Malaysian Applied Biology
https://doi.org/10.55230/mabjournal.v54i3.3242

Research Article

Shorea macrophylla (Engkabang) Endosperm Extract Elevates Lipogenesis and Adipogenesis in 3T3-L1 Mouse Adipocyte Cells

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ABSTRACT

The *Shorea macrophylla* endosperm, also known as illipe nuts, has a high lipid content, making it a favoured dietary choice for wild animals. Its composition is an interesting aspect to investigate, as it is still unknown to date despite its superior high-quality lipid profile. In this research, we conducted endosperm extraction using various solvents to investigate potential lipogenesis-inducing compounds. The acetone showed the highest endosperm extraction efficiency at 38.30% while other solvents achieved efficiency above 30%, except for ethanol and methanol. GCMS analysis revealed common compounds across extracts, with methyl stearate and methyl elaidate being among the most prevalent, except in acetone extraction. Molecular docking experiments demonstrated good binding scores between these compounds and lipogenesis-related proteins, particularly highlighting methyl elaidate's potential. *In vitro* studies conducted on 3T3-L1 cells confirmed an increase in triglyceride levels with methanol and diethyl ether extracts. Both experimental and computational analyses strengthen the potential of methyl elaidate as a pivotal lipogenesis inducer, with potential implications for enhancing fat storage in meat quality when incorporated into vertebrate diets. The effective lipogenesis-inducing elements mentioned can then be incorporated in the diet of livestock with the hope of increasing the amount of fat stored in meat and improving its overall quality.

Key words: 3T3-L1 cells, cytotoxicity, molecular docking, phytochemistry, *Shorea macrophylla*

INTRODUCTION

Lipogenesis, which occurs in the liver and adipose tissue, involves the production of fatty acids and the synthesis of triglycerides (Ameer *et al*., 2014). It is the primary pathway of fatty acid and triglyceride deposition in meat animals, and an increase in lipogenesis and intramuscular fats enhances meat tenderness and juiciness (Ladeira *et al*., 2018). Without proper regulation, lipogenesis can result in meat that is Dark, Firm, and Dry (DFD) under high-carbohydrate diet conditions, which is typically not favoured by consumers (Viljoen *et al*., 2002). There are a plethora of plant extracts that have been proven effective in improving lipid profiles of the host and inducing lipogenesis, namely fenugreek, olive, and oil palm (Heshmat-Ghahdarijani *et al*., 2020; González-Hedström *et al*., 2021; Kedir *et al*., 2023; Abdennebi *et al*., 2025). Some of the bioactive compounds identified to be the major orchestrators of *de novo* lipogenesis are quercetin, polyphenols, apigenin, kaempferol, ginsenoside, silibinin, and narigenin (Kussmann *et al*., 2023; Barbhuiya *et al*., 2024).

Engkabang (*Shorea macrophylla*) is primarily found in Southeast Asia (Chai, 1998; Ng *et al*., 2002). The engkabang endosperm, known as the illipe nut, is extremely high in fat content. The fat is rapidly used for seed development. If left unharvested, the locals collect these endosperms as soon as they fall to the ground to produce various food products (Chai, 1998). Many wild animals, including the wild empurau - one of Malaysia's most expensive fish—consume illipe nuts (Kamarudin *et al*., 2018), which have contributed to their distinctively unique flesh flavor, fragrance, and texture, as it is widely deemed that the high-quality fatty acid profiles of the engkabang had further enhanced the overall flavor of the fish (Lim *et al*., 2021; Lau *et al*., 2021a-b; Lau *et al*., 2022). Therefore, engkabang illipe nuts are highly valuable resources that warrant further evaluation and investigation.

Shorea macrophylla is listed as a protected plant under Sarawak's Wildlife Protection Ordinance of 1998 (Sarawak Government Gazette, 1998). Additionally, it is classified as vulnerable on the IUCN Red List but was updated to least concern in 2019 (IUCN, 2023). As a result, data and research on this plant species and the illipe nut remain limited to only a few genomic analyses, such as chloroplast and whole genome sequencing recently (Chew *et al*., 2022; Chung *et al*., 2025). Furthermore, the fruiting and flowering of *S. macrophylla* are irregular, with bulk fruiting occurring only once every few years, making fruit harvesting increasingly difficult (Chew *et al*., 2022). To further evaluate the bioactive components of engkabang and understand its contribution as a food source and in the Borneo rainforest food chain, it is essential to determine whether *S. macrophylla* possesses secondary metabolites and bioactive compounds that could trigger the lipogenesis pathway, leading to enhanced intramuscular fat deposition in meat. This study aims to identify lipogenesis-inducing compounds from the endosperm of *S. macrophylla* that could potentially activate the *de novo* lipid biosynthesis pathway via PPARγ or other lipogenesis-related proteins.

Article History
Accepted: 16 June 2025
First version online: 30 September 2025
Cite This Article:
Chung, H.H., Chew, I.Y.Y., Lau, M.M.L. & Sim, S.F. 2025. *Shorea macrophylla* (Engkabang) endosperm extract elevates lipogenesis and adipogenesis in 3T3-L1 mouse adipocyte cells. Malaysian Applied Biology, 54(3): 59-75. https://doi.org/10.55230/mabjournal.v54i3.3242

MATERIALS AND METHODS

Endosperm extraction

The endosperms of *Shorea macrophylla* at full maturity were obtained in a dried condition from a local aquaculture farm. This is because the flowering and fruiting of this plant is somewhat unpredictable, and they only flower and fruit in a 5-7 year interval. Hence, collecting fresh endosperms of engkabang faces many obstructions. The aquaculture farm usually keeps tonnes of supply of dried engkabang endosperms as they use them for fish feed enrichment. Identification of endosperm was done by a park ranger from Sarawak Forestry Department, and a voucher specimen named HB008123 has been deposited in the herbarium of the Faculty of Resource Sciences and Technology, Universiti Malaysia Sarawak (UNIMAS), Malaysia. The samples were stored in a ziplock bag and kept in a -20°C freezer until further action. The engkabang fruits (seed husks) were first broken before obtaining the endosperms, then the endosperms were ground into smaller pieces and were weighed up to 20 g for each extraction. Several solvents were used for the endosperm extraction, such as hexane, dichloromethane, methanol, acetone, ethanol, diethyl ether, isopropanol, and ethyl acetate. The Soxhlet extraction process was carried out for 8 hr, emulating that of Muleta *et al*. (2022). The 200 mL solvents were then separated from the extract under pressure at 1 Psi and a temperature of 70°C for 4.5 hr. The extracts were stored in amber bottles and left in a fume hood at room temperature (25°C) overnight (at least 8 hr) for further solvent evaporation. The experiment was conducted in triplicate (three biological & technical replicates each). The initial weight of the amber bottles and the final weight of the amber bottles with extracts were weighed to obtain the total extract weight, and the extraction efficiency was calculated.

Gas chromatography-mass spectrometry (GCMS)

A total of 1 mg of the crude extract was then redissolved back with 1 mL of methanol. The crude extracts were subjected to GCMS analysis. The profiling for the potential agonists in *S. macrophylla* extracts was performed using a GC-MS (Model: Shimadzu single quadrupole GCMS-QP2020 NX) equipped with a fused, non-polar silica column made of 5% phenyl polysilphenylene-siloxane named BPX-5 of 30 m length, 0.25 mm diameter, and 0.25 µm thickness. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. A total of 1 µL of the extract was injected into the instrument at 280°C. The initial temperature of the column oven was set at 50°C and held for 1 min, and was then increased to 260°C at a speed of 4.5°C/min throughout the process. The final temperature was set at 280°C and held for another 10 min. This condition has been optimized for all solvents used in this study. The total run time of the GC-MS was 45 min. Interpretation of the mass spectrum of the plant extracts was conducted using the database of the National Institute of Standards and Technology (NIST) library, which has more than 62,000 spectral patterns.

