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Original Article

# *Sygyzium claviflorum* fruit extract preadipocyte differentiation inhibition in 3T3-L1 cells

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**الملخص**

**أهداف البحث:** دفعت المخاوف بشأن العدد المتزايد من الأفراد الذين يعانون من السمنة المفرطة والمخاطر الصحية المرتبطة بها إلى استكشاف الخيارات العلاجية. وبالمثل، تهدف هذه الدراسة إلى إنشاء سمات مضادة للدهون في مستخلص فاكهة سيجيزيوم كلافيفلوروم في خلايا ٣ت٣-إل١.

**طرق البحث:** تم التعرف على المركبات البوليفينولية في فاكهة سيجيزيوم كلافيفلوروم باستخدام التحليل اللوني السائل عالي الأداء للمرحلة العكسية. وفي الوقت نفسه، تم استخدام الخلايا الشحمية ٣ت٣-إل١ الفأرية، وقياس مستويات الليبتين، وأنواع الأكسجين التفاعلية، ومحتويات الدهون والدهون الثلاثية خلال تقييمات النشاط المضاد للدهون. في الوقت نفسه، تم تحديد تأثيرات فاكهة سيجيزيوم كلافيفلوروم على عوامل النسخ الدهنية باستخدام تفاعل البلمرة المتسلسل الكمي في الوقت الحقيقي.

**النتائج:** أشارت النتائج إلى ثلاثة مركبات بوليفينولية في فاكهة سيجيزيوم كلافيفلوروم، بما في ذلك فلافونويد واحد (نارينجين) واثنين من الأحماض الفينولية (سرينجيك وبكوماريك). على الرغم من أن علاجات فاكهة سيجيزيوم كلافيفلوروم (٢٥٠-١٠٠٠ ميكروغرام/مل) لم تؤد إلى سمية الخلايا، إلا أنها خفضت بشكل كبير تراكم الدهون المعتمد على الجرعة، وإنتاج الأكسجين التفاعلي، ومستويات الدهون الثلاثية واللبتين بالنسبة للخلايا الشحمية المتمايزة بالتحكم. علاوة على ذلك، قامت فاكهة سيجيزيوم كلافيفلوروم بقمع بروتين ربط العناصر التنظيمية للستيرول-١، العنصر التنظيمي للستيرول، ومستقبلات غاما المنشط بالبيروكسيسوم ، والتعبيرات الجينية لبروتين سي/ايبب ألفا المرتبطة بالمحسن خلال الخلية البريدية الشحمية والتمايز إلى الخلايا الشحمية.

**الاستنتاجات:** كشفت النتائج عن خصائص فاكهة سيجيزيوم كلافيفلوروم المضادة للدهون، مما يشير إلى إمكاناته كعلاج طبيعي لإدارة السمنة. ومع ذلك، هناك حاجة إلى مزيد من الدراسات لتوضيح التفاعلات التي تؤدي إلى تأثيرات فاكهة سيجيزيوم كلافيفلوروم المضادة للدهون والمكونات النشطة المسؤولة عن الخاصية.

**الكلمات المفتاحية:** تكوين الشحوم؛ مضاد للدهون؛ في المختبر؛ بدانة؛ الخلايا التحضيرية؛ فاكهة سيجيزيوم كلافيفلوروم

**Abstract**

**Objective:** Concerns over the increasing number of obese individuals and the associated health risks have prompted therapeutic option explorations. Similarly, this study aimed to establish *Sygyzium claviflorum* fruit extract (SCFE) anti-adipogenic attributes in 3T3-L1 cells.

**Methods:** The polyphenolic compounds in SCFE were identified with Reverse phase-high performance liquid chromatography (RP-HPLC). Meanwhile, murine 3T3-L1 preadipocytes, measuring leptin levels, reactive oxygen species (ROS), and lipid and triglyceride (TG) contents were utilized during anti-adipogenic activity assessments. Concurrently, the effects of SCFE on adipogenic transcription factors were established with quantitative real-time-polymerase chain reaction (qRT-PCR).

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**Results:** The RP-HPLC results indicated three polyphenolic compounds in SCFE, including one flavonoid (naringin) and two phenolic acids (syringic and p-coumaric). Although SCFE treatments (250−1000 μg/mL) did not result in cell toxicity, they significantly reduced dose-dependent lipid accumulation, ROS production, and TG and leptin levels relative to control-differentiated adipocytes. Moreover, SCFE suppressed sterol regulatory element binding protein-1 (SREBP-1), peroxisome proliferator-activated receptor-gamma (PPAR-γ), and CCAAT/enhancer-binding protein-alpha (C/EBP-α) gene expressions during preadipocyte differentiation into adipocytes.

**Conclusion:** The findings revealed the anti-adipogenic properties of SCFE, indicating its potential as a natural obesity management remedy. Nevertheless, more studies are necessary to elucidate the reactions resulting in SCFE anti-adipogenic effects and the active constituents responsible for the property.

**Keywords:** Adipogenesis; Anti-adipogenic; *In vitro*; Obesity; Preadipocytes; *Sygyzium claviflorum*



## Introduction

Obesity is characterized by excess adipose tissue buildup. The condition is a worldwide health issue due to its complex relationship to various metabolic disturbances, including type 2 diabetes mellitus, hypertension, and non-alcoholic fatty liver disease.[1,2] Obesity pathophysiology is intricately associated with adipocytes, the primary cellular component of adipose tissue.[1] Adipocytes are required to maintain energy balance by storing excess energy in the form of triglyceride (TG).[2] Nonetheless, the physiological function is dysregulated in obese individuals, resulting in pathological adipocyte growth and adipose tissue dysfunction.[3]

Mature adipocyte derivation from preadipocytes is critical for adipose tissue growth and maturation. Although adipocyte differentiation promotes adipocyte development, the process also generates reactive oxygen species (ROS), which exerts oxidative stress in the cellular environment.[4] A discernible rise in adipokines secretion, including adiponectin and leptin, is also observed during the activity.[5] Complex reactions between transcription factors, such as CCAAT/enhancer-binding protein-alpha (C/EBP-α), sterol regulatory element-binding protein-1 (SREBP-1), and peroxisome proliferator-activated receptor-gamma (PPAR-γ), manage adipocyte differentiation.[6] The transcription factors are crucial to coordinating the gene expressions related to adipogenesis and lipid metabolism and regulating critical adipocyte developmental and functional aspects.[6]

