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IN VITRO EVALUATION OF SCAVENGING AND ANTI-ADIPOGENIC ACTIVITY OF *Momordica Cochinchinensis* (LOUR). SPRENG FRUIT EXTRACTS ON 3T3-L1 ADIPOCYTES

Mohd Nazri Abdul Rahman[1,2*], Amin Ismail[2,3], Azrina Azlan[2,3], Ahmad Fazli Abdul Aziz[4], Nor Hayati Muhammad[5]

[1]*Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[2]*Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Research Centre of Excellent, Nutrition and Non-Communicable Diseases, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*KPJ Selangor Specialist Hospital, Jalan Singa 20/1, Seksyen 20, 40300 Shah Alam, Selangor, Malaysia*
[5]*Centre of Foundation Studies, Universiti Teknologi MARA, Cawangan Selangor, Kampus Dengkil, 43800 Dengkil, Selangor, Malaysia*

*Corresponding Author: mdnazri@ums.edu.my

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Abstract

Gac fruit, scientifically known as *Momordica cochinchinensis* (Lour) Spreng, is rich in potent bioactive compounds, particularly carotenoids such as β-carotene, lycopene, and lutein. This study investigated the effects of gac fruit extract fractions (peel, pulp, and aril) on the scavenging, cytotoxic, and anti-adipogenic activities in 3T3-L1 adipocytes. The study assessed the DPPH radical scavenging activity of gac extracts through serial dilution at a concentration of 1000 µg/mL. The viability of 3T3-L1 preadipocytes was measured using the MTT assay. Differentiated adipocytes were treated with gac extracts at concentrations of 75, 150, and 300 µg/mL for 7 days. The impact on lipid accumulation and adipogenesis inhibition was determined through Oil Red O staining and triglyceride content analysis. The IC_{50} values for DPPH radical scavenging were 573.40 µg/mL for peel, 525.46 µg/mL for pulp, and 817.33 µg/mL for aril extracts. No toxicity was observed in 3T3-L1 cells at concentrations up to 200 µg/mL. At 200 µg/mL, gac extracts reduced 3T3-L1 cell viability while promoting growth and proliferation. Treatment with gac extracts significantly reduced lipid accumulation and inhibited 3T3-L1 cell differentiation in a dose-dependent manner. Among the gac extract fractions, pulp notably decreased intracellular triglyceride content in adipocytes, surpassing aril and peel extracts. In conclusion, gac fruit extract fractions (peel, pulp, and aril) effectively inhibited adipogenesis in 3T3-L1 adipocytes, as evidenced by reduced lipid accumulation, triglyceride content, and cell viability. These findings unveil valuable insights into bioactive compounds from *Momordica cochinchinensis* and their potential for addressing obesity prevention and treatment.

INTRODUCTION

Gac (*Momordica cochinchinensis* L. Spreng), a member of the Momordica species, has a long history of use in managing both type I and II diabetes. Belonging to the melon family, gac is exceptionally rich in bioactive compounds, including essential fatty acids, β-carotene, lycopene, and lutein [1]. It is renowned for its potent antioxidant properties due to its high carotenoid concentration, primarily β-carotene and lycopene [2]. Gac is emerging as a top source of carotenoids, notably beta-carotene and lycopene. Lycopene levels in gac can reach up to 308 µg/g in the seed

membrane, surpassing other lycopene-rich fruits and vegetables by approximately tenfold. Additionally, the gac aril (pulp) boasts an even higher mean lycopene level of 2227 μg/g in fresh material. Furthermore, the aril contains substantial levels of fatty acids, ranging from 17% to 22% by weight.

The utilization of *in vitro* models, such as the 3T3-L1 pre-adipocyte and 3T3-F442A cell lines, has provided significant insights into the study of adipogenesis and the underlying transcriptional processes governing fat cell differentiation. These cell lines offer advantages such as their uniform cell populations and the ability to propagate cells. However, it is essential to acknowledge that the molecular mechanisms observed in these cell lines may not precisely replicate those happening in human pre-adipocytes. Additionally, the adipogenic capacity of pre-adipocyte cell lines tend to decline as the passage number increases [3].

For more than three decades, the 3T3-L1 adipocyte cell line has played a pivotal role in advancing research on metabolic diseases. The 3T3-L1 system has been instrumental in deepening our understanding of the fundamental cellular mechanisms underpinning conditions like diabetes, obesity, and related disorders [4]. These cells are widely employed to investigate two key aspects: adipogenesis and insulin response. However, it is noteworthy that their capacity for adipogenesis diminishes as they spend more time in culture [5].

Studies utilizing 3T3-L1 cells have delved into the expression of specific mRNA molecules during the differentiation process. These investigations have demonstrated that the adipose conversion observed in this cell line faithfully recapitulates the process seen in living organisms. Initially possessing a fibroblastic morphology, these cells undergo differentiation into adipocytes that exhibit both the structural and biochemical characteristics of mature adipocytes. Typically, the differentiation of 3T3-L1 cells into adipocytes is induced by treatment with pro-differentiation agents, which include insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX) [6-7]. This approach has proven invaluable in studying the intricate processes of adipocyte development and function.

To our knowledge, there have been no previous studies examining the adipogenic impacts of gac fruit extract (GFE) fractions, which include peel, pulp, and aril, in any *in vitro*, *in vivo*, or human intervention research. Consequently, this study stands as the inaugural exploration into the antioxidant potential and anti-adipogenic effects of GFE when applied *in vitro*, utilizing 3T3-L1 cell lines as the experimental model.

MATERIALS AND METHODS

Gac Fruit Extract (GFE) Preparation

Gac fruit, specifically the Malaysian cultivar of *Momordica cochinchinensis* L. Spreng, was provided by the International Tropical Fruits Network in Selangor, Malaysia. The accurate species identification and confirmation were conducted by the Biodiversity Unit at the Institute of Bioscience, Universiti Putra Malaysia, located in Serdang, Selangor, Malaysia. A voucher specimen (SK 3164/17) was retained for reference. During the harvesting season in February to April of 2018, a total of eleven ripe fruits were randomly collected. These fruits collectively weighed 5.342 kg, with an average individual weight of approximately 485.64 grams (± 143.52 g).

Fresh gac fruits were handled in one of two ways: they were either stored at a temperature of 5°C for a period ranging from 3 to 5 days or they were immediately employed for experimentation. Subsequently, the peel, pulp, aril, and seeds were meticulously separated and promptly frozen at -80°C in preparation for lyophilization, a process carried out using the Christ Beta 2-8 LD plus lyophilizer manufactured by GmbH in Germany. Following lyophilization, the resulting freeze-dried samples of Gac peel, pulp, and aril were mechanically ground to reduce their particle size. These ground samples were then securely sealed in airtight containers, specifically Falcon tubes or Schott bottles equipped with screw caps and were further shielded by aluminum foil. These samples were stored at a temperature of -20°C, preserved for future analyses and investigations.

Gac Fruit Extraction Yields Percentage

The gac fruit fractions, including the peel, pulp, and aril, were subjected to extraction using a method based on the procedures outlined by Auisakchaiyoung and Rojanakorn [8] and Tran *et al.* [9], with certain adaptations. In brief, approximately 2 grams of gac powder were accurately weighed into a beaker. Subsequently, they were subjected to extraction with 100 mL of an extraction solvent composed of *n*-hexane/acetone/ethanol (in a volume-to-volume ratio of 50:25:25) for a duration of 30 minutes, utilizing a magnetic stirrer for assistance. Following this extraction step, 15 mL of water was introduced.

The upper layer of the solution was separated and filtered through filter paper with a pore size of 0.45 μM, directing it into a round-bottomed flask. Subsequently, an aliquot of 10 mL from the extract was subjected to evaporation to achieve dryness using a rotary evaporator. The resulting extract was then carefully shielded from light by wrapping it with aluminum foil and stored at a temperature between 4°C and 5°C until it was ready for further utilization. Throughout the study, the experiments were consistently conducted in triplicate to ensure the reliability of the results. The extraction yield percentage of the gac fruit was calculated using the following formula:

Extraction Yield (%) = $[(W_1 / W_0) \times 100] /$ Weight of Dried Sample ……...……. **(Equation 1)**

Where;
W0 = Weight of Empty Flask
W1 = Weight of Flask with the Extract

1,1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Capacity

The assessment of scavenging activity in the extracts was conducted by evaluating their ability to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, following a method akin to that described by Auisakchaiyoung and Rojanakorn [8], with minor modifications. For the DPPH assay, an initial stock solution was prepared by dissolving 24 milligrams of DPPH in 100 mL of methanol, and this solution was stored at -20°C until required. To create the working solution, 10 mL of the stock solution was combined with 45 mL of methanol.

The assessment of the hydrogen atom or electron-donation capacity of the respective extracts, along with select pure compounds, was determined by measuring the decolourization of a purple-coloured methanol solution of DPPH. The scavenging activity of the extracts, reliant on their ability to scavenge the stable DPPH free radical, was subsequently quantified. In the experimental procedure, 0.1 mL of the ethanolic extract was introduced into 3 mL of a 0.001M DPPH solution in methanol. After a 30-minute incubation period, the absorbance at 517 nm was measured, and the percentage of inhibition activity was calculated using the formula:

Percentage of inhibition activity (%) = $[(A_o - A_e)/A_o] / 100$ …… (**Equation 2**)

Where;
A_o = absorbance without extract
A_e = absorbance with extract

The determination of the inhibition concentration at 50% (IC_{50}), which signifies the amount of the sample extracted into a 1 mL solution required to reduce the initial DPPH concentration by 50%, was derived from the plot showing the percentage disappearance versus concentration (where concentration refers to the milligrams of fruit extracted into 1 mL solution). The IC_{50} value for DPPH was calculated using the equation y = mx + c, and the linearity (R-squared, denoted as R^2) obtained from the graph. R^2 serves as a statistical metric that indicates the proportion of variance in a dependent variable explained by one or more independent variables in a linear regression model. An R^2 value approaching 1 reflects a high degree of linearity in the graph. In the context of IC_{50} determination, a lower IC_{50} value indicates a more effective inhibition concentration, signifying a stronger scavenging effect.

Cell Culture and Simulation

The 3T3-L1 (ATCC® CL-173™) cell lines, which are a type of mouse embryonic fibroblast cell, were sourced from the Institute of Bioscience at Universiti Putra Malaysia (UPM). For all experiments conducted, the cells used had a passage number ranging from P26 to P34.

Cell Culture Experimental Group

There are four groups involved which group 1 considers as control, groups 2 to 4 are the treatment group.

Group 1: 3T3-L1 (ATCC® Cat. No. CL-173™), in DMEM containing 10% (v/v) FBS until 2 days post-confluence.
** Induced to differentiate by the presence of differentiation medium (MDI)*
Group 2: 3T3-L1 + Dexamethasone (1 µmol/l or 250 nM) + 75 µg/mL GFE
Group 3: 3T3-L1 + 0.52 mmol/l 3-isobutyl-1-methylxantine + 150 µg/mL GFE
Group 4: 3T3-L1 + (0.17 µmol/l or 5 µg/mL) insulin + 300 µg/mL GFE

**Note: GFE's concentration (75, 150 and 300 µg/mL) were dependent on the 50% of growth inhibition concentration (IC_{50}) values of GFE serial concentration* (7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000 µg/mL)*, which were determined using a graph that represents the percentage of viable cells plotted against their respective concentrations.*

For groups 2 to 4 (Group 2 to Group 4), treatment was given for 48 hours, and the cells were cultured for 2 days in α-DMEM with 10% FBS + 5 g/mL insulin after the medium had been changed. The cells were then maintained in a CO_2 incubator until they had fully developed adipocyte morphology.

