using paraffin. The tissue was then cut using a rotary microtome machine. The tissue was immersed in a water bath at 38°C for 3 minutes. This was followed by drying and staining with Hematoxylin and Eosin. The final step involved mounting the tissue and allowing it to rest overnight (Feng et al., 2019; Lesmana et al., 2021; Oksal et al., 2020; Syamsunarno et al., 2014). Tissue preparations that were mounted were then observed using a light microscope connected to Optilab Viewer 3 software by PT Miconos, Indonesia, with a magnification of 20 times. Total adipocytes in adipose tissue were calculated using Image J software (Lesmana et al., 2021; Oksal et al., 2020; Syamsunarno et al., 2021).

Western Blotting Analysis

The fat tissue used for western blotting analysis was iWAT. The tissue was taken from rats, weighed, and measured in g units; the sample was then stored at -80 °C for analysis preparation. To determine the level of UCP-1 protein, we used the western blotting method using monoclonal rabbit (#U6382) from Sigma Aldrich (Lesmana et al., 2021). The tissue used came from IWAT. Western blotting is appropriate for determining protein levels and the signalling process of targeted protein activity (Begum et al., 2022). This study's western blotting antibody was UCP-1 (E9ZZV) XP Rabbit mAB, Cell Signaling Technology - 72298S, Massachusetts, USA.

Western blotting technique was conducted by preparing the sample by adding 5 µL of buffer to the sample. The next volume was equalized in each lane by adding double-distilled H₂O to a volume of 15 µL per well. The sample was then heated using a dry plate at 100°C for about 5 minutes. Prepare the rack for gel printing, then add the stacking gel slowly until it reaches the appropriate height on the mark on the glass plate. H₂O was added to the gel and then waited for about 15-30 minutes until the gel hardened, then removed the water and stacking gel (Sanjaya et al., 2023).

The next step was to pour the running buffer into the electrophoresis device. Cover the gel entirely and slowly remove the comb. Add 6 µL of marker to the sample in each well. The transfer buffer was then added to the blotting equipment, ensuring the buffer covered the sandwich. Place the electrode on the sandwich, then make sure the Polyvinylidene Fluoride (PVDF) membrane was between the gel and the positive electrode and the transfer was carried out for 90 minutes (Sanjaya et al., 2023).

RESULTS AND DISCUSSIONS

In Silico Study

The results of the *in silico* test obtained the docking value between Mitragynine and the β 1 adrenergic receptor was -189.55. Other values obtained were LGScore 3.94, maxsub 0.30, and confidence score 0.69. The ligand Mitragynine that was tested for docking in this study formed hydrogen bonds to residues Asn1006A, Ser1007A, Asn1022A, Glu1202A, Arg1222A, Tyr1224A, Asn1363A, and Tyr1367A. Meanwhile, the bond distance between Mitragynine and the residues found was, on average, less than 3.2 Å. The results of the *in silico* test obtained the docking value between epinephrine and the β 1 adrenergic receptor was -149.39. Other values obtained are LGScore 3.94, 0.30, and confidence score

0.49. The epinephrine ligand tested for docking in this study forms hydrogen bonds to residues Val1139A, Thr1143A, Ser1228A, Ser1232A, Asn1363A,

Asn1006A, Ser1007A, Asn1022A, Asn1034A, and Arg1035A. The results of the docking test in 3-dimensional (3D) form can also be seen in Figure 1.

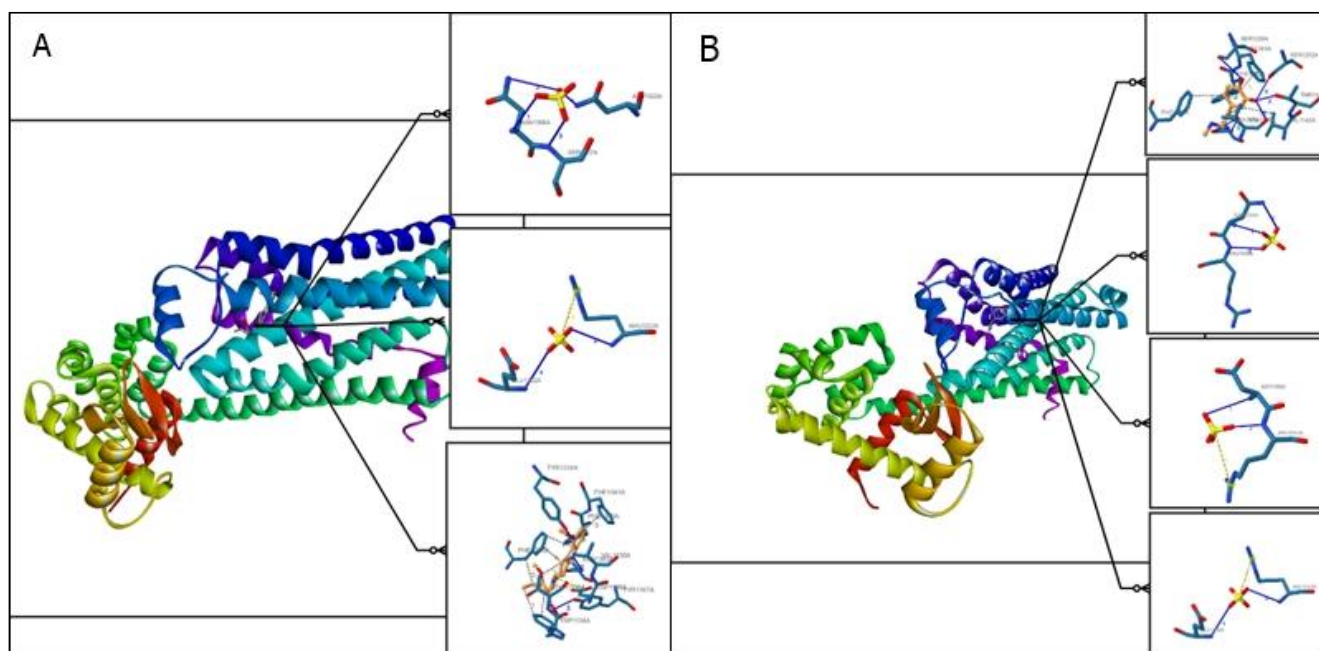


Figure 1: 3D Docking result of hydrogen Residues Interaction between Mitragynine and β1 Adrenergic Receptor (A), epinephrine and β1 Adrenergic Receptor (B)

The docking analysis results, obtained using the H-Dock server, yielded an interaction value of -189.55, which was further supported by the LGScore of 3.94, maxsub of 0.30, and a confidence score of 0.69. These results indicate the potential for optimal binding between the ligand Mitragynine and the β1 adrenergic receptor. Compared to the docking results using the native ligand epinephrine, Mitragynine produces a more optimal affinity potential because Mitragynine has a higher docking affinity value (Table 3). Meanwhile, residues similar to epinephrine are Asn1006A, Ser1007A, and Asn1022A. The RMSD value of all amino residues formed is $<2.5 \text{ \AA}$. The RMSD value indicates a good range of docking values (Bashir et al., 2024). Epinephrine is a native ligand that can activate the β1 adrenergic receptor (agonist). Previous research has indicated that drug candidates acting as β1 adrenergic receptor agonists via the G protein activation pathway involve the identification of the Asp121 and Asn329 residues.

Meanwhile, to produce a ligand into a full agonist, it is strengthened by a bond to Ser211 (Vilar et al., 2017). In this study, Serine residues with the code Ser1007A were also found. The results of other studies added that the potential for ligands that have the potential to interact with β1 adrenergic receptors were found to have one of the oxygen atoms forming a hydrogen bond with the Asn310 residue in the receptor (Islam et al., 2021). Some studies explain that the tendency for more hydrogen bonds than hydrophobic ones can indicate that the ligand has the potential to activate (Wu et al., 2021). Compared to previous studies, the residues found in this study are similar, indicating that the study's results answer the study's purpose to analyze the potential for Mitragynine to interact with β1 adrenergic receptors for thermogenesis stimulation. The potential for activation by Mitragynine is through the G protein pathway.

Meanwhile, the in silico test results between Mitragynine and the β_3 adrenergic receptor obtained a docking value of -179.76. Other values obtained were LGScore 5.44, maxsub 0.36, and confidence score 0.64. This study's Mitragynine ligand tested for docking formed hydrogen bonds to the Ser208R and Asn332R residues. Meanwhile, the epinephrine docking test results on the β_3 Adrenergic receptor

obtained a value of -131.87. The results of other test values were LG score 5.44, max sub 0.36, and confidence score 0.41. Meanwhile, the residues that form hydrogen bonds with the epinephrine ligand are Asp117R, Tyr204R, Asn332R, and Tyr336R. The results of the docking test in 3D form can also be seen in Figure 2.