Molecular docking

The endosperm extract substances discovered by GCMS analysis were grouped according to their abundance level. Compounds with 1% abundance level and above are chosen for molecular docking analysis. The chemicals were designed as ligands for docking with lipogenesis-related proteins. For the molecular docking investigation, the compounds' SMILES structures were taken from the PubChem database. Protein such as Acetyl-CoA Carboxylase (ACC) (pdb id: 3FF6) (Bhadauriya *et al*., 2013; Huang *et al*., 2015; Wu *et al*., 2021), ATP-Citrate Lyase (ACLY) (pdb id: 6O0H) (Miladiyah & Nuryadi, 2022; Wang *et al*., 2022), Carbohydrate Responsive Element Binding Protein (ChREBP) (pdb id: 5F74) (Hou *et al*., 2017; Sijbesma *et al*., 2020; Gillella & Sumithra, 2022), Fatty Acid Synthase (FASN) (pdb id: 4PIV) (John *et al*., 2015; Angeles & Hudkins, 2016; Pulla & Ahil, 2021), Glucose Transporter 1 (GLUT1) (pdb id: 5EQI) (Almahmoud *et al*., 2019; Sun *et al*., 2023), Peroxisome Proliferator-Activated Receptor γ (PPARγ) (pdb id: 2PRG) (Stalin *et al*., 2016; Penas *et al*., 2017; Prasad *et al*., 2018), Stearoyl-CoA Desaturase (SCD) (pdb id: 4YMK) (Huang *et al*., 2020) and Sterol Regulatory Element Binding Protein (SREBP) (pdb id: 6K9M) (Chen *et al*., 2020) were used in the docking analysis. The proteins were downloaded in pdb format from the RCSB PDB website (https://www.rcsb.org/). The docking study was carried out using the UCSF Chimera software version 1.16 and the AutoDock Vina programme.

Preparation of proteins and compounds

The downloaded protein files were opened in the USCF Chimera (https://www.cgl.ucsf.edu/chimera/), and protein as receptors were prepped for docking by removing water molecules and ligands that were still connected to the receptors and replacing them with polar hydrogen atoms. All protonatable residues in proteins were assigned the common protonation state at physiological pH. Each ligand was constructed using the SMILES structure. The structures of the compounds were first energy-minimised using the Tripos force, then electrostatic charge was assigned using the Gasteiger-Hückel method. These conformations were used as beginning points for docking.

Molecular docking analysis and visualization

Following the preparation of the receptor and ligand, the active site of the protein was determined to identify the locations for grid box settling. The docking was carried out using Autodock Vina, which uses the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm. The validations were carried out by re-docking the original ligand back into the pocket to validate the docking technique. The output ligand conformation after docking and the original ligand conformation were highly superimposed, indicating the high reproducibility of the docking technique for receptor-ligand complexes. All docking analyses for the same protein used the same grid box information. For the docking analysis, rosiglitazone (a drug that targets peroxisome proliferator-activated receptor gamma (PPAR-γ), which is related to lipid metabolism) was used as a positive control. The docking result was stored as a.py (Python file) for further analysis. The docking conformation of the ligands was selected by choosing the posture with the best binding position (the largest negative Gibbs' free energy of binding). Furthermore, amino acid interactions between receptors and ligands were investigated. LigPlot+ v.2.2 was utilised for the identification of hydrophobic interactions (Wallace *et al*. 1996).

3T3-L1 cell viability assay

3T3-L1 mouse adipocyte cell culture was used in this study. 3T3-L1 cells are commonly utilised for researching adipogenesis and adipocyte biochemistry because of their ability to differentiate from fibroblasts to adipocytes (Zebisch *et al*., 2012). In this study, 3T3-L1 cell culture was used to identify if there is any fat accumulation after being treated with plant extracts. By referring to Azahari *et al*. (2015) protocol, in 96 plates, 100 µL of 3.0 x 10^4 preadipocyte cells were seeded with preadipocyte media for 48 hr at 5% CO_2 and 37°C. After two days, the 100 µL preadipocyte medium was replaced with differentiation media and incubated for 72 hr at 5% CO_2 at 37°C. Following the differentiation of preadipocyte cells into mature adipocyte cells, all eight solvent extracts with final concentrations of 0.25, 0.025, 0.0025, 0.00025, and 0.000025 mg/mL were made with maintaining medium and treated in each well, with the final volume of extract with medium being 200 µL. For 24 hr, the cell culture treatment was incubated. The culture was discarded after 24 hr, and the cells were washed twice with 100 µL 1X PBS, 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) stock solution (5 mg/mL in PBS) was added to each well and incubated at 37°C for 4 hr. The MTT solution was removed after 4 hr, and 100 µL DMSO was added to each well to precipitate the salt and create a purple precipitate. The experiment was conducted in triplicate. Rosiglitazone in the same concentration was utilized as a positive control, whereas 0.1% DMSO was used as a negative control. The 96-well plate was covered with aluminium foil and incubated at room temperature for 1 hr. The relative cell viability was determined with a microplate reader and colorimetric absorbance at 570 nm. The wavelength of reference is 630 nm.

Oil Red O assay

Oil Red O assay was carried out as described by Fang *et al*. (2008). To quantify the effect of plant extracts on lipid accumulation in 3T3-L1 cells, the cells were treated with fresh plant extracts in the maintenance medium every other day from day 3 until day 7 of differentiation. On day 7 of differentiation, the medium was removed, and the cells treated with and without plant extracts were washed with 1X PBS and fixed with 4% paraformaldehyde for 30 min. The cells were then rinsed with distilled water and then incubated with Oil Red O solution (0.5% w/v in 60% isopropanol) for 20 min at room temperature. Finally, the dye retained in the 3T3-L1 cells was eluted with isopropanol and quantified by measuring the optical absorbance at 540 nm.

Adipogenesis assay

The sample preparation procedures were identical to the extract treatment procedures described above. Triglyceride levels were measured using the Adipogenesis Kit (Sigma) according to the manufacturer's protocol (Zheng *et al*., 2020). The medium was withdrawn after 5 days and washed once with 100 µL of room temperature 1X PBS. The plate was then sealed and incubated for 30 min at 95°C with 100 µL of Lipid Extraction Buffer added to each well. The plate was then allowed to cool to room temperature before being shaken for 1 min to homogenise the solution. To convert triglycerides to glycerol and fatty acids, 2 µL of Lipase solution was applied to each sample well and reference well and incubated for 10 min at room temperature. Each well received 50 µL of the Master Reaction Mix, which consists of 46 µL adipogenesis assay buffer, 2 µL adipogenesis probe, and 2 µL adipogenesis enzyme mix. At 37°C, the mixture was shaken horizontally for 30 min. During the incubation, the plate was covered with aluminium foil. Finally, the colorimetric absorbance at 570 nm was measured.

RESULTS AND DISCUSSION

Extraction efficiency of *Shorea macrophylla* endosperm in various solvents

Soxhlet extraction was performed on ground engkabang fruit using a variety of solvents with varying polarities in this investigation. In ascending order of their polarity index, the solvents used were hexane, diethyl ether, dichloromethane, isopropanol, ethyl acetate, acetone, methanol, and ethanol, with hexane being the least polar and ethanol being the most polar (Sadek, 2002). The extraction efficiency was calculated by dividing the extract weight at the end of the Soxhlet extraction by the initial sample weight. Figure 1 depicts the extraction efficiency. Acetone has the highest extraction efficiency (up to 38.30%) of all solvents, whereas ethanol has the lowest extraction efficiency (8.80%).

Both alcohols, methanol (10.64%) and ethanol (8.80%), have relatively poor extraction efficiency (statistically significant) when compared to the other six solvents, which have extraction efficiencies ranging from 30.77 to 38.30%. Different solvents with varied polarity indices resulted in different extraction efficiencies (Karseno *et al*., 2022). The extraction efficiency is influenced by various solvents' parameters such as viscosity, surface tension, and polarity (Alshammari *et al*., 2021). Alshammari *et al*. (2021) utilized ethaline to successfully extract five major phenolic compounds from olive oil, namely vanilic acid, tyrosol, ferulic acid, apigenin, and p-coumaric acid.