3T3-L1 Adipocytes Differentiation

The differentiation of 3T3-L1 adipocytes was carried out following a protocol outlined by Etesami *et al.* [10]. Starting from two days after reaching confluence, denoted as day 0, the cells were subjected to culture in DMEM supplemented with a hormonal mixture comprising 0.5 mM IBMX, 1.0 µM DEX, 1.0 µg/mL insulin, and 2.0 µM pioglitazone to induce differentiation. Following the differentiation process, the medium was substituted with DMEM referred to as the post-differentiation medium (post-DM), which contained 1.0 µg/mL insulin. This post-DM medium was maintained for an additional 2 days. Subsequently, the cells were transitioned

to regular DMEM, with medium changes occurring every 2 days. Throughout this period, the 3T3-L1 preadipocytes were exposed to the specified concentrations (75, 150, and 300 µg/mL) of extracts from day -2 to day 6, as illustrated in Figure 1.

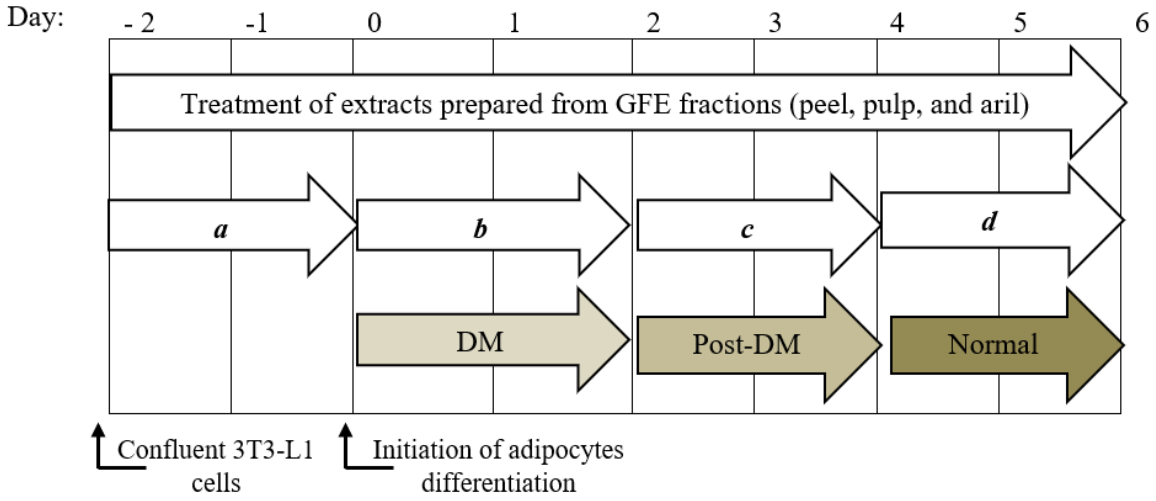


Figure 1. Schematic diagram of 3T3-L1 differentiation and the treatment of GFE DM, differentiation medium consists of foetal bovine serum (FBS)-Dulbecco's modified Eagle's medium (DMEM) including 3-isobutyl-1-methylxanthine (500 µM), dexamethasone (5.2 µM), and insulin (167 nM); post-DM, post-differentiation medium consists of FBS-DMEM including insulin (167 nm). Small alphabets ***a, b, c,*** and ***d*** represent the cycle of medium changes every 2 days for every stage from reaching the confluency to pre-adipocytes to adipocyte differentiation.

Cell Proliferation and Viability

The assessment of 3T3-L1 cell proliferation and cell viability was conducted through the utilization of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To examine the capacity of GFE fractions, including peel, pulp, and aril, to inhibit cell growth, a 96-well microtiter plate was employed for the cultivation of 3T3-L1 cells. In brief, a DMSO solution with a concentration of 100 mg/mL was used to prepare the GFE fractions. Following incubation periods of 24, 48, and 72 hours, the old medium in the 96-well plate, containing varying doses of GFE (ranging from 7.8 µg/mL to 1000 µg/mL), was removed.

Following the removal of the medium, each well was treated with 20 µL of MTT solution, which was prepared at a concentration of 5 mg/mL in PBS. This mixture was then incubated for an additional 4 hours in an incubator. Subsequently, 100 µL of DMSO was added to each well after the 4-hour incubation period. The purpose of introducing DMSO was to lyse the cells after the removal of the medium, allowing for the measurement of absorbance. The absorbance was quantified at 570 nm using a microplate reader. The 50% growth inhibition concentration (IC_{50}) values were determined from a graph representing the percentage of viable cells plotted against their respective concentrations. The calculation of percentages of viable cells was performed using the following equation:

$$\text{Percentage of viable cells} = \frac{Absorbance\ treatment}{Absorbance\ control} \times 100\% \quad \text{(Equation 3)}$$

Oil Red-O Staining

The assessment of the inhibitory effects of GFE fractions on 3T3-L1 adipogenesis was carried out using Oil Red-O (ORO) staining. In a nutshell, both DMSO (used for control) and GFEs at varying concentrations, ranging up to 1000 µg/mL, were applied to 3T3-L1 cells over a 7-day period during adipogenesis. On the 7th day, the cells were fixed with a 10% formalin solution for 1 hour at room temperature following a PBS wash. Subsequently, the cells were subjected to ORO staining for 10 minutes at room temperature, followed by rinsing with 2.4 mL of 60% isopropanol and four subsequent washes with distilled water. Images of each dish were captured under a microscope (Olympus Corporation, Tokyo, Japan) for further analysis and visualization.

Cell Morphological Changes and Intracellular Triglyceride Content

To investigate the potential anti-adipogenic properties of Gac fruit extract (GFE), its effects on cell morphology and triglyceride content were examined. These observations were conducted using an inverted microscope. The results indicated significant alterations in lipid accumulation in

3T3-L1 adipocytes when treated with GFE aril at concentrations of 75, 150, and 300 µg/mL.

Triglyceride Content Measurement

The quantification of triglyceride content in 3T3-L1 adipocytes was carried out using the AdipoRed assay reagent from Lonza, MD, USA, following the manufacturer's recommended procedure. In brief, both DMSO (used for control) and GFE at varying concentrations, up to 1000 µg/mL, were applied to 3T3-L1 cells over a 7-day period during the differentiation process. On the 7th day, the 96-well plates containing 3T3-L1 adipocytes were rinsed with 0.2 mL of PBS. Subsequently, the cells were incubated with 140 µL of AdipoRed for 15 minutes at room temperature. The fluorescence emitted from the supernatant was then measured using a spectrophotometer (Molecular Devices, CA, USA) at wavelengths of 485 nm and 535 nm for further analysis.

Statistical Analysis

All the data are presented in the format of mean ± standard deviation (SD) based on triplicate measurements (n=3). Statistical analysis was conducted using the Statistical Packages for Social Sciences (SPSS) software, version 26, developed by SPSS Inc. in Chicago, IL, USA. The significance of differences between the various groups was assessed through a one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Extraction Yields and Antioxidant Activities of GFE

Table 1 presents the extraction yields of gac fruit extract fractions, including peel, pulp, and aril. Notably, the peel demonstrated the highest extraction yield at 38.96%, followed by the aril with 28.82%, and finally, the pulp with 18.85%. These extraction yields were influenced by the moisture and carotenoid content present in each gac fruit fraction. Interestingly, despite the pulp having the greatest dry sample weight compared to the peel and aril, it exhibited the lowest extraction yield. This variation could be attributed to factors such as carotenoid density and moisture content among the different fractions. The slightly lower extraction yield of the aril, despite its richness in carotenoids and "gac oils," may be attributed to its sticky nature. These findings align with previous research that discussed the carotenoid content within gac fruit extract fractions, highlighting the importance of considering the composition and properties of each fraction when extracting bioactive compounds from gac fruit. Furthermore, the choice of solvent composition, specifically n-hexane: acetone: ethanol (HAE) in a 50:25:25 (v/v/v) ratio, significantly influenced the extraction efficiency of bioactive compounds from various gac fruit parts. These insights provide valuable information for optimizing the extraction process and can guide future studies focused on isolating and characterizing bioactive components from gac fruit extracts.

Table 1. The extraction yields of gac fruit extract fraction (peel, pulp, and aril) using solvent *n*-hexane: acetone: ethanol (HAE) in ratio 50:25:25 (*v/v/v*).

Sample	Weight of dried sample (g)	Weight of empty flask, g (W_0)	Weight of flask + extract, g (W_1)	W_1-W_0 (g)	% Yield (wt/wt-dry weight)
Peel	153.57±0.13	645.30±1.12	705.13±1.24	59.83	38.96^{A}
Pulp	239.70±1.12	502.20±1.27	547.39±1.33	45.19	18.85^{C}
Aril	210.63±0.79	506.15±0.98	566.86±1.09	60.71	28.82^{B}

Results are expressed as means ± standard deviations (n=3).
$^{A-C}$: Values with different superscripts within a row are significantly different ($p<0.05$).

The scavenging activities of gac fruit extracts (GFEs) were assessed using the DPPH scavenging capacity assay, and the results are summarized in Table 2. As reference standards, the IC_{50} values of well-known antioxidants against DPPH radicals, namely BHT and gallic acid, were determined to be 182.02 mg/L and 145.86 mg/L, respectively. Table 3 presents the IC_{50} values for the various gac fruit fractions. Notably, the gac pulp exhibited an IC_{50} value of 525.46 mg/L, while the gac peel displayed an IC_{50} value of 573.40 mg/L. On the other hand, the aril fraction demonstrated the highest IC_{50} value among the three fractions, with a value of 817.33 mg/L. These results underscore the antioxidant potential of gac fruit extracts, with the gac pulp fraction showing the most potent DPPH scavenging activity among the fractions examined. These findings offer valuable insights into the scavenging capacity of gac fruit extracts and suggest their potential applications in functional foods or the formulation of natural antioxidants.

The DPPH scavenging capacity, represented as the percentage of inhibition or IC_{50} (the concentration needed to inhibit 50% of radicals), was determined using the linear equation $y = mx + c$, derived from the graph depicted in Figure 2. In this equation, the y-value corresponds to 50 ($y = 50$), indicating the concentration required to achieve a 50% scavenging effect by the gac fruit extracts (GFEs). The x-

value represents this concentration. Based on these calculations, the IC_{50} values for the gac fruit fractions were determined as follows: gac pulp with an IC_{50} of 525.46 mg/L, gac peel with an IC_{50} of 573.40 mg/L, and gac aril with an IC_{50} of 817.33 mg/L (Table 3). These results reveal that the GFE from the pulp fraction possesses the highest scavenging capacity, followed by the GFE from the peel fraction. In contrast, the aril fraction exhibits the lowest efficacy in scavenging radicals (pulp > peel > aril). It is interesting to note that these findings contradict previous research on the antioxidant capacity of gac fruit fractions (peel, pulp, and aril) based on their ability to inhibit lipase activity *in vitro*. According to the previous study, the efficacy of the gac fruit fractions in inhibiting lipase activity was reported as peel > aril > pulp. This inconsistency suggests that various factors or mechanisms within the gac fruit fractions may influence their antioxidant capacity and inhibitory effects on lipase activity. These findings shed light on the diverse bioactive properties of different gac fruit fractions and emphasize the potential of gac pulp and peel as valuable sources of antioxidants. Further investigations are needed to explore the underlying mechanisms and specific bioactive components contributing to the observed variations in antioxidant capacity and inhibitory effects.