Obesity management is a significant challenge for medical interventions.[7] Moreover, pharmaceutical approaches have often been ineffective and not safe.[8] The questionable efficacy and possible adverse effects associated with anti-obesity medications have also raised concerns.[7] Consequently, developing alternative anti-obesity therapies with minimum side effects and long-term efficacy is urgently required. Harnessing the potential of natural compounds for obesity management has gained much attention, specifically polyphenols, which have exhibited obesity and associated health condition mitigation effects.[9] The compounds reportedly exert advantageous properties through numerous biochemical targets and pathways, collectively providing significant health advantages.[9]

*Syzygium claviflorum* (Roxb.) Wall. ex Steud. (*S. claviflorum*), or *Eugenia claviflora* Roxb., belongs to the *Myrtaceae* family.[10] In Malaysia, the species is known as 'jambu arang' or 'keriang dot'. The plant possesses well-known distinctive-flavored fruits. Nevertheless, research on the nutritional and medicinal properties of *S. claviflorum* is limited.

Recent studies indicated that *S. claviflorum* fruit pulp and seed extracts are rich in polyphenols offering potent antioxidant properties.[11] Nonetheless, the specific health-promoting effects of *S. claviflorum*, particularly for managing obesity, are still unknown. The present study analyzed the phytochemical composition and anti-adipogenic attributes of *S. claviflorum* fruit extract (SCFE) in 3T3-L1 cells. SCFE effects on the expression of critical genes required in adipogenesis and lipid metabolism were also determined.

## Materials and Methods

### *Harvesting the plant specimens*

The *S. claviflorum* fruits employed in this study were harvested from residential fruit trees in Alor Setar, Kedah (N 6° 7′ 29.2800" E 100° 22′ 4.1556″) in July 2022. Specimen identification was performed by a botanist from Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia, and the sample voucher (AuRIns-20220 322) was deposited in the Herbarium of AuRIns, UiTM Puncak Alam.

Dirt was removed from the harvested *S. claviflorum* fruits with multiple washes of distilled water before manually removing the peel and seeds. Finally, the fruit pulp samples were freeze-dried, crushed into powder, and stored at −20 °C before subjecting them to further procedures.

### *Preparing the SCFE*

The extraction methodology outlined by Ahmed et al.[11] was applied in the present study with slight adjustments. Firstly, an ethanolic extract was acquired by adding 10 g of the powdered peel to 100 mL of 80:20 (w/v) an ethanolic aqueous solvent system. After being shaken for 24 h at room temperature, the mixture was filtered through Whatman number 1 filter paper [Millipore, Burlington, Massachusetts, United States of America (USA)]. For an optimized extraction, the residue was redissolved in the solvent. The filtrates from the procedure, which was performed in triplicates, were then combined. The resultant mixture was concentrated with a Rotavapor R-215 (Büchi, Flawil, Switzerland) set to 45 °C and 150 rpm for 90 min. Finally, the extract was lyophilized and kept at −20 °C.

*The SCFE phytochemical analysis*

The present study established the phytochemicals in the SCFE samples with reverse phase-high performance liquid chromatography (RP-HPLC). The methodology reported by Kong et al.[12] was utilized. A C18 column (5 μm particle size; 150 mm × 4.6 mm) [Agilent, California (CA), USA] adjusted to 30 °C was employed. This study also utilized ultrapure water added with 0.3 % formic acid and acetonitrile (ACN) as the mobile phase at an injection volume and flow rate of 10 μL and 1.0 mL/min, respectively.

Caffeic acid phenethyl ester (CAPE), gallic, syringic, cinnamic, and p-coumaric acids, which were phenolic acids, and six flavonoids; chrysin, quercetin, naringin, kaempferol, galangin, and pinocembrin (Sigma−Aldrich, Steinheim, Germany) (see Figure 1) were employed as the standards in this study. P-coumaric and cinnamic acids were determined at 300 and 280 nm, respectively, while the other standards were detected at 260 nm. The gradient sequence applied was 0−3 min, ACN: 10 %, 3−25 min, ACN: 10%−20 %, 25−45 min, ACN: 20%−45 %, 45−55 min, ACN: 45%−60 %, 55−60 min, ACN: 60%−95 %, 60−65 min, ACN: 95 %, 65−70 min, ACN: 95%−10 %, 70−72 min, ACN: 10 %. Chromatograms from triplicate assessments of the standards and the SCFE were compared.

*Adipocyte cell cultivation and differentiation*

The 3T3-L1 murine preadipocytes procured from the American Type Culture Collection (Manassas, Virginia, USA) employed in this study were grown in a glucose-rich Dulbecco's modified Eagle medium (DMEM) containing 1 % penicillin-streptomycin and 10 % fetal bovine serum (FBS). The guideline reported by Choi et al.[13] was applied during adipocyte differentiation initiation, as presented in Figure 2.

Upon confluence, a differentiation medium replaced the medium employed. FBS (10 %), 3-isobutyl-1-methylxanthine (0.5 mM), dexamethasone (0.1 μM), and insulin (1 μg/mL) were employed, which was maintained for two days. Subsequently, a regular medium of 1 μg/mL insulin and 10 % FBS was utilized for cell culture for six days. A fresh medium was supplied every two days.

In this study, the influences of SCFE on adipocyte differentiation were evaluated with and without SCFE addition to the differentiation medium two days post-confluent. N-acetyl-L-cysteine (NAC) (5 mM) was employed as the positive control. After eight days, the cells were harvested.

*Cell viability assay*

The current study established cell viability via the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. 96-well plates incorporated with 250, 500, and 1000 μg/mL of the SCFE was employed to place the 3T3-L1 preadipocytes for 24 h. Subsequently, cell absorbance at 570 nm was determined post-incubating the cells for 4 h with an MTT solution.

Differentiation media added with the SCFE 250, 500, and 1000 μg/mL of were employed to procure mature adipocytes. The MTT assay was conducted to establish cell viability after eight days. Viable cell percentages were calculated according to Equation (1).

$$\text{Cell viability (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}\right) \times 100\% \quad \text{(Eq. 1)}$$

*Lipid accumulation determination via oil red O (ORO) staining*

Minor modifications to the guidelines described by Chung and Hyun[14] was applied in the present study for the ORO staining to assess the SCFE lipid inhibitory properties on differentiated adipocytes. Differentiation of the 3T3-L1 preadipocyte was induced in a 24-well plate containing 250, 500, and 1000 μg/mL of the SCFE. The cells were fixed and rinsed in 10 % formaldehyde for 1 h and phosphate-buffered saline (PBS), respectively, before drying them after eight days.