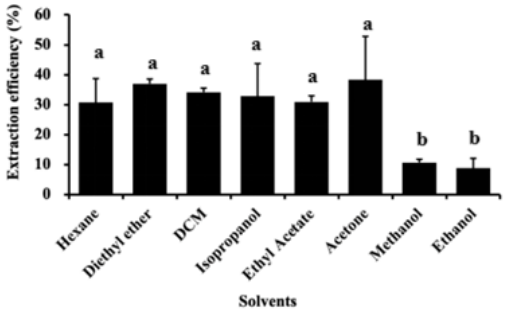

Fig. 1. Extraction efficiency by different solvents. Statistically significantly different groups were marked with different letters.

Table 1. List of compounds with abundance level in the area (%). Compounds that are available in all extract fractions are tabulated. Polar compounds are represented in bold word format.

Solvent	Compound	Pubchem CID	Abundance level (%)
Hexane (low polarity)	Methyl stearate (fatty acid ester)	8201	24.69
	Methyl elaidate (unsaturated fatty acid)	5280590	16.92
	Bis(2-ethylhexyl) adipate (phthalate ester, plasticizer)	7641	10.45
	Hexacosane (alkane)	12407	10.44
	Methyl palmitate (fatty acid ester)	8181	8.88
	Bis(2-ethylhexyl) phthalate (plasticizer)	8343	3.16
	3-Ethoxy-4-hydroxyphenylacetonitrile (nitrile group)	590922	1.14
	Methyl arachidate (fatty acid ester)	14259	1.11
Dichloromethane (moderate polarity)	Methyl stearate (fatty acid ester)	8201	42.68
	Methyl elaidate (fatty acid ester)	5280590	28.09
	Methyl palmitate (fatty acid ester)	8181	15.60
	Methyl arachidate (fatty acid ester)	14259	1.94
	Oxiran-2-ylmethyl tetradecanoate (ester compound)	346148	1.45
	Methyl linoleate (fatty acid ester)	5284421	1.11
Methanol (high polarity)	Methyl stearate (fatty acid ester)	8201	36.25
	Methyl elaidate (fatty acid ester)	5280590	35.50
	Methyl palmitate (fatty acid ester)	8181	17.14
	Methyl linoleate (fatty acid ester)	5284421	1.66
	Methyl arachidate (fatty acid ester)	14259	1.50
Acetone (high polarity)	Glycidyl palmitate (ester of palmitic acid with a glycidyl group)	347736	23.78
	Methyl stearate (fatty acid ester)	8201	20.58
	Methyl elaidate (fatty acid ester)	5280590	13.74
	Glycidyl oleate (ester of oleic acid with a glycidyl group)	5354568	13.5
	Methyl palmitate (fatty acid ester)	8181	8.06
	Glycidyl stearate (ester of stearic acid with a glycidyl group)	62642	2.98
	Methyl arachidate (fatty acid ester)	14259	1.36
	Methyl behenate (fatty acid ester)	13584	1.1
Isopropanol (moderate polarity)	Methyl stearate (fatty acid ester)	8201	36.88
	Methyl elaidate (fatty acid ester)	5280590	24.17
	Methyl palmitate (fatty acid ester)	8181	13.83
	Methyl arachidate (fatty acid ester)	14259	1.73
Ethanol (high polarity)	Methyl stearate (fatty acid ester)	8201	44.97
	Methyl elaidate (fatty acid ester)	5280590	32.96
	Methyl palmitate (fatty acid ester)	8181	17.03
	Ethyl stearate (fatty acid ester)	8122	1.40
	Methyl arachidate (fatty acid ester)	14259	1.08
	Ethyl oleate (fatty acid ester)	5363269	1.05
Ethyl Acetate (moderate polarity)	Methyl stearate (fatty acid ester)	8201	45.16
	Methyl elaidate (fatty acid ester)	5280590	29.67
	Methyl palmitate (fatty acid ester)	8181	21.81
	Methyl arachidate (fatty acid ester)	14259	1.36
Diethyl ether (low polarity)	Methyl stearate (fatty acid ester)	8201	40.3
	Methyl elaidate (fatty acid ester)	5280590	25.8
	Methyl palmitate (fatty acid ester)	8181	14.58
	Butylated hydroxytoluene (synthetic antioxidant used in preservation)	31404	2.52
	Methyl arachidate (fatty acid ester)	14259	2.09

Referring to Table 1, low-polarity solvents such as hexane and diethyl ether have a far higher extraction efficiency than high-polarity solvents such as methanol and ethanol. According to Rguez *et al*. (2023), flavonoids and alkaloids are extracted from solvents with a wide range of polarity indexes; nevertheless, the extracted flavonoids from high polarity solvents are quantitatively greater than those from low polarity solvents. Terpenes, glycosides, and steroids, on the other hand, are lacking

in the diethyl ether fraction but present in high-polarity solvents. Aside from the examples above, Selvamuthukumaran and Shi (2017) stated that ethanol and methanol have relatively higher polarity, and tannins, polyphenols, flavonol, terpenoids, and alkaloids are examples of compounds extracted from ethanol, whereas anthocyanin, terpenoids, and saponins are examples of compounds extracted from methanol.

Bis(2-ethylhexyl) adipate, hexacosane, bis(2-ethylhexyl) phthalate, and 3-Ethoxy-4-hydroxyphenylacetonitrile were discovered to be in abundance levels, 10.45%, 10.44%, 3.16%, and 1.14% in hexane fractions, respectively. *Benincasa hispida* also yields bis(2-ethylhexyl) adipate and bis(2-ethylhexyl) phthalate (Du *et al*., 2006). These two chemicals, however, are plasticizers that are typically absorbed from environmental contamination. However, they are categorized as synthesized compounds; finding these phthalates in plants is quite rare. Du *et al*. (2006) hypothesized that their accumulation in plants was caused by the presence of the former in the aquatic environment due to leaching, which was then taken up by the plant and stored in its fruit. Hexacosene has also been discovered to exhibit analgesic and antinociceptive effects in other plant species (Githinji *et al*., 2011; Githinji *et al*., 2012). While Alharbi *et al*. (2022) extracted 3-Ethoxy-4-hydroxyphenylacetonitrile from Frankincense, its characteristics were not studied further.

Using dichloromethane as a solvent, oxiran-2-ylmethyl tetradecanoate and methyl linoleate were found at abundances of 1.45% and 1.11%, respectively, while methyl linoleate was found at 1.66% in the methanol extract. Prasath *et al*. (2019) and Xia *et al*. (2020) reported antioxidant capacity for oxiran-2-ylmethyl tetradecanoate, also known as myristic acid glycidyl ester, in the GCMS database. The acetone extract, which has the highest crude extraction efficiency, contains the most abundant compound, which was glycidyl palmitate, at 23.78%, followed by glycidyl oleate, glycidyl stearate, and methyl behenate, at 13.5%, 2.98%, and 1.10%, respectively. Methyl linoleate, glycidyl palmitate, glycidyl oleate, glycidyl stearate, and methyl behenate are all fatty acid methyl esters and fatty acid derivatives derived from lipid-rich sources (Zhang *et al*., 2021). Through *in silico* research, Jahan *et al*. (2022) offered glycidyl oleate as a potential efficient analgesic drug.