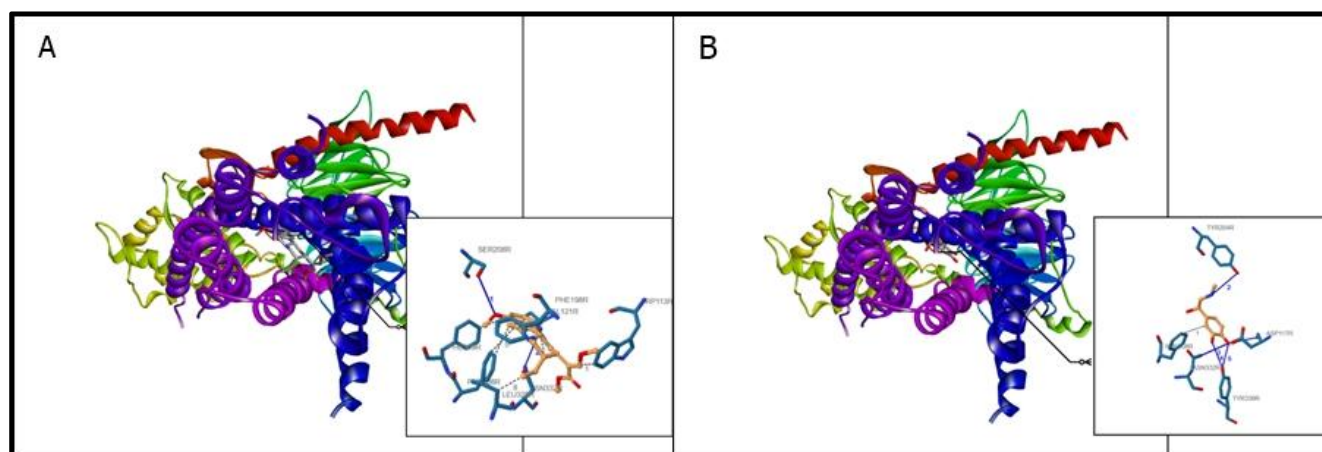


Figure 2: 3D Docking Result of Hydrogen Residues Interaction between Mitragynine and β_3 Adrenergic Receptor (A), epinephrine and β_3 Adrenergic Receptor (B)

The results of the Mitragynine docking test in this study showed a higher affinity value for the β_3 adrenergic receptor when compared to epinephrine. At the same time, similar residues found were Asn332R with a bond distance of $> 3 \text{ \AA}$ (see Table 3). The RMSD value indicates that the affinity formed tends to be unstable. Previous studies have explained stable affinity between ligands and β_3 adrenergic receptors if the RMSD value is $0.6 - 1.5 \text{ \AA}$ (Luan et al., 2023). Previous studies have also suggested that the presence of the Asn332 residue, which forms hydrogen bonds, highlights the potential of the ligand as an agonist for the β_3 adrenergic receptor. The presence of hydrophobic bonds then functions as a conformational bond strengthener (Luan et al., 2023). Other studies have noted that the ligand's binding to the β_3 adrenergic receptor is facilitated by the interaction between the chiral hydroxyl group and the amine of the phenylethanolamine core with Asn332.

Furthermore, the hydroxyl group also forms a bond with the Asp117 residue (Huang et al., 2024). Therefore, the results of this docking study suggest that Mitragynine has the potential to act as an agonist for β_3 adrenergic receptors. However, the binding stability, as indicated by the RMSD value, demonstrates that the interaction is unstable.

***M. speciosa* Extract Preparations and Characterizations**

The results of kratom extraction were obtained as much as 15 g in concentrated form. Meanwhile, the characterisation test results using HPLC showed a chromatogram comparison between the *M. speciosa* extract sample and pure Mitragynine. The expected result parameters in this study are as follows: the amount of Mitragynine alkaloid content contained in the *M. speciosa* extract was obtained. The linearity curve obtained in the study was obtained, namely having the equation $y = 8.4783x - 14.532$ (0.9978)

Table 3. The Comparison of Docking Results between Mitragynine and Epinephrine against $\beta 1$ and $\beta 3$ adrenergic receptors

Receptors Target	Mitragynine					
	Docking Score	LGScore	MaxSub	Confidence Score	Hydrogen Bonds	Distance (Å)
β1 Adrenergic	-189.55	3.94	0.30	0.69	MG – Asn1006A	2.30
					MG - Ser1007A	2.19
					MG – Asn1022A	2.06
					MG – Glu1202A	2.72
					MG – Arg1222A	2.76
					MG – Tyr1224A	2.94
					MG – Asn1363A	3.19
					MG – Tyr1367A	1.86
β3 Adrenergic	-179.76	5.44	0.36	0.64	MG – Ser208R	3.72
					MG – Asn332R	3.37
Receptors Target	Epinephrine					
	Docking Score	LGScore	MaxSub	Confidence Score	Hydrogen Bonds	Distance (Å)
β1 Adrenergic	-149.39	3.94	0.30	0.49	Epi – Val1139A	2.88
					Epi – Thr1143A	2.60
					Epi – Ser1228A	2.46
					Epi – Ser1232A	2.16
					Epi – Asn1363A	2.11
					Epi – Asn1006A	2.94
					Epi – Ser1007A	2.19
					Epi – Asn1022A	2.06
					Epi – Asn1034A	1.86
					Epi – Arg1035A	2.16
					Epi – Asp117R	2.95
β3 Adrenergic	-131.87	5.44	0.36	0.41	Epi – Tyr204R	3.71
					Epi – Asn332R	3.28
					Epi – Tyr336R	2.37

When the extracted sample was compared with the results of pure Mitragynine, the peak wave was found to be at an absorbance of more than 99% at 6.02 minutes (Figure 3). At the same time, the Mitragynine content contained in the *M. speciosa* extract was 84 ± 0.002 mg / g. The LoD value obtained was $1.103 \mu\text{g} / \text{ml}$, and the LoQ was $3.342 \mu\text{g} / \text{ml}$.

The study performed an analysis of Mitragynine concentrations in *M. speciosa* extract to determine the amount of Mitragynine present per gram of extract. The extraction and analysis of Mitragynine in the *M. speciosa* extract were carried out using methanol as the solvent. The use of methanol in extraction for *in vivo* studies does not have the potential to trigger toxicity to the point of causing death in rats. The result was proven in previous studies using extracts with methanol solvents in acute

and chronic administration models to rats (Kamsani et al., 2019).

The use of methanol in the extraction process of *Mitragyna speciosa* enhances the phenolic and flavonoid content of *M. speciosa* leaves, as methanol effectively interacts with polar and semipolar phenolic compounds, promoting their diffusion from plant tissue (Karunakaran et al., 2022). The extraction process of *M. speciosa* using methanol solvent produces a total of phenolics of 419.50 ± 2.50 mg/g when compared to extraction using ethanol, which is only 295.75 ± 1.25 mg/g (Karunakaran et al., 2022). However, using methanol is not recommended by WHO in manufacturing extracts for human administration because of the risk of triggering irritation to the digestive mucosal lining (World Health Organization, 1997).

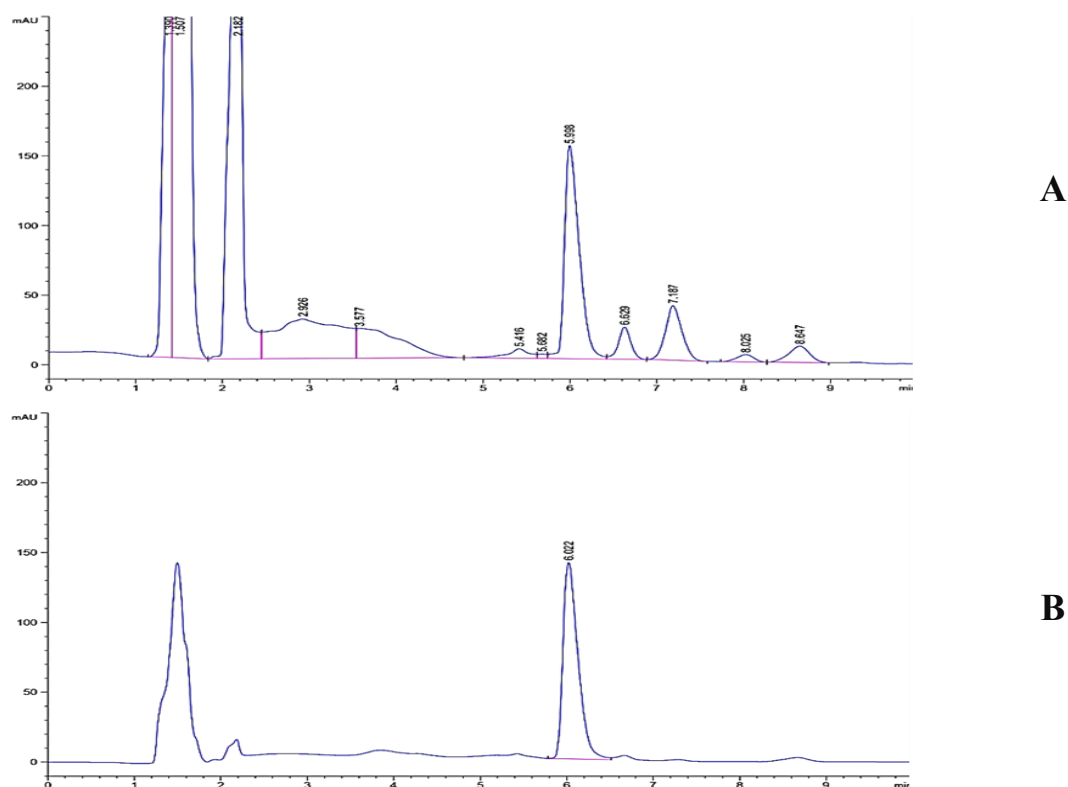


Figure 3. Chromatography between *M. speciosa* extract sample (A) and Mitragynine (B)

Meanwhile, other solvents, such as ethanol for herbal products, should be at most 10% for adult-specific products (Vojvodić et al., 2023). Controlling methanol levels is also needed to prevent cell damage and maintain healthy protein production (Joseph et al., 2022). Therefore, to ensure that no remaining solvent residue, such as methanol or ethanol, remains, additional testing is needed to manufacture extracts using a more sensitive analysis method, namely GC-MS (Pandey et al., 2011). This study did not use GC-MS testing because it used rat subjects, which were still safe for nanoparticle experiments made from *M. speciosa* extract.

The objective of analyzing Mitragynine levels in the *M. speciosa* extract was successfully accomplished in this study. The test results revealed the purity of Mitragynine in the *M. speciosa* extract (R^2), with a linearity curve equation of 0.9978. The results of this study are consistent with the findings of the conducted survey by Beng et al., 2011, namely the purification of Mitragynine using the crystallization method with an R^2 value of 0.99, which means that

the calibration range showed promising results (López-Fernández et al., 2022). Meanwhile, the purity obtained in this study was higher (84%) compared to the survey by Beng et al., 2011 using chloroform (70%).