A 0.5 % ORO dissolved in isopropanol (60 % v/v) at room temperature was employed to stain the dried 3T3-L1 preadipocytes for 30 min. The samples were then washed with water and dried. An Olympus CKX53 microscope (Tokyo, Japan) was utilized for observing and photographing the stained lipid droplets. Quantification of lipid accumulation was performed by solubilizing the stained lipid droplets with isopropanol. The absorbance of the samples was determined at 490 nm with a microplate reader. The lipid accumulation percentage was established with Equation (2).

$$\text{Lipid accumulation (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}\right) \times 100\% \quad \text{(Eq. 2)}$$

This study applied the procedure reported by Choi et al. (2017) during the NBT assay to investigate the effects of the SCFE samples on ROS generation. The 3T3-L1 preadipocytes were induced to differentiate into adipocytes in 96-well plates post−confluence. SCFE of 250, 500, and 1000 μg/mL were then employed to treat the sample cells. The NBT solution was added on the eighth day and left for 90 min. The cells were subjected to dissolution with formazan. Equation (3) was applied to calculate ROS formation percentages upon determining cell absorbance at 570 nm.

$$\text{ROS (\%)} = \left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}\right) \times 100\% \quad \text{(Eq. 3)}$$

*TG and leptin level evaluations*

A commercial colorimetric test kit (Elabscience, Houston, Texas, USA) was utilized to determine the TG levels in cell lysates in this study per the manufacturer's instructions. Post−confluence, the 3T3-L1 preadipocyte cells were treated with 250, 500, and 1000 μg/mL of SCFE in 6-well plates during the eight days of differentiation. The cell lysates were

then scraped and centrifuged at 1000×*g* for 10 min after being subjected to PBS wash several times.

Isopropanol was added to the cell lysates to resuspend them. The 1-sec pulse sonication technique was then performed. Supernatants were procured by centrifuging the lysates at 10 000×*g* for 10 min. The TG deposition results were recorded as TG (mg) per cellular protein (mg). Furthermore, the Lowry technique was employed to determine the total protein content in the lysate cells.

*Leptin enzyme-linked immunosorbent (ELISA) assay*

The leptin levels in the adipocytes evaluated in the present study were determined with a mouse leptin immunoassay kit (Elabscience, Houston, Texas, USA) following the manufacturer's recommendations. After an eight-day treatment period, the supernatant from the culture was collected. The cells were rinsed with PBS, carefully harvested utilizing trypsinization, and centrifuged at 1000×*g* for 10 min. Subsequently, cellular pellets were washed thrice with cold PBS before being subjected to three freezing and thawing cycles. The supernatants were analyzed for leptin levels following centrifugation at 1500×*g* for 10 min. Finally, a standard curve was generated with leptin concentrations between 0 ng/mL and 20 ng/mL to calculate the leptin levels.

*RNA extraction and quantitative real-time-polymerase chain reaction (qRT-PCR)*

The current study isolated RNA from untreated and treated cells (on day 8) with an SV Total RNA extraction kit [Promega, Wisconsin (WI), USA] to assess the gene expression associated with adipocyte differentiation. Subsequently, the GoScript™ Reverse Transcriptase system (Promega, WI, USA) was employed to synthesize cDNA from the total RNA. The procedures applied followed the guidelines provided by the manufacturers.

The desired genes were amplified with an Applied Biosystems StepOne system (Life Technologies, MA, USA) utilizing 200 ng/reaction of cDNA incorporated with SYBR Green-based qPCR Master Mix (Promega, WI, USA). A total of 40 denaturation cycles at 95 °C for 15 s followed by annealing and extension at 60 °C for 60 s were performed. Relative gene expression was established according to the $2^{-\Delta\Delta Ct}$ technique with β-actin as the internal control. All samples were examined in triplicates and quantified per the relative standard curve approach. The primer sequences employed in this study are listed in Table 1.

*Statistical analysis*

The statistical analyses conducted in the current study were performed with GraphPad Prism version 7.0. The evaluations included the one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test to identify significant differences among experimental groups. The data procured were recorded as mean ± standard deviation (SD), obtained from triplicate runs. Differences demonstrating $p < 0.05$ were considered statistically significant.

## Results

*RP-HPLC*

Figure 3(A) and (B) illustrate the chromatograms of the standards and SCFE, respectively. The SCFE contained phenolic acids; syringic (RT: 10.446 min) and p-coumaric (RT: 15.634 min), and a flavonoid compound, naringin (RT: 24.698 min).

*The effects of the SCFE on 3T3-L1 cell viability*

The 3T3-L1 preadipocytes and mature adipocytes in this study were exposed to SCFE at varying concentrations (250,

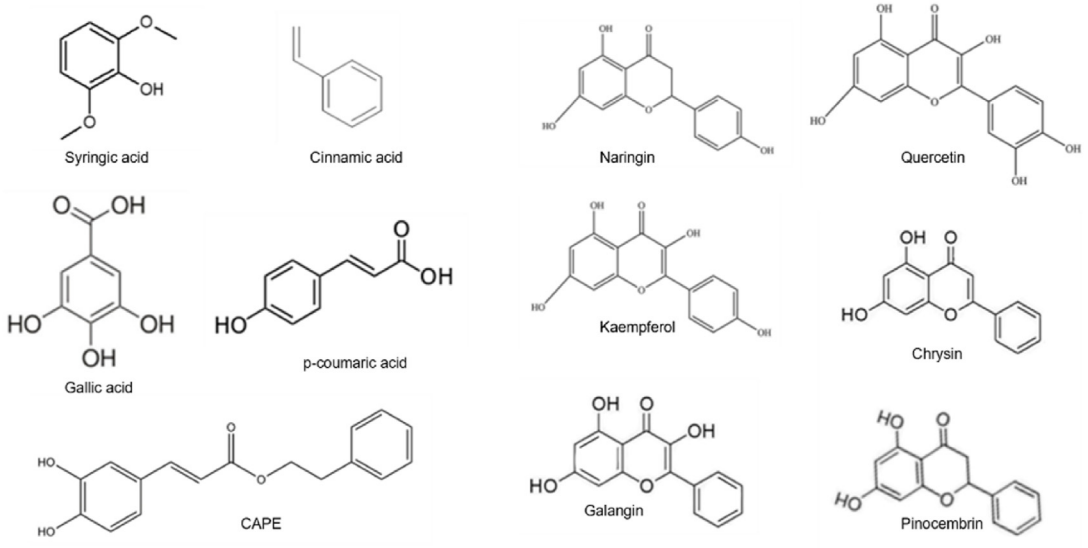


**Figure 1:** Chemical structures of the five phenolic acids and six flavonoid standards used in the high-performance liquid chromatography (HPLC) analysis.

