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# Potency of Rosella calyx as a candidate for anti-obesity treatment based on *in silico* and *in vitro* studies with human umbilical cord mesenchymal stem cells

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ABSTRACT

*Background:* Obesity is a complex medical condition with an increasing global prevalence. It is influenced by various genetic, environmental, and behavioral factors, contributing to higher risks of chronic diseases. The current therapy for obesity uses a multidisciplinary approach, but often leads to undesirable side effects. Therefore, potential natural alternatives such as anti-obesity agents are needed.
*Objective:* This study aimed evaluate the potential of Rosella calyx extract in inhibiting adipocyte maturation using *in silico* and *in vitro* approaches with human umbilical cord mesenchymal stem cells (hUC-MSCs).
*Method:* The content of Rosella extract compound was identified using GC–MS. An *in silico* analysis was conducted to predict the potential of the compounds, protein targets, and molecular pathways. Furthermore, the effect of extract on the differentiation of adipocytes from mesenchymal stem cells in the human umbilical cord was evaluated *in vitro*.
*Result:* The results showed that GC–MS identified four major compounds in Rosella extract, namely Triacetin, Pentadecanol, Phenol, and Propionic Acid. Based on *in silico* analysis, these compounds have the potential to inhibit adipocyte maturation through interactions with target proteins such as PPARG, LEP, INS, ADIPOQ, SIRT1, and POMC. *In vitro* studies confirmed that Rosella extract effectively inhibited the differentiation of human umbilical cord stem cell-derived adipocytes at a concentration of 250 $\mu$g/ml.
*Conclusion:* Rosella calyx extract showed promising anti-obesity effects by inhibiting adipocyte differentiation pathways, primarily targeting PPAR$\gamma$. These results provide a scientific basis for the development of extract as a potential therapeutic agent in the management of obesity.


## 1. Introduction

Obesity remains a pressing global health issue with increasing prevalence over the years. It occurs when there is an imbalance between energy intake and expenditure, leading to the accumulation of excess fat in the body. Obesity can impact various bodily systems, from the molecular level to clinical symptoms, increasing the risk of chronic diseases such as type 2 diabetes, high blood pressure, heart disease, and metabolic syndrome (Silveira Rossi et al., 2022). The development entails a combination of genetic, environmental, and behavioral factors. Adipose cells play a crucial role, but as storage units and endocrine organs that release hormones and other substances with a significant influence on appetite, metabolism, and insulin

*Abbreviations:* GCMS, gas chromatography-mass spectrometry; SAR, structure-activity relationship; ADMET, absorption, distribution, metabolism, excretion, and toxicity; hUC-MSCs, human umbilical cord mesenchymal stem cells; PRP, platelet rich plasma; PBS, phosphate-buffered saline; PPARG, peroxisome proliferator-activated receptor gamma; LEP, leptin; INS, insulin; ADIPOQ, adiponectin; SIRT1, sirtuin 1; POMC, ProOpiomelanocortin; CREB1, cAMP response element-binding protein 1; NFKB1, nuclear factor Kappa B subunit 1; PKA, protein kinase A; Epac, cAMP-directly activated exchange protein; MMP-9, matrix metalloproteinase-9

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function. This can lead to insulin resistance, increased inflammation, and abnormal cholesterol levels (Piché et al., 2020). Effective management of obesity requires a multifaceted approach, including dietary changes, increased physical activity, behavioral interventions, medications, and in some cases, bariatric surgery (Baker et al., 2022). The objective is to achieve sustainable weight loss and improve overall quality of life by addressing the underlying causes and associated health risks.

In the context of weight loss medication, the drugs used aim to influence various biological processes that regulate appetite, metabolism, and body fat. Appetite suppressants such as phentermine and topiramate work by altering brain chemistry to reduce hunger and increase feelings of fullness (Pati et al., 2023). Another drug, orlistat inhibits enzymes that break down fat, reducing the absorption and overall calorie intake (Katimbwa et al., 2022). Other drugs, such as metformin, enhance insulin sensitivity and decrease glucose production, contributing to weight loss (Yerevanian and Soukas, 2019). Injections such as GLP-1 analogs mimic natural hormones that regulate appetite (Kokkinos et al., 2019). However, these medications often have undesirable side effects, necessitating the need for a safe, natural alternative.

*Hibiscus sabdariffa*, also known as Rosella, shows potential as anti-obesity agent due to the bioactive compounds, including flavonoids, polyphenols, and anthocyanins showed by Ujianti's study. This study explores the potential of Rosella (*Hibiscus sabdariffa*) extract as an anti-steatohepatis. The plant is rich in bioactive compounds, such as flavonoids, polyphenols, and anthocyanins, which are known to regulate metabolic pathways and reduce inflammation (Ujianti et al., 2023a). *In vitro* studies have showed the plant effectiveness in reducing plasma lipid levels and promoting weight loss. The bioactive compounds play a role in regulating the biomolecular pathways associated with the prevention of obesity and inflammation (Ujianti et al., 2022).

