ORIGINAL ARTICLE

Antioxidant and Anti-Adipogenic Activities of *Momordica cochinchinensis* (Lour). Spreng Fruit Extracts

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ABSTRACT

Introduction: *Momordica cochinchinensis* (Lour) Spreng, known as gac fruit, is rich in bioactive compounds like carotenoids (β-carotene, lycopene, and lutein). This study assessed the antioxidant, cytotoxic, and anti-adipogenic properties of gac fruit extracts (GFE) from different fractions (peel, pulp, aril), using 3T3-L1 adipocytes. **Method:** Gac extracts' DPPH radical scavenging was tested with 1000µg/mL dilutions. 3T3-L1 pre-adipocyte viability was measured via MTT assay. Differentiated adipocytes were treated (75, 150, 300 µg/mL) with GFE for 7 days. Inhibitory effects on adipogenesis and lipid accumulation were studied through Oil Red O staining. Triglyceride content was quantified and compared to controls. **Results:** IC_{50} values against DPPH radicals were 660µg/mL (peel), 560µg/mL (pulp), and 820µg/mL (aril). 3T3-L1 cell viability was unaffected up to 200µg/mL. However, 200µg/mL GFE decreased viability, inhibiting growth. Gac extracts effectively reduced lipid accumulation and hindered cell differentiation dose-dependently. Pulp extract notably reduced intracellular triglycerides, surpassing aril and peel effects. **Conclusion:** Gac fruit extract fractions (peel, pulp, and aril) efficiently inhibited adipogenesis in 3T3-L1 cells, evidenced by lowered lipid accumulation, triglyceride content, and cell viability. This study highlights gac fruit extracts' potential therapeutic use against obesity.
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INTRODUCTION

Momordica cochinchinensis Spreng, commonly known as gac fruit, has a rich historical background of traditional use in the management of diabetes, including both type I and II diabetes. Classified within the Momordica species and belonging to the melon family, the gac fruit is renowned for its remarkable abundance of bioactive compounds, prominently featuring essential fatty acids, β-carotene, lycopene, and lutein (1). These compounds endow gac fruit with exceptional nutritional and antioxidant properties, primarily attributed to its elevated carotenoid content, which encompasses β-carotene and lycopene (2).

Gac fruit stands out as an extraordinary source of carotenoids, particularly notable for its exceptionally high levels of beta-carotene and lycopene. The lycopene content within gac fruit is approximately tenfold higher than that found in other lycopene-rich fruits and vegetables, reaching astonishing levels of up to 308 µg/g in the seed membrane. Furthermore, the aril (pulp) of gac fruit surpasses even these levels, with lycopene concentrations measuring a remarkable 2227 µg/g of fresh material. Additionally, the aril is rich in fatty acids, constituting approximately 17% to 22% of its total weight.

In the realm of scientific investigation, the use of in vitro models has proven invaluable, especially in the context of adipogenesis research, for which 3T3-L1 pre-adipocyte and 3T3-F442A cell lines have emerged as key tools. While these cell lines offer advantages such as homogenous populations and the capacity for passaging,

it is important to acknowledge that the molecular events observed in these cell lines may not precisely replicate those occurring in human pre-adipocytes. Furthermore, the adipogenic potential of pre-adipocyte cell lines often diminish with increasing passage numbers (3).

The 3T3-L1 adipocyte cell line has played a pivotal role in metabolic disease research for over three decades. This model has significantly enriched the understanding of fundamental cellular mechanisms underlying diabetes, obesity, and related disorders (4). It is frequently employed to investigate adipogenesis and insulin response, although it is noted that the adipogenic potential of 3T3-L1 cells wanes over time in culture (5). Studies employing 3T3-L1 cells have explored the expression of specific mRNA during differentiation, illustrating that the process of adipose conversion in this cell line faithfully replicates what is observed *in vivo*. Initially displaying a fibroblastic morphology, these cells undergo differentiation into adipocytes that exhibit both morphological and biochemical characteristics akin to mature adipocytes. This differentiation process is typically induced through treatment with pro-differentiative agents like insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX) (6-7).

Remarkably, no prior studies have delved into the effects of gac fruit extract (GFE) fractions, specifically from the peel, pulp, and aril, on adipogenesis, neither *in vitro* nor *in vivo*, including human intervention studies. Consequently, this study represents pioneering research exploring the potential anti-obesity effects of gac fruit extract (GFE) on adipogenesis, employing 3T3-L1 cell lines as a model system. By pursuing these objectives, this study aims to advance understanding of the potential benefits offered by gac fruit extract fractions in the ongoing battle against obesity and its associated metabolic disorders.