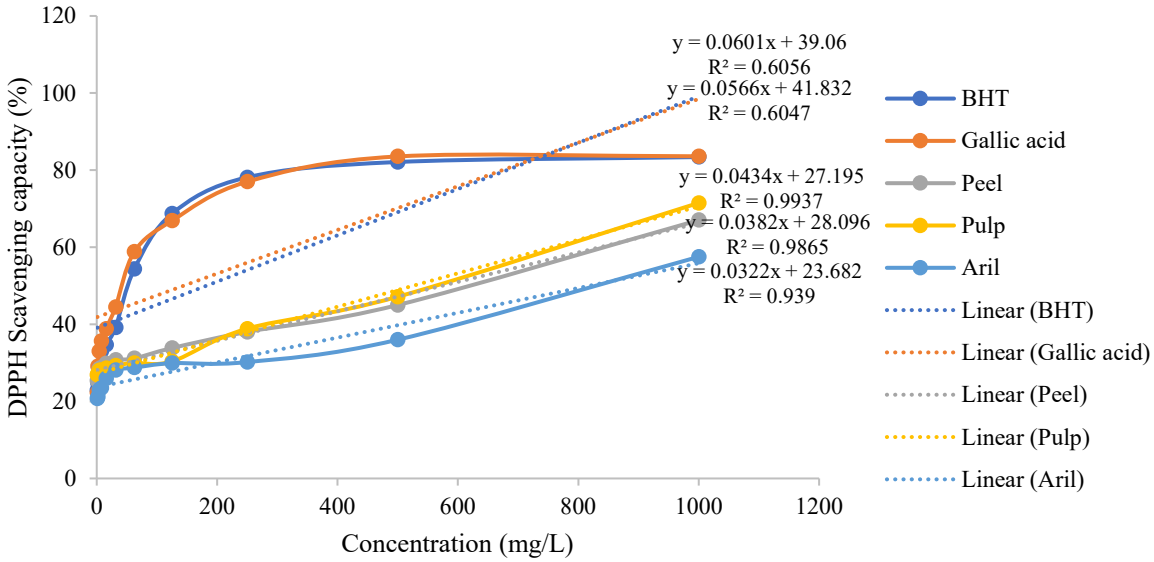


Figure 2. The scavenging capacity (IC50) of gac extracts (peel, pulp, and aril) was measured using the DPPH scavenging capacity assay, with BHT and gallic acid serving as reference standards. The broken lines in the graph represent the IC50 values for each sample fraction.

Table 2. The scavenging capacity of gac fruit extract fraction (peel, pulp, and aril) using solvent *n*-hexane: acetone: ethanol (HAE) in ratio 50:25:25 (*v/v/v*).

Sample	Concentration (mg/L)										
	0.9	**1.95**	**3.91**	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	**500**	**1000**
BHT	22.89±0.03	24.72±0.45	28.60±0.26	32.98±0.12	34.69±0.17	39.18±0.23	54.35±0.32	68.69±1.43	78.13±1.22	82.11±0.36	83.39±0.97
Gallic acid	22.27±1.25	29.15±1.32	33.06±2.24	35.68±1.56	38.87±2.65	44.50±3.12	58.82±0.09	66.88±0.18	76.99±1.27	83.55±1.16	83.59±1.22
Peel	25.40±0.89	27.04±1.13	28.47±2.21	28.77±1.14	29.86±1.86	30.75±1.11	31.17±2.11	33.83±0.66	38.03±1.23	45.00±1.64	67.03±1.34
Pulp	26.85±0.77	27.11±2.14	28.07±1.15	28.12±1.78	28.58±2.16	29.28±2.10	29.99±2.12	30.38±0.34	38.87±1.46	47.15±1.84	71.49±1.57
Aril	20.70±0.86	21.17±0.15	22.95±2.11	23.50±1.92	25.93±2.11	28.15±1.33	28.76±0.65	29.91±0.51	30.20±1.75	36.00±1.73	57.51±1.88

*1 mg/L = 1 µg/mL = 1 ppm
*BHT and gallic acid were used as antioxidant reference solution.
Results are expressed as means ± standard deviations (n=3).

Table 3. The inhibition concentration at 50 percent (IC_{50}) of gac fruit extract fraction (peel, pulp, and aril) using solvent *n*-hexane: acetone: ethanol (HAE) in ratio 50:25:25 (*v/v/v*).

Gac fruit part	Equation, y = mx + c	Linearity, R-squared (R^2)	DPPH radical scavenging activity (IC_{50}, mg/L)
BHT	y = 0.0601x + 39.06	0.6056	182.03
Gallic acid	y = 0.0566x + 41.832	0.6047	145.86
Peel	y = 0.0382x + 28.096	0.9865	573.40
Pulp	y = 0.0434x + 27.195	0.9937	525.46
Aril	y = 0.0322x + 23.682	0.939	817.33

Table 4. Percent of cell viability (%CV) of 3T3-L1 after treated with gac fruit extract (GFE) fractions (peel, pulp, and aril) for 24 hrs, 48 hrs, and 72 hrs, measured at OD 590 nm.

Extract	Concentration (µg/ml)							
	7.8	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**	**1000**
Incubation, 24 hrs								
Peel	115.73±2.12	110.60±2.33	108.67±3.21	106.26±2.10	106.22±1.10	100.61±1.09	93.24±1.19	81.59±1.34
Pulp	82.90±1.24	71.76±1.22	70.29±2.21	66.20±1.87	65.51±1.56	64.42±2.19	63.76±1.82	60.06±1.56
Aril	97.40±1.65	96.34±1.91	92.75±1.43	90.53±1.65	87.05±1.32	84.50±3.10	81.48±1.26	73.39±1.66
Incubation, 48 hrs								
Peel	120.78±2.33	112.05±2.09	100.27±2.14	99.85±1.32	99.67±1.34	99.65±2.51	98.02±1.75	90.11±1.76
Pulp	105.55±1.32	90.40±2.86	90.07±1.33	87.43±1.54	82.87±1.82	81.99±2.43	80.54±1.87	76.67±1.98
Aril	97.97±1.76	95.85±1.46	93.75±2.13	92.01±1.67	84.30±1.95	83.72±2.11	79.86±1.65	79.69±1.76
Incubation, 72 hrs								
Peel	159.34±2.34	143.56±2.43	116.80±2.43	106.78±1.73	102.25±1.22	100.13±2.12	99.99±2.11	98.71±2.12
Pulp	131.18±2.13	116.12±2.13	104.01±1.26	88.97±1.56	87.46±1.32	80.48±1.06	80.37±2.34	78.81±2.13
Aril	92.43±1.13	91.48±1.54	90.77±3.21	89.12±1.22	88.34±1.56	84.79±1.91	82.41±2.09	78.14±2.24

Results are expressed as means ± standard deviations (n=3).

Table 5. Percent of cell viability (%CV) of 3T3-L1 after treated with gac fruit extract (GFE) fractions (peel, pulp, and aril) for the 24 hrs, 48 hrs, and 72 hrs, measured at OD 630 nm.

Extract	Concentration (µg/ml)							
	7.8	**15.6**	**31.3**	**62.5**	**125**	**250**	**500**	**1000**
				Incubation, 24 hrs				
Peel	113.65±0.19	107.97±2.13	105.96±0.44	105.95±1.84	104.51±2.32	99.81±1.11	95.68±1.98	85.39±1.89
Pulp	83.14±1.10	74.78±1.78	71.75±1.09	69.88±1.47	68.04±2.04	67.68±1.64	66.80±1.84	63.40±2.18
Aril	97.53±1.14	97.23±1.89	95.60±2.12	92.63±1.99	92.46±2.15	91.32±1.82	83.83±1.59	79.07±2.99
				Incubation, 48 hrs				
Peel	124.43±2.10	112.43±1.99	101.86±2.11	100.61±2.65	99.89±1.87	99.63±1.48	97.14±2.11	94.50±1.98
Pulp	104.20±2.06	90.26±2.11	89.46±1.75	86.45±2.19	82.69±1.69	80.64±1.39	78.98±1.89	76.10±2.15
Aril	99.07±1.87	96.96±2.09	94.24±2.34	92.08±2.45	84.18±1.52	84.16±1.87	81.38±1.75	80.06±2.07
				Incubation, 72 hrs				
Peel	182.84±1.19	156.43±1.77	123.03±1.09	113.94±2.67	107.19±2.19	106.59±1.03	106.28±2.19	102.50±1.95
Pulp	140.93±1.65	121.46±1.48	108.58±2.81	93.76±1.32	92.44±2.04	84.73±1.75	83.95±1.60	83.82±1.68
Aril	93.61±2.07	92.91±1.36	92.90±2.87	91.64±0.79	89.44±2.17	86.29±1.98	84.34±1.67	79.52±1.44

Results are expressed as means ± standard deviations (n=3).

Effect of GFE on Cell Viability of 3T3-L1 Cells

To evaluate the impact of gac fruit extract (GFE) on the viability of 3T3-L1 preadipocytes, an MTT assay was conducted after 24 hours, 48 hours, and 72 hours of incubation of the cultured 3T3-L1 cells with GFE fractions (peel, pulp, and aril). The optical density (OD) was measured at 590 nm and 630 nm using a microplate reader. The cell viability percentage (%CV) was then calculated based on Equation 3.

In the aforementioned equation, OD sample represents the optical density of the treated cells, OD blank corresponds to the background optical density of the blank wells, and OD control represents the optical density of the control cells without GFE treatment. The resulting %CV value indicates the percentage of viable cells in comparison to the control. By employing the MTT assay and calculating the %CV, we aimed to evaluate the impact of GFE fractions on the viability of 3T3-L1 pre-adipocytes across various incubation periods. The results of the viability assessment of 3T3-L1 cells after treatment with gac fruit extract (GFE) fractions (peel, pulp, and aril) for 24 hours, 48 hours, and 72 hours, measured at optical densities of 590 nm and 630 nm, are presented in Table 4 and Table 5, respectively.

The results obtained indicate that the exposure of 3T3-L1 cells to gac fruit extract fractions (peel, pulp, and aril) did not lead to any toxic effects on cell viability, even at concentrations up to 1000 µg/mL. However, a minor reduction in cell viability was observed when cells were exposed to a concentration of 250 µg/mL. Based on these findings, subsequent experiments were carried out using concentrations up to 200 µg/mL of gac fruit extracts. These results suggest that gac fruit extracts have a non-toxic influence on the viability of 3T3-L1 cells at the tested concentrations, rendering them suitable for further investigations and experiments pertaining to their potential impacts on adipogenesis.

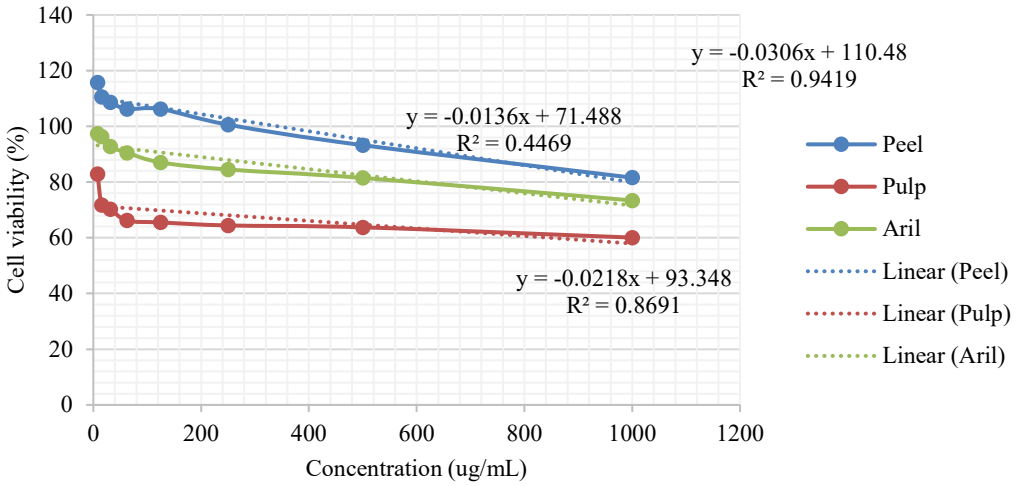
The results displayed in Figure 3a-f illustrate the impact of gac peel, pulp, and aril extracts on cell viability at various time points (24 h, 48 h, and 72 h) and concentrations (up to 1000 µg/mL serial concentration). In general, there were no significant differences observed in cell viability following treatment with gac extracts. However, at higher concentrations of GFE, there was a reduction in the number of viable cells, albeit without any indications of apoptosis or cytotoxic effects. Notably, it is crucial to mention that the inhibition concentration at 50 percent (IC_{50}) could not be determined, as the equation $y = mx + c$ yielded a negative value, with y representing the value of 50 and x representing the concentration of the extract. This suggests that the tested concentrations did not reach a level where 50% inhibition was observed. These findings imply that gac peel, pulp, and aril extracts, even at higher concentrations, do not exert significant adverse effects on cell viability, implying their potential safety and suitability for further investigations. Further experiments and analysis may be required to explore other aspects of the extract's biological activity and ascertain the specific concentration required for 50% inhibition.