Although Roselle's anti-obesity effects are well-supported, studies specifically targeting its influence on adipocyte maturation remain limited. To bridge this gap, this study aimed to comprehensively examine the application of Rosella calyx extract on stem cells derived from the umbilical cord to prevent adipocyte maturation. The results will offer insights into prevention strategies to counter excessive fat accumulation and ultimately reduce the incidence of obesity. This study directly apply Rosella extract on stem cells to prevent transformation into mature adipocytes. The innovative approach offers a new path in obesity studies and herbal therapy, focusing on the modification of adipogenesis as a strategic intervention target.

## 2. Method

### 2.1. Extract material

The Rosella calyx extract was analyzed using **GC–MS** to identify its *bioactive compounds*.Using GC–MS, chemical compounds in the ethanol extract of calyx Rosella were evaluated with a Shimadzu GCMS-QP 2010 Ultra instrument with an Rtx-5MS (5 % diphenyl/95 % dimethyl polysiloxane) stationary phase, a column length of 30 m, and a diameter of 0.25 mm. The carrier gas was ultra-high purity helium with a pressure of 40.7 kPa, an injection volume of 5 $\mu$L, an injector temperature of 250 °C, an ion source temperature of 200 °C, an interface temperature of 280 °C, and a splitless mode. The column was programmed from 60 °C, held for 1 min, then increased to 330 °C at a rate of 10 °C/min and held for 5 min.

### 2.2. In silico *analysis*

#### 2.2.1. Database search

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain compound details such as structural form, ID, and Simplified Molecular-input line-entry System (SMILES).

### 2.3. Prediction of compound potential with structure-analysis relationship (SAR) approach

SAR is an *in-silico* approach used to compare the input compounds with those already available in the Way2Drug database. The more similar the compound structure, the higher the potential value, with a cutoff score of 0.5. SAR in this study used the Way2Drug PassOnline web server.

### 2.4. Prediction of compound potential with ADMET approach

The canonical SMILES structure of each compound was selected and entered into the Admet Lab 2.0 web server to make predictions.

### 2.5. Target prediction and graph topology analysis protein

SuperPred predicted genes or proteins that Rosella extract could target. Experimental results of a compound interaction with a gene in the SuperPred database can be used to predict the role of extracts in obesity. The targetable genes or proteins were predicted using the SuperPred database.

The compiled target list was entered into the STRING database multiple protein menu. Text mining, experiments, and databases served as active interaction sources (Yang et al., 2021). The target list from the previous stage was compiled and then inputted into the multiple protein menu from the STRING database (https://string-db.org/) for protein interaction analysis. The visualization results were conducted using Cytoscape v.9.0 software.

### 2.6. In vitro *analysis*

#### 2.6.1. Mesenchymal cell culture and treatment

This study received approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Prof.Dr.Hamka, under the number KEPKK/FK/071/17/2022. Mesenchymal stem cells from human umbilical cord (HUC-MSCs) were cultivated in 12-well culture plates using Amem medium supplemented with 10 % Platelet Rich Plasma (PRP). Cells were incubated at a temperature of 37 °C with 5 % CO2. The culture medium was replaced every 2–3 days to ensure optimal cell growth.

Before any interventions, a standardized protocol was followed for subculturing cells. This entailed rinsing cells with phosphate-buffered saline (PBS), followed by a 2-minute incubation with 1 mL of 0.05 % trypsin-EDTA. Cells were then transferred to a 15 mL tube containing 10 mL of Amem medium supplemented with 10 % PRP and centrifuged at a speed of 1200 rpm for 10 min at a temperature of 20 °C. The supernatant was discarded, and cell pellet was resuspended in 1 mL of medium (Pawitan et al., 2015). Viability was assessed using Trypan Blue assay, then cells were seeded in a 12-well culture plate at a density of 5000 cells/cm2 per well and incubated at 37 °C with 5 % CO2. Adipogenesis medium induction was performed and replaced every 2–3 days. The induction of adipogenesis was performed concurrently with the application of sea cucumber extract at concentrations of 250 $\mu$g/ml and 350 $\mu$g/ml Rosella extract (Abdulhamid et al., 2014).

## 3. Results

### 3.1. Gas chromatography-mass spectrometry (GC–MS) analysis

As observed in the GC–MS analysis, the ethanol extract from Rosella contains four unique compounds, details of which are shown in Fig. 1. These compounds, consisting of Triacetin, Phenol, Propionic Acid, and Pentadecanol, constitute the primary molecules identified in extract.