When compared with the pure Mitragynine test, sample testing showed that the peak wave was at an absorbance of more than 99% at 6.02 minutes. These results align with the study, which explains that the peak wave of chromatography results for the Mitragynine compound is at a value above 99.9% and 6 minutes (Mudge & Brown, 2017; Parthasarathy et al., 2013). The amount of Mitragynine content contained in the extract per g of sample is 84 mg or equivalent to 0.084 g, with the LoD and LoQ values obtained are 1.10 and 3.34, respectively. Thus, for the manufacture of nanoparticles with a composition of *M. speciosa* as much as 20 mg, the Mitragynine content is around 1.68 mg.

The amount of Mitragynine is still within the normal limits of administration or is not toxic if it is less than 15 mg or 100 mg/kg BW per induction for rat

research subjects with an average weight requirement of 200 g (Sabetghadam et al., 2013). The objective of analyzing the Mitragynine levels in the *Mitragyna speciosa* extract was successfully achieved in this study. The test results indicated that the purity of Mitragynine in the *M. speciosa* extract (R^2) was characterized by a linearity curve equation of 0.9978.

These findings are consistent with the results of the previous study by Beng et al., 2011, namely the purification of Mitragynine using the crystallization method with an R^2 value of 0.99, which means that the calibration range showed promising results (López-Fernández et al., 2022).

Meanwhile, the purity obtained in this study was higher (84%) than in Beng et al., 2011 using chloroform (70%). When compared with the pure Mitragynine test, sample testing showed that the peak wave was at an absorbance of more than 99% at 6.02 minutes. These results align with the study, which explains that the peak wave of chromatography results for the Mitragynine compound is at a value above 99.9% and 6 minutes [58,59]. The amount of Mitragynine content contained in the extract per g of sample is 84 mg or equivalent to 0.084 g, with the LoD and LoQ values obtained at 1.10 and 3.34, respectively. Thus, for the manufacture of nanoparticles with a composition of *M. speciosa* as much as 20 mg, the Mitragynine content is around 1.68 mg. The amount of Mitragynine is still within the normal limits of administration or is not toxic if it is less than 15 mg or 100 mg/kg BW per induction for rat research subjects with an average weight requirement of 200 g [60].

Particle Size Analysis

The nanoparticle size analysis results indicated that the MG-Ch/Peg7/Al9 formulation, which contained a higher alginate concentration, exhibited a smaller size (Z-Average) of 189 ± 62.1 nm compared to the other formulations. In addition, the MG-

Ch/Peg7/Al9 formula also obtained a smaller polydispersity index (PDI) value. However, the test results for all sizes between nanoparticles differed significantly. The complete results of the size analysis can be seen in Table 4.

Table 4 Particle Size Analysis of Nanoparticles

Nanoparticle Formulas	Z-Average (nm)	PDI
F1 (MG-Ch/Al5)	218 ± 69.2	2.15
F2 (MG-Ch/Peg3/Al7)	230 ± 73.3	2.37
F3 (MG-Ch/Peg7/Al9)	189 ± 62.1	1.68
F4 (MG-Ch/Peg12)	214 ± 64.1	1.98

The formulation of making and characterization test of nanoparticles aims to determine which formulation is best for further studies, namely *in vivo* studies. This study used biopolymeric materials such as chitosan, alginate, and PEG to produce nanoparticles with a polymeric structure. Previous studies used alginate and chitosan to produce nanoparticle packaging with a polymeric matrix structure because it is more stable than other materials. The results obtained when nanoparticles only contain chitosan and alginate produce sizes between 200-300 nm. When PEG was added, there was a decrease in the size diameter of around 12-30% (Masalova et al., 2013). The combination of alginate and chitosan serves to shield active compounds or pharmaceutical ingredients from the action of digestive enzymes in the stomach (S. Li et al., 2022). When alginate is combined with PEG, hydrogen bonds are formed between the electropositive amino hydrogen molecules of chitosan and the electronegative oxygen atoms (Amin & Boateng, 2022).

The results obtained in this study from the manufacture of nanoparticles using chitosan, alginate, and PEG, namely the smallest size was found in the formula with a PEG content of 75 mg and Alginate 90 mg (MG-Ch/Peg7/Al9) measuring 189 ± 62.1 nm. The

PDI value obtained was 1.68 compared to other formulas. The smaller size and PDI values compared to other formulas indicate that the nanoparticles are very stable because they are supported by a suitable sonication method using a probe (Oksal et al., 2020). The amount of alginate was more significant than other formulas. Based on this, the combination of PEG6000 and alginate may cause the size of MG-Ch/Peg7/Al9 nanoparticles to be much smaller than the MG-Ch/Al5 and MG-Ch/Peg3/Al7 formulas. PEG is predicted to cause nanoparticles to become stable colloids, especially in water (Masalova et al., 2013). The size of nanoparticles used in this study differs from previous studies. Similar studies targeting nanoparticles to adrenergic receptors used nanoparticle sizes in the 13–50 nm (Qiao et al., 2017b). However, there are studies explaining that each adrenergic receptor has a different affinity size for the native ligand of the neurotransmitter group, namely adrenergic α_2 0.3 – 3 nm, α_1 10 – 30 nm, β_1 or 3 more than 10,000 nm, and β_2 around 100 nm (Wu et al., 2021).

Therefore, standard tests are still needed to ensure that nanoparticles with chitosan, alginate, and PEG materials can stimulate adrenergic receptors, as shown in the docking test results, namely by conducting *in vitro*. The standard test employed for this analysis was Inductively Coupled Plasma Mass Spectrometry (ICP-MS), utilizing the DRC-e ICP-MS instrument to examine tissues containing adrenergic target receptors (Qiao et al., 2017a). The method compares nanoparticles containing PEG and nanoparticle levels in plasma and correlates the effects based on the type of receptor stimulated (Qiao et al., 2017a). However, this study has not been carried out as in previous studies.

SEM Analysis

The SEM analysis conducted in this study is compared with SEM results from previous studies that used chitosan nanoparticles and conventional *M. speciosa* extracts (Jintawiwat et al., 2023; Muchtaridi

et al., 2023; Oksal et al., 2020). This study used chitosan, alginate, and PEG biopolymer materials so that the structure formed was nanoparticles with a matrix structure and medicinal ingredients (Elmowafy et al., 2023; Zielińska et al., 2020). The image of the nanoparticle structure formed was visualized in a 2D illustration as in Figure 4 (A). The SEM test results obtained in the study were in the MG-Ch/Al5 formula; the nanoparticles appeared to be spherical and close to each other between other nanoparticles. The same happened to the MG-Ch/Peg3/Al7 formula nanoparticles. Meanwhile, the nanoparticles were semicrystalline in the MG-Ch/Peg7/Al9 and MG-Ch/Peg12 formulas. Namely, they were spherical, close to each other, and in groups.

The results obtained in the MG-Ch/Al5 formula were that the nanoparticles appeared spherical and close to each other. Similar things also happened to the MG-Ch/Peg3/Al7 formula nanoparticles. In the MG-Ch/Peg7/Al9 and MG-Ch/Peg12 formulas, the nanoparticles were semicrystalline in shape, where some nanoparticles were spherical and close to each other but in groups. The presence of chitosan and alginate in the nanoparticles will help the formation of spheres because the combination of the two materials provides a compaction effect (Sanches et al., 2020).

Meanwhile, the formation of clustered spherical nanoparticles can be caused by adding PEG to the nanoparticles. As is known, PEG can increase the biocompatibility of nanoparticles to increase stability and provide a conformational effect (Padín-González et al., 2022; Shi et al., 2021). In addition, nanoparticles that are given additional PEG will increase the conjugation effect between nanoparticles as ligands and target receptors (Padín-González et al., 2022). A comparison of SEM tests is still needed between *M. speciosa* extract nanoparticles and nanoparticle components as previously conducted in previous studies [63,71]. The purpose of the SEM test

to analyze the shape of nanoparticles was achieved. However, the SEM experiment still needs to see the shape of nanoparticles that only contain chitosan,

alginate, PEG, and *M. speciosa* extract, and it has become a suggestion for further research.