Molecular docking analysis of compounds with lipogenic-related protein

Several lipogenesis proteins were chosen before the molecular docking investigation of the identified drugs. These proteins are Acetyl-CoA Carboxylase (ACC) (Jones *et al*., 2017), ATP-Citrate Lyase (ACLY) (Lucenay *et al*., 2016), Carbohydrate Responsive Element Binding Protein (ChREBP) (Iizuka *et al*., 2004), Fatty Acid Synthase (FASN) (Wang *et al*., 2004), Glucose Transporter 1 (GLUT1) (Macotela *et al*., 2009), Peroxisome Proliferator-Activated Receptor γ (PPARγ) (Rosen *et al*., 1999), Stearoyl-CoA Desaturase (SCD) (Cheng *et al*., 2016) and Sterol Regulatory Element Binding Protein (SREBP) (Ji *et al*., 2006). Table 2 shows the docking scores of all identified compounds against lipogenic proteins. The docking scores of positive controls, rosiglitazone, a commercialised lipogenic-inducing drug, are also included in the docking analysis table. Referring to Table 2, bis (2-ethylhexyl) phthalate was found to be able to interact with four of the eight lipogenic proteins examined, with a score consistently lower than -7.5 kcaL/moL, which is better than the positive control score. Bis(2-ethylhexyl) adipate and butylated hydroxytoluene both interacted with three lipogenic factors at a score lower than -7.0 kcaL/moL. Glycidyl oleate and hexacosane were the two substances that interacted with two lipogenic factors, each with a score lower than -7.0 kcaL/moL.

It is essential for molecular docking analysis to take into account the amino acid interaction between the lipogenic protein's catalytic groove and the selected compound (Charoenjotivadhanakul *et al*., 2023). Bis(2-ethylhexyl) adipate, glycidyl palmitate, glycidyl stearate, and methyl elaidate were discovered to have the most interactions with the list of lipogenic proteins, interacting with six out of eight lipogenic factors. Seven of the 16 compounds analysed were shown to interact with SREBP, the master regulator of the lipogenesis pathway, all at the HIS435 location, as shown in Table 3, showing the importance of this amino acid in lipogenesis modulation. It is noteworthy that the docking score of bis(2-ethylhexyl) phthalate was found to be lower (-8.9 kcaL/moL) than the score for rosiglitazone (-8.6 kcaL/moL), which indicates that the former has better interactions with the SCD protein than the positive control. Besides having a strong binding affinity, bis(2-ethylhexyl) phthalate also has an interaction with the amino acid residue, ASN261, with a 1.849Å hydrogen bond. All of the discovered amino acid residues are consistent with the findings of Huang *et al*. (2020).

SREBP protein also demonstrated low docking scores (< -7.0 kcaL/moL) with 12 of the tested compounds. SREBP protein plays an important function in hepatic triglyceride and cholesterol synthesis (Ji *et al*., 2006). Three SREBPs with overlapping roles regulate lipid production in animals' livers and other organs. In contrast to the SCD protein, the docking result with the SREBP protein has seven compounds with hydrogen bond interactions (Table 3). At various hydrogen bond lengths, all seven compounds interacted with the same amino acid, HIS435. Chen *et al*. (2020) discovered that HIS435 is one of the SREBP protein's key bindings, which is related to its involvement in triglyceride and cholesterol synthesis.

ACLY enzymatic activity has been shown to enhance lipid droplet production in adipose tissue, which in turn increases cell proliferation (Lucenay *et al*., 2016). In the docking analysis, four compounds attained docking scores equal to or less than -7.0 kcaL/moL: ethyl oleate (-7.0 kcaL/moL), bis(2-ethylhexyl) adipate (-7.2 kcaL/moL), butylated hydroxytoluene (-7.6 kcaL/moL), and bis(2-ethylhexyl) phthalate (-8.4 kcaL/moL). According to Miladiyah and Nuryadi (2022) and Wang *et al*. (2022), amino acid interactions are typically established with residues GLY342, THR348, ASN349, and GLY380, which are crucial in the inhibitory activity of ATP citrate lyase in lipid metabolism.

Aside from the active sites studied in the domain, there are additional allosteric sites that may occasionally affect protein activity. When a regulatory trigger, such as the binding of a small-molecule effector or inhibitor, occurs at a distance from the active region of a protein or protein complex, allosteric regulation of protein activity occurs (Laskowski *et al*., 2009). Various stimuli, such as ligand binding, protein modification, or light absorption, may result in changes in the distant region (Fauser *et al*., 2022). These modifications are transmitted to the active site via a vast network of important amino acids in the protein structure and can either boost or decrease protein function (Dokholyan, 2016).

https://doi.org/10.55230/mabjournal.v54i3.3242

Table 2. Docking score of docking analysis of compounds with the lipogenesis-related protein. Bolded numbers are docking analysis with a lower than -7.0 kcaL/moL docking score. ACC: Acetyl-CoA Carboxylase; ACLY: ATP-Citrate Lyase; ChREBP: Carbohydrate Responsive Element Binding Protein; FASN: Fatty Acid Synthase; GLUT1: Glucose Transporter 1; PPARγ: Peroxisome Proliferator-Activated Receptor γ; SCD: Stearoyl-CoA Desaturase; SREBP: Sterol Regulatory Element Binding Protein

Compounds / Protein	ACC	ACLY	ChREBP	FASN	GLUT1	PPARγ	SCD	SREBP
Rosiglitazone *SMILES:* *CN(CCOC1=CC=C(C=C1)CC2C(=O)NC(=O)S2)C3=CC=CC=N3*	-6.4	**-8.0**	-6.9	**-8.6**	**-8.6**	**-9.4**	**-8.6**	**-9.0**
3-Ethoxy-4-hydroxyphenylacetonitrile *SMILES: CCOC1=C(C=CC(=C1)CC#N)O*	-4.8	-6.5	-5.8	-6.4	-6.1	-5.7	**-7.3**	-6.5
Bis(2-ethylhexyl) adipate *SMILES: CCCCC(CC)COC(=O)CCCCC(=O)OCC(CC)CCCC*	-4.3	**-7.2**	-5.1	**-7.4**	-6.4	-6.2	**-8.0**	**-7.5**
Bis(2-ethylhexyl) phthalate *SMILES: CCCCC(CC)COC(=O)C1=CC=CC=C1C(=O)OCC(CC)CCCC*	-4.7	**-8.4**	-5.8	**-7.9**	-6.5	-6.6	**-8.9**	**-8.3**
Butylated hydroxytoluene *SMILES: CC1=CC(=C(C(=C1)C(C)(C)C)O)C(C)(C)C*	-4.6	**-7.6**	-6.3	**-7.4**	**-7.0**	-6.4	-6.2	**-7.8**
Ethyl Oleate *SMILES: CCCCCCCCC=CCCCCCCCC(=O)OCC*	-3.9	**-7.0**	-4.9	-6.5	-5.9	-5.9	-6.0	**-7.1**
Ethyl stearate *SMILES: CCCCCCCCCCCCCCCCCC(=O)OCC*	-3.5	-6.6	-4.5	-6.2	-5.4	-6.1	-6.5	**-7.1**
Glycidyl oleate *SMILES: CCCCCCCCC=CCCCCCCCC(=O)OCC1CO1*	-4.0	-6.8	-5.0	-6.4	-5.9	-6.1	**-7.2**	**-7.0**
Glycidyl palmitate *SMILES: CCCCCCCCCCCCCCCC(=O)OCC1CO1*	-4.1	-6.5	-5.3	-6.2	-5.5	-6.0	-6.0	-6.9
Glycidyl stearate *SMILES: CCCCCCCCCCCCCCCCCC(=O)OCC1CO1*	-3.4	-6.5	-5.0	-6.1	-5.7	-5.9	**-7.1**	-6.9
Hexacosane *SMILES: CCCCCCCCCCCCCCCCCCCCCCCCCC*	-3.4	-6.6	-4.1	-6.1	-5.5	-6.0	**-7.6**	**-7.7**
Methyl arachidate *SMILES: CCCCCCCCCCCCCCCCCCCC(=O)OC*	-3.4	-6.6	-4.7	-6.3	-5.5	-5.7	-6.1	**-7.1**
Methyl behenate *SMILES: CCCCCCCCCCCCCCCCCCCCCC(=O)OC*	-3.7	-6.7	-4.8	-6.1	-5.7	-5.9	-6.8	**-7.2**
Methyl elaidate *SMILES: CCCCCCCCC=CCCCCCCCC(=O)OC*	-3.6	-6.6	-4.8	-6.2	-5.5	-6.3	**-7.1**	**-7.0**
Methyl linoleate *SMILES: CCCCCC=CCC=CCCCCCCCC(=O)OC*	-4.0	-6.9	-5.2	-6.9	-5.8	-6.1	**-7.5**	**-7.4**
Methyl palmitate *SMILES: CCCCCCCCCCCCCCCC(=O)OC*	-3.7	-6.4	-5.1	-6.3	-5.4	-6.2	-6.8	-6.7
Methyl stearate *SMILES: CCCCCCCCCCCCCCCCCC(=O)OC*	-3.3	-6.7	-4.6	-6.2	-5.5	-5.6	-6.3	**-7.0**
Oxiran-2-ylmethyl tetradecanoate *SMILES: CCCCCCCCCCCCCC(=O)OCC1CO1*	-3.7	-6.3	-5.2	-6.1	-5.3	-5.9	-6.1	-6.7

