Date: Mar 06, 2025
To: "irena ujianti" irenaujianti@uhamka.ac.id
From: "South African Journal of Botany" support@elsevier.com
Subject: Decision on submission to South African Journal of Botany

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Manuscript Number: SAJB-D-24-03770R1

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

Dear Dr. ujianti,

I am pleased to inform you that your manuscript has been accepted for publication.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate and value your contribution to South African Journal of Botany. We regularly invite authors of recently published manuscript to participate in the peer review process. If you were not already part of the journal's reviewer pool, you have now been added to it. We look forward to your continued participation in our journal, and we hope you will consider us again for future submissions.

Kind regards,

Srinivasa Chary Pendota
Review Board Editor
South African Journal of Botany
Follow us on Twitter: https://twitter.com/SajbOf

February 10, 2025

Dear Editor-in-Chief
South African Journal of Botany

We are sincerely grateful for the opportunity to revise and improve our manuscript titled "Potency of Kalix Rosella as a Candidate for Anti-obesity Based on In silico and In vitro with Mesenchymal Stem Cells from Human Umbilical Cord" with Manuscript Number, SAJB-D-24-03770, in accordance with the comments and suggestions provided by the reviewers. We greatly appreciate the constructive feedback, which has helped us enhance the quality and clarity of our work.

In this revised version, we have carefully addressed all the reviewers' comments and incorporated the suggested revisions. Furthermore, we have undertaken professional proofreading by a certified expert to ensure that the language and presentation meet the high standards of your esteemed journal.

We extend our heartfelt thanks to you and the reviewers for the thorough evaluation, thoughtful guidance, and the valuable opportunity to improve our manuscript. It is an honor for us to have the chance to contribute to your prestigious journal.

We hope that this revised version meets your expectations and look forward to your favorable consideration. Please feel free to contact us if any additional information or further revisions are required.

Thank you once again for your generous support and guidance.

Sincerely,

Irena Ujianti
Department of Medical Physiology
Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. HAMKA
Jakarta, Indonesia
irenaujianti@uhamka.ac.id

Dear Reviewer #1,

Thank you for your insightful feedback and recommendations to improve our manuscript, titled *"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."*

As per your suggestions, we have thoroughly revised the manuscript. The following changes have been implemented:

1. **Typographical and Grammatical Error Corrections:**
    - We have meticulously reviewed the manuscript and addressed all identified typographical and grammar issues. To ensure the highest language quality and clarity, we also sought the assistance of a professional proofreading service. Attached to this submission is the proofreading certificate as evidence of this process.
2. **Revisions in Content and Language:**
    - The introduction, methods, results, and discussion sections were carefully reviewed to enhance coherence, readability, and alignment with scientific writing standards.
    - Redundant sentences and repetitive phrases have been removed, while new formulations were added to improve the logical flow of ideas. For example:
        - Ambiguous phrases such as "*RosellRosella calyx extract showsshowed promising potential*" were clarified to "*Rosella calyx extract has shown significant potential...*".
        - Paragraph transitions and connections between the results and their interpretations were strengthened.
3. **Structural Improvements:**
    - Key sections were reorganized to improve readability and scientific rigor. For instance, the methods section was refined to clearly delineate the in silico and in vitro procedures.
    - Tables and figures were revisited to ensure accuracy and consistency with the text, including captions and descriptions.
4. **Technical Improvements to Scientific Validity:**
    - Terminologies and acronyms such as PPARG, SIRT1, and CREB1 were explicitly defined where first mentioned to ensure clarity for a diverse readership.
    - Statistical results linked to the figures (e.g., Figures 5 and 6) were refined for better presentation and correlation to the narrative in the discussion.
5. **Proofreading Certification:**
    - To further enhance the manuscript, a professional proofreading service reviewed the document. We believe their involvement has addressed any remaining linguistic inconsistencies comprehensively. The certificate for this service is included as part of our submission.

We are confident that the revisions have significantly improved the overall quality of this manuscript in terms of both scientific rigor and language accessibility. Your constructive feedback was invaluable in achieving these improvements.

Once again, we thank you for your effort in reviewing our manuscript and providing detailed feedback. Please feel free to provide further input or recommendations to ensure our work meets the highest standards of publication.

Sincerely,

Irena Ujianti
On behalf of all authors

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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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. Obesity is a serious health condition with aan increasing global prevalence that continues increase globally.. The pathogenesis of obesity involvesincludes multifactorial interactions between genetic, environmental, and behavioral factors. The current therapy for obesity encompassesuses a multidisciplinary approach, but often leads to undesirable side effects. Therefore, potential natural alternatives such as anti-obesity agents are needed.