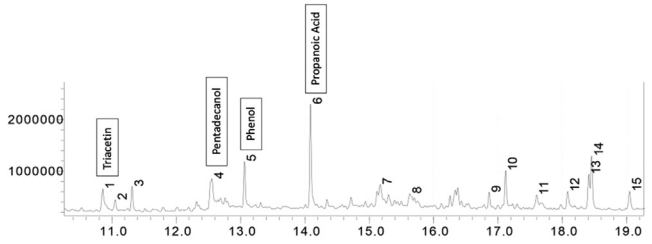


**Fig. 1.** Chromatogram for high-performance gas chromatography analysis of Rosella extract.

**Table 1**
GC–MS analysis in Rosella extract.

| No. | Compound | Formula | Retention Time (min) | Area (%) | Number in Picture |
|---|---|---|---|---|---|
| 1 | Triacetin | $C_9H_{14}O_6$ | 10.86 | 4.52 | 1 |
| 2 | Pentadecanol | $C_{15}H_{32}O$ | 12.55 | 9.35 | 4 |
| 3 | Phenol | $C_6H_6O$ | 18.86 | 8.58 | 5 |
| 4 | Propinoic Acid | $C_3H_6O_2$ | 12.82 | 20.06 | 6 |

A total of 15 compound components were obtained, but only 4 showed specific biological activity for anti-obesity, as shown in Table 1.

### 3.2. Quantitative structure-activity relationship (QSAR) analysis

The compounds found in Rosella extract may have the potential to treat obesity, according to the SAR (Structure-Activity Relationship) analysis. SAR is an *in-silico* technique that compares the compounds in Rosella extract to therapeutic compounds already in the Way2-Drug database (Ujianti et al., 2024). The higher the potential value, the more similar the structure of the compound. As shown in Fig. 2, Rosella extract has showed potential as an effective treatment for obesity. Extract appears to work through multiple pathways to address the complex issue of obesity.

### 3.3. Toxicity analysis compound

The analysis of the toxicity levels in each bioactive sample, using the AdMet Lab2.0 web server, showed that all four compounds present in the sea cucumber extract adhere to Lipinski's rule, as shown in Fig. 3.

### 3.4. Predicting protein targets

Fig. 4 shows the target pathway network for obesity treatment using sea cucumber. In this network, Peroxisome Proliferator-Activated Receptor Gamma (PPAR$\gamma$) is a key gene that serves as a central interaction point for various proteins in the process of obesity development. The network captures the complexity and interconnectedness of metabolic signaling pathways.

Table 2 presents a network analysis for several obesity-related genes. PPAR$\gamma$, LEP, and INS have high Betweenness Centrality values, showing an important role in connecting various components within the network. These genes also have high Closeness Centrality values, suggesting a close connection to other genes in the network. Meanwhile, NF-$\kappa$B1 has a high Clustering Coefficient, showing a significant role in the formation of interconnected gene groups. PPAR$\gamma$, LEP, and INS also have high Degree, suggesting connectedness to many other genes in the network. NF-$\kappa$B1 has a high Topological Coefficient, showing many connections with other highly connected genes.

Fig. 5 shows the molecular function and KEGG Pathway analysis for the genes associated with obesity process. This analysis can provide information about the molecular functions and metabolic pathways associated with the genes. This diagram presents the results of an enrichment analysis of genes linked to biological processes and key signaling pathways involved in Rosella extract's mechanism for inhibiting adipogenesis. Pathways such as the adipocytokine signaling pathway and biological processes like the regulation of lipid localization play significant roles in adipocyte differentiation and lipid metabolism regulation. In the biological processes category, findings like the response to hormones and regulation of lipid localization indicate that Rosella extract affects hormone receptors and lipid distribution. For key signaling pathways, the adipocytokine signaling pathway influences the regulation of insulin sensitivity and inflammation. This action helps lower the risk of excessive fat formation while improving metabolic efficiency. The analysis provides a clear visual summary of the molecular pathways involved in targeting adipogenesis.

### 3.5. In vitro *analysis*

As shown in Fig. 6, there are observable differences in the morphology between the positive control (A) and the treatment (C and D).