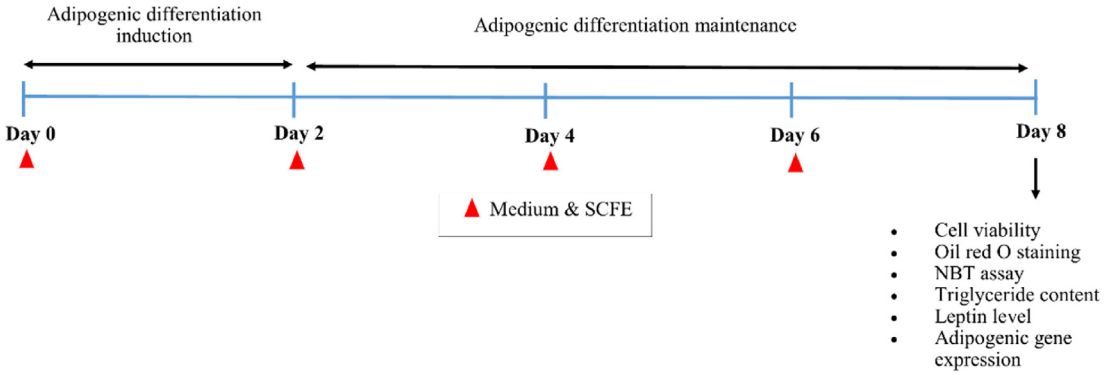


**Figure 2:** Schematic timeline of the experiment.

**Table 1: Primer sequences used for quantitative reverse transcription PCR (qRT-PCR) analysis of adipogenic gene expression in 3T3-L1 adipocytes.**

| Gene | Forward (5′-3′) | Reverse (5′-3′) |
|---|---|---|
| PPARγ | TTTTCAAGGGTGCCAGTTTC | AATCCTTGGCCCTCTGAGAT |
| C/EBPα | TTACAACAGGCCAGGTTTCC | GGCTGGCGACATACAGTACA |
| SREBP-1 | TGTTGGCATCCTGCTATCTG | AGGGAAAGCTTTGGGGTCTA |
| β-actin | CTGTCCCTGTATGCCTCTG | ATGTCACGCACGATTTCC |

500, and 1000 µg/mL) to assess the effects of the extract on cell viability. Based on Figure 4(A) and (B), the SCFE treatment did not induce cytotoxicity in either the 3T3-L1 preadipocytes or mature adipocytes. Furthermore, cell viability consistently exceeded 90 % in all concentrations employed. The non-cytotoxic doses were employed in subsequent experiments to identify SCFE anti-adipogenic attributes.

*The effects of the SCFE on lipid accumulation and ROS production during adipocyte differentiation*

In the mature adipocyte samples evaluated in this study, intracellular lipid accumulation was assessed via ORO staining to determine SCFE anti-adipogenic activities. Microscopic analysis revealed consistent lipid droplet reductions with increasing SCFE concentration [see Figure 5(A)]. The lipid levels diminished significantly ($p < 0.05$) compared to the control group [see Figure 5(B)], recording 78.64 %, 73.96 %, and 55.34 %, when 250, 500, and 1000 µg/mL of the SCFE were supplied, respectively. At the highest SCFE concentration, 1000 µg/mL, the lipid reduction (55.34 %) was comparable to the cells treated with NAC, the positive control (45.38 %) ($p > 0.05$).

In this study, the NBT assay was conducted to evaluate the effects of the SCFE on ROS production during adipogenic differentiation. Treatment with 250, 500, and 1000 µg/mL of the SCFE inhibited ROS production considerably ($p < 0.05$), documenting 59.36 %, 46.40 %, and 33.86 %, respectively, relative to the control [see Figure 5(C)]. At 1000 µg/mL, the SCFE reduced ROS formation by 33.86 %, which was comparable to the NAC (25.26 %) ($p < 0.05$). The data indicated that SCFE inhibitory effects on lipid accumulation and ROS production throughout adipogenesis are dose-dependent.

*The effects of the SCFE on TG and leptin levels during adipocyte differentiation*

The intracellular TG and leptin contents in the adipocytes assessed in this study were quantified eight days following adipogenic differentiation. The cells treated with the SCFE and positive control (NAC) exhibited notably less TG accumulation than the control samples [see Figure 6(A)]. The cells documented TG levels of 1.79, 1.85, and 1.49 1.50 mmol/L post-treatment with 250, 500, and 1000 µg/mL of the SCFE, respectively, while the NAC-treated cells had 1.50 mmol/L. Meanwhile, the control group recorded 2.33 mmol/L of TG. Nevertheless, the 1000 µg/mL SCFE treatment yielded no notable variation ($p > 0.05$) compared to the NAC-treated cells regarding TG levels.

The leptin levels of the SCFE-treated cell were significantly lower ($p < 0.05$) than that of the control cells in a dose-dependent manner [see Figure 6(B)]. The cells recorded diminished leptin levels (0.47, 0.40, and 0.16 ng/mL) with increasing SCFE concentrations (250, 500, and 1000 µg/mL). The control group exhibited 0.63 ng/mL leptin content. Treatment with 1000 µg/mL of the SCFE resulted in a more substantial reduction in leptin levels (0.16 ng/mL) relative to the positive control (NAC) (0.23 ng/mL). The observations suggested that the SCFE effectively reduces TG accumulation and leptin levels during adipogenic differentiation, with the highest concentration (1000 µg/mL) demonstrating comparable effects to the positive control.