Objective: This research aimsstudy 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).to comprehensively examine the potential of Rosella calyx extract (Hibiscus sabdariffa) in preventing adipocyte maturation using an in silico and in vitro approach.

Method: The content of the 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 involved. Furthermore, the effect of the Rosella extract on the differentiation of adipocytes from mesenchymal stem cells fromin the human umbilical cord (hUCHUC-MSCs) was evaluated *in vitro*.

Result: The GC-MS results showed that GC-MS identified four major compounds in the RosellRosellae extract, namely Triacetin, Pentadecanol, Phenol, and Propionic Acid. Based on *inIn silico* analysis showed that, 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 the Rosella extract effectively inhibited the differentiation of human umbilical cord stem cell-derived adipocytes at a concentration of 250 µg/ml.

Conclusion: Rosell: Rosellae calyx extract showsshowed promisingsignificant anti-obesity effects by inhibiting adipocyte differentiation pathways, primarily targeting PPARGthrough the inhibition of adipogenesis, with PPARG and SIRT1 as the main mediators. These findingsresults provide a scientific basis for the development of Roselle extract as a potential therapeutic agent in the management of obesity.

Keywords: calyx rosella; ethanol extract; *H ibiscus sabdariffa*; *in silico*; *in vitro;* adipogenesis; stemcell

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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INTRODUCTION

Obesity remains a pressing global health issue with increasing prevalence over the years.~~Obesity is a serious and complex health condition that continues to affect more people worldwide.with an increasing global prevalence~~ (Mahojan et al., 2021~~)~~) .~~It~~ occurs when there is an imbalance between ~~the~~ energy ~~we consume~~intake and ~~the energy we use~~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 ~~like~~such as type 2 diabetes, high blood pressure, heart disease, and metabolic syndrome.~~((~~Silveirra Rossi, et al., 2023~~)).~~ The development ~~of obesity involves~~entails a combination of genetic, environmental, and behavioral factors~~ that contribute to this fat buildup.~~. Adipose cells play a ~~vital~~crucial role, ~~not just~~but as storage units~~, but also as~~ and endocrine organs that release hormones and other substances ~~that can~~with a significant influence on appetite, metabolism, and insulin function. This can lead to insulin resistance, increased inflammation, and abnormal cholesterol levels.~~((~~Piché, M., et al., 2021~~)).~~ Effective management of obesity requires a multifaceted approach, including dietary changes, increased physical activity, behavioral interventions, medications, and in some cases, bariatric surgery.~~((~~Baker, J.S., et al., 2022~~)).~~ The ~~goal~~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 ~~like~~such as phentermine and topiramate work by altering brain chemistry to reduce hunger and increase feelings of fullness.~~((~~Pati, B., et al., 2023~~) Orlistat~~). Another drug, orlistat inhibits enzymes that break down fat, reducing ~~its~~the absorption and overall calorie intake.~~((~~Katimbwa, D.A., et al., 2023~~)).~~ Other drugs, such as metformin, enhance insulin sensitivity and decrease glucose production, contributing to weight loss.~~((~~Yerevanian, A., & Soukas, A. A., 2019). ~~Hormone injections like~~Injections such as GLP-1 analogs mimic natural hormones that regulate appetite.~~((~~Kokkinos, A., et al., 2019~~)).~~ However, these medications often have undesirable side effects, ~~which is why~~necessitating the need for a safe, natural alternative~~ is needed~~.

Hibiscus sabdariffa, also known as ~~Roselle~~Rosella , shows potential as ~~an~~ anti-obesity agent due to ~~its~~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, I., et al., 2023~~)).~~ *In vitro* ~~research on Roselle calyx has demonstrated its~~studies have showed the plant effectiveness in reducing plasma lipid levels and promoting weight loss. ~~These~~The bioactive compounds play a role in regulating the biomolecular pathways ~~involved in~~associated with the prevention of obesity and inflammation.~~ (~~Ujianti, I., et al., 2022~~)).~~

Although Roselle's anti-obesity effects are well-supported, studies specifically targeting its influence on adipocyte maturation remain~~Studies related to the prevention of adipocyte maturation by Roselle~~Rosella~~ extract are still~~ limited. To bridge this gap~~Therefore~~, this study ~~aims~~aimed to comprehensively examine the application of ~~Roselle~~Rosella calyx extract on stem cells derived from the umbilical cord to prevent adipocyte maturation. ~~This step is crucial~~

as a The results will offer insights into prevention strategystrategies to counter excessive fat accumulation and ultimately reduce the incidence of obesity. To the best of our knowledge, our researchThis study y is the first to directly apply the role of RoselleRosella extract on stem cells with the goal of preventing theirto prevent transformation into mature adipocytes. InnovativeThe innovative approach offers a new path in obesity researchstudies and herbal therapy, focusing on the modification of adipogenesis as a strategic intervention target.