MATERIALS AND METHODS

Preparation of gac fruit extract (GFE)

Gac fruit (*Momordica cochinchinensis* L. Spreng) sourced from a Malaysian cultivar was obtained through the International Tropical Fruits Network in Selangor, Malaysia. The fruits were meticulously identified and authenticated as the correct species by the Biodiversity Unit at the Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. A voucher specimen (SK 3164/17) was meticulously collected and preserved for future reference. During the harvesting season, which occurred between February and April 2018, a total of eleven ripe fruits were randomly selected. The combined weight of these collected fruits amounted to 5.342 kg, with each fruit averaging approximately (485.64 ± 143.52) grams.

Fresh gac fruits were subjected to one of two handling methods: they were either stored at a temperature of 5°C for a period ranging from 3 to 5 days or immediately utilized for experimental purposes. Following this, the peel, pulp, aril, and seeds were expertly separated and promptly frozen at -80°C in preparation for lyophilization. The lyophilization process was executed using the Christ Beta 2-8 LD plus lyophilizer, a high-quality equipment manufactured by GmbH in Germany. After lyophilization, the resulting freeze-dried samples of gac peel, pulp, and aril were mechanically ground to reduce their particle size effectively. These ground samples were then hermetically sealed in airtight containers, specifically Falcon tubes or Schott bottles equipped with screw caps and were additionally shielded by aluminium foil. These meticulously preserved samples were securely stored at a temperature of -20°C, ensuring their integrity for subsequent analyses and further investigations.

Gac Fruit Extraction Yields Percentage

Carotenoid extraction from the gac samples, including the peel, pulp, and aril, were performed using a modified version of the method outlined by Auisakchaiyoung & Rojanakorn (8) and Tran et al. (9). Approximately 2 grams of gac powder were accurately weighed and placed into a beaker. Subsequently, 100 mL of the extraction solvent, consisting of *n*-hexane/acetone/ethanol in a ratio of 50:25:25 (v/v/v), was added to the beaker. The mixture was then stirred using a magnetic stirrer for 30 minutes, after which 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 the filtered liquid into a round-bottomed flask. Following this, a 10 mL aliquot from the extract underwent evaporation to achieve dryness through the use of a rotary evaporator. The resulting extract was then meticulously shielded from light by wrapping it with aluminium foil and stored within a temperature range of 4°C to 5°C until it was ready for subsequent utilization. To ensure the robustness and reliability of the results, all experiments throughout the study were consistently conducted in triplicate. The extraction yield percentage of the gac fruit was calculated using the following formula:

$$\text{Extraction Yield (\%)} = \frac{[(W_1 / W_0) \times 100]}{\text{Weight of Dried Sample}} \quad \text{..... (Equation 1)}$$

Where;
W_0 = Weight of the Empty Flask
W_1 = Weight of the Flask with the Extract

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

The assessment of antioxidant activity in the extracts was conducted by evaluating their capacity to scavenge stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, employing a method akin to that outlined

by Auisakchaiyoung and Rojanakorn [8], with minor adjustments. To prepare the DPPH assay, an initial stock solution was created by dissolving 24 milligrams of DPPH in 100 mL of methanol, and this solution was stored at -20°C until needed. For the working solution, 10 mL of the stock solution was mixed with 45 mL of methanol.

The evaluation of the hydrogen atom or electron-donation capacity of the respective extracts, in addition to select pure compounds, was determined by monitoring the decolourization of a purple-coloured methanol solution of DPPH. Subsequently, the antioxidant activity of the extracts, hinging on their ability to scavenge the stable DPPH free radical, was 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 (%)
= [(Ao - Ae)/Ao] / 100 ..... (Equation 2)

Where;
Ao = absorbance without extract
Ae = absorbance with extract

The determination of the inhibition concentration at 50% (IC_{50}), denoting the quantity of the sample extracted into a 1 mL solution required to reduce the initial DPPH concentration by 50%, was derived from the plot illustrating the percentage disappearance versus concentration. Here, the concentration refers to the milligrams of fruit extracted into 1 mL of the solution. The IC_{50} value for DPPH was calculated through the equation y = mx + c, considering the linearity (R-squared, represented as R^2) obtained from the graph. R^2 serves as a statistical metric indicating 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 signifies a high degree of linearity in the graph. In the context of IC_{50} determination, a lower IC_{50} value signifies a more effective inhibition concentration, indicating a stronger antioxidant effect.

Cell culture and stimulation

The 3T3-L1 cell line (ATCC® CL-173™), which is a type of mouse embryonic fibroblast cells, was obtained from the Institute of Bioscience, Universiti Putra Malaysia (UPM). Initially, this cell line was sourced from the American Type Culture Collection (ATCC) located in Rockville, MD, USA, and more precisely acquired from The Global Bioresource Center at University Blvd, Manassas, VA, United States. For all experiments conducted in this study, the cells used fell within the passage range of P26 to P34.