*The influences of the SCFE on adipogenic transcription factor expressions*

Gene expression levels of primary adipogenic transcription factors, including C/EBP-α, PPAR-γ, and SREBP-1,

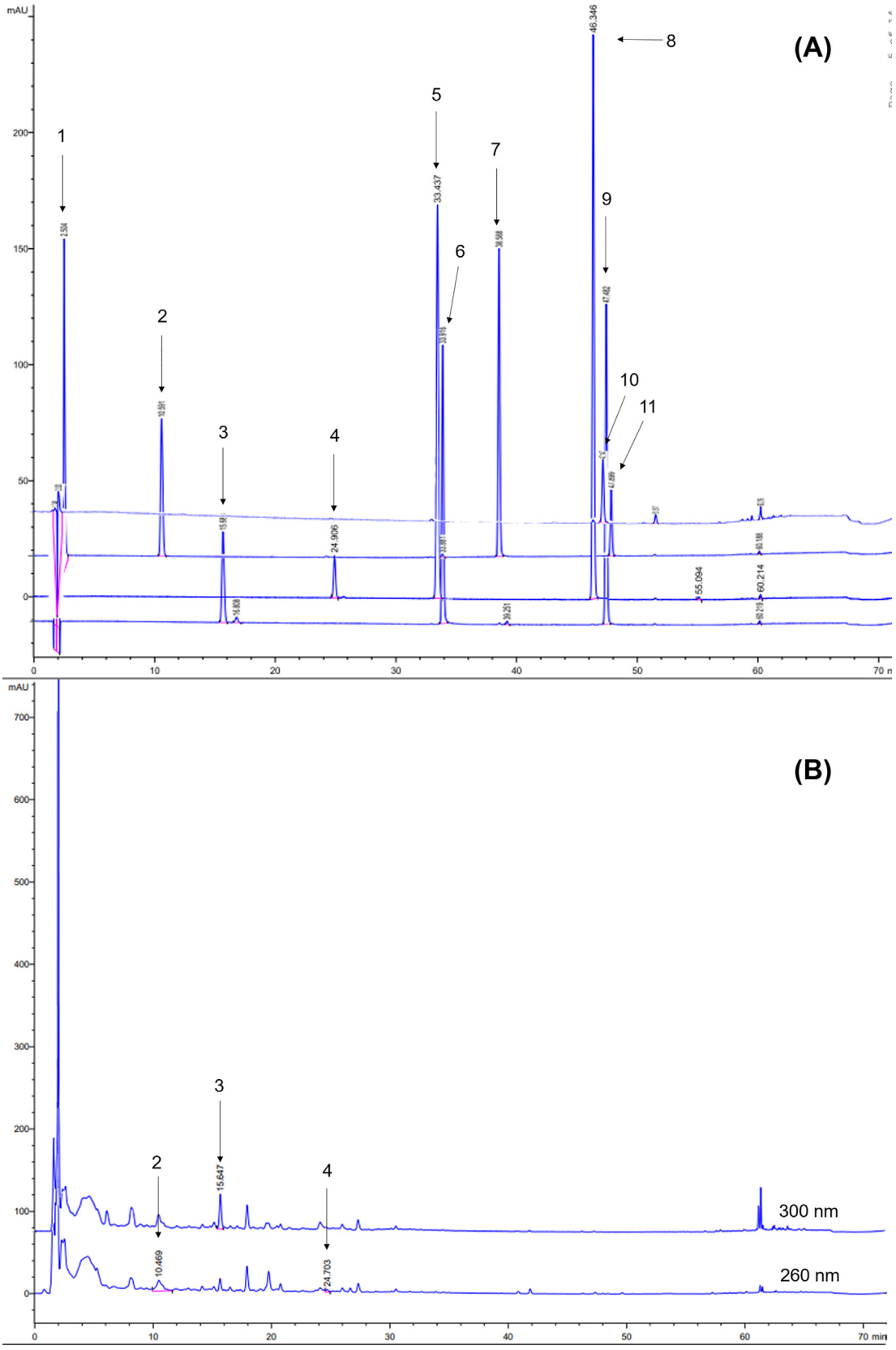


**Figure 3:** HPLC chromatograms of (A) the standard compounds and (B) SCFE. Peaks correspond to the following polyphenols present in the SCFE: 1, gallic acid; 2, syringic acid; 3, p-coumaric acid; 4, naringin; 5, cinnamic acid; 6, quercetin; 7, kaempferol; 8, chrysin; 9, galangin; 10, CAPE; 11, pinocembrin.