METODH¶

METHOD¶

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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 c alyx 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 µL, 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 minute, then increased to 330°C at a rate of 10°C/minute and held for 5 minutes.¶

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In Silico Analysis

Extract Material¶

ByUsing GC-MS, chemical compounds in the ethanol extract of c alixalyx Rosellea 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 µL, 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 minute, then increased to 330°C at a rate of 10°C/minute and held for 5 minutes.¶

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). The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain detailed information on the compound.

Prediction of Compound Potential with Structure- Analysis Relationship (SAR) Approach

SAR is an *in-silico* approach by comparingused to compare the input compounds with compounds that arethose already available in the Way2Drug database. The more similar the compound'scompound structure, the higher the potential value. The, with a cutoff score used isof 0.5. The SAR in this report usesstudy used the Way2Drug PassOnline web server.

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~~. ADMET~~ to make predictions ~~were performed using the Admet Lab 2.0 web server database~~.

Target Prediction and Graph Topology Analysis Protein

SuperPred predicted genes or proteins that rosella extract could target. ~~There are experimental~~Experimental results of a ~~compound's~~compound interaction with a gene in the SuperPred database~~, which~~ 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 ~~is then~~was entered into the STRING ~~database's~~database multiple protein menu. Text mining, experiments, and databases ~~are~~served as active interaction sources. The target list from the previous stage ~~is~~was compiled and then inputted into the multiple protein menu from the STRING database (https://string-db.org/) for protein interaction analysis ~~(https://string-db.org/)~~. The visualization results ~~are~~were ~~then imported into~~conducted using ~~the~~ Cytoscape v~~.~~–9~~.~~0 software.

In Vitro Analysis

Mesenchymal Cell Culture and Treatment

~~The~~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~~HUC-MSCs) were cultivated in 12-well culture plates using Amem medium supplemented with 10% Platelet Rich Plasma (PRP). ~~The cells~~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.

~~Prior to~~Before any interventions, a standardized protocol was followed for subculturing ~~the~~ cells. This ~~involved~~entailed rinsing ~~the~~ cells with phosphate-buffered saline (PBS), followed by a 2-minute incubation with 1 mL of 0.05% trypsin-EDTA. ~~The cells~~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 minutes at a temperature of 20°C. The supernatant was discarded, and ~~the~~ cell pellet was resuspended in 1 mL of medium~~.~~ ~~(~~ (Pawitan et al., 2015~~) Cell viability~~). Viability was assessed using Trypan Blue assay~~. Subsequently, the~~, 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. ~~The adipogenesis~~Adipogenesis medium induction was performed and replaced every 2-3 days. The induction of adipogenesis was ~~done~~performed concurrently with the application of sea cucumber extract at concentrations of 250 µg/ml and 350 µg/ml ~~roselle~~Rosella extract~~.~~ (Abdulhamid, Z., et al., 2014~~)~~).

RESULTS

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

As observed in the GC-MS analysis, the ethanol extract from ~~roselle~~Rosella contains four unique compounds, details of which ~~can be referred to~~are shown in Figure 1~~ in the supplementary material~~. These compounds, consisting of Triacetin, Phenol, Propionic Acid, and Pentadecanol, constitute the primary molecules identified in ~~the~~ extract.

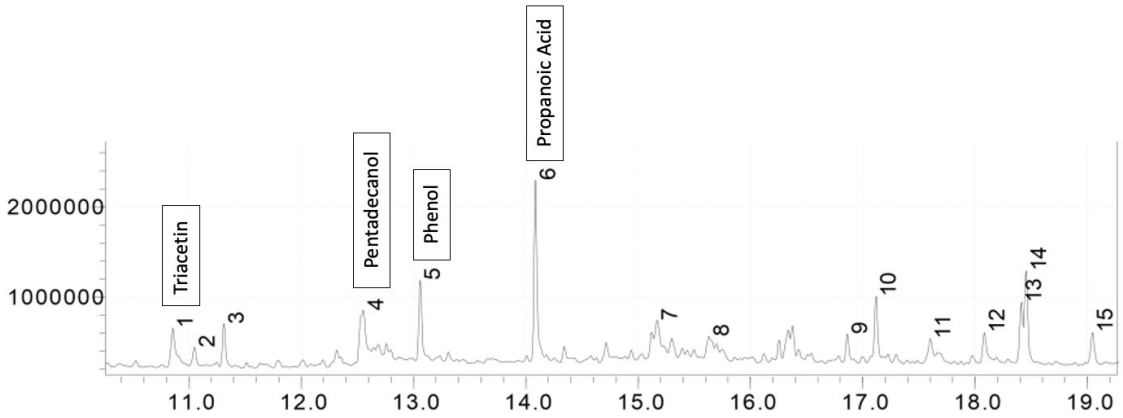

Figure 1. Chromatogram for High-~~-~~Performance Gas Chromatography Analysis of ~~the Roselle~~Rosella Extract