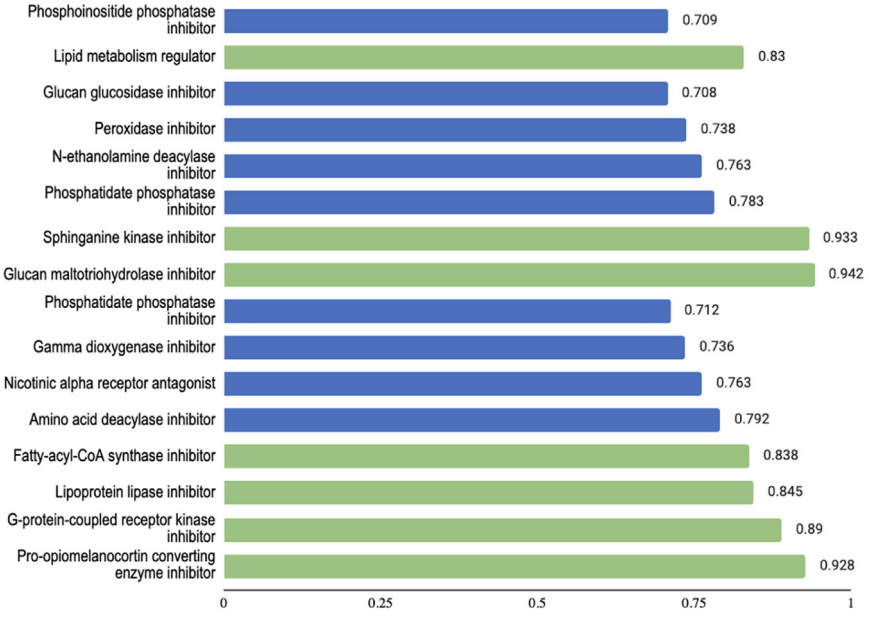


**Fig. 2.** Prediction of Rosella extract potential as obesity treatment by structure analysis quantitative (SAR).

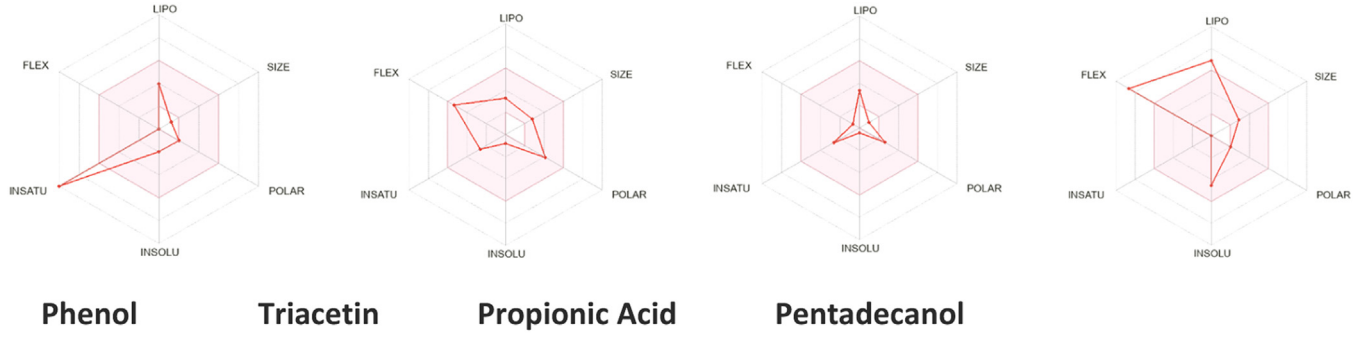


**Fig. 3.** Toxicity analysis compound of Rosella potential as an anti-obesity agent.

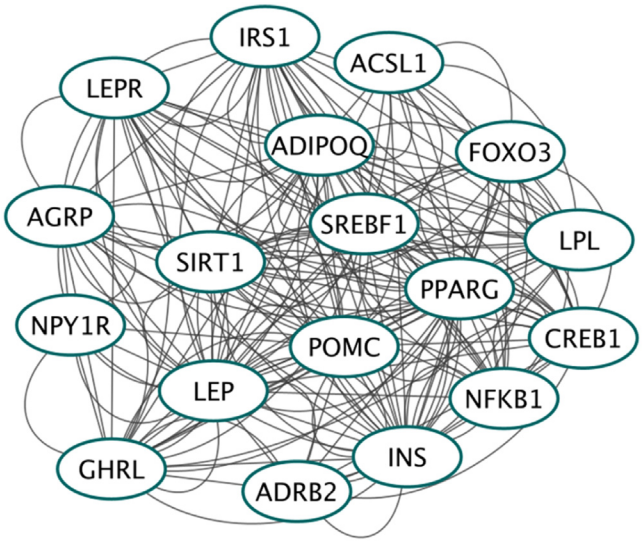


**Fig. 4.** Protein Interaction Network of Metabolism-Related Genes.
The figure represents a network of interactions among metabolism-related genes and proteins. Each node (oval) represents a gene or protein, and the edges (lines) connecting the nodes depict known functional interactions. Nodes such as *PPARγ* (*PPARG*), *LEP*, and *INS* exhibit high Betweenness Centrality values, indicating their critical role as key connectors within the network.

Image A shows a higher level of cell density differentiation. Conversely, C has a lower cell density compared to A.

In Fig. 7 with 20x magnification, the positive control (A) shows the morphology of mature adipocyte cells after adipogenesis induction process. Cells appear round or spherical in shape with a full and lipid droplets cytoplasm that appears white or transparent. These lipid droplets are a characteristic of mature adipocyte cells that have accumulated lipids within the cytoplasm. Additionally, cells appear larger in size compared to cells before differentiation into adipocytes, compared to the negative control (B). This shows an increase in volume and lipid accumulation within the cytoplasm during the adipocyte maturation process. Meanwhile, stem cells given adipogenesis induction with 250 $\mu$g/ml Rosella extract (C) and 350 $\mu$g/ml Rosella extract appear to have smaller adipocyte morphology and do not develop optimally compared to normal adipocyte cells. Cells have an irregular structure and do not show the typical morphology of mature adipocyte cells. The accumulation of lipid droplets in the cytoplasm was also inhibited.

Based on the data shown in Fig. 8, the negative control treatment had a viability percentage of 99.33 %, while the positive control treatment had a viability percentage of 98.67 %. Rosella treatment with a concentration of 250 $\mu$g/ml had a viability percentage of 96.67 %. Rosella treatment with a concentration of 350 $\mu$g/ml had a lower viability percentage of 77.33 %compared to the negative control.