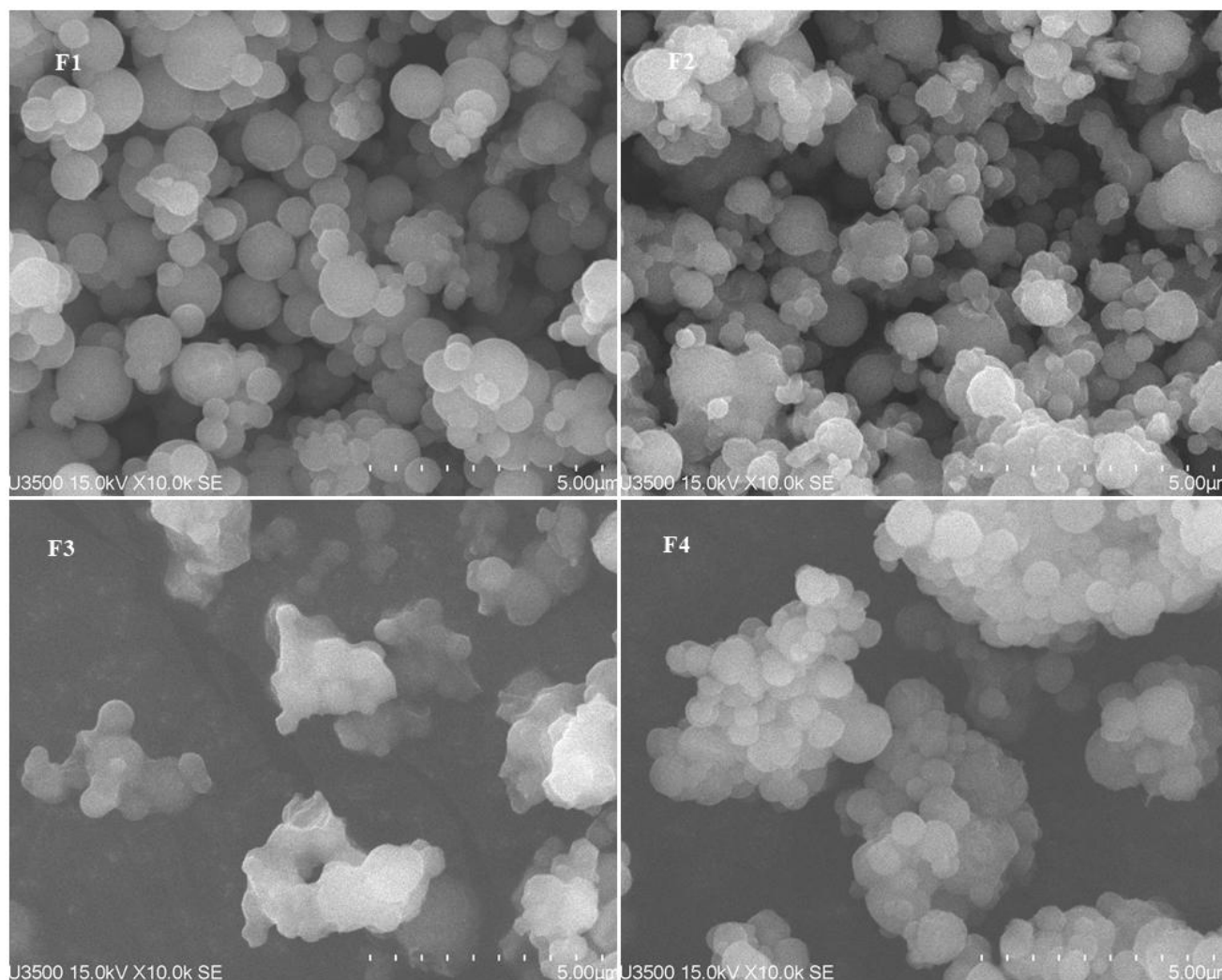


Figure 4. The results of SEM of *M. speciosa* extract encapsulated in nanoparticles with 10×10^3 times magnification from the formulas of F1 (MG-Ch/Al5), F2 (MG-Ch/Peg3/Al7), F3 (MG-Ch/Peg7/Al9), dan F4 (MG-Ch/Peg12)

In Vivo Study Results

Rats Body Weight

The results of this study, as determined by the T-test, revealed no significant increase in body weight for the HFD + Npm10 group (pre-test: 278.43 ± 5.83 g vs. post-test: 289.29 ± 8.56 g, $p = 0.061$) and the HFD + Npm20 group (pre-test: 260.57 ± 12.49 g vs. post-test: 279.29 ± 12.95 g, $p = 0.057$). However, a significant increase in body weight was observed in the HFD group between the pre- and post-examinations, with a p-value of 0.012. The detailed results of the

weight development analysis are presented in Figure 6.

Nanoparticle testing was conducted to analyze the results related to the stimulation of thermogenesis activity in HFD-induced rats. Typically, the weight of rats will increase with age. As age increases, the weight of rats increases due to the addition of mass and number of muscle cells (Quirós Cognuck et al., 2020). Previous studies that observed the development of rats' weight with HFD induction found that significant weight gain in rats would occur after

administration for a week or in the third week (Kovacs & Hajnal, 2022; Ster et al., 2002). This study found that the group receiving only HFD experienced significant weight gain from the start to the end of the study. Therefore, the results are in agreement with

previous studies, which have shown that HFD induction in rats is highly effective in significantly increasing WAT, particularly in the perirenal, epididymal, and retroperitoneal regions (Zhao et al., 2022).

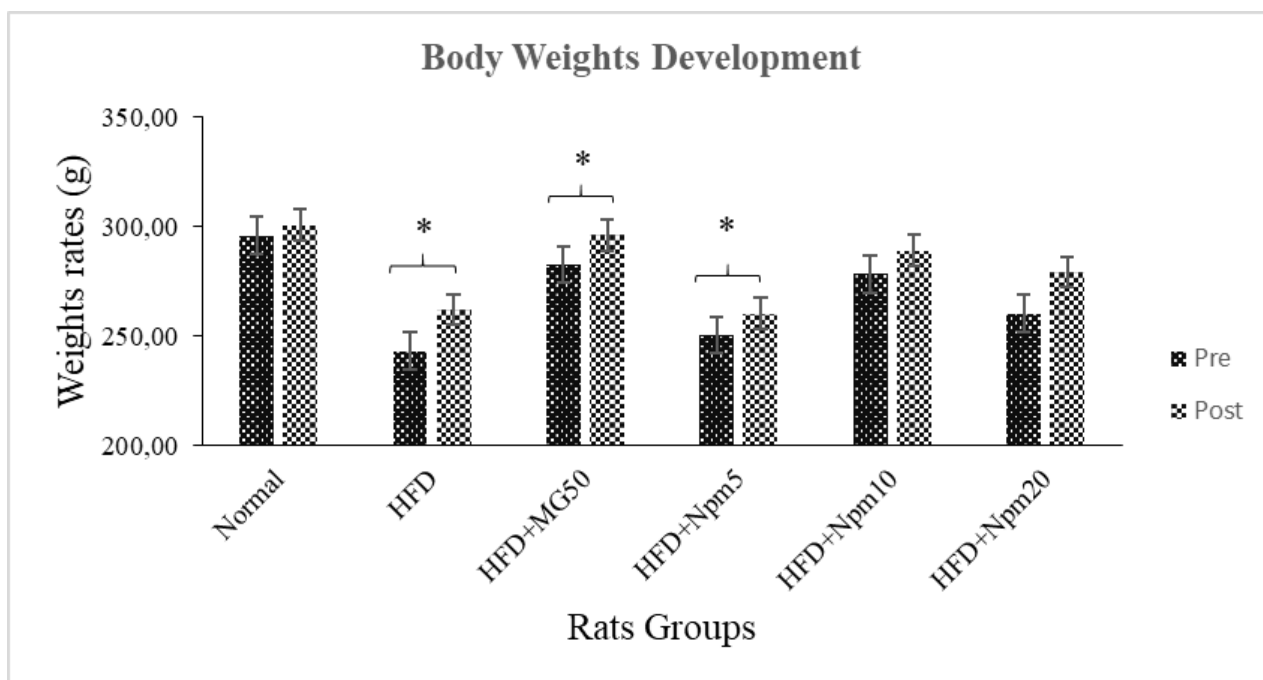


Figure 5 The development of rats body weight between pre and post study based on T-test analysis (*) indicated significance with p-value < 0.05

The HFD rat group receiving conventional *M. speciosa* extract at a dose of 50 mg/kg BW (HFD+MG50) also experienced significant weight gain from the beginning to the end of the study. However, no significant weight increase was observed in the HFD groups treated with *M. speciosa* nanoparticles at doses of 10 mg/kg BW (HFD+Npm10) and 20 mg/kg BW (HFD+Npm20) by the end of the study. These findings are consistent with previous studies using *M. speciosa* extract at a dose of 50 mg/kg BW, which has been shown to reduce weight gain and food intake in rats fed a regular diet (Kumarnsit et al., 2006).

Therefore, the absence of weight gain in rats in this study could be achieved using a minimum dose of 10 mg/kg BW until the end of the study. The HFD rats group with conventional *M. speciosa* extract (HFD + MG50) in the study experienced significant weight gain between the beginning and the end of the study. Thus, the administration of *M. speciosa* extract 50 mg/kg

BW in this study is different from the findings of previous studies using the same dose (Kumarnsit et al., 2006). By the end of the study, all HFD rat groups receiving *M. speciosa* extract and nanoparticles showed significant weight gain. This outcome may be attributed to the chronic effects of *M. speciosa* administration in diabetic rats, similar to previous studies where *M. speciosa* extract was given at a dose of 100 mg/kg BW for 5 weeks (P. Zhang et al., 2023).

The absence of weight gain in HFD rats with *M. speciosa* nanoparticles could be caused by Mitragynine in *M. speciosa* being delivered targeted to the β_1 adrenergic receptor. This process then forms thermogenesis activity that oxidizes lipids in tissues in BAT and WAT. The author's assumption is also based on findings from previous studies explaining that agonist induction (norepinephrine) of 0.25 mg/kg BW in rats can activate β_1 or β_3 adrenergic receptors, thereby triggering a decrease in food intake in mice

and preventing weight gain in rats (Racotta & Soto-Mora, 1993). Another study that supports this is the use of Calafate extract at a dose of 50 mg/kg BW, which results in weight suppression in HFD-induced rats. No weight gain is supported by the presence of UCP-1 activity in IWAT, increased energy burning, and fatty acids (FA) oxidation in BAT (Ramirez et al., 2021).

The authors also suspect that there is a possibility of decreased food intake because *M. speciosa* nanoparticles are antagonistic to opioid receptors. This prediction is also based on previous studies that explain that the trial of administering agonists to μ -opioid receptors causes an increase in eating in rats while administering antagonists to μ -opioid receptors decreases food intake in rats (Nogueiras et al., 2012). However, additional tests, such as *in vitro* studies, are still needed to ensure that the alkaloid that is delivered explicitly to adrenergic is Mitragynine. In addition, to avoid more vital stimulation of μ -opioid receptors by the alkaloid 7-HydroxyMitragynine, as reported in previous studies (Obeng et al., 2021). Examination of opioid receptors *in vitro* also needs to be done to ensure that *M. speciosa* nanoparticles are antagonistic to μ -opioid receptors. In vitro tests can be done by analyzing the comparison of activity between $\beta 1$ and μ -opioid adrenergic receptors using the receptor-binding assay and GTPyS Functional Assay (Obeng et al., 2021).

Rat's Body Temperature

The body temperature analysis of the rats, conducted using a paired T-test, revealed a significant increase between the pre- and post-study measurements in the HFD group treated with 10 mg/kg BW *M. speciosa* extract nanoparticles (p-value = 0.021). Table 5 refers to the results of the complete development of rats' body temperature. The results of the group comparisons, based on the Kruskal-Wallis post hoc Mann-Whitney test, indicated significant

differences between the Normal Group and the HFD+MG50, HFD+Npm5, HFD+Npm10, and HFD+Npm20 groups, with p-values of 0.004, 0.008, 0.001, and 0.006, respectively. In addition, a significant difference was found between HFD vs HFD+MG50, HFD+Npm5, HFD+Npm10, and HFD+Npm20 values (Figure 7).