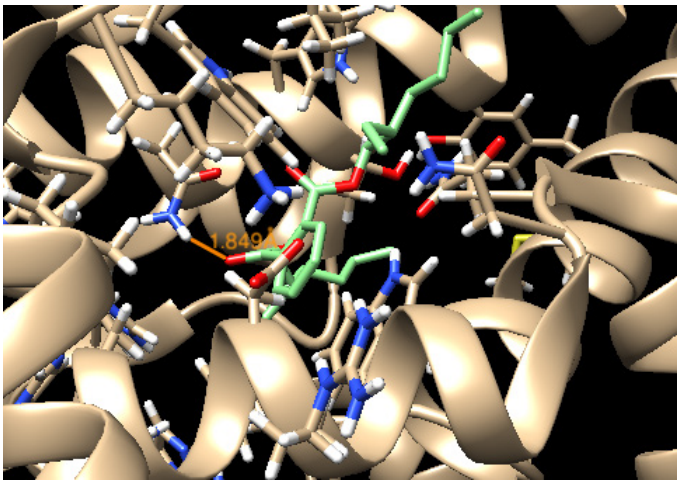

Fig. 2. Docking of bis(2-ethylhexyl) phthalate into stearoyl-CoA desaturase (SCD) protein.

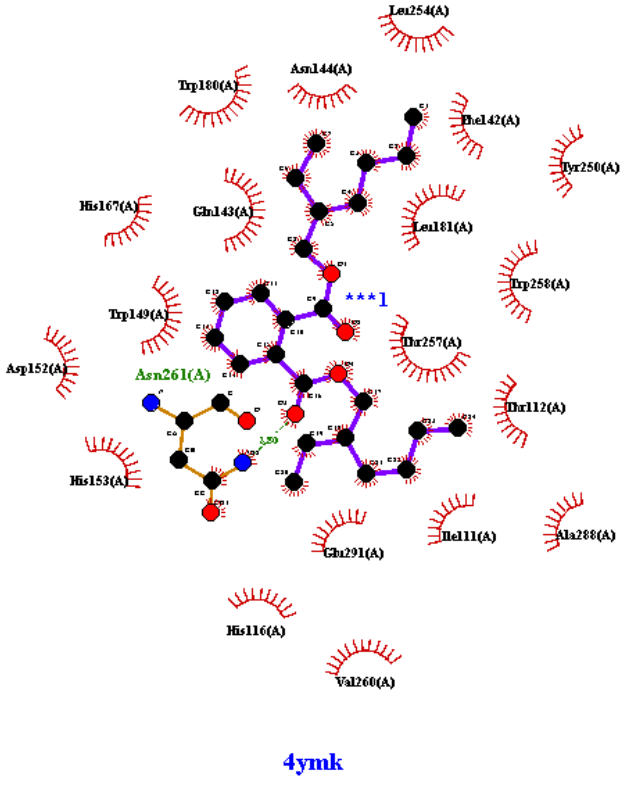

Fig. 3. Several hydrophobic interactions were identified between bis(2-ethylhexyl) phthalate with stearoyl-CoA desaturase (SCD) using Ligplot.

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Table 3. Hydrogen bond formation between the amino acids of proteins with ligands. The numbering in brackets indicates the hydrogen bonding length in Å. ACC: Acetyl-CoA Carboxylase; ACLY: ATP-Citrate Lyase; ChREBP: Carbohydrate Responsive Element Binding Protein; FASN: Fatty Acid Synthase; GLUT1: Glucose Transporter 1; PPARγ: Peroxisome Proliferator-Activated Receptor γ; SCD: Stearoyl-CoA Desaturase; SREBP: Sterol Regulatory Element Binding Protein

Compounds / Proteins	ACC	ACLY	ChREBP	FASN	GLUT1	PPARγ	SCD	SREBP
Rosiglitazone	GLY2126 *(2.192)*.	GLY309 *(2.402)*, GLY380 *(2.240)*.	ASN124 *(2.434)*.	ILE2068 *(1.994)*.	ASN288 *(2.169)*, GLN283 *(2.604)*.	TYR473 *(2.361)*, GLN286 *(1.878)*, SER289 *(2.009)*.	TRP258 *(2.071)*.	-
3-Ethoxy-4-hydroxyphenylacetonitrile	-	THR348 *(2.290)*, GLY380 *(2.324)*.	ASN123 *(2.270)*, ARG58 *(2.021)*.	TYR2034 *(2.349)*, LEU2069 *(2.342)*, GLY2061 *(2.351)*, LYS1995 *(1.983)*, GLY1897 *(2.275)*.	GLN283 *(2.469)*, TRP388 *(2.238)*.	-	ASN144 *(2.249)*, HIS153 *(2.454)*.	-
Bis(2-ethylhexyl) adipate	GLU2230 *(2.056)*.	-	ARG128 *(2.175, 2.482)*, ARG129 *(2.012)*, ASN175 *(2.214)*, LYS122 *(2.202)*.	ALA2062 *(2.176)*.	ASN415 *(2.536, 2.419)*, ASN288 *(1.964, 2.724, 2.324)*, TRP388 *(2.437, 2.453)*, GLN283 *(2.595)*.	-	ASN261 *(2.101)*, HIS153 *(2.454)*.	HIS435 *(2.238)*.
Bis(2-ethylhexyl) phthalate	GLY2202 *(2.103)*.	-	ARG58 *(2.188)*, LYS51 *(2.193)*.	-	GLN283 *(2.278)*.	-	ASN261 *(1.849)*.	-
Butylated hydroxytoluene	-	-	-	-	-	-	-	-
Ethyl Oleate	GLY2202 *(2.098)*.	GLY342 *(2.237, 2.584)*, GLY380 *(2.266)*.	ARG58 *(2.089)*, LYS51 *(2.169, 2.445)*, ARG128 *(2.055, 2.528)*, ARG129 *(2.400, 2.333, 2.441)*.	SER2021 *(2.234)*.	THR137 *(2.721)*.	-	-	-

Table 3. Continued...