Effect of GFE on Anti-Adipogenic Properties and Intracellular Triglyceride Content of Adipocytes in 3T3-L1 Cells

To assess the effects of gac fruit extracts (GFEs) on the differentiation of 3T3-L1 pre-adipocytes into adipocytes, confluent cells were exposed to various concentrations (75, 150, and 300 µg/mL) for cell morphology evaluation, and concentrations (7.8, 15.6, 31.3, 62.5, 125, 250, 500, and 1000 µg/mL) for quantifying triglyceride content. The inhibitory impact of gac extracts on 3T3-L1 adipogenesis was determined on the seventh day using Oil Red-O (ORO) staining. The anti-adipogenic properties of gac fruit extract (GFE) fractions (peel, pulp, and aril) on 3T3-L1 adipogenesis were assessed by observing morphological changes and intracellular lipid accumulation under an inverted microscope. The levels of intracellular lipid accumulation were subsequently visualized.

Figure 4.1, Figure 4.2 (Plate 1), Figure 4.3 (Plate 2), and Figure 4.4 (Plate 3) illustrate the observed cell morphological changes and intracellular lipid accumulation visualized under an inverted microscope. Comparing the various Gac fruit extract (GFE) fractions, including peel, pulp, and aril, their impact on lipid accumulation and the differentiation of 3T3-L1 cells was assessed. The findings revealed a significant decrease in lipid accumulation in 3T3-L1 cells following GFE treatment. Furthermore, GFE exhibited an inhibitory effect on the differentiation of 3T3-L1 cells, with the extent of this effect correlating with the concentration of GFE applied.

(a)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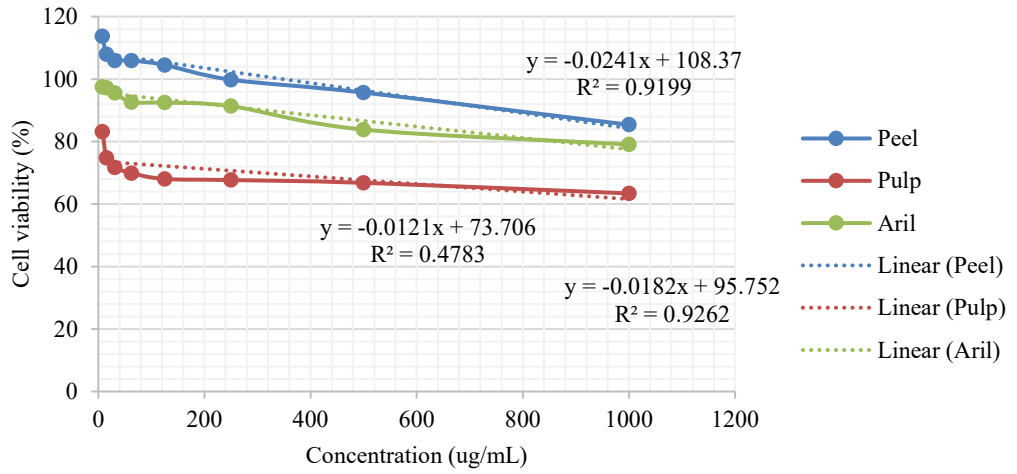
R² = 0.9419
y = -0.0136x + 71.488
R² = 0.4469
y = -0.0218x + 93.348
R² = 0.8691
Cell viability (%)
Concentration (ug/mL)
Peel
Pulp
Aril
Linear (Peel)
Linear (Pulp)
Linear (Aril)

(b)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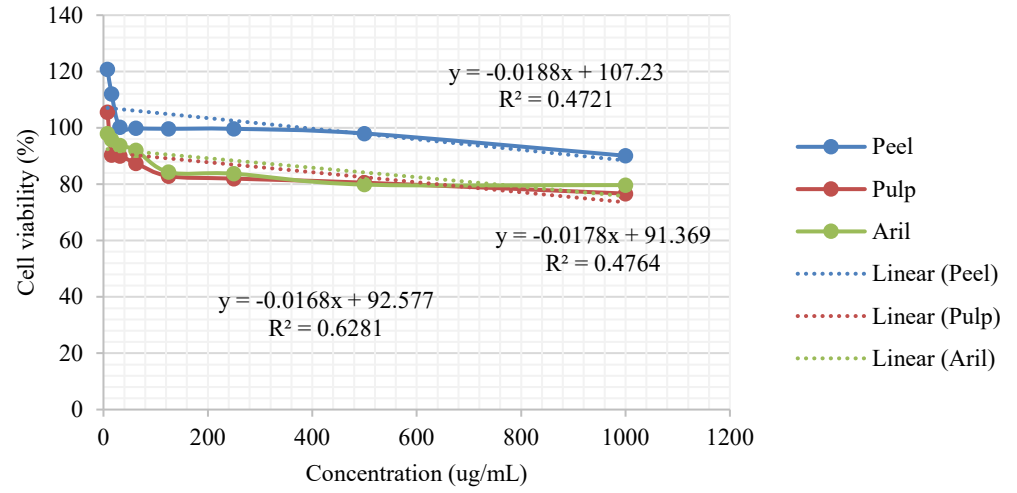
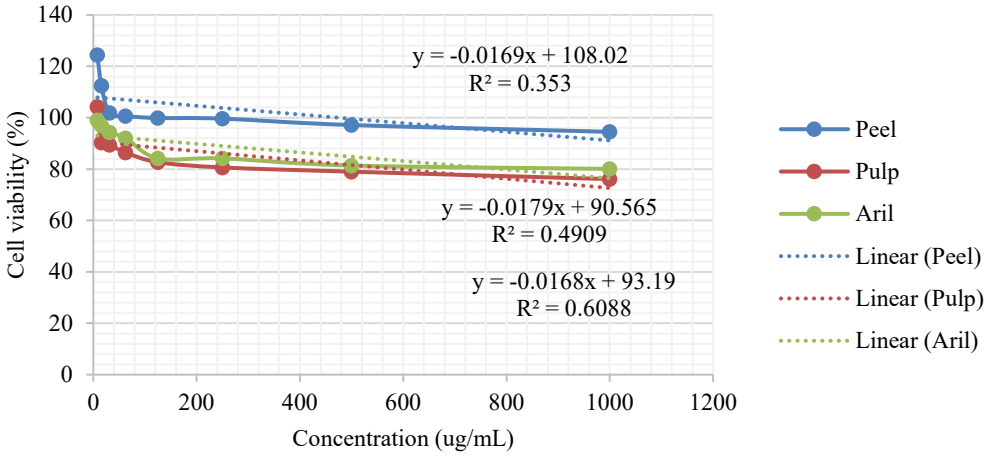
R² = 0.9199
y = -0.0121x + 73.706
R² = 0.4783
y = -0.0182x + 95.752
R² = 0.9262
Cell viability (%)
Concentration (ug/mL)
Peel
Pulp
Aril
Linear (Peel)
Linear (Pulp)
Linear (Aril)

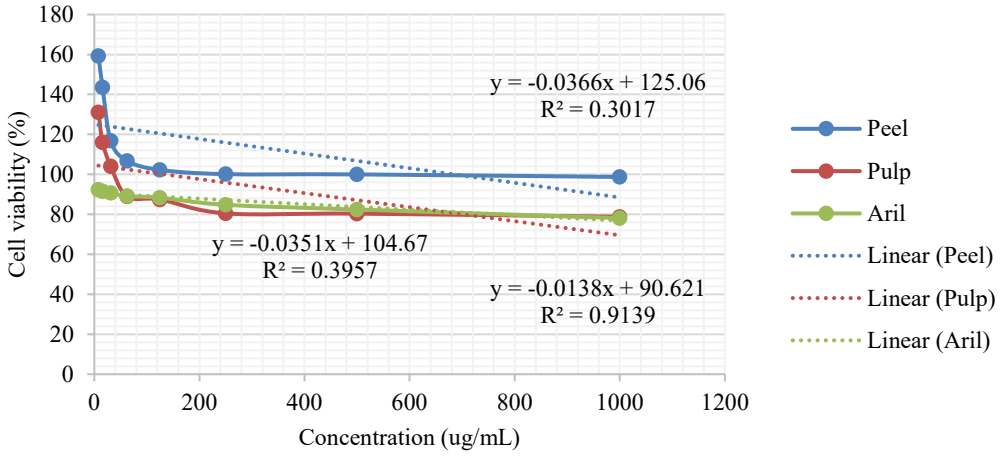
(c)


y = -0.0188x + 107.23
R² = 0.4721
y = -0.0178x + 91.369
R² = 0.4764
y = -0.0168x + 92.577
R² = 0.6281
Cell viability (%)
Concentration (ug/mL)
Peel
Pulp
Aril
Linear (Peel)
Linear (Pulp)
Linear (Aril)

(d)



(e)



(f)

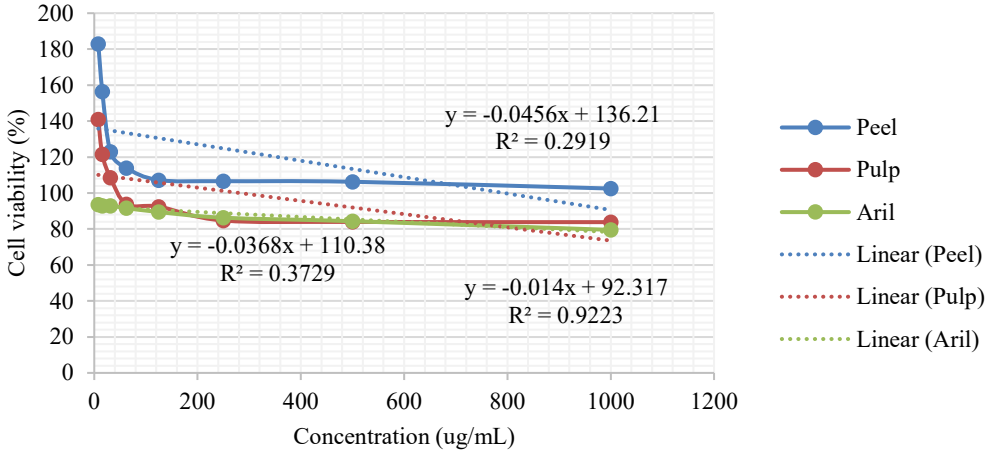


Figure 3. Effects of gac fruit extracts on cell viability and differentiation of 3T3-L1 pre-adipocytes, (a) after 24 hours incubation (measured at optical density, OD 590 nm); (b) after 24 hours incubation (measured at optical density, OD 630 nm); (c) after 48 hours incubation (measured at optical density, OD 590 nm); (d) after 48 hours incubation (measured at optical density, OD 630 nm); (e) after 72 hours incubation (measured at optical density, OD 590 nm); (f) after 72 hours incubation (measured at optical density, OD 630 nm).