Higher body temperature conditions can indicate the production of thermogenesis produced by extracts and nanoparticles *M. speciosa*. Physiologically heat production is caused by the activity of the UCP-1 that takes place in the BAT through the electron transport mechanism and changes in proton gradiensi through the mitochondrial membrane (Ying et al., 2023). This temperature increase was also supported by the expression of the higher UCP-1 protein in the Western Blotting test. Higher body temperature conditions can be caused by lipolysis activity in the form of burning energy stored in IWAT and used by Bat to activate UCP-1 to produce body heat. The use of energy in this IWAT will cause browning so that lipid droplets stored in adipocytes are reduced. The formation of browning in WAT can also increase insulin sensitivity thereby increasing tolerance to glucose as evidenced in previous studies using induction deferasirox with a dose of 20 mg/kg BW in HFD rats (Nazari et al., 2022).

Histological Analysis of iBAT

The expected parameter of the Adiposa iBAT histology analysis is a group of HFD rats given by nanoparticle *M. speciosa* induction, which has a total of more adipocytes than HFD groups without induction. The study findings obtained in this study were the picture of the Adipose iBAT tissue in the HFD+Npm5, HFD+Npm10 group, and HFD+Npm20 have more dense multilocular droplets than HFD groups. The results of histology analysis are presented in Figure 8.

Table 5. The development of rat body temperature between time of measurements

Groups (n=7)	Pre Study (°C)	Post Study (°C)	p-Values
Normal	35.8 ± 0.39	35.2 ± 1.38	0.270
HFD	35.9 ± 0.66	35.2 ± 0.71	0.103
HFD+MG50	36.3 ± 0.88	36.3 ± 0.33	0.934
HFD+Npm5	35.9 ± 1.07	36.2 ± 0.23	0.567
HFD+Npm10	35.8 ± 0.38	36.5 ± 0.32	0.021*
HFD+Npm20	35.9 ± 0.74	36.3 ± 0.31	0.268

Note: (*) indicated significance with p-value < 0.05 based on paired-T test

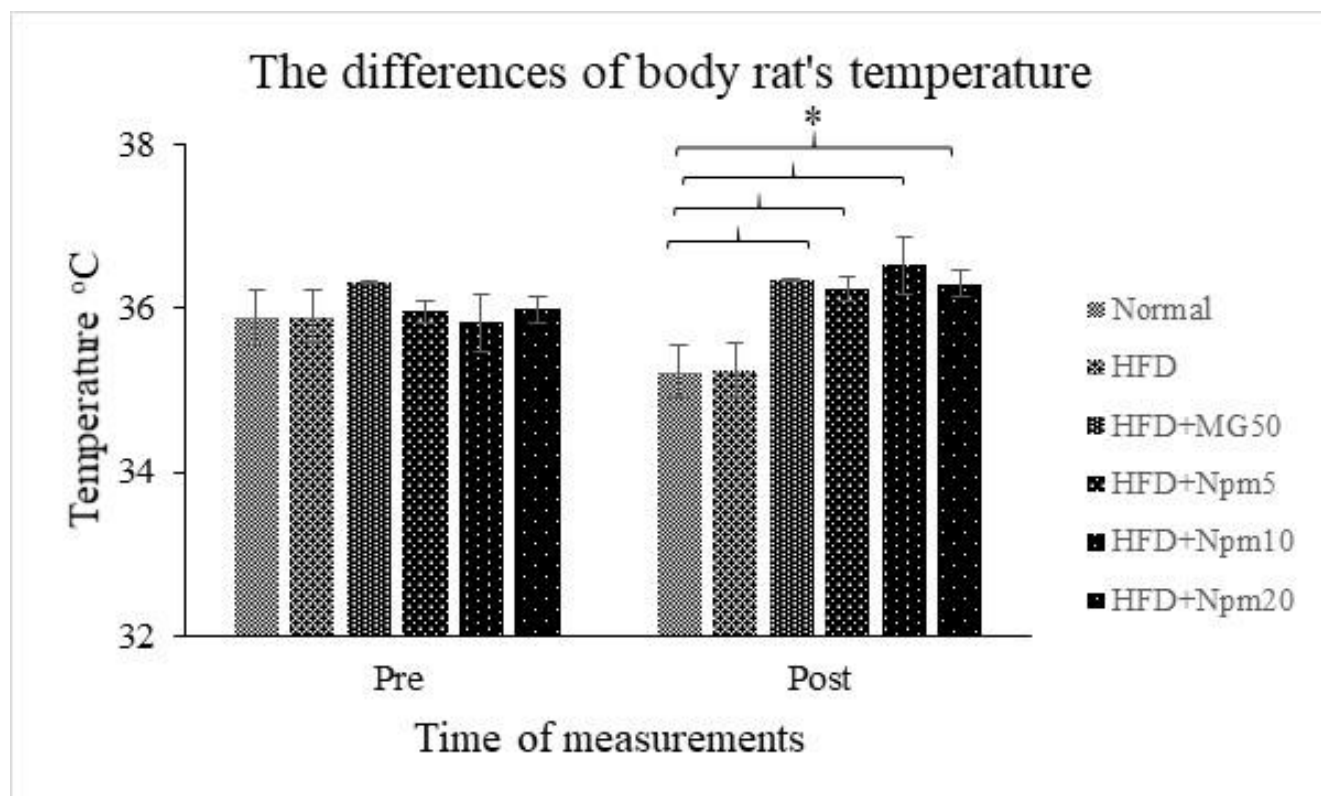


Figure 6. The difference of rat's body temperature means between groups based on *Kruskal Wallis* test, (*) significance with p-value < 0.05

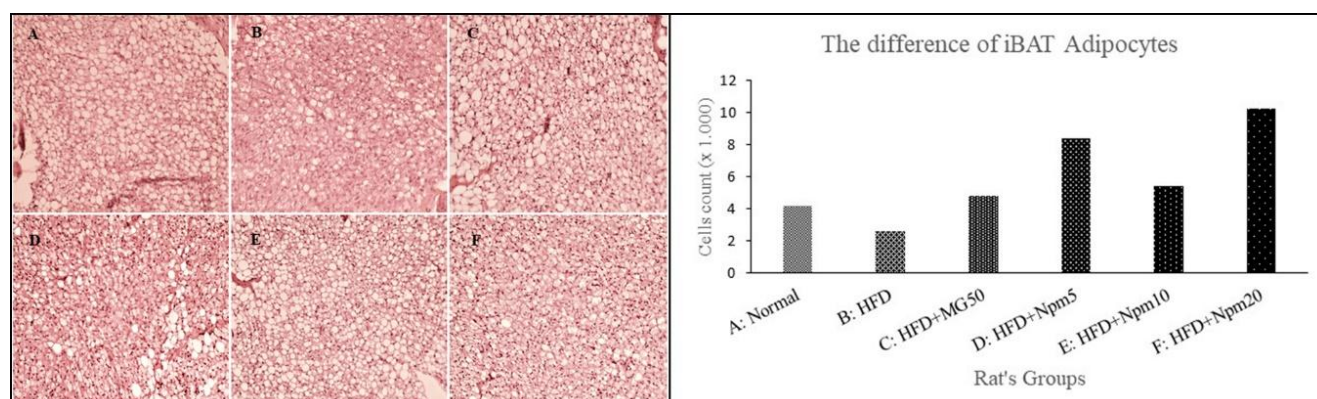


Figure 7. The histological results of iBAT adipocytes between rats groups of (A) Normal (B) HFD (C) HFD+MG50 (D) HFD+Npm5 (E) HFD+Npm10 (F) HFD+Npm20

In this study, the total adipocytes in iBAT were higher in HFD groups with *M. speciosa* extract nanoparticles at 5 mg/kg BW, 10 mg/kg BW, and 20 mg/kg BW. This result indicates that Nanoparticles of *M. speciosa* extract are predicted to improve adiposity in iBAT.

Feeding a high-fat diet leads to a reduction in adipocytes within the BAT. Histological analysis revealed that the number of adipocytes in the HFD rat groups was lower compared to those in the HFD group treated with *M. speciosa* extract nanoparticles. The reduction in BAT adipocytes is attributed to the decreased expression of Vascular Endothelial Growth Factor A (VEGF-A) and pro-angiogenic cytokine proteins, which promote capillary depletion and hypoxia in BAT (Syamsunarno et al., 2021).

In the physiological concept, FA and glucose, when not used, will be stored as reserves in the form of triglycerides by hepatocytes in WAT. When activation stimulates non-shivering thermogenesis, triglycerides are excreted from hepatocytes and transferred to VLDL. This VLDL is then sent to the BAT for heat production materials. Thus, LDL levels will be reduced for heat production (Ying et al., 2023). In this study, LDL levels that are in line with the condition of the adipocytes in iBAT, namely in the HFD group with *M. speciosa* extract nanoparticles 10 mg/kg BW, but not suitable for groups that obtain nanoparticles with a dose of 20 mg/kg BW.

Western Blotting Analysis of UCP-1

The Western Blot analysis of the IWAT tissues revealed significant differences between the HFD group (0.99 ± 0.003 AU) and the HFD+MG50 group (4.05 ± 0.472 AU), with a p-value of 0.002. Similarly, significant differences were observed between the HFD group and the HFD+Npm5 group (3.46 ± 0.436 AU) with a p-value of 0.008, the HFD group and the HFD+Npm10 group (4.01 ± 0.159 AU) with a p-value of 0.016, and the HFD group and the HFD+Npm20

group (4.34 ± 0.152 AU) with a p-value of 0.004 (Figure 9). The provision of conventional extracts of *M. speciosa* with a dose of 50 mg/kg BW in this study stimulates the activity of the UCP-1. The results of this study support the previous research, which suggests that Mitragynine from *M. speciosa* has the potential to activate adrenergic receptors (Obeng et al., 2022).

This study's results also complement the previous study's findings that explain that the induction of agonists (norepinephrine) of 0.25 mg/kg BW in rats can stimulate adrenergic receptors β to suppress food intake and weight in rats (Racotta & Soto-Mora, 1993).