## 4. Discussion

Rosella has been shown in several studies to inhibit the development of obesity-related diseases. However, the scientific evidence explaining how Rosella helps manage obesity, particularly the ability to inhibit the maturation of fat cells, is still limited (Singab et al., 2024; Ujianti et al., 2023a; Ujianti et al., 2023b). This study used tissue pharmacology to explore and predict the potential active components and underlying mechanisms of Rosella in addressing obesity (Ujianti et al., 2022). Validation was carried out through *in vitro* functional tests and the morphological changes in stem cells to induce adipogenesis were examined. GC–MS, which identified Triacetone, Pentadecanol, Phenol, and Propionic Acid in Rosella extract, confirmed the presence of anti-obesity effects both *in silico* and *in vitro* studies.

The protein-protein interaction (PPI) network is a fundamental principle in biological organization that shows the significance of basic cellular processes (Tomkins and Manzoni, 2021). Inhibiting a protein within the PPI network can disrupt multiple mechanisms. In this study, PPAR$\gamma$, LEP, INS, and ADIPOQ were found to be the top five targets in the constructed PPI network. CREB1 can interact with PPAR$\gamma$, INS, ADIPOQ, and SIRT1. Inhibiting CREB in relation to PPAR$\gamma$ has a substantial impact on the process of adipogenesis and adipocyte maturation. CREB1 is typically active in the early stages of

**Table 2**
Topological parameters of 8 interconnected targets on Obesity in the PPI network.

| Name | Betweenness Centrality | Closeness Centrality | Clustering Coefficient | Degree | Topological Coefficient |
|---|---|---|---|---|---|
| PPARγ | 0.05 | 0.94 | 675 | 32 | 0.67 |
| LEP | 0.08 | 0.94 | 0.64 | 32 | 0.65 |
| INS | 0.05 | 0.94 | 675 | 32 | 0.67 |
| ADIPOQ | 0.02 | 0.85 | 0.76 | 28 | 0.71 |
| SIRT1 | 0.02 | 0.85 | 0.77 | 28 | 0.72 |
| NF-κB1 | 0.00 | 0.68 | 0.94 | 18 | 0.79 |
| POMC | 0.03 | 0.77 | 0.74 | 24 | 0.70 |
| CREB1 | 0.02 | 0.74 | 0.80 | 22 | 0.73 |

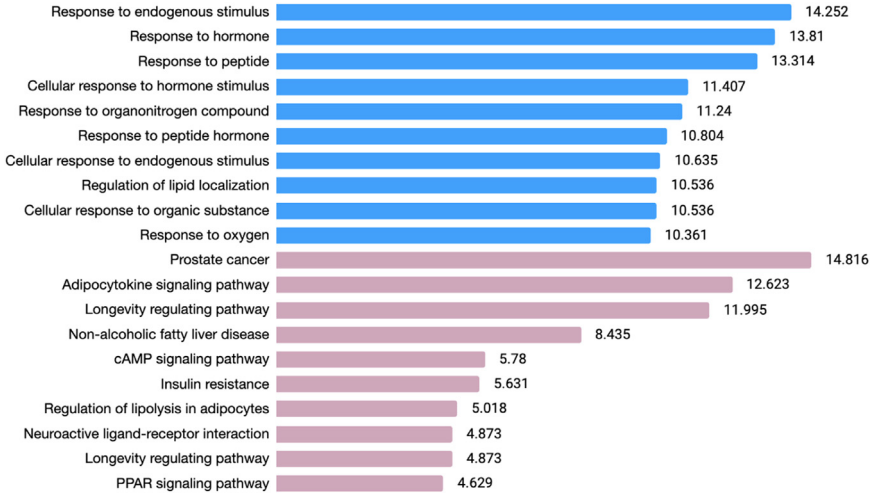


**Fig. 5.** Molecular Function and KEGG Pathway Analysis. Enrichment analysis of gene sets highlighting biological processes (blue bars) and pathways (pink bars) related to the inhibition of adipogenesis by Rosella extract.

adipogenesis, stimulating the expression of PPAR$\gamma$ and C/EBP$\beta$, before PPAR$\gamma$ takes over to complete adipocyte differentiation (Ambele et al., 2020). When PPAR$\gamma$ is inhibited, the disruption of the transcriptional cascade inhibits the expression of adipocyte-specific genes. Consequently, cells are trapped in the preadipocyte stage, leading to the accumulation of partially differentiated cells. Inhibiting PPAR$\gamma$ can also reduce lipogenesis and alter the adipokine secretion profile (Jakab et al., 2021). Long-term implications include disruption of mature adipocyte formation. Overall, these genes may play crucial roles in inhibiting adipogenesis of Rosella in obesity based on the network pharmacological analysis. This is in line with a study on the pharmacology of obesity networks (Wang et al., 2020).