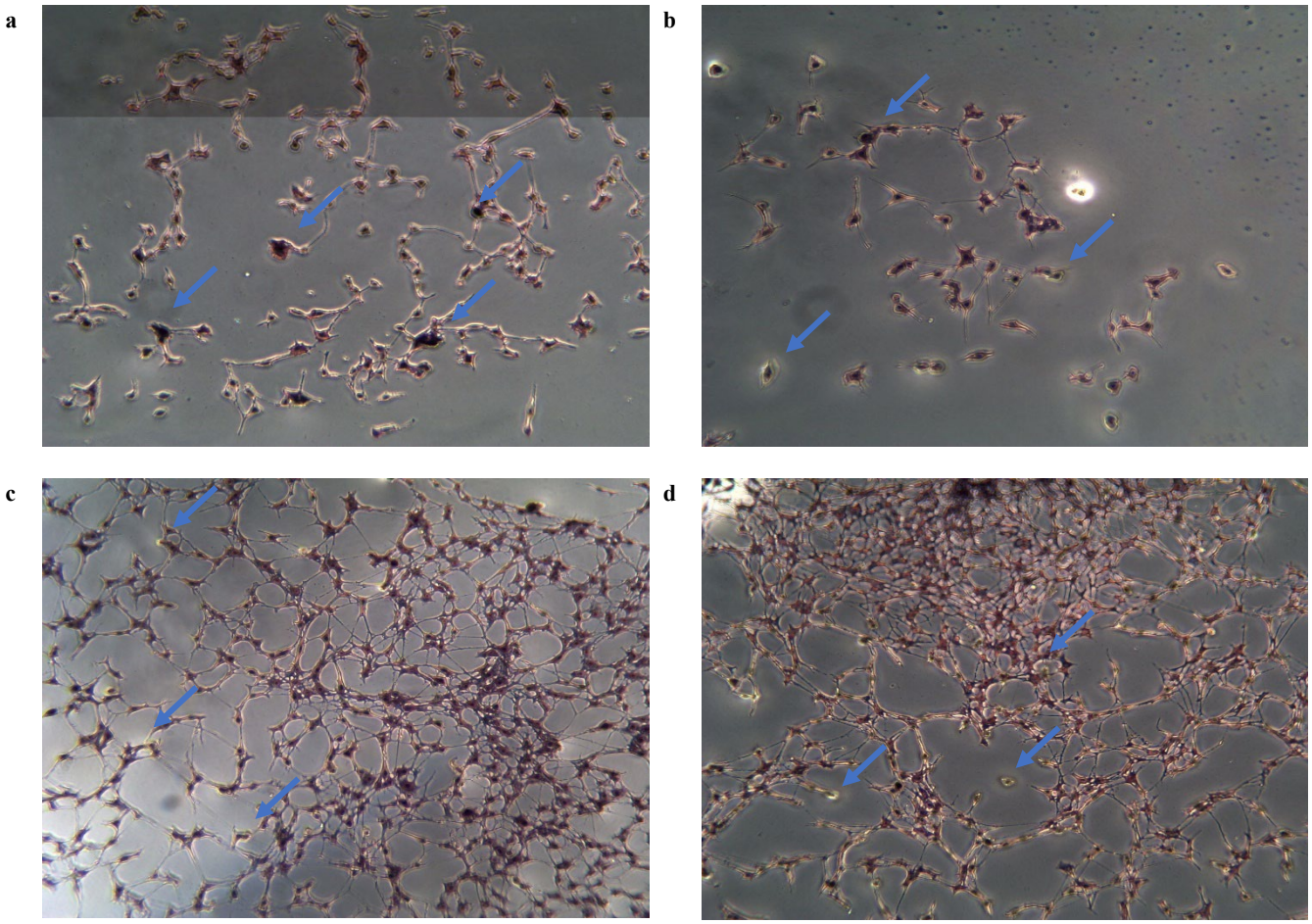
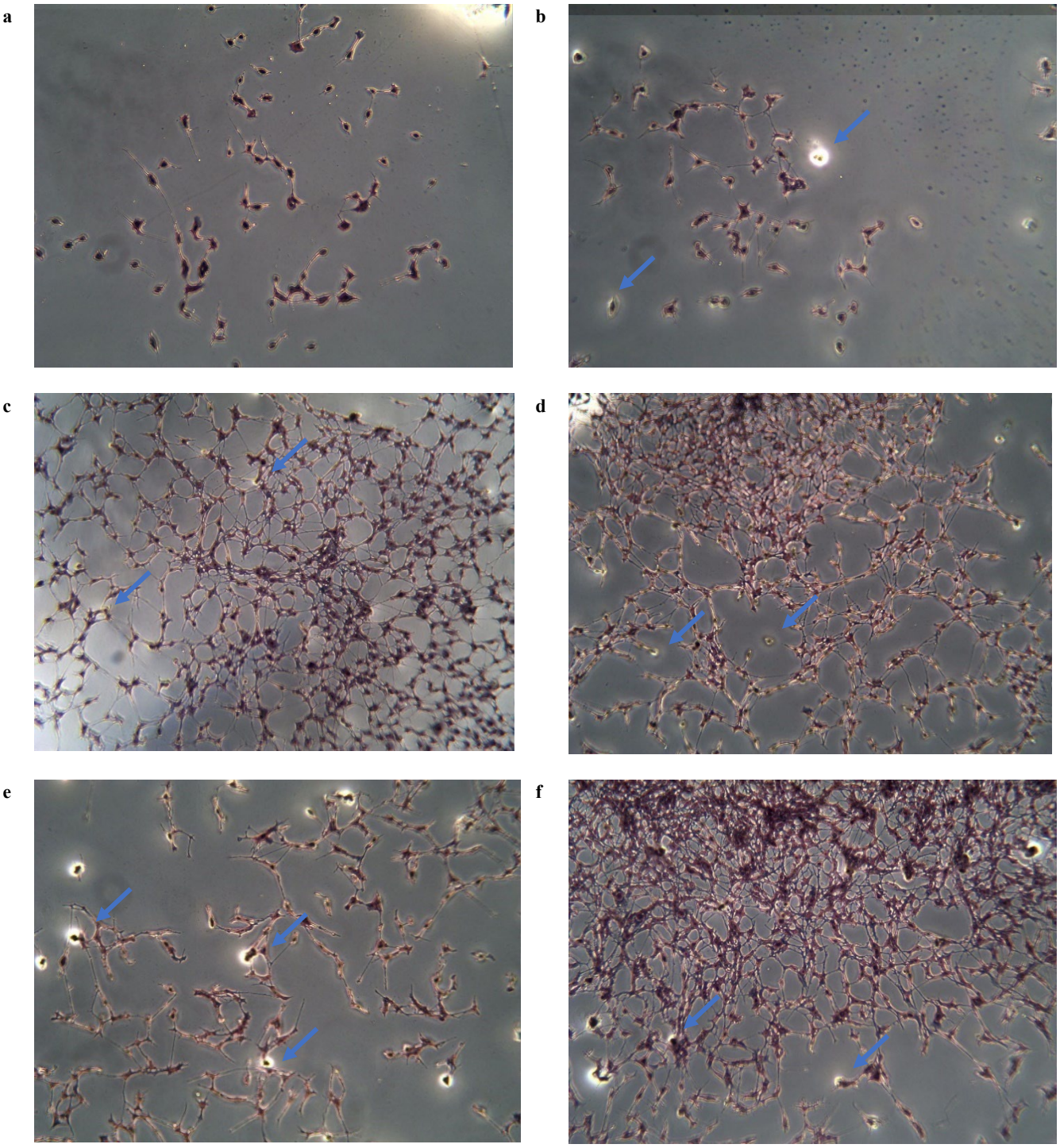


Figure 4.1. Microscopic images on the effect of GFE supplementation against lipid droplet accumulation. Adipocytes with lipid droplet accumulations (blue arrows) appeared in yellow circled after being stained with Oil Red O reagent. (Magnification 200x). (a) Control (untreated); (b) GFE Aril 75 µg/mL; (c) GFE Aril 150 µg/mL; (d) GFE Aril 300 µg/mL.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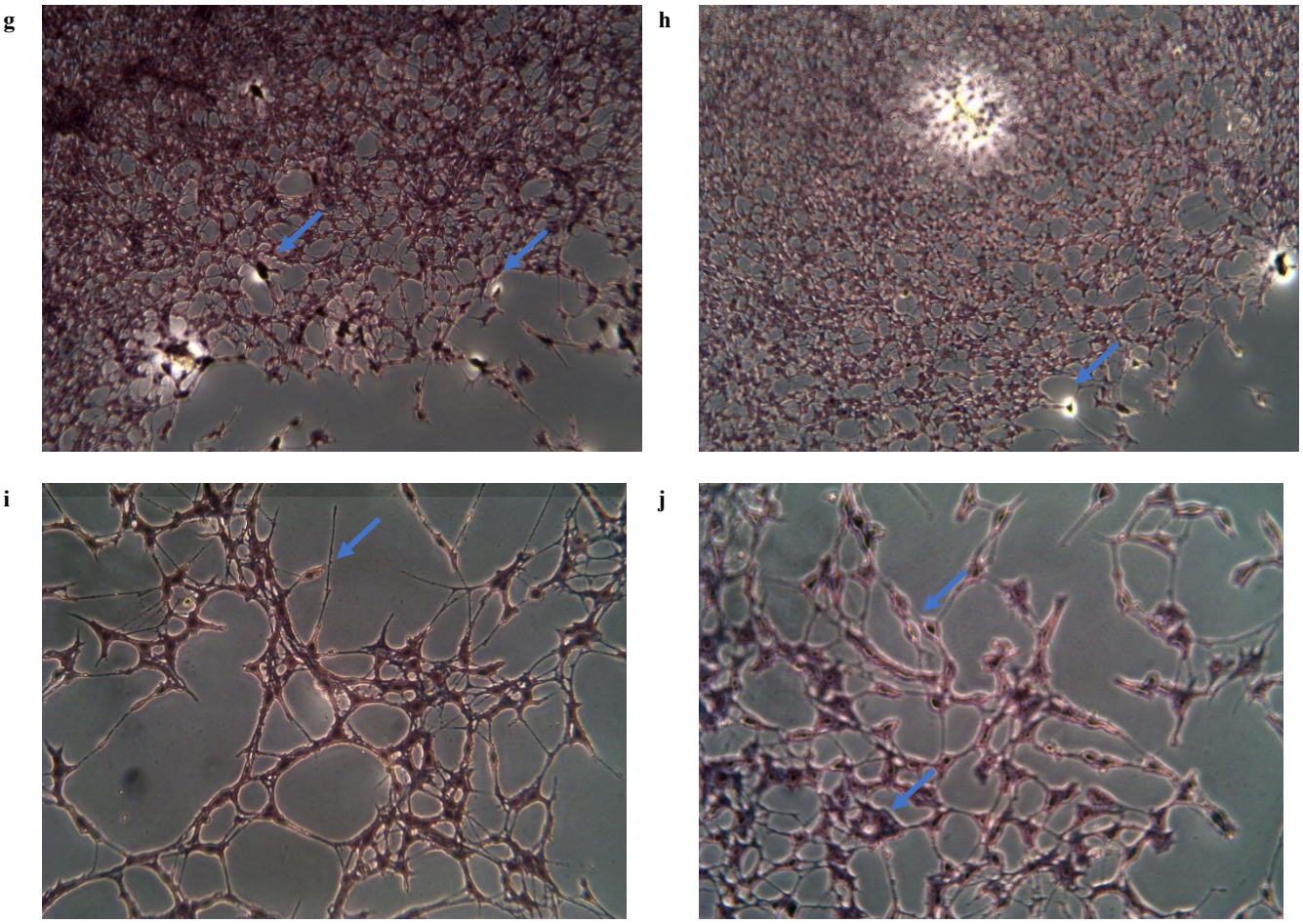
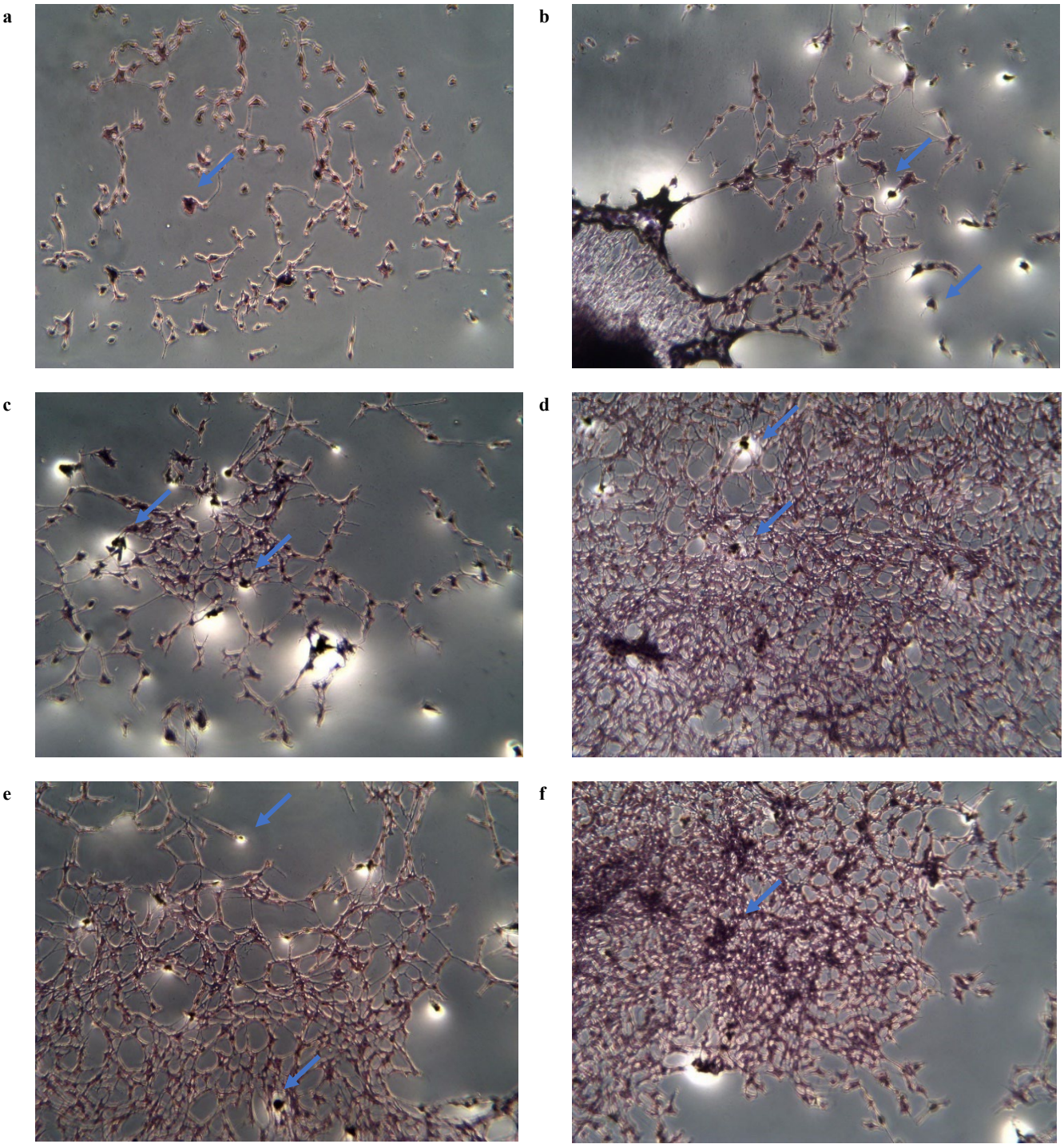