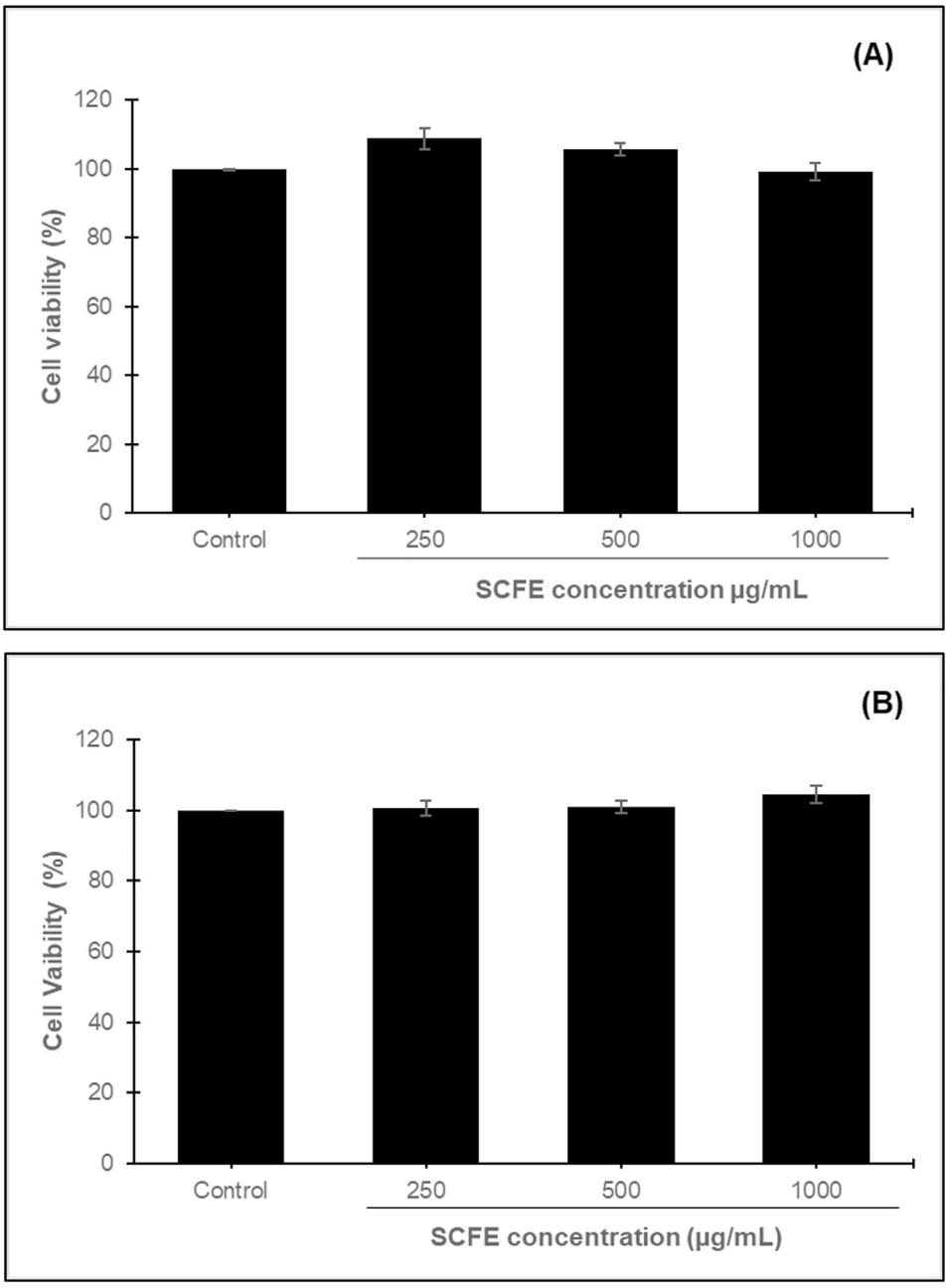


**Figure 4:** Effect of SCFE on the viability of (A) 3T3-L1 preadipocytes treated for 24h and (B) 3T3-L1 mature adipocytes treated for 8 days, as determined by MTT assay. The control group represents cells treated with 0.5 % dimethyl sulfoxide (DMSO). Results are shown as the mean ± standard deviation (SD) of three independent experiments.

were assessed utilizing qRT-PCR. The effects of the SCFE on target genes involved in adipogenesis were determined in the evaluation. The results revealed that the SCFE considerably suppressed C/EBP-α, PPAR-γ, and SREBP-1 gene expressions in 3T3-L1 cells in a dose-dependent manner compared to the control group (see Figure 7). Nevertheless, the inhibitory properties of 500 and 1000 µg/mL SCFE on C/EBP-α and PPAR-γ expressions were similar to the NAC ($p > 0.05$). The findings indicated that the SCFE reduced the expression of adipogenic transcription factors essential for adipocyte differentiation.

## Discussion

Adipogenesis, the process where precursor cells differentiate into mature adipocytes, is pivotal in the formation of adipose tissue. Dysregulated adipogenesis, commonly observed in obesity, contributes to metabolic complications, including insulin resistance and inflammation.[15,16] Consequently, targeting adipogenesis exhibits the potential to address obesity-related disorders.

Natural products, particularly polyphenol-rich substances, have gained attention due to their promising anti-