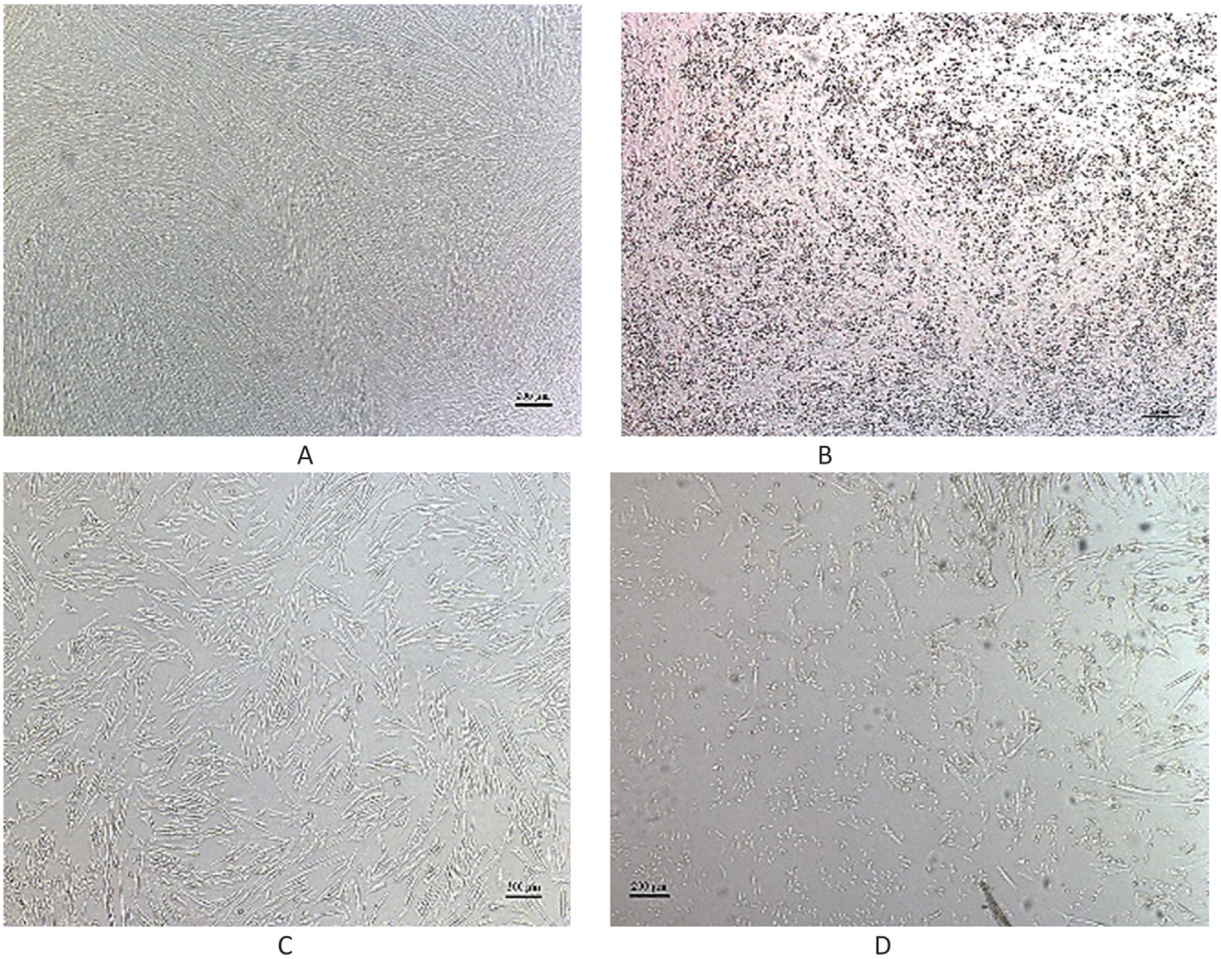


**Fig. 6.** (A) Positive control with differentiated medium, (B) Negative control with undifferentiated medium; (C) Differentiated medium with 250 $\mu$g/ml Rosella extract; (D) Differentiated medium with 350 $\mu$g/ml Rosella extract. Treatment duration: 14 days. Scale bar represents 100 $\mu$m in magnification 4x.