Figure 4.2. Microscopic images on the effect of GFE supplementation against lipid droplet accumulation. Adipocytes with lipid droplet accumulations appeared in yellow circled (blue arrows) after being stained with Oil Red O reagent. (Magnification 200x). **Plate 1:** (a) Control; (b) Aril 75 µg/mL; (c) Aril 150 µg/mL; (d) Aril 300 µg/mL; (e) Peel 75 µg/mL; (f) Peel 150 µg/mL; (g) Peel 300 µg/mL; (h) Pulp 75 µg/mL; (i) Pulp 150 µg/mL; (j) Pulp 300 µg/mL.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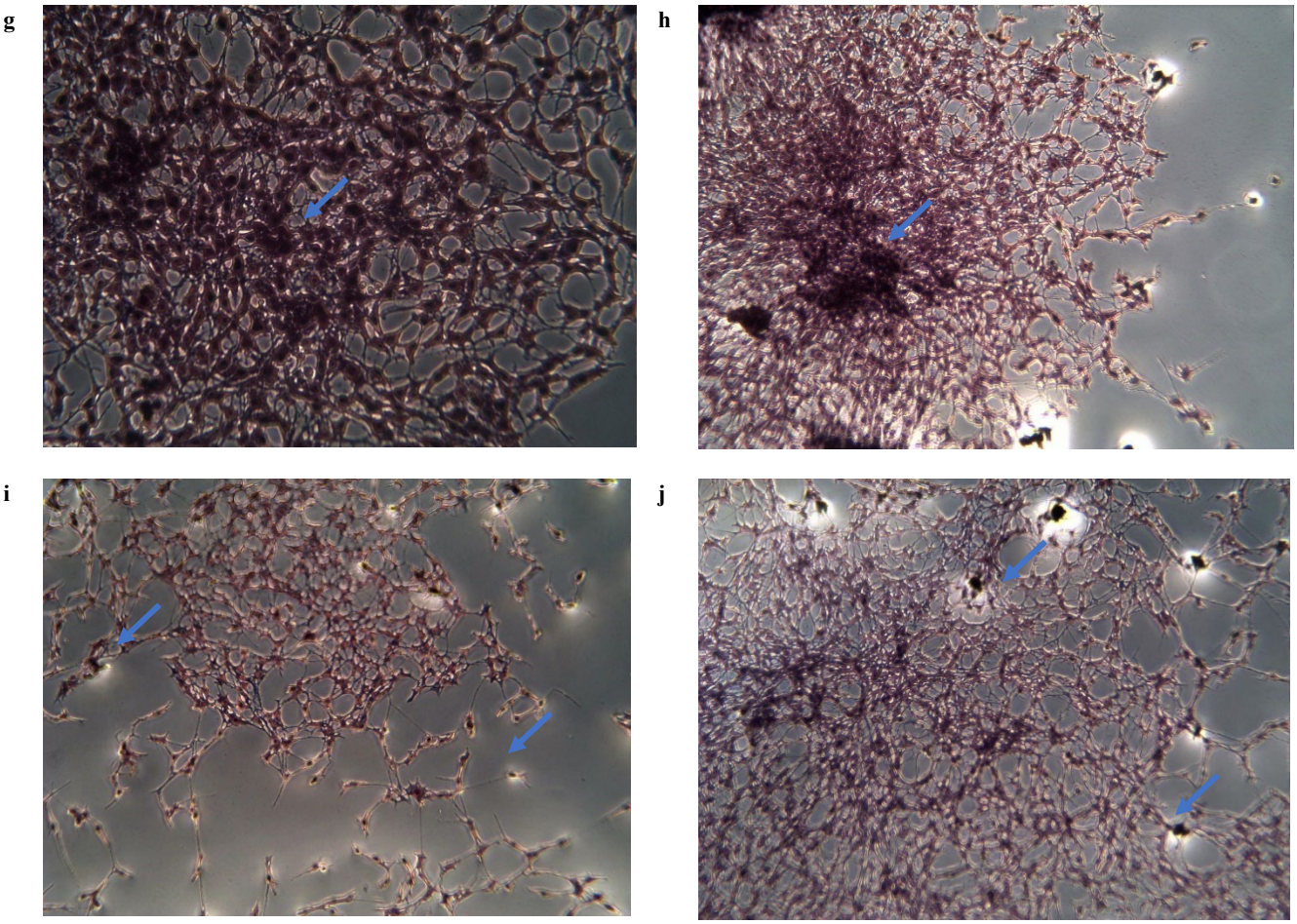
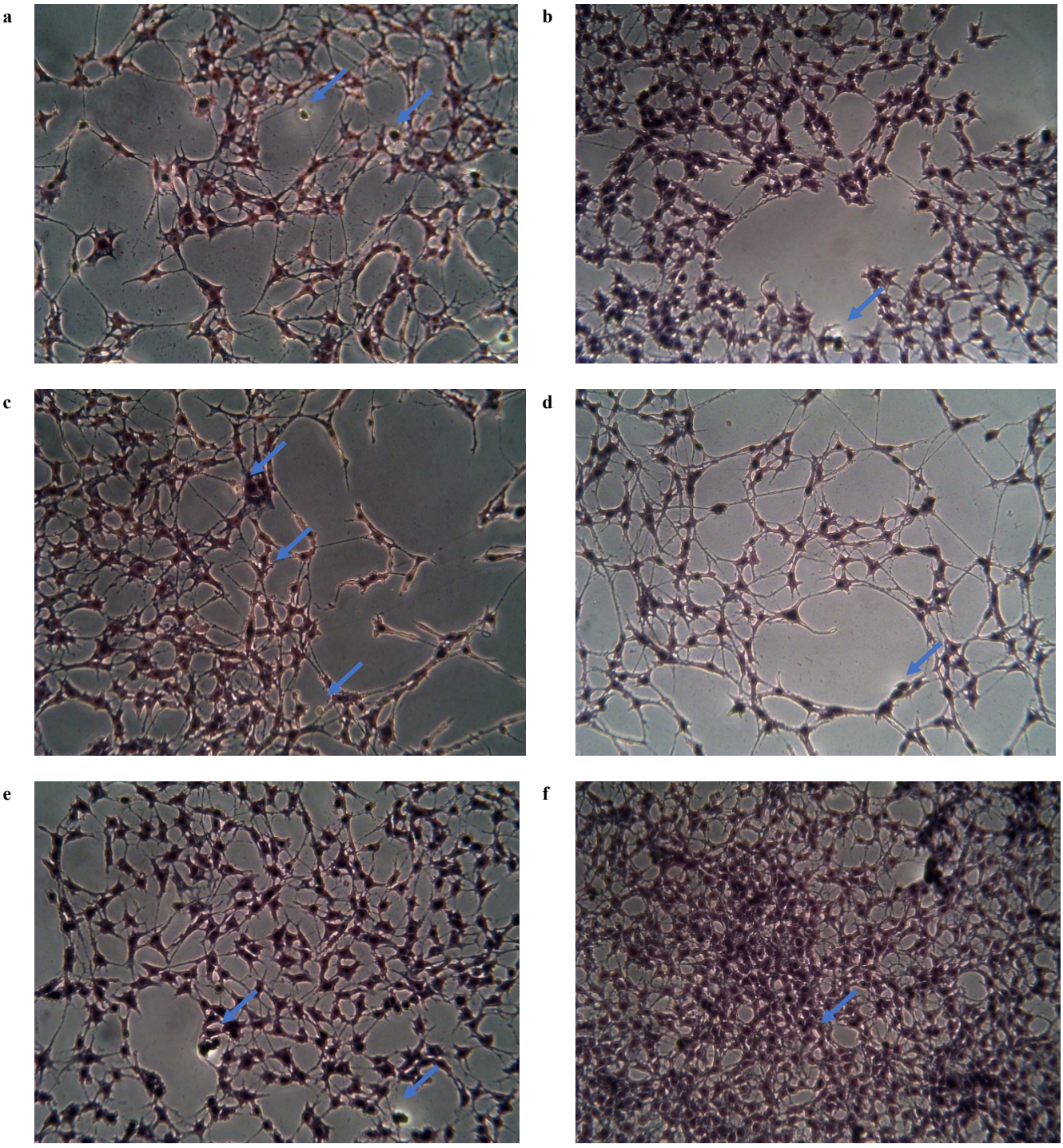