Ethyl stearate	GLY2162 *(2.518)*, GLY2163 *(2.508)*.	THR348 *(2.067)*, ASN349 *(2.019)*.	ARG128 *(2.223)*.	-	ASN415 *(2.370, 2.652)*, ASN288 *(2.272, 2.629, 2.628)*, GLN283 *(2.690, 2.467)*.	-	-	HIS435 *(2.087)*.
Glycidyl oleate	PHE2160 *(2.129)*.	THR348 *(2.497)*, ASN349 *(2.428)*.	ARG128 *(2.100, 2.134)*, ARG129 *(2.214)*, LYS51 *(2.317)*.	GLY1897 *(2.210)*, PHE1896 *(2.324)*.	GLN283 *(2.684)*, ASN415 *(2.467)*, ASN288 *(2.714)*.	SER289 *(2.046)*.	-	-
Glycidyl palmitate	-	-	ASN124 *(2.737)*, ARG128 *(2.564)*, ARG129 *(2.110, 2.370)*.	SER2021 *(1.954)*.	TRP388 *(2.439)*, THR137 *(2.377)*.	HIS449 *(2.358)*.	ASN261 *(2.858)*, TRP258 *(2.375)*.	-
Glycidyl stearate	GLY2162 *(1.917)*.	-	ARG129 *(2.234)*.	SER2021 *(2.044)*, GLY1895 *(2.342)*.	ASN415 *(2.470)*, ASN288 *(2.417)*.	-	-	HIS435 *(2.266)*.
Hexacosane	-	-	-	-	-	-	-	-
Methyl arachidate	GLY2202 *(2.123)*.	GLY380 *(2.134)*, THR348 *(1.908)*, ASN349 *(1.989)*.	ARG128 *(2.718, 2.371, 2.332, 2.171)*, ASN124 *(2.367, 2.566)*, LYS51 *(2.328)*, TYR130 *(2.453)*, ARG129 *(1.010, 2.491, 2.170, 2.356)*.	PHE1896 *(1.868)*.	ASN415 *(2.574)*, ASN288 *(2.500)*.	-	-	-
Methyl behenate	GLY2162 *(1.874)*, GLY2163 *(2.495)*.	GLY380 *(2.199)*.	ARG129 *(2.289, 2.355, 2.332)*, LYS51 *(2.299)*.	-	TRP388 *(2.402)*.	-	-	-
Methyl elaidate	GLY2162 *(2.212)*, GLY2163 *(2.451)*.	GLY342 *(2.175)*, GLY380 *(2.176)*.	LYS51 *(2.328)*.	SER2021 *(2.314, 2.501)*, GLY1897 *(2.276, 2.025)*, PHE1896 *(2.102, 2.203)*.	ASN415 *(2.551)*, ASN288 *(2.393)*.	HIS323 *(2.344)*.	-	HIS435 *(2.155)*.

https://doi.org/10.55230/mabjournal.v54i3.3242

https://doi.org/10.55230/mabjournal.v54i3.3242

Table 3. Continued...

Methyl linoleate	-	GLY380 *(2.389, 2.284)*, GLY342 *(2.366)*.	ARG58 *(2.293, 2.015)*, ARG128 *(1.936)*.	PHE1896 *(2.196)*, GLY1897 *(2.076)*.	ASN415 *(2.540)*.	SER289 *(2.327)*.	-	HIS435 *(2.346)*.
Methyl palmitate	-	-	ARG128 *(2.408)*, ARG129 *(2.327, 2.124), LYS51 (2.479)*.	GLY1897 *(2.218)*, PHE1896 *(2.025)*.	ASN415 *(2.519, 2.353)*, ASN288 *(2.292, 2.605)*.	-	-	HIS435 *(2.247)*.
Methyl stearate	GLY2162 *(2.559)*.	GLY380 *(2.272)*.	ASN124 *(2.302)*, ARG128 *(2.427)*.	GLY1897 *(2.167)*, PHE1896 *(2.139)*.	ASN288 *(2.444, 2.390)*, ASN415 *(2.394)*.	-	-	-
Oxiran-2-ylmethyl tetradecanoate	-	-	ASN124 *(2.259)*, ARG128 *(2.655)*, ARG129 *(2.269, 2.250)*.	-	TRP412 *(2.450)*, TRP388 *(2.410)*.	-	-	HIS435 *(2.422)*.

Cytotoxicity assay

The MTT assay was performed to determine the cytotoxicity of the extract towards adipocyte cells at various concentration gradients. Figure 4(A) demonstrates that the positive control, Rosiglitazone, has no effect on cell viability at concentrations ranging from 0.000025 mg/mL to 0.0025 mg/mL; however, at concentrations of 0.025 mg/mL and above, over half of the cells lose viability. On the other hand, even at the highest tested concentration with hexane extract, the cells survived with 100% cell viability (Figure 4(B)). Cell viability was maintained at 100% for all concentrations except 0.25 mg/mL for dichloromethane, methanol, acetone, isopropanol, and diethyl ether extracts. To minimise harmful effects on adipocyte cells, the proposed effective concentration of extracts was 0.025 mg/mL, with no negative effect on cell survival being observed.

When it comes to medications, cytotoxicity is critical for ensuring the correct and safe dosage. The cytotoxicity assay is typically performed by assessing the effect of a drug on the proliferation of a population of cells, with an estimate of cell number as the endpoint (Plumb, 2004). The quantities of extracts used on the 3T3-L1 cells in this study range from 0.000025 to 0.25 mg/mL. Although different solvents are used to extract the endosperm, the cell viability of each extract after one week of treatment shows similar patterns. Most extracts, including the positive control, methanol, acetone, ethanol, isopropanol, ethyl acetate, and diethyl ether, have a generally poor cell viability, ranging from 5.75% to 58.14%, reducing cell survival by 50 to 95% at 0.25 mg/mL concentration. Rosiglitazone, the positive control, has a cell survival of up to 57.70% at 0.025 mg/mL, which is comparatively low when compared to the tested extracts.

When compared to previous studies on the cytotoxicity of 3T3-L1 cells, Rhyu *et al*. (2014) tested on pear pomace aqueous extracts and Mohan *et al*. (2020) tested on *Artabotrys suaveolens* methanolic extracts, both of which showed relatively high cell viability (above 90%) even at concentrations of 400 g/mL and 250 g/mL, respectively. However, the extracts examined in this study at 0.25 mg/mL had low cell viability, as previously stated, with the exception of hexane and dichloromethane, which both attained cell viability of approximately 90%.

However, when compared to the results of Nishina *et al*. (2017), the cell viability of 3T3-L1 cells after treatment with hexane, ethyl acetate, and methanol extracts of *Vitex trifolia* L. showed distinctive patterns. The cell viability diminishes in an inversely proportional manner as the concentration of the extracts treated increases. In the case of hexane extracts, there is a clear decrease in cell viability (from 83% to 5%) at 100 µg/mL extract concentration compared to 30 µg/mL extract concentration. In this study, the cell viability for the hexane extract fell at 250 µg/mL extract concentration, but maintained cell viability above 100%. A similar phenomenon was observed for the combination of *Nigella sativa* and *Zingiber zerumbet* hexane extracts on human leukemia HL60 cells, whereby the cell viability plummeted to below 60% at extract concentration 250 µg/mL and higher (500 & 1000 µg/mL) (Nawi *et al*., 2014).