The extract results are similar to the administration of *M. speciosa* extract nanoparticle therapy at 5 mg/kg BW, 10 mg/kg BW, and 20 mg/kg BW. UCP-1 activity was significantly higher than the HFD group without additional therapy. Other studies that use nanoparticle of Eucalyptus extract 214 mg/kg BW found a similar mechanism, namely the activation of the UCP-1 in groups that obtain nanoparticle extract eucalyptus, but resulted in an improvement in the profile of lipid triglycerides, HDL, cholesterol, and LDL in HFD groups (Escobar-Chaves et al., 2023). This study did not include an analysis of lipid profiles. Consequently, further assessment of lipid profiles is required to confirm the effects of UCP-1 activity.

CONCLUSIONS

Based on the *in silico* test, Mitragynine has the potential to be an agonist of adrenergic receptors β_1 and β_3 , indicated by a higher affinity value and residue similar to ligand native epinephrine. The weight of the rat does not increase only in the group of HFD rats given nanoparticles of *M. speciosa* extracts with a dose of 10 and 20 mg/kg BW. Meanwhile, nanoparticles with a dose of 5, 10, and 20 mg/kg BW can stimulate UCP-1 activity and produce higher body temperature in rats given HFD feed.

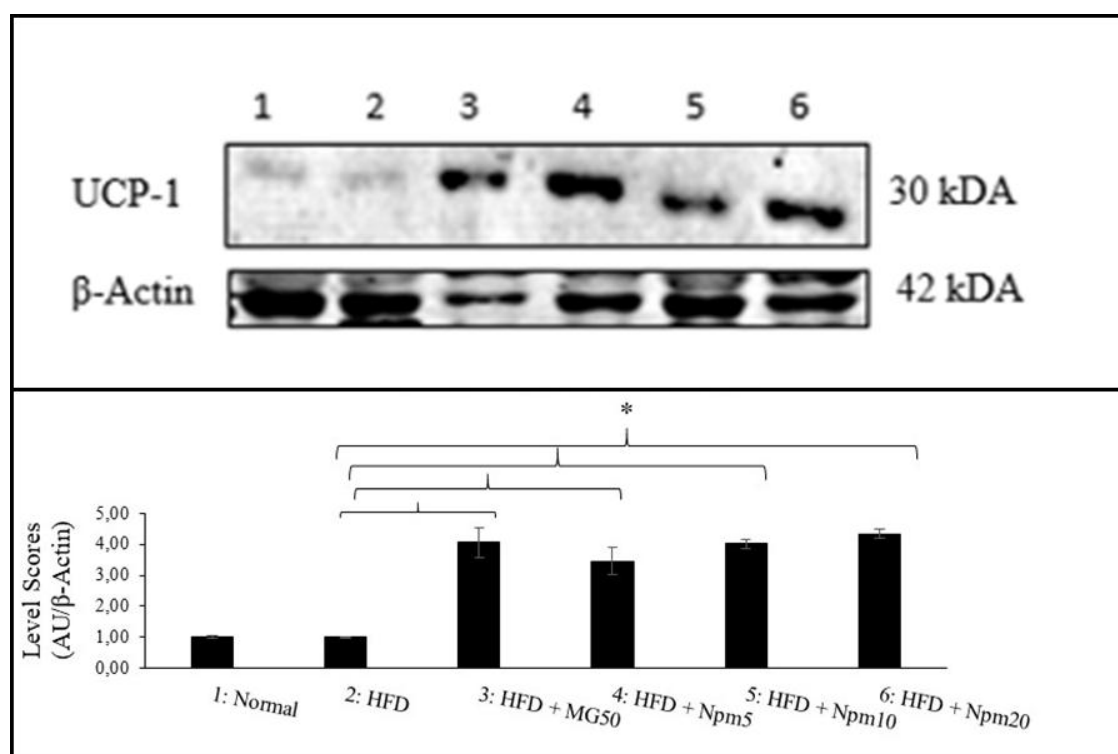


Figure 8. The difference of UCP-1 protein levels western blotting based on ANOVA test, (*) indicated significance values < 0.05

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests in this study.

REFERENCES

- Abd El-Aziz, N. M., Khalil, B. E., & El-Gamal, N. N. (2023). Structure prediction, docking studies and molecular cloning of novel *Pichia kudriavzevii* YK46 metalloprotease (MetPr) for improvement of feather waste biodegradation. *Scientific Reports* 13(1), 1–16.
- Amin, M. K., & Boateng, J. S. (2022). Enhancing Stability and Mucoadhesive Properties of Chitosan Nanoparticles by Surface Modification with Sodium Alginate and Polyethylene Glycol for Potential Oral Mucosa Vaccine Delivery. *Marine Drugs* 20(3), .
- Barthe, M., Lefebvre, F., Itturioz, X., Moine, L., Tsapis, N., & Fischmeister, R. (2020). PEGylated-Ligands as tools to study β -adrenergic receptor function on surface and T-tubule membranes. *Archives of Cardiovascular Diseases Supplements* 12(2), 241.
- Bashir, M. I., Aziz, N. H. K. A., Noor, D. A. M., Ibrahim, U. I., & Zahari, Z. (2024). In-silico Molecular Docking of two Bioactive Constituents (Quercetin 3-Glucuronide and Quercitrin) from *Polygonum minus* Leaves into Monoamine Oxidase - A Crystal Structure. *Biomedical and Pharmacology Journal* 17(3), 1785–1793.
- Begum, H., Murugesan, P., & Tangutur, A. D. (2022). Western Blotting: A Powerful Staple In Scientific and Biomedical Research. *BioTechniques* 73(1), 58–69.
- Beng, G. T., Hamdan, M. R., Siddiqui, M. J., Mordi, M. N., & Mansor, S. M. (2011). A simple and cost effective isolation and purification protocol of mitragynine from *mitragyna speciosa* korth (ketum) leaves. *Malaysian Journal of Analytical Sciences* 15(1), 54–60.
- Borst, S. E., Oliver, R. J., Sego, R. L., & Scarpace, P. J. (1994). α -Adrenergic receptor-mediated thermogenesis in brown adipose tissue of rat. *General Pharmacology* 25(8), 1703–1710.
- Cheaha, D., Reakkamnuan, C., Nukitram, J., Chittrakarn, S., Phukpattaranont, P., Keawpradub, N., & Kumarnsit, E. (2017). Effects of alkaloid-rich extract from *Mitragyna speciosa* (Korth.) Havil. on naloxone-precipitated morphine withdrawal symptoms and local field

- potential in the nucleus accumbens of mice. *Journal of Ethnopharmacology* 208 129–137.
- Chien, M. Y., Ku, Y. H., Chang, J. M., Yang, C. M., & Chen, C. H. (2016). Effects of herbal mixture extracts on obesity in rats fed a high-fat diet. *Journal of Food and Drug Analysis* 24(3), 594–601.
- Elmowafy, M., Shalaby, K., Elkomy, M. H., Alsaidan, O. A., Gomaa, H. A. M., Abdelgawad, M. A., & Mostafa, E. M. (2023). Polymeric Nanoparticles for Delivery of Natural Bioactive Agents: Recent Advances and Challenges. In *Polymers* (Vol. 15, Issue 5).
- Escobar-Chaves, E., Acin, S., Muñoz, D. L., Fernández, M., Echeverri, A., Echeverri, F., Orozco, J., & Balcázar, N. (2023). Polymeric nanoformulation prototype based on a natural extract for the potential treatment of type 2 diabetes mellitus. *Journal of Drug Delivery Science and Technology* 81 104264.
- Fain, J. N., & Garcia Sainz, J. A. (1983). Adrenergic regulation of adipocyte metabolism. *Journal of Lipid Research* 24(8), 945–966.
- Federation, W. O. (2022). *World Obesity Atlas 2022*.
- Feng, K., Zhu, X., Chen, T., Peng, B., Lu, M., Zheng, H., Huang, Q., Ho, C. T., Chen, Y., & Cao, Y. (2019). Prevention of Obesity and Hyperlipidemia by Heptamethoxyflavone in High-fat Diet-induced Rats. *Journal of Agricultural and Food Chemistry* 67(9), 2476–2489.
- Flores-Bocanegra, L., Raja, H. A., Graf, T. N., Augustinović, M., Wallace, E. D., Hematian, S., Kellogg, J. J., Todd, D. A., Cech, N. B., & Oberlies, N. H. (2020). The Chemistry of Kratom [*Mitragyna speciosa*]: Updated Characterization Data and Methods to Elucidate Indole and Oxindole Alkaloids. *Journal of Natural Products* 83(7), 2165–2177.
- Huang, R., Yu, Q., Tamalunas, A., Stief, C. G., & Hennenberg, M. (2024). Ligand–Receptor Interactions and Structure–Function Relationships in Off-Target Binding of the β 3-Adrenergic Agonist Mirabegron to α 1A-Adrenergic Receptors. In *International Journal of Molecular Sciences* (Vol. 25, Issue 13).
- Islam, M. A., Rallabandi, V. P. S., Mohammed, S., Srinivasan, S., Natarajan, S., Dudekula, D. B., & Park, J. (2021). Screening of β 1- and β 2-Adrenergic Receptor Modulators through Advanced Pharmacoinformatics and Machine Learning Approaches. In *International Journal of Molecular Sciences* (Vol. 22, Issue 20).
- Jintawiwat, R., Punamontarakul, N., Hirunyasiri, R., Jarupoom, P., Kulphaisal, P., Pankasemsuk, T., Supasin, S., & Kawee-ai, A. (2023). *Testing of a Prototype Combining Ultrasound and Pulsed Electric Field on Extracted Valuable Compounds of Mitragyna speciosa Leaves*.
- Joseph, J. A., Akkermans, S., & Van Impe, J. F. M. (2022). Processing Method for the Quantification of Methanol and Ethanol from Bioreactor Samples Using Gas Chromatography-Flame Ionization Detection. *ACS Omega* 7(28), 24121–24133.
- Kamsani, N. E., Zakaria, Z. A., Md Nasir, N. L., Mohtarrudin, N., & Mohamad Alitheen, N. B. (2019). Safety Assessment of Methanol Extract of *Melastoma malabathricum* L. Leaves following the Subacute and Subchronic Oral Consumptions in Rats and Its Cytotoxic Effect against the HT29 Cancer Cell Line. *Evidence-Based Complementary and Alternative Medicine: ECAM* 2019 5207958.
- Karunakaran, T., Goh, Y. S., Santhanam, R., Murugaiyah, V., Abu Bakar, M. H., & Ramanathan, S. (2022). RP-HPLC-DAD Analysis of Mitragynine Content in *Mitragyna speciosa* Korth. (Ketum) Leaf Extracts Prepared Using Ultrasound Assisted Extraction Technique and Their Cytotoxicity. In *Separations* (Vol. 9, Issue 11).
- Kataoka, N., Takeuchi, T., Kusudo, T., Li, Y., Endo, Y., & Yamashita, H. (2020). Lack of UCP1 stimulates fatty liver but mediates UCP1-independent action of beige fat to improve hyperlipidemia in Apoe knockout mice. *Biochimica et Biophysica Acta - Molecular Basis of Disease* 1866(7), 165762.
- Khoirul Rista Abidin, Ariffaldi Nurhidayatulloh, & Nurmah. (2024). In Silico Identification of Bajakah Root (*Spatholobus littoralis* Hassk) Alkaloid Compounds to Stimulate Lipolysis Through Inhibition of Phosphodiesterase-4. *Science & Technology Asia* 29(3 SE-), 269–279.
- Kovacs, P., & Hajnal, A. (2022). Short-term high-fat diet consumption increases body weight and body adiposity and alters brain stem taste information processing in rats. *Chemical Senses* 47bjac020.
- Kumarnsit, E., Keawpradub, N., & Nuankaew, W. (2006). Acute and long-term effects of alkaloid extract of *Mitragyna speciosa* on food and water intake and body weight in rats. *Fitoterapia* 77(5), 339–345.
- Lee, Z., Llanes, E. J., Sukmawan, R., Thongtang, N., Quang, H., & Ho, T. (2021). *Prevalence of plasma lipid disorders with an emphasis on LDL cholesterol in selected countries in the Asia-Pacific region*. 1–12.
- Lesmana, R., Siannoto, M., Nugraha, G. I., Goenawan, H., Feinisa, A. K., Pratiwi, Y. S., Veronica, F., Tarawan, V. M., Susianti, S., & Supratman, U. (2021). Nutmeg extract potentially alters characteristics of white adipose tissue in rats. *Veterinary Medicine and Science* 7(2), 512–520.
- Li, H., Huang, E., Zhang, Y., Huang, S.-Y., & Xiao, Y. (2022). HDock update for modeling protein-RNA/DNA complex structures. *Protein Science* 31(11), e4441.