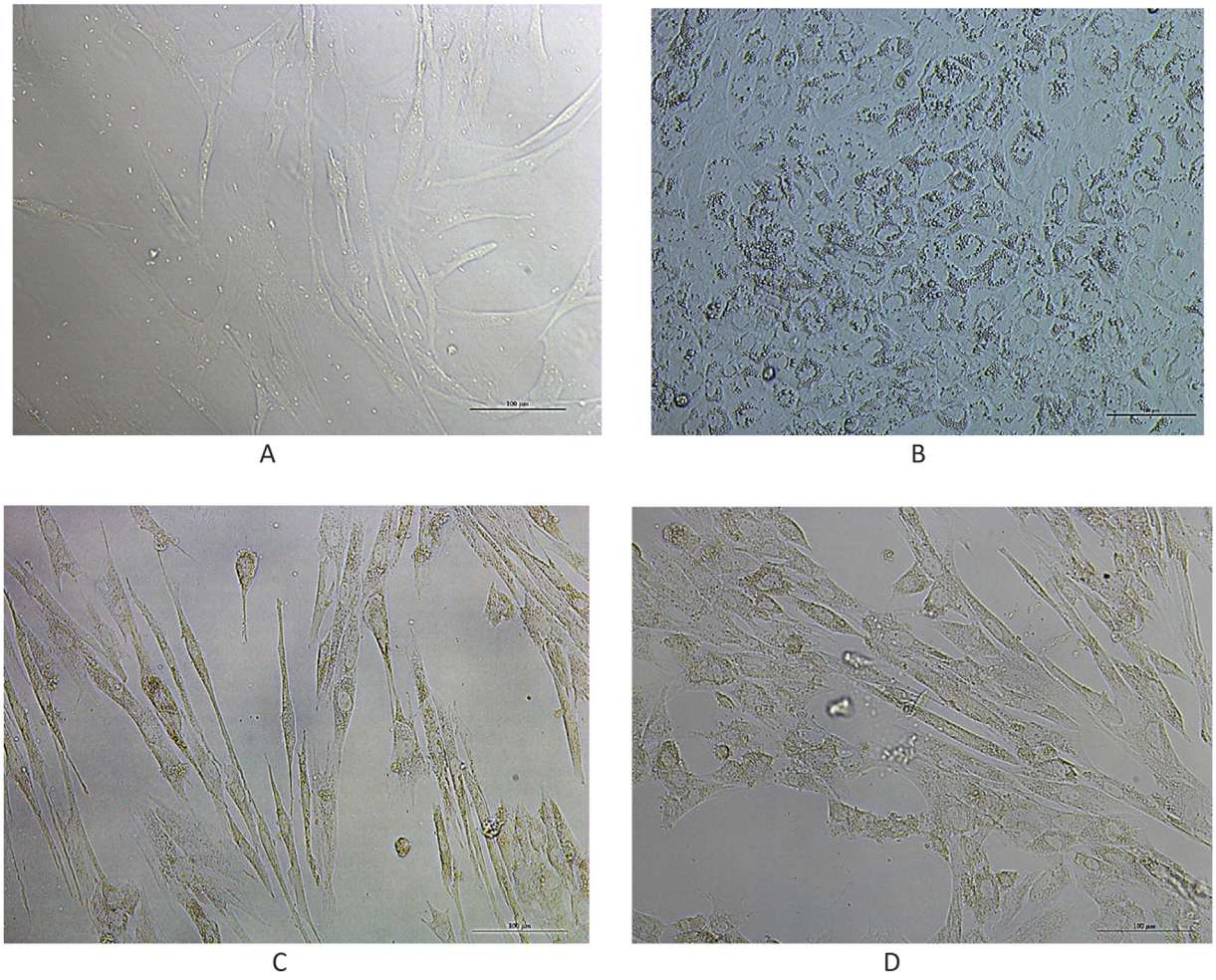


**Fig. 7.** (A) Positive control with differentiated medium, (B) Negative control with undifferentiated medium; (C) Differentiated medium with 250 $\mu$g/ml Rosella extract; (D) Differentiated medium with 350 $\mu$g/ml Rosella extract. Treatment duration: 14 days. Scale bar represents 100 $\mu$m in magnification 20x.

In the *in silico* study, the cAMP signaling pathway was found to be significantly enriched in the KEGG analysis. The cAMP signaling pathway is the primary regulator of adipogenesis, the process by which fat cells are formed (Kim, 2023). The cAMP activates two key effectors namely protein kinase A (PKA) and the cAMP-directly activated exchange protein (Epac) (Valentine, 2022). Epac and PKA work together to enhance adipogenesis by regulating the Rap GTPase and downstream factors in preadipocytes. Furthermore, cAMP signaling regulates the expression and activity of key transcription factors, such as PPAR$\gamma$, which are crucial for adipocyte differentiation (Benchoula, 2021). The results suggest that Rosella suppresses the formation of fat cells by inhibiting the activities of two key proteins, CREB and C/EBP$\beta$. This, in turn, leads to decreased expressions of C/EBP$\alpha$ and PPAR$\gamma$, as well as other genes implicated in fat cell development. This is consistent with a study conducted by Zhang et al., which showed inhibition of CREB activation during adipocyte maturation, although the study used berberine extract (Zhang et al., 2015). Additionally, Aranaz et al. reported that polyphenols can decrease the expression of PPAR$\gamma$ target genes implicated in adipogenesis and lipid metabolism in fat cells (Aranaz et al., 2019).