Cell Culture Experimental Group

In this study, four distinct groups were involved, with Group 1 serving as the control, while Groups 2 to 4 represented the treatment groups:

Group 1: 3T3-L1 cells (ATCC® Cat. No. CL-173™) were cultured in DMEM containing 10% (v/v) FBS until reaching a state of confluence 2 days post-confluence. Subsequently, these cells were induced to differentiate through the addition of a differentiation medium (MDI).

Group 2: 3T3-L1 cells were treated with Dexamethasone (1 µmol/l or 250 nM) and supplemented with 75 µg/mL of GFE.

Group 3: 3T3-L1 cells were exposed to 0.52 mmol/l of 3-isobutyl-1-methylxantine and simultaneously treated with 150 µg/mL of GFE.

Group 4: 3T3-L1 cells received insulin at a concentration of 0.17 µmol/l (or 5 µg/mL) along with 300 µg/mL of GFE.

It is important to note that the concentrations of GFE used in Groups 2 to 4 (75, 150, and 300 µg/mL) were determined based on the 50% growth inhibition concentration (IC_{50}) values obtained from a serial concentration of GFE (ranging from 7.8 to 1000 µg/mL). These IC_{50} values were determined by plotting the percentage of viable cells against their respective GFE concentrations.

For Groups 2 to 4, treatment was administered for 48 hours. Following this treatment period, the cells were cultured in α-DMEM with 10% FBS and 5 g/mL insulin for an additional 2 days after changing the medium. The cells were then maintained in a CO_2 incubator until they exhibited full adipocyte morphology, indicating the completion of the differentiation process.

Cell Proliferation and Viability

Cell proliferation and viability of 3T3-L1 cells were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The objective was to investigate how various GFE fractions, including peel, pulp, and aril, affected cell growth. The experimental setup involved cultivating 3T3-L1 cells in a 96-well microtiter plate and treating them with different concentrations of GFE (ranging from 7.8 µg/mL to 1000 µg/mL) after 24, 48, and 72 hours of incubation. After the medium containing GFE was removed, each well received 20 µL of MTT solution (5 mg/mL in PBS) and underwent an additional 4-hour incubation. Subsequently, 100 µL of DMSO was added to lyse the cells, enabling absorbance measurement at 570 nm using a microplate reader. The percentage of viable cells was calculated using the formula:

Viable cells (%) =
Absorbance treatment x 100% ..... (Equation 3)
Absorbance control

This allowed for the determination of the 50% growth inhibition concentration (IC_{50}) values, shedding light on the inhibitory effects of GFE fractions on cell growth.

Oil Red-O Staining

To assess the inhibitory effects of GFE fractions on 3T3-L1 adipogenesis, Oil Red-O (ORO) staining was employed as a vital technique. In brief, both dimethyl sulfoxide (DMSO) for the control group and GFEs at various concentrations, ranging up to 1000 µg/mL, were administered to 3T3-L1 cells during a 7-day adipogenesis period. On the 7th day, the cells underwent fixation using a 10% formalin solution for 1 hour at room temperature, preceded by a thorough PBS wash. Subsequently, ORO staining was applied to the cells for 10 minutes at room temperature, after which they were rinsed with 2.4 mL of 60% isopropanol, followed by four consecutive washes with distilled water. To further analyze and visualize the results, images of each dish were captured under an Olympus microscope (Tokyo, Japan). This comprehensive approach allowed for the evaluation of the impact of GFE fractions on 3T3-L1 adipogenesis.

Cell Morphological Changes and Intracellular Triglyceride Content

To explore the potential anti-adipogenic properties of GFE, the present study scrutinized its impact on cell morphology and triglyceride content. These assessments were carried out using an inverted microscope, revealing noteworthy changes in lipid accumulation within 3T3-L1 adipocytes when subjected to GFE aril treatments at concentrations of 75, 150, and 300 µg/mL.

Triglyceride Content Measurement

The quantification of triglyceride content within 3T3-L1 adipocytes was conducted utilizing the AdipoRed assay reagent from Lonza, MD, USA, following the recommended procedure provided by the manufacturer. In summary, both dimethyl sulfoxide (DMSO) for the control group and GFE at various concentrations, reaching up to 1000 µg/mL, were administered to 3T3-L1 cells during a 7-day differentiation process. On the 7th day, the 96-well plates containing the 3T3-L1 adipocytes underwent a PBS rinse of 0.2 mL. Subsequently, the cells were exposed to 140 µL of AdipoRed for a 15-minute incubation period 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 in-depth analysis. This approach facilitated the accurate assessment of triglyceride content and its potential alterations due to GFE treatments.