- Li, S., Zhang, H., Chen, K., Jin, M., Vu, S. H., Jung, S., He, N., Zheng, Z., & Lee, M.-S. (2022). Application of chitosan/alginate nanoparticle in oral drug delivery systems: prospects and challenges. *Drug Delivery* 29(1), 1142–1149.
- López-Fernández, O., Domínguez-Valencia, R., Santos, E., Pateiro, M., Munekata, P., Campagnol, P., & Lorenzo, J. M. (2022). Comparison Between HPLC-PAD and GC-MS Methods for the Quantification of Cholesterol in Meat. *Food Analytical Methods* 15 1–14.
- Luan, J., Hu, B., Wang, H., Liu, H., Wang, S., Chen, L., Li, W., Wang, J., & Cheng, M. (2023). Insights into β 3-adrenoceptor agonism through comprehensive in silico investigation. *Computational Biology and Chemistry* 104(January), .
- Maradonna, F., & Carnevali, O. (2018). Lipid metabolism alteration by endocrine disruptors in animal models: An overview. *Frontiers in Endocrinology* 9(November), 1–14.
- Marianti, A., Amalina, N. D., Mursiti, S., Sitompul, F. N., Futri, S., Negara, L. G., Sholehah, I. K., Sri, D., & Astari, P. D. (2024). *Pharmacokinetics Profile of Chitosan Nanoparticles in Chronic Lead-induced Toxicity Rats Model*. 16(1), 107–115.
- Masalova, O., Kulikouskaya, V., Shutava, T., & Agabekov, V. (2013). Alginate and chitosan gel nanoparticles for efficient protein entrapment. *Physics Procedia* 40 69–75.
- Meyer, C. W., Ootsuka, Y., & Romanovsky, A. A. (2017). *Body Temperature Measurements for Metabolic Phenotyping in Mice*. 8(July), 1–13.
- Muchtaridi, M., Suryani, A. I., Wathoni, N., Herdiana, Y., Mohammed, A. F., Gazzali, A. M., Lesmana, R., & Joni, I. M. (2023). Chitosan/Alginate Polymeric Nanoparticle-Loaded α -Mangostin: Characterization, Cytotoxicity, and In Vivo Evaluation against Breast Cancer Cells. In *Polymers* (Vol. 15, Issue 18).
- Mudge, E. M., & Brown, P. N. (2017). Determination of Mitragynine in Mitragyna speciosa Raw Materials and Finished Products by Liquid Chromatography with UV Detection: Single-Laboratory Validation. *Journal of AOAC INTERNATIONAL* 100(1), 18–24.
- Nazari, M., Ho, K. W., Langley, N., Cha, K. M., Kods, R., Wang, M., Laybutt, D. R., Cheng, K., Stokes, R. A., Swarbrick, M. M., & Gunton, J. E. (2022). Iron chelation increases beige fat differentiation and metabolic activity , preventing and treating obesity. *Scientific Reports* 1–12.
- Nogueiras, R., Romero-Picó, A., Vazquez, M. J., Novelle, M. G., López, M., & Diéguez, C. (2012). The opioid system and food intake: Homeostatic and hedonic mechanisms. *Obesity Facts* 5(2), 196–207.
- Noor, Y., Jasaputra, D. K., Gunadi, J. W., Lesmana, R., & Safira, R. A. (2023). The Effect of Turmeric and Mangosteen Peel Extract on PPAR α Gene Expression in the Retina of HFD-Induced Rat Model. *Journal of Medicine and Health* 5(2), 156–168.
- Novindriani, D., Novindriana, D., Wijianto, B., & Andrie, M. (2021). Studies on the Sedative Effect of Mitragyna speciosa Korth. as an Endemic Plant in West Borneo, Indonesia. *Letters in Applied NanoBioScience* 11(2), 3344–3349.
- Obeng, S., Kamble, S. H., Reeves, M. E., Restrepo, L. F., Patel, A., Behnke, M., Chear, N. J.-Y., Ramanathan, S., Sharma, A., León, F., Hiranita, T., Avery, B. A., McMahon, L. R., & McCurdy, C. R. (2020). Investigation of the Adrenergic and Opioid Binding Affinities, Metabolic Stability, Plasma Protein Binding Properties, and Functional Effects of Selected Indole-Based Kratom Alkaloids. *Journal of Medicinal Chemistry* 63(1), 433–439.
- Obeng, S., Leon, F., Patel, A., Zuarth Gonzalez, J. D., da Silva, L. C., Restrepo, L. F., Gamez-Jimenez, L. R., Ho, N. P., Guerrero Calvache, M. P., Pallares, V. L. C., Helmes, J. A., Shiomitsu, S. K., Soto, P. L., McCurdy, C. R., McMahon, L. R., Wilkerson, J. L., & Hiranita, T. (2022). Interactive Effects of m-Opioid and Adrenergic- α 2 Receptor Agonists in Rats: Pharmacological Investigation of the Primary Kratom Alkaloid Mitragynine and Its Metabolite 7-Hydroxymitragynine. *J Pharmacol Exp Ther* 383(3), 182–198.
- Obeng, S., Wilkerson, J. L., León, F., Reeves, M. E., Restrepo, L. F., Gamez-Jimenez, L. R., Patel, A., Pennington, A. E., Taylor, V. A., Ho, N. P., Braun, T., Fortner, J. D., Crowley, M. L., Williamson, M. R., Pallares, V. L. C., Mottinelli, M., Lopera-Londoño, C., McCurdy, C. R., McMahon, L. R., & Hiranita, T. (2021). Pharmacological Comparison of Mitragynine and 7-Hydroxymitragynine: In Vitro Affinity and Efficacy for μ -Opioid Receptor and Opioid-Like Behavioral Effects in Rats. *The Journal of Pharmacology and Experimental Therapeutics* 376(3), 410–427.
- Oksal, E., Pangestika, I., Muhammad, T. S. T., Mohamad, H., Amir, H., Kassim, M. N. I., & Andriani, Y. (2020). In vitro and in vivo studies of nanoparticles of chitosan-Pandanus tectorius fruit extract as new alternative treatment for hypercholesterolemia via Scavenger Receptor Class B type 1 pathway. *Saudi Pharmaceutical Journal* 28(10), 1263–1275.
- Padín-González, E., Lancaster, P., Bottini, M., Gasco, P., Tran, L., Fadeel, B., Wilkins, T., & Monopoli, M. P. (2022). Understanding the Role and Impact of Poly (Ethylene Glycol) (PEG) on Nanoparticle Formulation: Implications for COVID-19 Vaccines. *Frontiers in Bioengineering and Biotechnology* 10.
- Pandey, S., Pandey, P., Kumar, R., & Singh, N. P. (2011). Residual solvent determination by head space gas chromatography with flame ionization detector in omeprazole API. *Brazilian Journal of*