Figure 4.3. Microscopic images on the effect of GFE supplementation against lipid droplet accumulation. Adipocytes with lipid droplet accumulations appeared in yellow circled (blue arrows) after being stained with Oil Red O reagent. (Magnification 200x). **Plate 2:** (a) Control; (b) Aril 75 µg/mL; (c) Aril 150 µg/mL; (d) Aril 300 µg/mL; (e) Peel 75 µg/mL; (f) Peel 150 µg/mL; (g) Peel 300 µg/mL; (h) Pulp 75 µg/mL; (i) Pulp 150 µg/mL; (j) Pulp 300 µg/mL.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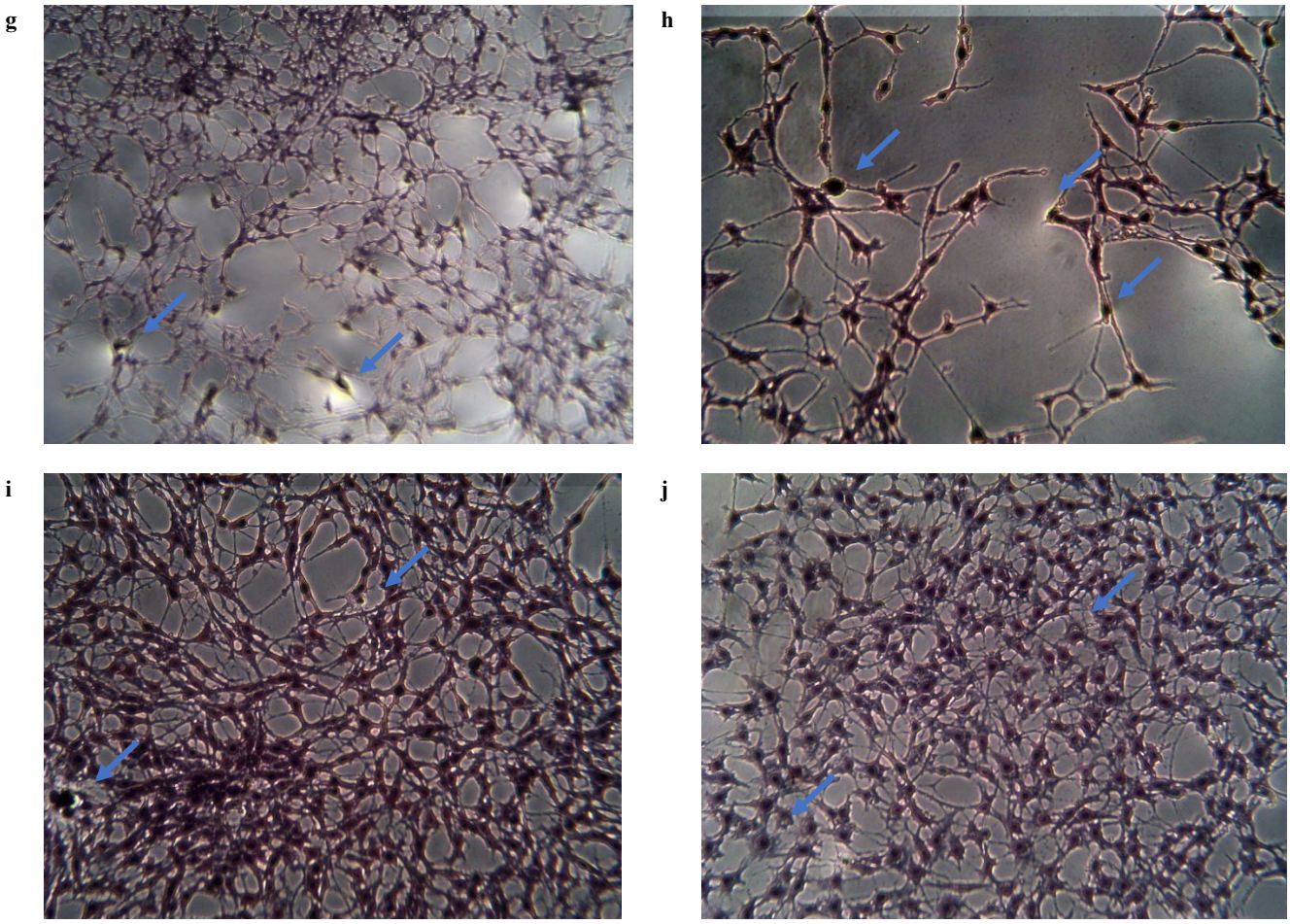

Figure 4.4. Microscopic images on the effect of GFE supplementation against lipid droplet accumulation. Adipocytes with lipid droplet accumulations appeared in yellow circled (blue arrows) after being stained with Oil Red O reagent. (Magnification 200x). **Plate 3:** (a) Control; (b) Aril 75 µg/mL; (c) Aril 150 µg/mL; (d) Aril 300 µg/mL; (e) Peel 75 µg/mL; (f) Peel 150 µg/mL; (g) Peel 300 µg/mL; (h) Pulp 75 µg/mL; (i) Pulp 150 µg/mL; (j): Pulp 300 µg/mL.

Intracellular triglyceride content was quantified using the AdipoRed assay. A triglyceride standard curve was generated in accordance with the AdipoRed assay reagent and the manufacturer's protocol. The study revealed that treatment with GFE fractions (peel, pulp, and aril) at concentrations up to a maximum of 1000 µg/mL did not significantly inhibit the intracellular accumulation of triglycerides in 3T3-L1 adipocytes when compared to the control (Figure 5). Moreover, there were no observable beneficial effects of gac fruit extract (GFE) fractions in reducing the percentage of intracellular triglyceride content in adipocytes across all tested doses, ranging from 7.8 µg/mL to 1000 µg/mL, except for the GFE aril fraction. Notably, the GFE aril fraction exhibited a positive impact in reducing the accumulation of intracellular triglycerides in 3T3-L1 cells at concentrations ranging from 125 to 500 µg/mL.

Treatment with GFE at serial concentrations (7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000 µg/mL) did not lead to a significant reduction in triglyceride (TG) contents. The data represents the mean percentage levels compared to non-treated cells (Control). Results are presented as means ± SD of at least three independent experiments and were analyzed using analysis of variance (ANOVA) with Duncan's multiple range tests. Groups sharing the same alphabet indicate no significant difference between them ($p < 0.05$).

Inconsistencies in triglyceride concentrations were observed in the GFE fractions (peel, pulp, and aril) at different concentrations ranging from 7.8 µg/mL to 1000 µg/mL. Surprisingly, the control group (cells not treated with GFE) showed the highest triglyceride concentration at the lowest GFE concentration of 7.8 µg/mL. However, as the GFE concentration increased to the range of 250 µg/mL to 1000 µg/mL, cells treated with GFE exhibited higher intracellular lipid accumulation compared to the untreated group (Figure 6). Based on these findings, it is hypothesized that the highest concentration of GFE capable of inhibiting intracellular triglyceride accumulation is likely below 250 µg/mL, possibly within the range of 125 µg/mL to 200 µg/mL. Further investigations and experiments are warranted to confirm this hypothesis and determine the

optimal GFE concentration for effectively inhibiting intracellular triglyceride accumulation in 3T3-L1 cells.

The comparison between different types of GFE fractions revealed that the extract derived from gac pulp exhibited the highest efficacy in inhibiting triglyceride accumulation in 3T3-L1 cells, followed by the GFE peel, while the GFE aril showed the least effectiveness (pulp > peel > aril). This difference in effectiveness may be attributed to the higher carotenoid content in the peel and aril fractions. Interestingly, the peel and aril also contain high-fat content compared to the aril alone. Furthermore, previous studies have also focused on the extraction of gac oils from various parts of the fruit, including the peel, aril, and seeds [11–15]. These studies further support the significance of different components in the peel and aril fractions, which may contribute to their potential effects on triglyceride accumulation. These findings highlight the importance of considering different fractions of the gac fruit when evaluating their potential effects and extracting bioactive components such as carotenoids and oils. Further research is warranted to explore the specific bioactive compounds responsible for the observed effects and their mechanisms of action in inhibiting triglyceride accumulation in adipocytes.

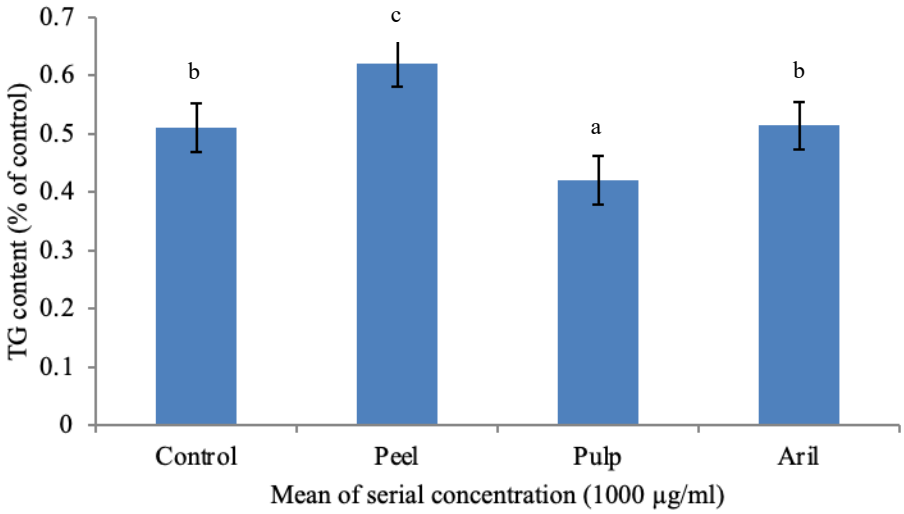

Figure 5. Effects of GFE fractions (peel, pulp, and aril) on TG contents of 3T3-L1 adipocytes.

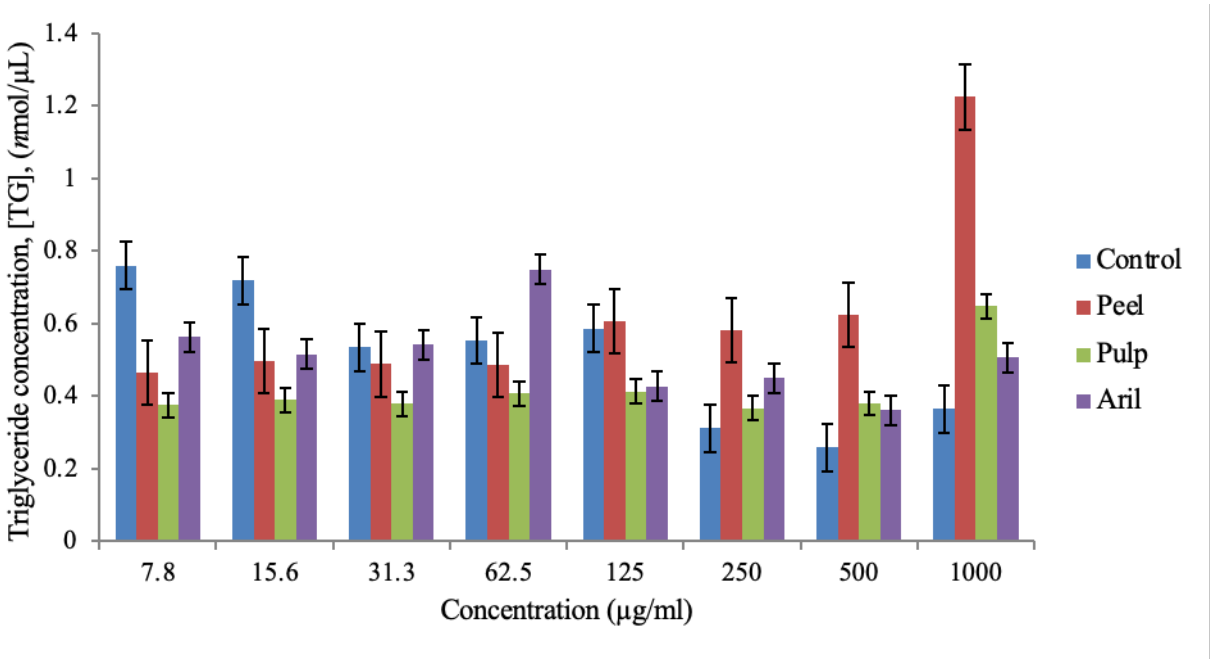

Figure 6. The levels of triglyceride concentration in sample (*m*mol/µL or mM) at different GFE fractions (peel, pulp, and aril) at concentrations 7.8 – 1000 µg/mL.