Statistical Analysis

The study presents data following a standard format, including mean values and standard deviations (SD) from triplicate measurements (n=3). Statistical analysis utilized SPSS version 26 software for a one-way analysis of variance (ANOVA) and subsequent LSD tests for post-hoc analysis. A significance threshold of $p<0.05$ ensured robust identification of group differences.

RESULTS

Extraction yields and antioxidant activities of GFE

The extraction yields of gac fruit extracts varied significantly among the different fractions, with noteworthy implications for the study. Specifically, the peel exhibited the highest extraction yield at 38.96%, followed by the aril at 28.82% and the pulp at 18.85% (Table I). These differences can be attributed to variations in moisture and carotenoid content in each fraction, with the pulp, despite having the highest weight of the dried sample, yielding the lowest. This discrepancy may be due to factors such as the density of carotenoids and the moisture content in the different fractions.

Table I: Extraction yields of gac fruit extracts using n-hexane: acetone: ethanol (HAE) solvent in a ratio of 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 (w/w-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]

The results are expressed as means ± standard deviations (n=3). Different superscripts [a, b, c] within a row indicate significant differences between values ($p<0.05$). This notation highlights the statistically significant variations observed among the measured parameters.

The unique characteristics of the aril, rich in carotenoids and "gac oils," contributed to its slightly lower extraction yield compared to the peel. This finding aligns with previous research discussing carotenoid content in gac fruit extract fractions (peel, pulp, and aril). These results underscore the importance of considering the composition and characteristics of each fraction when extracting bioactive compounds from gac fruit. The extraction yields of gac fruit extract fractions were determined using the *n*-hexane: acetone: ethanol (HAE) solvent in a ratio of 50:25:25 (v/v/v). These findings offer valuable insights into the extraction process, aiding future studies seeking to isolate and characterize bioactive constituents from gac fruit extracts.

Furthermore, the antioxidant activities of the GFE were evaluated using the DPPH scavenging capacity assay. The IC_{50} values for BHT and gallic acid, established antioxidants against DPPH radicals, were determined as 182.02 mg/L and 145.86 mg/L, respectively (Table II). Table III presents the IC_{50} values for different gac fruit fractions, with the pulp fraction demonstrating the

Table II: The antioxidant activity of gac fruit extract fraction (peel, pulp, and aril) using solvent n-hexane: acetone: ethanol (HAE) in a 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^{bE}± 0.03	24.72^{cE}± 0.45	28.60^{bDE}± 0.26	32.98^{aD}± 0.12	34.69^{bD}± 0.17	39.18^{bD}± 0.23	54.35^{bC}± 0.32	68.69^{aB}± 1.43	78.13^{aB}± 1.22	82.11^{aA}± 0.36	83.39^{aA}± 0.97
Gallic acid	22.27^{bH}± 1.25	29.15^{aH}± 1.32	33.06^{aG}± 2.24	35.68^{aFG}± 1.56	38.87^{aF}± 2.65	44.50^{aE}± 3.12	58.82^{aD}± 0.09	66.88^{aC}± 0.18	76.99^{aB}± 1.27	83.55^{aA}± 1.16	83.59^{aA}± 1.22
Peel	25.40^{aE}± 0.89	27.04^{bD}± 1.13	28.47^{bD}± 2.21	28.77^{bD}± 1.14	29.86^{cD}± 1.86	30.75^{cD}± 1.11	31.17^{cD}± 2.11	33.83^{bCD}± 0.66	38.03^{bC}± 1.23	45.00^{bB}± 1.64	67.03^{cA}± 1.34
Pulp	26.85^{aE}± 0.77	27.11^{bE}± 2.14	28.07^{bD}± 1.15	28.12^{bD}± 1.78	28.58^{cD}± 2.16	29.28^{cdD}± 2.10	29.99^{cdD}± 2.12	30.38^{cD}± 0.34	38.87^{bC}± 1.46	47.15^{bB}± 1.84	71.49^{bA}± 1.57
Aril	20.70^{cD}± 0.86	21.17^{dD}± 0.15	22.95^{cD}± 2.11	23.50^{cCD}± 1.92	25.93^{dC}± 2.11	28.15^{dB}± 1.33	28.76^{dB}± 0.65	29.91^{cB}± 0.51	30.20^{cB}± 1.75	36.00^{cB}± 1.73	57.51^{dA}± 1.88

*1 mg/L = 1 µg/ml = 1 ppm
*BHT and gallic acid were used as antioxidant reference solutions.
Results are expressed as means ± standard deviations (n=3).
$^{a-d}$: Values with different superscripts within a row significantly differ ($p<0.05$).
$^{A-H}$: Values with different superscripts within a column significantly differ ($p<0.05$).