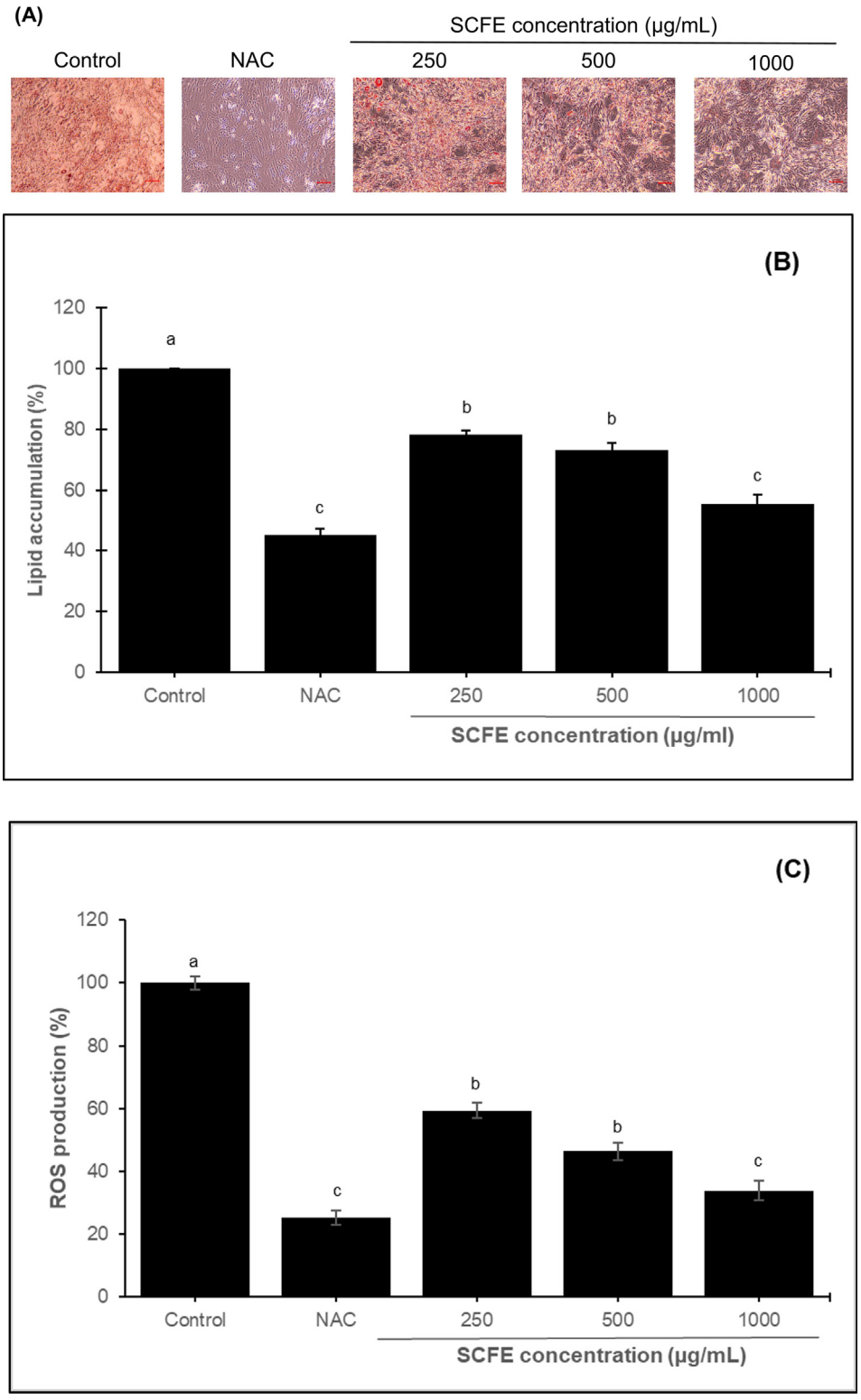


**Figure 5:** Effects of SCFE on the buildup of lipids and the generation of reactive oxygen species (ROS) production in 3T3-L1 adipocytes. Post−confluence 3T3-L1 cells were subjected to differentiation either without or with SCFE at concentrations of 250, 500, and 1000 µg/mL for 8 days. (A) Microscopic images of adipocytes stained with Oil red O (Magnification: 40 ×, scale bar: 100 µm). (B) Percentage of lipid accumulation after Oil Red O elution. (C) ROS production was determined by the nitroblue tetrazolium assay. The control group represents cells treated with 0.5 % DMSO, and "NAC" represents cells treated with 5 mM N-acetyl-L-cysteine as a positive control. Results are displayed as the mean ± SD of three independent experiments. Significant differences ($p < 0.05$) are shown by bars with different letters.

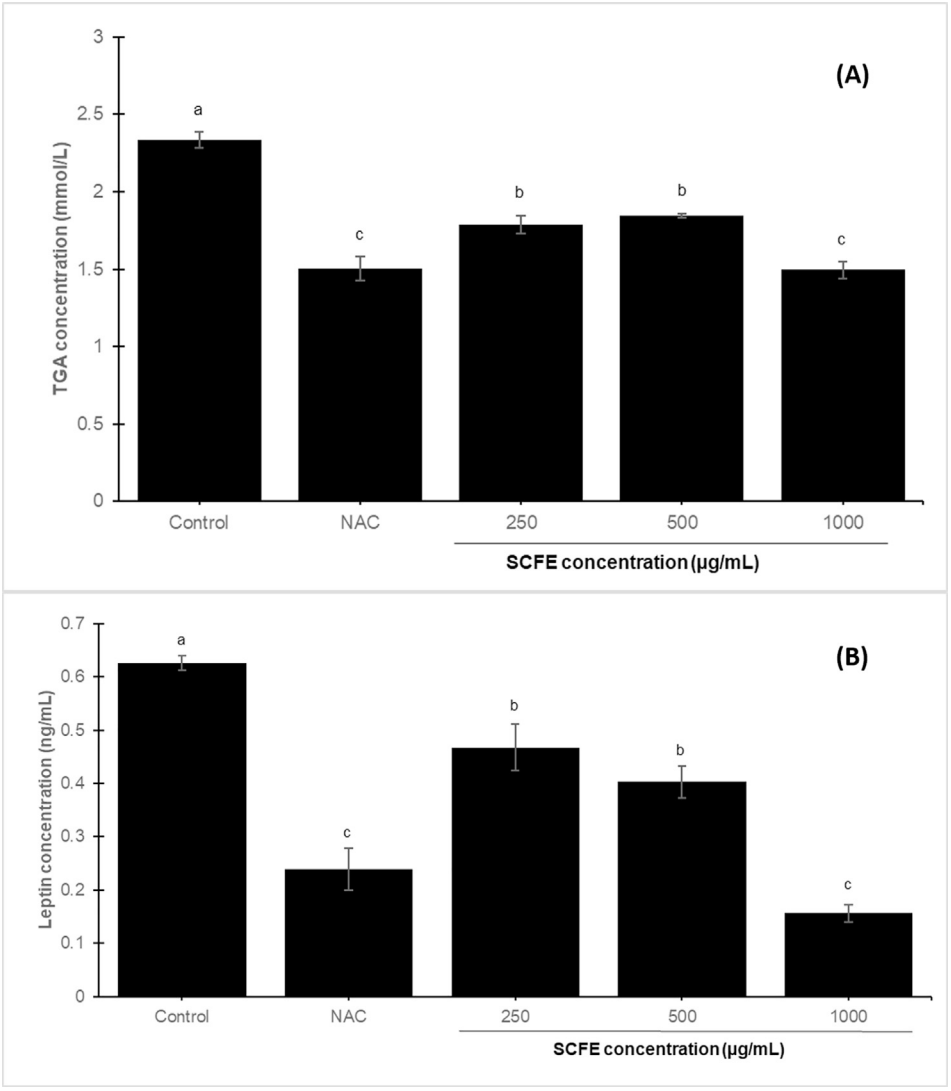


**Figure 6:** Effects of SCFE on (A) triglyceride and (B) leptin concentrations in 3T3-L1 adipocytes. Post-confluent 3T3-L1 cells were subjected to differentiation either without or with SCFE at concentrations of 250, 500, and 1000 µg/mL for 8 days. The control group represents cells treated with 0.5 % DMSO. "NAC" represents cells treated with N-acetyl-L-cysteine at 5 mM and serves as a positive control group. Results are presented as the mean ± SD of three independent experiments. Significant differences ($p < 0.05$) are shown by bars with different letters.

adipogenic effects.[17,18] The compounds are abundant in fruits, vegetables, and medicinal plants. Various biological activities, such as anti-inflammatory and antioxidant attributes, have also been reported.[18,19] Ahmed et al.[11] performed chromatographic profiling via HPLC of *S. claviflorum* fruit and seed methanolic extracts. The report revealed substantial phenolic compound levels in the extract. The study identified epicatechin, catechin hydrate, and gallic acid as the predominant compounds in the plant parts. Nonetheless, this study detected a distinct set of compounds; syringic and p-coumaric acids and quercetin, in the ethanolic extract of SCFE. The dissimilarities were attributable to variations in the extraction methods and solvents employed. Differences in geographical origins and maturity of the plants utilized might also contribute to the variations.

The SCFE did not exhibit cytotoxic effects on 3T3-L1 preadipocytes and mature adipocytes at concentrations between 250 and 1000 µg/mL. Consequently, a safe concentration range for subsequent experiments was established. The data also indicated that the effects observed during the differentiation were not confounded by cytotoxicity.