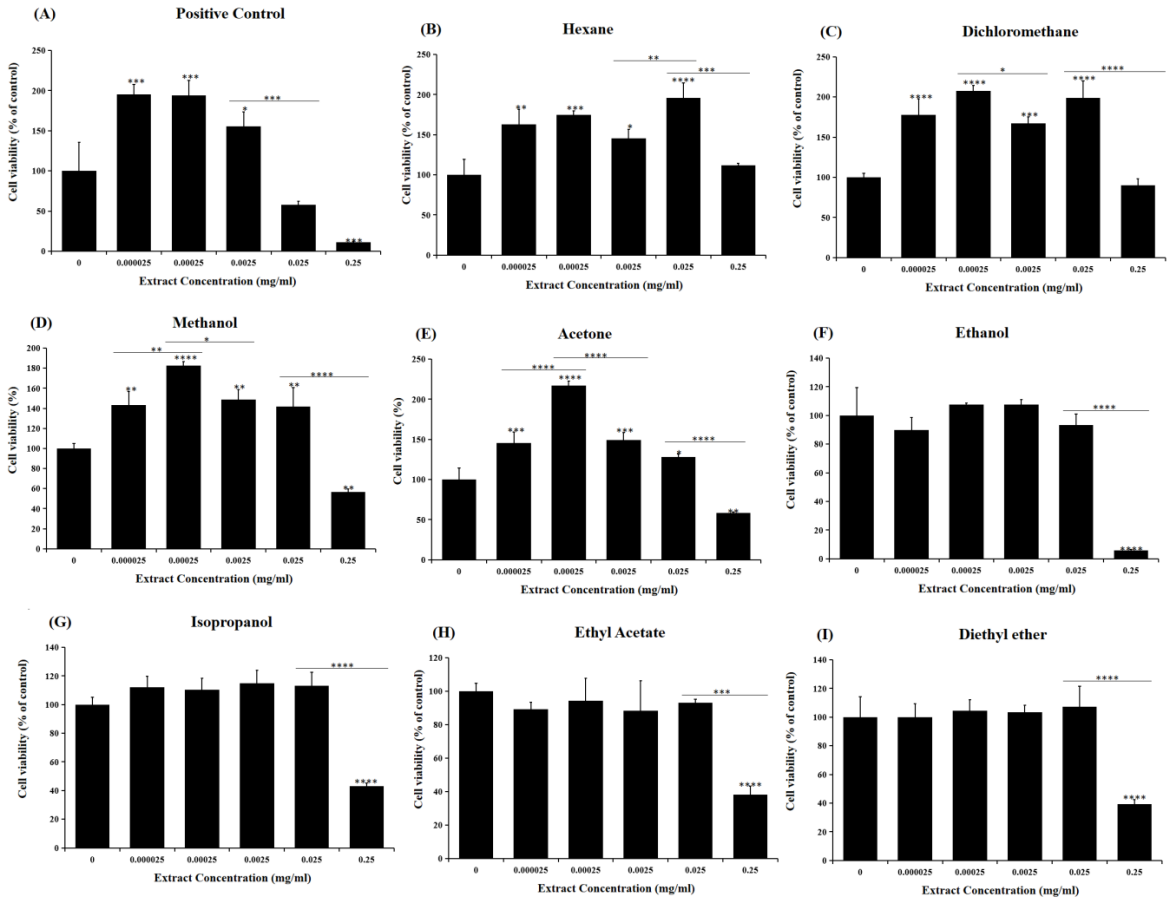

Fig. 4. MTT assay of 3T3-L1 adipocyte cells treated with extracts that are extracted using different types of solvents. Data are means ± S.D. of three replicates. **P*≤0.1, ***P*≤0.001, ****P*≤0.0001, and *****P*≤0.00001 when compared with negative control (0.1% DMSO).

Lipogenesis effect of extracts on 3T3-L1

The Oil Red O assay was utilised as a fat accumulation indicator in 3T3-L1 adipocyte cells to test the potential of different extracts at different concentration gradients. Figure 5 shows the results of Oil Red O staining on 3T3-L1 adipocyte cells treated with various extracts. Looking at Figure 5(A), the positive control, rosiglitazone, demonstrated increasing lipid accumulation in 3T3-L1 adipocyte cells when extract concentration increased from 0.000025 mg/mL to 0.25 mg/mL. Although hexane, ethanol, isopropanol, and diethyl ether extracts all showed slight decreasing trends in lipid accumulation as concentration increased from 0.000025 mg/mL to 0.025 mg/mL, there was a significant increase in lipid accumulation level at 0.25 mg/mL when compared to the non-treated control. The lipid accumulation in dichloromethane, acetone, and ethyl acetate extracts did not rise significantly with increasing extract concentration, and all tested concentrations of ethyl acetate extracts had lower lipid accumulation than the non-treated group.

Using the results of the MTT and Oil Red O staining assays as a guide, four extracts were chosen for adipogenesis assay analysis which are hexane, methanol, isopropanol, and diethyl ether. The positive control, as shown in Figure 6(A), increased triglyceride content with increasing extract concentration from 0.000025 mg/mL to 0.25 mg/mL. In contrast to hexane and isopropanol extracts, which do not enhance adipogenesis in 3T3-L1 cells, methanol and diethyl ether extracts, on the other hand, have shown an increase in triglyceride formation with an increase in extract concentration starting at 0.000025 mg/mL and peaking at 0.25 mg/mL

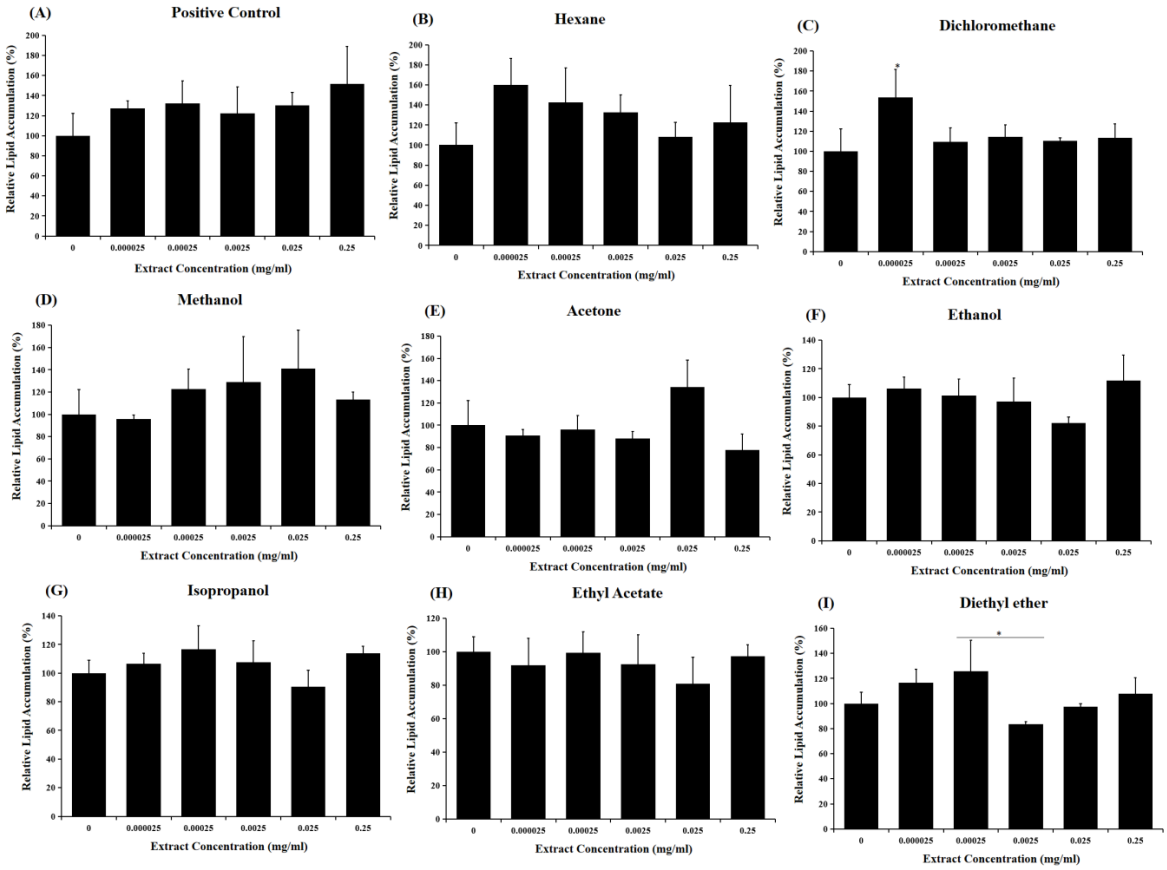

Fig. 5. ORO assay indicating lipid accumulation of adipocyte cells treated with extracts that are extracted using different types of solvents. Data are means ± S.D. of three replicates. **P*≤0.1, ***P*≤0.001, ****P*≤0.0001, and *****P*≤0.00001 when compared with negative control (0.1% DMSO).