Table III: 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 a 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

*R-squared (R^2) is a statistical measure representing the proportion of the variance for a dependent variable explained by an independent variable or variables in a linear regression model. R^2 is almost 1, reflecting the linear graph.
**IC_{50} value, the lowest the value, the better the inhibition concentration.

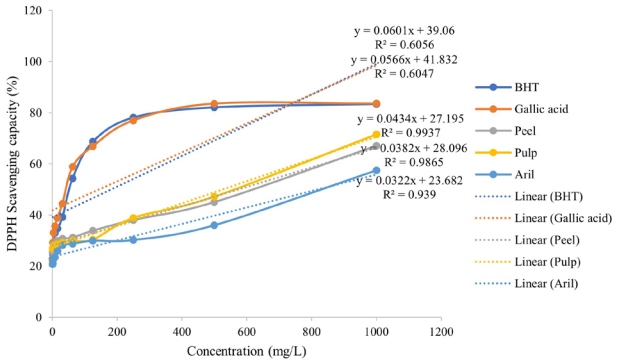

Figure 1: Inhibition concentration at 50 per cent (IC_{50}) and the linear equation, y = mx + c, and R^2 of gac extracts (peel, pulp, and aril) measured using the DPPH scavenging capacity. BHT and gallic acid were used as references.

most potent DPPH scavenging activity among the tested fractions. Specifically, gac pulp had an IC_{50} of 525.46 mg/L, while gac peel exhibited an IC_{50} of 573.40 mg/L. The aril fraction displayed the highest IC_{50} value, at 817.33 mg/L. These results underscore the antioxidant potential of gac fruit extracts, with the pulp fraction emerging as the most effective in scavenging DPPH radicals. This information is valuable for considering the application of gac fruit extracts in functional foods or natural antioxidant formulations.

It is worth noting that the IC_{50} values were determined based on a linear equation derived from the graph shown in Figure 1. The results indicate that the GFE from the pulp fraction possesses the highest antioxidant activity, followed by the GFE from the peel fraction, with the aril fraction exhibiting the lowest efficacy in scavenging radicals (pulp > peel > aril). This finding contradicts previous research on the antioxidant activity of gac fruit fractions 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 discrepancy suggests that different factors or mechanisms within the gac fruit fractions may influence antioxidant activity and inhibitory effects on lipase activity. These findings highlight the diverse bioactive properties of different gac fruit fractions and underscore the potential of gac pulp and peel as valuable sources of antioxidants. Further investigations are necessary to explore the underlying mechanisms and specific bioactive components contributing to these observed differences in antioxidant activity and inhibitory effects.

Effect of GFE on cell viability of 3T3-L1 cells

The MTT assay results indicated that exposure to GFE fractions, even at concentrations up to 1000 µg/mL, did not exhibit any toxic effects on the viability of 3T3-L1 cells. However, a slight reduction in cell viability was observed when cells were exposed to a concentration of 250 µg/mL. Based on these findings, further experiments were conducted using concentrations up to 200 µg/mL of gac fruit extracts. These results suggest that GFE fractions have a non-toxic effect on the viability of 3T3-L1 cells within the tested concentration range, making them suitable for subsequent investigations related to their potential effects on adipogenesis.

The results provide valuable insights into the impact of gac peel, pulp, and aril extracts on cell viability at various time points and concentrations. Overall, the data indicates that these extracts do not significantly affect cell viability, even at higher concentrations. It's noteworthy that the inhibition concentration at 50 per cent (IC_{50}) could not be determined due to the negative value obtained from the equation y = mx + c, indicating that the concentrations tested did not reach a level where 50% inhibition was observed. These findings suggest that gac peel, pulp, and aril extracts, even at higher concentrations, do not exhibit substantial adverse effects on cell viability, thus indicating their potential safety and suitability for further investigations. Further experiments and analyses may be needed to explore other aspects of the extract's biological activity and to determine the specific concentration required for 50% inhibition.

Effect of GFE on anti-adipogenic properties and intracellular triglyceride content of adipocytes in 3T3-L1 cells

Upon comprehensive analysis, the outcomes revealed that subjecting 3T3-L1 adipocytes to a range of GFE concentrations (from 7.8 to 1000 µg/mL) did not significantly hinder intracellular triglyceride accumulation when compared to the control group. Notably, the GFE aril fraction demonstrated an intriguing exception by exhibiting a positive effect in reducing intracellular triglyceride content (%) in 3T3-L1 adipocytes, particularly at concentrations spanning from 125 to 500 µg/mL. These intriguing findings suggest that while the GFE fractions obtained from the peel and pulp of the gac fruit did not exert substantial effects on intracellular triglyceride accumulation in 3T3-L1 adipocytes, the GFE aril fraction displayed promising anti-adipogenic potential within a specific concentration range. To gain deeper insights, further investigations are warranted to uncover the underlying mechanisms responsible for these observed effects and to identify the specific bioactive components within the GFE aril fraction that contribute to its anti-adipogenic activity. These endeavours will undoubtedly advance the understanding of the intricate interactions between gac fruit extracts and the regulation of adipogenesis.