- Pharmaceutical Sciences* 47(2), 379–384.
- Parthasarathy, S., Ramanathan, S., Murugaiyah, V., Hamdan, M. R., Mohd Said, M. I., Lai, C. S., & Mansor, S. M. (2013). A simple HPLC-DAD method for the detection and quantification of psychotropic mitragynine in *Mitragyna speciosa* (ketum) and its products for the application in forensic investigation. *Forensic Science International* 226(1–3), 183–187.
- Payab, M., Aletaha, A., & Aminjan, H. H. (2019). *Effect of the herbal medicines in obesity and metabolic syndrome: A systematic review and meta - analysis of clinical trials. September* 1–20.
- Pedroso-Santana, S., & Fleitas-Salazar, N. (2020). Ionotropic gelation method in the synthesis of nanoparticles/microparticles for biomedical purposes. *Polymer International* 69(5), 443–447.
- Qiao, Y., Zhu, B., Tian, A., & Li, Z. (2017a). PEG-coated gold nanoparticles attenuate β -adrenergic receptor-mediated cardiac hypertrophy. *International Journal of Nanomedicine* 12 4709–4719.
- Qiao, Y., Zhu, B., Tian, A., & Li, Z. (2017b). PEG-coated gold nanoparticles attenuate β -adrenergic receptor-mediated cardiac hypertrophy. *International Journal of Nanomedicine* 12 4709–4719.
- Quirós Cognuck, S., Reis, W. L., Silva, M., Debarba, L. K., Mecawi, A. S., de Paula, F. J. A., Rodrigues Franci, C., Elias, L. L. K., & Antunes-Rodrigues, J. (2020). Sex differences in body composition, metabolism-related hormones, and energy homeostasis during aging in Wistar rats. *Physiological Reports* 8(20), e14597.
- Racotta, R., & Soto-Mora, L. M. (1993). Specificity of alpha- and beta-adrenergic inhibition of water and food intake. *Physiology and Behavior* 53(2), 361–365.
- Rafferty, R., O'Brien, F. J., & Cryan, S. A. (2013). Chitosan for gene delivery and orthopedic tissue engineering applications. *Molecules* 18(5), 5611–5647.
- Rahbani, J. F., Scholtes, C., Lagarde, D. M., Hussain, M. F., Roesler, A., Dykstra, C. B., Bunk, J., Samborska, B., O'Brien, S. L., Tripp, E., Pacis, A., Angueira, A. R., Johansen, O. S., Cinkornpumin, J., Hossain, I., Lynes, M. D., Zhang, Y., White, A. P., Pastor, W. A., ... Kazak, L. (2022). ADRA1A-Gq signalling potentiates adipocyte thermogenesis through CKB and TNAP. *Nature Metabolism* 4(11), 1459–1473.
- Ramanathan, S., Parthasarathy, S., Murugaiyah, V., Magosso, E., Tan, S. C., & Mansor, S. M. (2015). Understanding the physicochemical properties of mitragynine, a principal alkaloid of *Mitragyna speciosa*, for preclinical evaluation. *Molecules* 20(3), 4915–4927.
- Ramirez, L. A., Quezada, J., Duarte, L., Concha, F., Escobillana, L., Rincon-Cervera, M. A., Perez-Bravo, F., Elorza, A. A., Bravo-Sagua, R., & Garcia-Diaz, D. F. (2021). The administration of an extract from *Berberis microphylla* stimulates energy expenditure, thermogenesis and mitochondrial dynamics in mice brown adipose tissue. *Food Bioscience* 41 100988.
- Rosenbaum, M., & Leibel, R. L. (2013). *Adaptive thermogenesis in humans. 34*(0 1), 1–15.
- Sabetghadam, A., Ramanathan, S., Sasidharan, S., & Mansor, S. M. (2013). Subchronic exposure to mitragynine, the principal alkaloid of *Mitragyna speciosa*, in rats. *Journal of Ethnopharmacology* 146(3), 815–823.
- Sanches, M. P., Gross, I. P., Saatkamp, R. H., Parize, A. L., & Soldi, V. (2020). Chitosan-sodium alginate polyelectrolyte complex coating pluronic® F127 nanoparticles loaded with citronella essential oil. *Journal of the Brazilian Chemical Society* 31(4), 803–812.
- Sanjaya, A., Lesmana, R., Goenawan, H., Setiawan, I., Sylviana, N., Pratiwi, Y. S., Dewi, F. N., & Supratman, U. (2023). The functional relationship of Yap/Taz with autophagy functions in sarcopenia associated with aging. *Nutrition and Healthy Aging* 8 31–39.
- Shi, L., Zhang, J., Zhao, M., Tang, S., Cheng, X., Zhang, W., Li, W., Liu, X., Peng, H., & Wang, Q. (2021). Effects of polyethylene glycol on the surface of nanoparticles for targeted drug delivery. *Nanoscale* 13(24), 10748–10764.
- Stein, C. (2013). Opioids, sensory systems and chronic pain. *European Journal of Pharmacology* 716(1), 179–187.
- Ster, L., Durand, G., Taouis, M., Dagou, C., Dagou, C., Durand, G., & Dela-, J. (2002). *N -3 Polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. April.*
- Suryani, A. I., Wathoni, N., Muchtaridi, M., & Joni, I. M. (2022). *IN VIVO EVALUATION OF ALFA MANGOSTIN CHITOSANALGINATE NANOPARTICLES (NANO-AMKAL) AS ANTI-BREAST CANCER (Thesis).* Universitas Padjadjaran.
- Sveidahl Johansen, O., Ma, T., & Gerhart-Hines, Z. (2022). Leveraging GPCR signaling in thermogenic fat to counteract metabolic diseases. *Molecular Metabolism* 60 101474.
- Syamsunarno, M. R. A. A., Alia, F., & Anggraeni, N. (2021). *Ethanol extract from Moringa oleifera leaves modulates brown adipose tissue and bone morphogenetic protein 7 in high-fat diet mice. 14* 1234–1240.
- Syamsunarno, M. R. A. A., Iso, T., Yamaguchi, A., Hanaoka, H., Putri, M., Obokata, M., Sunaga, H., Koitabashi, N., Matsui, H., Maeda, K., Endo, K., Tsushima, Y., Yokoyama, T., & Kurabayashi, M. (2014). Fatty Acid Binding Protein 4 and 5 Play a Crucial Role in Thermogenesis under the Conditions of Fasting and Cold Stress. *PLOS ONE*

- α 3), e90825.
- Vilar, S., Sobarzo-Sanchez, E., Santana, L., & Uriarte, E. (2017). Molecular Docking and Drug Discovery in β -Adrenergic Receptors. *Current Medicinal Chemistry* 24(39), .
- Vojvodić, S., Čonić, B. S., & Torović, L. (2023). Safety assessment of herbal food supplements: Ethanol and residual solvents associated risk. *Journal of Food Composition and Analysis* 122 105483.
- Wathoni, N., Meylina, L., Rusdin, A., Abdelwahab Mohammed, A. F., Tirtamie, D., Herdiana, Y., Motoyama, K., Panatarani, C., Joni, I. M., Lesmana, R., & Muchtaridi, M. (2021). The potential cytotoxic activity enhancement of α -mangostin in chitosan-kappa carrageenan-loaded nanoparticle against mcf-7 cell line. *Polymers* 13(11), 1–13.
- Wathoni, N., Rusdin, A., Febriani, E., Purnama, D., Daulay, W., Azhary, S. Y., Panatarani, C., Joni, I. M., Lesmana, R., Motoyama, K., & Muchtaridi, M. (2019). Formulation and Characterization of α -Mangostin in Chitosan Nanoparticles Coated by Sodium Alginate, Sodium Silicate, and Polyethylene Glycol. *Journal of Pharmacy & Bioallied Sciences* 11(Suppl 4), S619–S627.
- WHO. (2021). *Obesity and overweight*.
- World Health Organization. (1997). Methanol Health and Safety Guide. *World Health Organization* 105(105), 1–31.
- Wu, Y., Zeng, L., & Zhao, S. (2021). Ligands of Adrenergic Receptors: A Structural Point of View. In *Biomolecules* (Vol. 11, Issue 7).
- Wulandari, I. . (2019). *Development of A Method For Synthesis of Fe3O4 Nanoparticles Through Surface Modification Based on Biocompatible Molecules as A Candidate Drug Delivery Agent*. Universitas Brawijaya.
- Ying, Z., Tramper, N., Zhou, E., Boon, M. R., Rensen, P. C. N., & Kooijman, S. (2023). Role of thermogenic adipose tissue in lipid metabolism and atherosclerotic cardiovascular disease: lessons from studies in mice and humans. *Cardiovascular Research* 119(4), 905–918.
- Ylitalo, R., Lehtinen, S., Wuolijoki, E., Ylitalo, P., & Lehtimäki, T. (2002). Cholesterol-lowering properties and safety of chitosan. *Arzneimittel-Forschung* 52(1), 1–7.
- Zargar, V., Asghari, M., & Dashti, A. (2015). A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives, and Applications. *ChemBioEng Reviews* 2(3), 204–226.
- Zhang, P., Wei, W., Zhang, X., Wen, C., Ovatlarnporn, C., & Olatunji, O. J. (2023). Antidiabetic and antioxidant activities of *Mitragyna speciosa* (kratom) leaf extract in type 2 diabetic rats. *Biomedicine & Pharmacotherapy* 162 114689.
- Zhang, Z., Yang, D., Xiang, J., Zhou, J., Cao, H., Che, Q., Bai, Y., Guo, J., & Su, Z. (2021). Non-shivering thermogenesis signalling regulation and potential therapeutic applications of brown adipose tissue. *International Journal of Biological Sciences* 17(11), 2853–2870.
- Zhao, Q., Liu, Z., Zhu, Y., Wang, H., Dai, Z., Yang, X., Ren, X., Xue, Y., & Shen, Q. (2022). Cooked Adzuki Bean Reduces High-Fat Diet-Induced Body Weight Gain, Ameliorates Inflammation, and Modulates Intestinal Homeostasis in Mice. In *Frontiers in nutrition* (Vol. 9, p. 918696).
- Zielińska, A., Carreiró, F., Oliveira, A. M., Neves, A., Pires, B., Venkatesh, D. N., Durazzo, A., Lucarini, M., Eder, P., Silva, A. M., Santini, A., & Souto, E. B. (2020). Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. In *Molecules* (Vol. 25, Issue 16).