Lipogenesis is a popular topic, with numerous studies exploring potential anti-diabetic drugs for treating obesity, cardiac, and liver diseases. It is the process by which fatty acids are synthesized from glucose, leading to fat storage within an organism (Ameer *et al*., 2014). Lipogenesis can be detrimental to human health as it results in fat accumulation, which can lead to obesity, cardiovascular disease, and various other health issues. However, fat build-up might not be harmful to animals, as meat with high-fat content is known to have a desirable texture favored by consumers (Schumacher *et al*., 2022).

Using the MTT results as a guide, four solvents are chosen for further lipogenesis analysis: hexane, methanol, isopropanol, and diethyl ether. Hexane, isopropanol, and diethyl ether exhibit a minor decrease followed by a significant increase in the relative lipid accumulation level of the cells in the Oil Red O staining examination. Furthermore, all of the solvent extract concentrations evaluated on 3T3-L1 cells accumulated lipid at a higher level than non-treated cells. The increase in triglyceride content after treatment with crude extracts of *Torreya nucifera* seed oil was similarly reported in the investigation by Koh *et al*. (2021). The relative lipid build-up increases gradually as the concentration of the treated extract increases for the methanol extract treatment. However, once the extract concentration reaches 0.25 mg/mL, there is a reduction in lipid level, which could be due to cell toxicity.

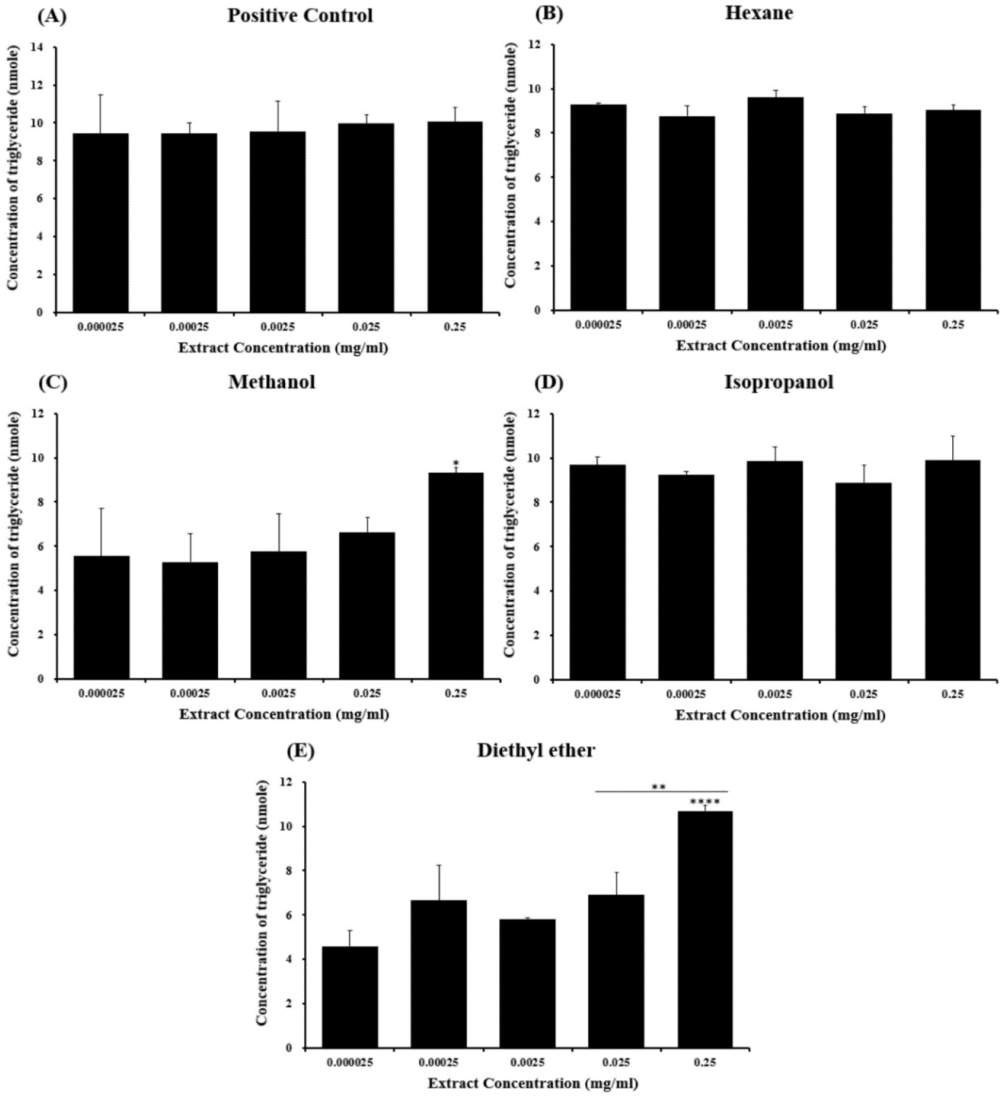

Fig. 6. Adipogenesis assay indicating the concentration of lipid accumulation of adipocyte cells treated with extracts that are extracted using different types of solvents. Data are means ± S.D. of three replicates. *P≤0.1, **P≤0.001, ***P≤0.0001, and ****P≤0.00001 when compared with negative control (0.1% DMSO).

When these four extracts were subjected to additional testing using an adipogenesis kit, it was discovered that hexane and isopropanol consistently attained triglyceride levels of nine nmole across all tested concentrations. Methanol and diethyl ether, on the other hand, caused a significant and dose-dependent increase in triglyceride levels. These findings add to the evidence that all of the extracts had beneficial effects on fat accumulation, resulting in an increase in triglyceride content within adipocyte cells.

The current amount of research into lipogenesis augmentation and stimulation is limited, as most studies focus on the opposing goal of lowering and inhibiting lipogenesis, targeting prospective pharmaceuticals for the treatment of diabetes and obesity-related health issues. Nonetheless, there are ongoing studies being undertaken on lipid build-up. Vahmani *et al*. (2015) carried out a study in which different fatty acids were supplied to cells, resulting in a considerable increase in total fatty acid content when compared to control cells, which is consistent with our findings. Other chemicals that induce lipogenesis include Orexin-A (Skrzypski *et al*., 2011) and tetrabromobisphenol-A (Chappell *et al*., 2018). These studies are largely concerned with the expression of lipogenic genes, such as ACC, FAS, PPARγ, and GLUT4 proteins, which are also included in the molecular docking analysis performed in this study. Due to all of the studied extracts (hexane, methanol, isopropanol & diethyl ether) contain higher triglyceride concentrations than the positive control, the chemicals shared by the four extracts could be the target molecules that triggered lipogenesis.

CONCLUSION

The study successfully extracted chemicals from engkabang endosperm using eight solvents with increasing polarity indexes. Methanol and ethanol, with higher polarity indexes, had the lowest extraction efficiency. Most bioactive compounds identified were fatty acids, specifically methyl stearate, methyl elaidate, methyl palmitate, and methyl arachidate. These compounds showed good docking results with the SREBP protein, a regulator of lipogenesis, with scores ranging from -6.7 to -7.1 kcaL/moL. The crude engkabang extracts were tested for cytotoxicity and lipogenic activity on 3T3-L1 cells. Cell viability remained above

90% at concentrations of 0.025 mg/mL or lower, deemed safe for future *in vivo* studies. Both methanol and diethyl ether extracts significantly increased lipid accumulation in 3T3-L1 cells, indicating heightened lipogenesis. Only the four identified bioactive compounds were present in these extracts, suggesting that lipogenic activity might be due to a minor component in the extracts or interactions between compounds.

ACKNOWLEDGEMENTS
The authors acknowledge that the Tun Zaidi Chair fully funded this research with a grant number UNI/F07/TZC/85864/2024 to H.H. Chung.

ETHICAL STATEMENT
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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