Interestingly, intriguing inconsistencies emerged in the levels of triglyceride concentrations within the GFE fractions (peel, pulp, and aril) across the broad spectrum of concentrations ranging from 7.8 to 1000 µg/mL. Of note, the control group, which comprised cells not subjected to GFE treatment, displayed a notably higher triglyceride concentration at lower GFE concentrations (ranging from 7.8 to 125 µg/mL). However, as the GFE concentration escalated to the range of 250 to 1000 µg/mL, the cells treated with GFE exhibited a higher degree of intracellular lipid accumulation in comparison to the untreated group (Table IV).

This intriguing observation has led to a hypothesis: the most effective concentration of GFE for inhibiting intracellular triglyceride accumulation likely lies below the 250 µg/mL threshold, possibly within the range of 125 to 200 µg/mL. However, it is imperative to emphasize that those further comprehensive investigations and experiments are indispensable to validate this hypothesis and pinpoint the precise optimal GFE concentration that can efficaciously impede intracellular triglyceride accumulation in 3T3-L1 cells. This endeavour holds substantial promise for shedding light on the intricate regulatory mechanisms governing lipid metabolism and adipogenesis.

The meticulous comparison between different types of GFE fractions has yielded valuable insights into their respective abilities to inhibit triglyceride accumulation in 3T3-L1 cells. Remarkably, the GFE derived from gac pulp emerged as the most potent in inhibiting triglyceride accumulation, followed by the GFE peel, while the GFE aril displayed the least effectiveness, with a ranking order of pulp > peel > aril. This notable variation in efficacy could potentially be attributed to the divergent carotenoid content within the peel and aril fractions.

Furthermore, it is intriguing to note that both the peel and aril fractions contain elevated levels of fat in comparison to the aril fraction alone. This finding introduces the possibility that fat content within these fractions may contribute to their differential effects on triglyceride accumulation. Additionally, prior research endeavours have delved into the extraction of gac oils from various parts of the fruit, encompassing not only the peel and aril but also the seeds (10-11). These studies, which have explored the distinct components present in the peel and aril fractions, further substantiate the notion that different constituents within these fractions may play

Table IV: The levels of triglyceride concentration in the sample (nmol/µL) or mM at different GFE fractions (peel, pulp, and aril) at concentrations 7.8 – 1000 µg/mL

Type of Sample	Sample concentration (µg/mL)							
	7.8	15.6	31.3	62.5	125	250	500	1000
Control	0.76^{a}±0.01	0.72^{a}±0.12	0.53^{a}±0.05	0.55^{b}±0.08	0.59^{a}±0.12	0.31^{c}±0.21	0.26^{c}±0.11	0.36^{c}±0.13
Peel	0.47^{bc}±0.02	0.50^{b}±0.20	0.49^{a}±0.07	0.49^{b}±0.09	0.61^{a}±0.15	0.58^{a}±0.17	0.62^{a}±0.16	1.22^{c}±0.18
Pulp	0.38^{c}±0.15	0.39^{c}±0.13	0.38^{b}±0.12	0.41^{b}±0.01	0.41^{b}±0.16	0.37^{bc}±0.09	0.38^{b}±0.09	0.65^{a}±0.09
Aril	0.56^{b}±0.03	0.51^{b}±0.05	0.54^{a}±0.01	0.75^{a}±0.02	0.43^{b}±0.17	0.45^{b}±0.21	0.36^{b}±0.08	0.51^{b}±0.12

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

pivotal roles in influencing triglyceride accumulation.

The implications of these findings underscore the necessity of taking into account the diverse fractions of the gac fruit when assessing their potential effects. This includes the careful extraction of bioactive components such as carotenoids and oils. The outcomes emphasize the need for further research endeavours aimed at elucidating the specific bioactive compounds responsible for the observed effects and gaining a deeper understanding of their mechanisms of action in the context of inhibiting triglyceride accumulation in adipocytes. Such investigations hold significant promise for shedding light on the intricate interplay between gac fruit components and lipid metabolism, offering potential insights for therapeutic applications.