Significant lipid buildup and TG contents in 3T3-L1 cell reductions post-exposure to the SCFE compared to the control cells were revealed during subsequent adipogenic differentiation process assessments. The dose-dependent lipid accumulation inhibition suggested that the SCFE might have impeded preadipocyte maturation into adipocytes, potentially involving mechanisms that disrupt lipid synthesis or storage. TG is a primary component of lipid droplets and the principal storage form of lipids in adipocytes, hence lipid accumulation and TG levels are closely

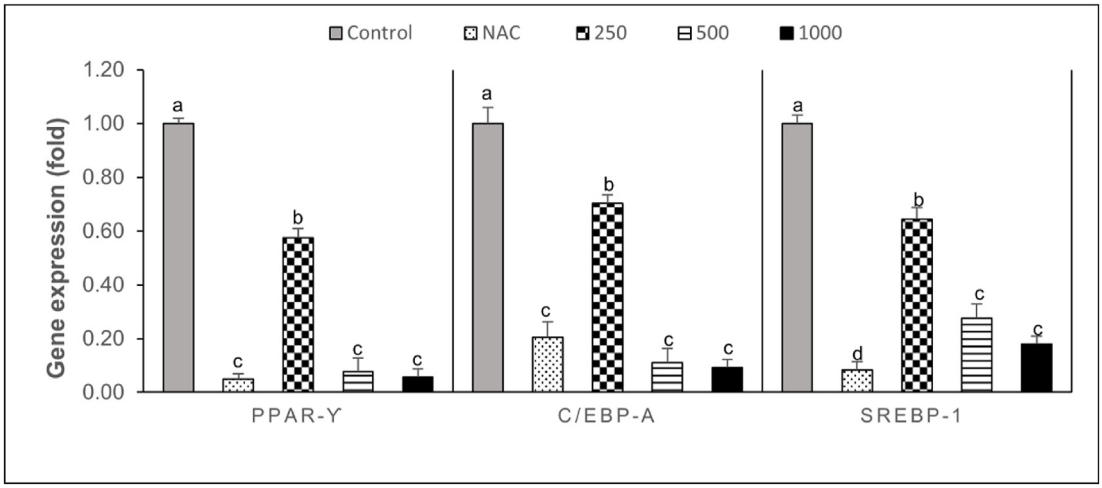


**Figure 7:** Effect of SCFE on the regulation of adipogenic gene expression in 3T3-L1 adipocytes. Post-confluent 3T3-L1 cells were subjected to differentiation either without or with SCFE at concentrations of 250, 500, and 1000 μg/mL for 8 days. The expression level of each gene was measured by qRT-PCR and normalized using β-actin as an internal control. The control group represents cells treated with 0.5 % DMSO. NAC represents cells treated with N-acetyl-L-cysteine at 5 mM and serves as a positive control group. Results are presented as the mean ± SD of three separate experiments. A significant difference ($p < 0.05$) is shown by bars with different letters.

linked.[20] The TG levels in the cells also increase as adipocytes mature and accumulate more lipids, which enhances lipid droplets and lipid storage capacity.[21] In terms of adipogenesis and obesity, increased lipid accumulation and TG content in adipocytes are primary adipocyte hypertrophy and hyperplasia characteristics, contributing to adipose tissue mass expansion.[22]

Heightened metabolic activity during adipogenesis generates ROS, which activates Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, endoplasmic reticulum stress, and inflammation-related immune cells.[23] The reactions also contribute to ROS production during preadipocyte transformation into mature adipocytes. The SCFE treatment employed in the current study substantially reduced ROS production, indicating its antioxidative properties. Polyphenol-rich extracts possess potent antioxidative attributes that could mitigate ROS construction and associated oxidative stress in adipogenesis and obesity. The effectiveness of berry extracts in reducing oxidative stress markers and enhancing antioxidant enzymatic activities in obese individuals has been reported, leading to improved metabolic parameters and decreased adiposity.[24] Similarly, green tea extracts, containing catechins, such as epigallocatechin-3-gallate (EGCG), exhibited robust antioxidative effects. EGCG, specifically scavenges ROS, inhibits lipid peroxidation and boosts antioxidant enzyme activity in adipocytes and obese animal models.[25]

Elevated leptin levels are often associated with obesity due to increased adipose tissue mass.[26] The SCFE treatment applied in this study led to reduced leptin levels during 3T3-L1 cell adipogenic differentiation, further supporting the anti-adipogenic effects of the extract. Leptin is an adipokine principally secreted by adipocytes. The substance is critical in regulating energy balance and body weight, which signals satiety to the brain and regulates food intake and energy expenditure.[5] In adipogenesis, preadipocyte differentiation into mature adipocytes is accompanied by leptin secretion increment, denoting adipose tissue expansion and lipid store accumulation.[5] Consequently, interventions that suppress adipogenic differentiation and lipid accumulation in adipocytes might also reduce leptin levels.

At the molecular level, the SCFE treatment demonstrated suppressive effects on the expression of primary adipogenic transcription factors, including C/EBP-α, PPAR-γ, and SREBP-1. The gene expression regulation revealed a potential adipocyte differentiation mechanism through which the SCFE inhibits. The PPAR-γ is a principal adipogenesis modulator that promotes preadipocyte differentiation into mature adipocytes by activating genes for lipid uptake and storage.[27] Similarly, C/EBP-α aids adipocyte maturation through lipid synthesis and storage-assisted adipogenic gene expression initiations.[28] Conversely, lipid metabolism is managed by SREBP-1, which enhances fatty acid and TG synthesis-related gene transcriptions.[29] The SCFE might disrupt the adipogenic gene regulatory network by downregulating C/EBP-α, PPAR-γ, and SREBP-1 expressions, hence impairing adipocyte maturation. The interference could lead to reductions in lipid synthesis and storage within adipocytes, inhibiting adipogenesis.

## Conclusion

The current study demonstrated the significant anti-adipogenic effects of the SCFE in impeding 3T3-L1 cell preadipocyte differentiation. The findings indicated promising therapeutic attributes of the SCFE as an intervention for managing obesity and its associated metabolic disorders. The results also advocate the employment of natural extracts in addressing global health issues. Nevertheless, identifying the action mechanisms and active components involved requires further examination.

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**Conflict of interest**

The authors declare no conflict of interest.

**Ethical approval**

This study did not require ethical approval.

**Authors contributions**

Conceptualization: RMZ. Data curation: MAA, SMM. Formal analysis: MAA, SMM. Funding acquisition: RMZ. Investigation: MAA, SMM. Supervision: RMZ. Validation: RMZ, AHAJ, MZAB. Writing – original draft: RMZ, MAA. Writing, reviewing and editing: RMZ, AHAJ, MZAB. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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