DISCUSSION

The primary objective of this study was to assess the scavenging capacity, toxicity profile, and anti-obesity potential of gac extracts enriched with carotenoids in 3T3-L1 pre-adipocyte cells. Extensive research has indicated that the antioxidant properties of various medicinal herbs are closely linked to their phytochemical composition, which includes compounds like flavonoids, carotenoids, terpenoids, and lignans [21]. Medicinal plants have been associated with the prevention and treatment of various diseases, including obesity, cancer, diabetes mellitus, osteoporosis, inflammatory disorders, and neurodegenerative diseases, owing to their ability to mitigate oxidative stress. As outlined by Park *et al.* [21], heightened oxidative stress has been linked to increased lipid accumulation in 3T3-L1 adipocytes. Therefore, our hypothesis was that carotenoids derived from gac fruits possess antioxidant properties capable of effectively hindering the adipogenic process in 3T3-L1 cells, drawing on previous findings related to fucoxanthin sourced from brown algae [16-20].

Prior research has established the anti-obesity properties of carotenoids, including fucoxanthin, which can inhibit the adipogenic process in 3T3-L1 cells. Building upon this existing body of work, the hypothesis posits that carotenoids extracted from gac fruits exhibit antioxidant properties and effectively impede the adipogenic program of 3T3-L1 cells. This hypothesis is grounded in the observed correlation between heightened oxidative stress and increased lipid accumulation in 3T3-L1 adipocytes [21]. Drawing parallels with the effects of fucoxanthin and other carotenoids, it is reasonable to anticipate similar anti-obesity effects from carotenoids found in gac extracts. However, further investigation and experimental validation are essential to elucidate the underlying antioxidant mechanisms and the potential anti-obesity effects of gac-derived carotenoids. Insights into the molecular pathways involved hold promise for the development of therapeutic strategies targeting obesity and related metabolic disorders.

To explore this hypothesis, *n*-hexane: acetone: ethanol (HAE) extracts were initially prepared from different parts of the gac fruit, including the peel, pulp, and aril. These extracts were then subjected to testing for their ability to scavenge DPPH radicals. In the course of this study, extraction yields of 38.96%, 28.82%, and 18.85% (w/w dry weight) were obtained from the gac peel, aril, and pulp, respectively. It's noteworthy to mention that a previous study by Chuyen *et al.* [22] reported carotenoid extraction yields of 271 mg/100 g DW and antioxidant capacity extraction yields of 737 µM TE/100 g DW. This study suggested that the recovery of carotenoids and antioxidants from gac peel could be achieved by employing ethyl acetate as the solvent at a ratio of 80:1 (mL solvent per g Gac peel) for a duration of 150 minutes at a temperature of 40.7°C.

In the DPPH assay, the results revealed that the IC_{50} value for DPPH scavenging was lowest in the gac pulp fraction compared to the peel and aril fractions. This indicates that the GFE derived from the pulp possesses a more potent scavenging capacity compared to the GFE from the peel, with the GFE from the aril fraction being the least effective at scavenging free radicals (pulp > peel > aril). These findings align with the study conducted by Abdulqader *et al.* [23], which reported that gac peel exhibited the highest ferric reducing power (140 mol FeSO4/g), while the gac aril demonstrated the highest scavenging activity (865 g/mL). These results collectively highlight the rich phytochemical composition of Malaysian-grown gac fruit, particularly its carotenoid content, and emphasize its robust antioxidant properties.

Obesity is a global health concern characterized by the excessive accumulation of fat, often resulting from an increase in the size and number of adipocytes (fat cells). Extensive research has been conducted on the anti-obesity properties of various medicinal plants and phytochemicals. These studies have explored the potential of natural compounds, including tea catechin, phytochemical-rich vegetables, Spirulina, *Orthosiphon stamineus* (Benth.) leaf extracts, *Morinda citrifolia* leaf extracts, curcumin, zeaxanthin, anthocyanins, carotenoid extracts from sweet potatoes, carotenoids themselves, fucoxanthin, lucidone from *Lindera erythrocarpa* Makino fruits, and other plant extracts, in inhibiting adipogenesis and combating obesity [24-39].

Indeed, the promising results observed with carotenoids and extracts from plants like *Momordica charantia* in inhibiting lipid accumulation in 3T3-L1 adipocytes emphasize their potential as valuable candidates for anti-obesity research. The wide range of plants and phytochemicals studied underscores the significance of exploring natural compounds as potential resources for preventing and managing obesity and its related health issues. Nonetheless, further extensive research is essential to unveil the precise mechanisms involved and to pinpoint potential therapeutic targets, as addressing the complexities of obesity requires a comprehensive understanding of its underlying processes.

The 3T3-L1 cell line serves as a widely recognized model for assessing anti-obesity properties, including the evaluation of cell viability and proliferation in response to various extracts and compounds [40]. In this study, the effect of gac fruit extract (GFE) on 3T3-L1 cell viability was investigated, with the highest GFE concentration of 1000 µg/mL used to assess cell viability. The results demonstrated that up to a concentration of 1000 µg/mL, the gac fruit extract fractions (peel, pulp, and aril) did not exhibit any detrimental effects on the viability of 3T3-L1 cells, while a decline in cell vitality was observed at exposure levels of 250 µg/mL. This observation is consistent with findings reported by Abdulqader *et al.* [41], where gac pulp, seed, and aril

extracts at a concentration of 1000 µg/mL displayed significant inhibitory effects on the viability of human retinal pigment epithelial (ARPE-19) cells. In another study conducted by Wimalasari *et al.* [42], a range of concentrations spanning from 2.75 to 22 mg/mL of red gac aril extract, prepared using hexane, acetone, and ethanol (HAE) as solvents, as well as a water extract, were evaluated for their impact on cell viability in breast cancer and melanoma cells. Their research revealed that the IC_{50} concentration for the crude water extract fell within the range of 0.49 to 0.73 mg/mL, leading to both apoptotic and necrotic cell death in a dose- and time-dependent manner.

3T3-L1 cells are commonly employed in obesity-related research to evaluate intracellular triglyceride accumulation. Previous studies have shown that various growth hormones and factors, such as 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), and insulin, can stimulate intracellular triglyceride accumulation in these cells [10]. In present study, treatment of 3T3-L1 adipocytes with GFE aril at concentrations of 75, 150, and 300 µg/mL resulted in a significant reduction in lipid accumulation. This suggests that gac fruit extracts have the ability to effectively reduce lipid accumulation in 3T3-L1 cells and inhibit their differentiation in a concentration-dependent manner. Notably, the GFE aril fraction exhibited a particularly favorable effect, significantly reducing intracellular triglyceride formation in 3T3-L1 cells when compared to other GFE fractions (peel, pulp, and aril) at concentrations ranging from 125 to 500 µg/mL.

A separate study by Saraphanchotiwitthaya & Sripalakit [39] found that *Morinda citrifolia* leaf extract at a concentration of 1 mg/mL effectively inhibited fat accumulation by 45% and reduced triglyceride content by 85%. Thus, the concentrations of GFE used in the study, up to 1000 µg/mL, do not appear to adversely affect cell viability; rather, it is likely the bioactive compounds within the extract that are responsible for these effects. The anti-adipogenic effects of gac fruit extract (GFE) fractions followed this order: pulp > peel > aril. This ranking is likely attributed to the higher carotenoid levels in the peel and aril compared to other parts of the fruit, as well as the greater fat content in the peel and aril. Additionally, various studies have explored the extraction of gac oils from different parts of the fruit, including the peel, aril, and seeds. According to Choi *et al.* [43], the total content of phenolic, flavonoid, and anthocyanin compounds, as well as their antioxidant activity, significantly impact their ability to inhibit the production of reactive oxygen species (ROS) and the accumulation of lipids during adipogenesis in 3T3-L1 cells compared to control cells. These findings underscore the potential anti-obesity effects of gac fruit extracts, especially the GFE aril fraction, and suggest that bioactive compounds such as carotenoids and phenolic compounds present in the extracts may contribute to their inhibitory effects on lipid accumulation and adipocyte differentiation in 3T3-L1 cells.

CONCLUSION

In conclusion, this research delved into the extraction yields and antioxidant activities of gac fruit extract fractions, specifically peel, pulp, and aril. The findings revealed intriguing insights into the extraction process. Notably, the peel exhibited the highest extraction yield, followed by the aril, while the pulp, despite having the greatest dry weight, displayed the lowest yield. This variation was attributed to factors such as carotenoid density and moisture content within the fractions. Additionally, the choice of solvent composition, particularly the *n*-hexane: acetone: ethanol (HAE) mixture, significantly influenced the extraction efficiency, highlighting the importance of solvent selection for future studies aiming to isolate bioactive compounds from gac fruit.

Moving on to the antioxidant activities of gac fruit extracts (GFEs), the DPPH scavenging capacity assay was employed. The results demonstrated substantial antioxidant potential within the GFEs, with the gac pulp fraction exhibiting the most potent DPPH scavenging activity, followed by the gac peel, and the aril fraction displaying the lowest efficacy. These findings indicated that GFEs hold promise as natural antioxidants, with potential applications in functional foods and natural antioxidant formulations.

Furthermore, the research explored the impact of GFEs on the viability of 3T3-L1 preadipocytes. The results indicated that GFE fractions, even at concentrations up to 1000 µg/mL, did not exert toxic effects on cell viability. However, a minor reduction in viability was observed at a concentration of 250 µg/mL, leading to the selection of concentrations up to 200 µg/mL for subsequent experiments. This non-toxic influence of GFEs on cell viability suggests their suitability for further investigations concerning their potential effects on adipogenesis.

The study then delved into the effects of GFEs on adipogenesis and intracellular triglyceride content in 3T3-L1 cells. The results highlighted that GFE treatment led to a significant reduction in lipid accumulation and inhibited the differentiation of 3T3-L1 cells, with the extent of these effects correlating with GFE concentration. Interestingly, GFE aril showed promise in reducing intracellular triglycerides at concentrations ranging from 125 to 500 µg/mL. However, there were inconsistencies in triglyceride concentrations at different GFE concentrations, indicating the need for further investigations to pinpoint the optimal concentration for effective triglyceride reduction.

In summary, this research underscores the potential anti-obesity effects of GFEs, particularly the GFE aril fraction. It suggests that bioactive compounds within GFEs, such as carotenoids, may contribute to inhibiting lipid accumulation and adipocyte differentiation in 3T3-L1 cells. These findings shed light on the diverse properties of different gac fruit fractions and emphasize the need for further research to elucidate the underlying mechanisms and specific bioactive components responsible for these effects.

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CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest associated with the publication of this manuscript.

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