DISCUSSION

The main objective of this study was to investigate the antioxidant activity, toxicity level, and anti-obesity effects of carotenoid-rich gac extracts on 3T3-L1 pre-adipocyte cells. Previous research has highlighted the relationship between the phytochemical composition of medicinal herbs, such as flavonoids, carotenoids, terpenoids, and lignans, and their antioxidant properties (20). These antioxidant properties have been linked to prevent and treat various diseases, including obesity, cancer, diabetes mellitus, osteoporosis, inflammatory disorders, and neurodegenerative diseases, by reducing oxidative stress. Previous studies have specifically explored the effects of fucoxanthin, a carotenoid derived from brown algae, on adipogenesis (15-19). These studies have proved that carotenoids, including fucoxanthin, possess anti-obesity properties and can inhibit the adipogenic program in 3T3-L1 cells. Building upon this existing research, it was hypothesized that carotenoids extracted from gac fruits would exhibit antioxidant properties and effectively inhibit the adipogenic program of 3T3-L1 cells. The rationale for this hypothesis is based on the observed relationship between enhanced oxidative stress and increased lipid formation in 3T3-L1 adipocytes (20). By drawing parallels with the effects of fucoxanthin and other carotenoids, it is plausible to anticipate similar anti-obesity effects from carotenoids in gac extracts. Further investigation and experimental validation are needed to explore the antioxidant mechanisms and potential anti-obesity effects of carotenoids derived from gac fruits. Understanding the molecular pathways involved in these effects will contribute to the development of therapeutic strategies targeting obesity and related metabolic disorders.

To investigate the antioxidant activity of gac fruit fractions (peel, pulp, and aril), *n*-hexane: acetone: ethanol (HAE) extracts were prepared, and their scavenging activities against DPPH radicals were evaluated. The extraction yields obtained in this study were 38.96%, 28.82%, and 18.85% (w/w dry weight) for gac peel, aril, and pulp, respectively. In a previous study, the carotenoid extraction yield and antioxidant activity extraction yield of gac peel were reported as 271 mg/100 g DW and 737 µM TE/100 g DW, respectively (21). The authors suggested an extraction method using ethyl acetate with a ratio of 80:1 (mL solvent per g gac peel) for 150 minutes at 40.7 °C to recover carotenoids and antioxidant activity from gac peel effectively. These findings highlight the variability in extraction yields and suggest that the choice of extraction solvent and conditions can influence the recovery of carotenoids and antioxidant activity from gac peel. Further optimization of the extraction process is required to maximize the yield of bioactive components and ensure their preservation for subsequent antioxidant and anti-obesity evaluations.

In the DPPH assay, results revealed that the gac pulp exhibited a lower IC_{50} value than the peel and aril fractions. This indicates that the gac pulp fractions possess a higher antioxidant activity than GFE from the peel. In contrast, GFE from the aril fraction showed the least effectiveness in scavenging free radicals (pulp > peel > aril). These findings align with the study conducted by Abdulqader *et al*. (22), where they reported that gac peel exhibited the highest ferric reducing power of 140 µmol FeSO4/g, while the gac aril exhibited the highest scavenging activity of 865 µg/mL. These results highlight that gac fruits grown in Malaysia are rich in phytochemicals, particularly carotenoids, and possess significant antioxidant properties.

Obesity has become a global health concern characterized by increased adipocyte size and number, resulting in fat accumulation. Numerous studies have explored the anti-obesity activity of various medicinal plants and phytochemicals. Examples include tea catechin (23), phytochemical-rich vegetables (24), Spirulina (25), *Orthosiphon stamineus* leaf extract (26), *Morinda citrifolia* leaf extracts (27), curcumin (28), zeaxanthin (29), anthocyanin and carotenoid extracts from sweet potatoes (30), carotenoids (31-32), fucoxanthin (15-19), lucidone from Lindera erythrocarpa Makino fruits (33), and other medicinal plants studied for their anti-adipogenic effects (34). Carotenoids, such as those found in carotenoid-rich plants and *Momordica charantia* extracts, have been reported to exhibit anti-obesity effects through the inhibition of lipid accumulation in 3T3-L1 adipocytes (35-38). These studies highlight the potential of carotenoids and plant extracts as promising candidates for anti-obesity research. The diverse range of plants and phytochemicals studied for their anti-obesity effects underscores the importance of exploring natural compounds to prevent and manage obesity. Further research is needed to elucidate the underlying mechanisms and identify potential therapeutic targets for combating obesity-related complications.