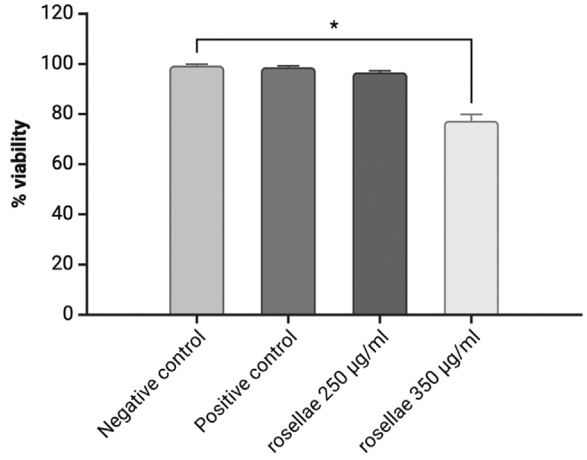


**Fig. 8.** Viability of human umbilical stem cells with induction of adipogenesis at various concentrations in 14 days.

This result was strengthened *in vitro* study, showing that the differentiation process of adipocytes from an immature to a mature form can be stopped by administering extract to stem cells derived from human umbilical cord. This inhibition was effective at a concentration of 250 $\mu$g/ml, while at a concentration of 350 $\mu$g/ml there was a significant level of cell death. Ray et al. found that the administration of 6-shogaol induced cell death in cancer stem cells. Although extract was different, shogaol is part of the active phytochemical compound that can exhibit anti-invasive effects in breast cancer cells. This is achieved by reducing the expression of MMP-9 through the activation of NF-$\kappa$B and PPAR$\gamma$ (Ray, 2015). Cell viability appears to decrease at a concentration of 350 $\mu$g/ml. Further comparison shows

that the study by Ray et al. used the compound 6-shagol, part of the active phytochemical components, to induce cell death in cancer stem cells. Meanwhile, this study used Rosella extract, which likely contains active phytochemical compounds and caused cell growth inhibition at similar concentrations. Both studies show cell growth inhibition effects related to the activation of NF-$\kappa$B and PPAR$\gamma$ pathways.

According to the *in silico* analysis, Rosella extract has the ability to inhibit adipogenesis process through the mechanism of inhibiting adipogenesis. Structural-activity analysis on Rosella extract shows potential as anti-obesity agent. Rosella extract components can inhibit key enzymes in the lipogenesis process, such as Fatty-acyl-CoA synthase, Lipoprotein lipase, and G-protein-coupled receptor kinase (Lakshmi and Thiyagarajan, 2018). Inhibition of these enzymes can reduce the formation and accumulation of lipids in the body. In addition, Rosella extract also has the potential to inhibit the activation of CREB and PPAR-$\gamma$ receptors, which play a role in adipocyte maturation (Haque et al., 2023). Overall, the analysis results showed that Rosella extract has a promising prospect as a drug or supplement candidate for addressing the problem of obesity.

The results of this study have interesting implications for the development of Rosella extract as a complementary therapy for obesity management. The ability to inhibit adipogenesis by modulating lipid metabolism pathways makes it a promising candidate. However, the limitations of the *in silico* and *in vitro* studies need to be addressed through *in vivo* validation to comprehensively show effectiveness and safety. Further development, including formulation optimization and clinical trials, will be required to realize the full therapeutic potential of Rosella extract in obesity management.

## 5. Conclusion

In conclusion, both *in silico* and *in vitro* studies demonstrated that Rosella extract exhibits potential as a natural anti-obesity agent by inhibiting adipocyte differentiation through the PPAR$\gamma$ pathway.

### Key findings

1. Chemical Composition- GC-MS analysis identified four major compounds in Rosella extract:
   * Triacetin
   * Pentadecanol
   * Phenol
   * Propionic Acid
2. *In Silico* Analysis: Identified key protein targets including:
   * PPARG (Peroxisome Proliferator-Activated Receptor Gamma)
   * LEP (Leptin)
   * INS (Insulin)
   * ADIPOQ (Adiponectin)
   * SIRT1 (Sirtuin 1)
3. *In Vitro* Results: Rosella extract effectively inhibited adipocyte differentiation at 250 $\mu$g/ml
   - Higher concentration (350 $\mu$g/ml) showed decreased cell viability (77.33 %)
   - Morphological changes observed in treated cells compared to control
4. Mechanism of Action: Works primarily through inhibition of adipogenesis
   - Modulates cAMP signaling pathway
   - Affects PPARG and SIRT1 as main mediators
   - Inhibits key enzymes in lipogenesis process
5. Safety & Toxicity: All four compounds comply with Lipinski's rule
   - 250 $\mu$g/ml concentration showed good safety profile
   - 350 $\mu$g/ml showed some toxicity effects

Conclusions: Rosella extract shows promising anti-obesity potential
- Works through multiple pathways to inhibit fat cell formation
- Most effective at 250 $\mu$g/ml concentration
- Further *in vivo* validation needed for clinical applications

Significance:

First study to directly apply Rosella extract on stem cells to prevent adipocyte maturation, offering a new approach in obesity research and herbal therapy.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### CRediT authorship contribution statement

**Irena Ujianti:** Writing – original draft, Investigation, Conceptualization. **Bety S Lakhsmi:** Investigation. **Zahra Nurushoffa:** Investigation. **Wawang S Sukarya:** Investigation. **Supandi:** Investigation. **Takashi Yashiro:** Project administration, Methodology.

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### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2025.03.009.

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