The 3T3-L1 cell line is a widely used model for assessing anti-obesity activity, including evaluating

cell viability and proliferation in response to various extracts and compounds (39). In this study, the effect of gac fruit extract (GFE) on the viability of 3T3-L1 cells was investigated using the highest concentration of GFE, which was 1000 µg/ml (equivalent to 1 mg/ml). The results revealed that GFE fractions (peel, pulp, and aril) had no significant impact on the viability of 3T3-L1 cells up to a concentration of 1000 µg/ml. However, cell vitality started to decline at exposure levels of 250 µg/ml. This finding is consistent with a study by Abdulqader *et al*. (40) that reported significant inhibition of cell viability in human retinal pigment epithelial cells (ARPE-19) treated with gac pulp, seed, and aril extracts at a concentration of 1000 µg/ml. Another study by Wimalasari *et al*. (41) evaluated the cell viability of breast cancer and melanoma cells treated with red gac aril extracts at concentrations ranging from 2.75 to 22 mg/ml. They found that the crude water extract had an IC_{50} concentration ranging from 0.49 to 0.73 mg/ml, inducing both apoptotic and necrotic cell death in a dose- and time-dependent manner. These findings suggest that the effects of gac fruit extracts on cell viability can vary depending on the cell type and concentration used. The present study observed no harmful effects on 3T3-L1 cell viability at concentrations up to 1000 µg/ml, indicating the potential safety of GFE fractions for further investigations related to anti-obesity activity.

Indeed, 3T3-L1 cells are widely utilized in studying intracellular triglyceride accumulation and lipid metabolism in obesity research. Growth hormones such as IBMX, dexamethasone, and insulin increase intracellular triglycerides, contributing to adipocyte differentiation (34). The study observed that treatment of 3T3-L1 adipocytes with GFE aril at concentrations of 75, 150, and 300 µg/ml resulted in reduced lipid accumulation. The gac fruit extracts exhibited a concentration-dependent inhibitory effect on lipid accumulation and the differentiation of 3T3-L1 cells. Specifically, the GFE aril fraction demonstrated a favourable effect in decreasing intracellular triglyceride formation in 3T3-L1 cells compared to the other GFE fractions (peel, pulp, and aril) at concentrations ranging from 125 to 500 µg/ml. This finding aligns with a study by Saraphanchotiwitthaya & Sripalakit (27), where *Morinda citrifolia* leaf extract at a concentration of 1 mg/ml effectively inhibited fat accumulation by 45% and reduced triglyceride content by 85%. It is important to note that the observed effects on cell viability are not solely attributed to the concentration of GFE but rather to the bioactive compounds within the extracts. The anti-adipogenic effects of GFE fractions followed the order: of pulp > peel > aril. This trend could be attributed to the higher levels of carotenoids present in the peel and aril compared to other parts of the fruit and the higher fat content in the peel and aril compared to the aril alone. Additionally, several studies have reported the extraction of gac oils from the peel, aril, and seeds. Choi *et al*. (42) found that the total content of phenolic compounds, flavonoids, anthocyanins, and their antioxidant activity played a significant role in inhibiting reactive oxygen species (ROS) production and lipid accumulation during adipogenesis in 3T3-L1 cells compared to control cells. These findings highlight the potential anti-obesity effects of gac fruit extracts, particularly the GFE aril fraction, and suggest that the bioactive compounds present in the extracts, such as carotenoids and phenolic compounds, may contribute to their inhibitory effects on lipid accumulation and adipocyte differentiation in 3T3-L1 cells.

CONCLUSION

The investigation into the effects of GFE fractions, including peel, pulp, and aril, on adipogenesis in 3T3-L1 cells has yielded noteworthy insights. GFE aril demonstrated the highest efficacy in inhibiting triglyceride accumulation in these adipocytes, particularly at concentrations ranging from 125 to 500 µg/mL. In contrast, the GFE fractions from peel and pulp did not significantly affect intracellular triglyceride accumulation. This intriguing variation in effectiveness may be linked to the differential carotenoid content present within these fractions, as well as the notable presence of fat content in the peel and aril fractions.

Furthermore, the study has revealed that while GFE fractions exhibited no substantial adverse effects on cell viability, they displayed potential anti-adipogenic properties, particularly in the case of GFE aril. Importantly, these effects were observed within a specific concentration range and did not result in cytotoxicity or apoptosis. The significance of these findings lies in their potential implications for understanding the interplay between gac fruit components and lipid metabolism. The results underscore the importance of considering different fractions of the gac fruit when evaluating their effects and extracting bioactive compounds. Further research endeavours are warranted to elucidate the precise bioactive compounds responsible for these observed effects and their underlying mechanisms of action.

In summary, the study provides valuable insights into the potential of gac fruit extract, particularly the aril fraction, as a candidate for further investigations and potential applications in addressing adipogenesis and triglyceride accumulation. These findings open new avenues for exploring the therapeutic potential of gac fruit components in the context of metabolic disorders and obesity-related conditions.

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