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

Table 1. GC-MS Analysis in Rosella Extract

No.	Compound	Formula	~~Retension~~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

Quantitative Structure-Activity Relationship (QSAR) Analysis

The compounds found in ~~roselle~~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 ~~roselle~~Rosella extract to therapeutic compounds already in the Way2Drug database. The higher the potential value, the more similar the structure of the compound. As shown in Figure 2, ~~roselle~~Rosella extract has ~~demonstrated~~showed potential as an effective treatment for obesity. ~~The extract~~Extract appears to work through multiple pathways to address the complex issue of obesity.

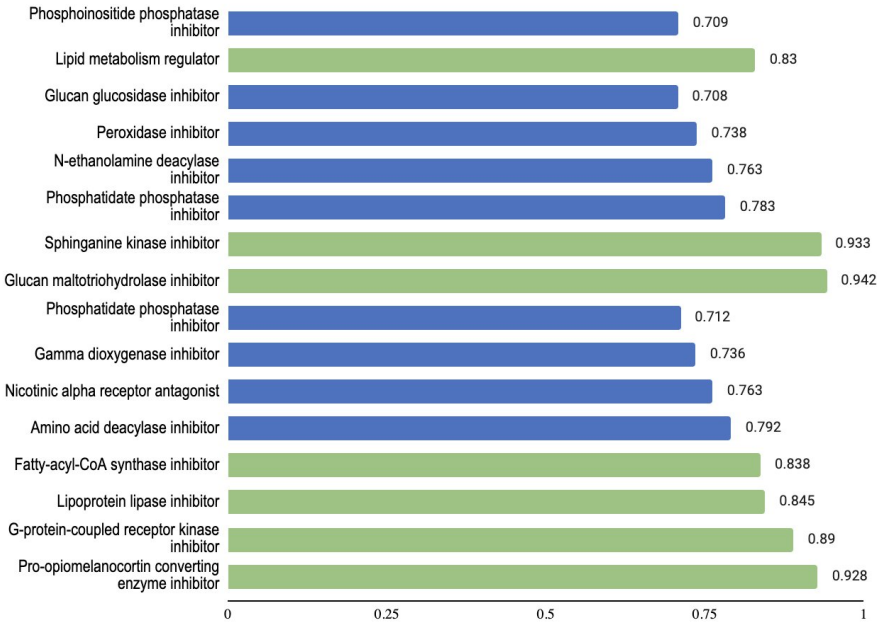

Figure 2. Prediction of Rosella ~~extract~~Extract Potential as Obesity Treatment by Structure Analysis Quantitative (SAR)

Toxicity Analysis Compound

The analysis of the toxicity levels in each bioactive sample, using the AdMet Lab2.0 web server, ~~reveals~~showed that all four compounds present in the sea cucumber extract adhere to Lipinski's rule, as ~~illustrated~~shown in Figure 3~~4~~.

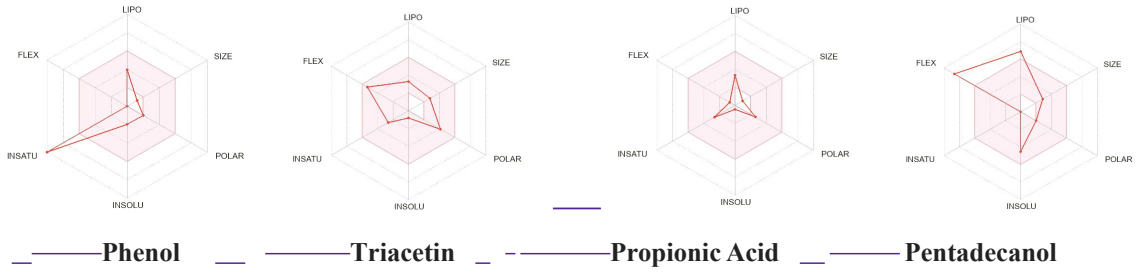

Figure 34. Toxicity Analysis Compound of ~~Rosella's~~Rosella Potential as an ~~Antiobesity~~Anti-obesity Agent

Predicting Protein Targets

Figure 4~~5~~ ~~depicts~~shows the target pathway network for obesity treatment using sea cucumber. In this network, Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) is a key gene that serves as a central interaction point for various proteins ~~involved~~ in the process of obesity development. The network captures the complexity and interconnectedness of metabolic signaling pathways.¶

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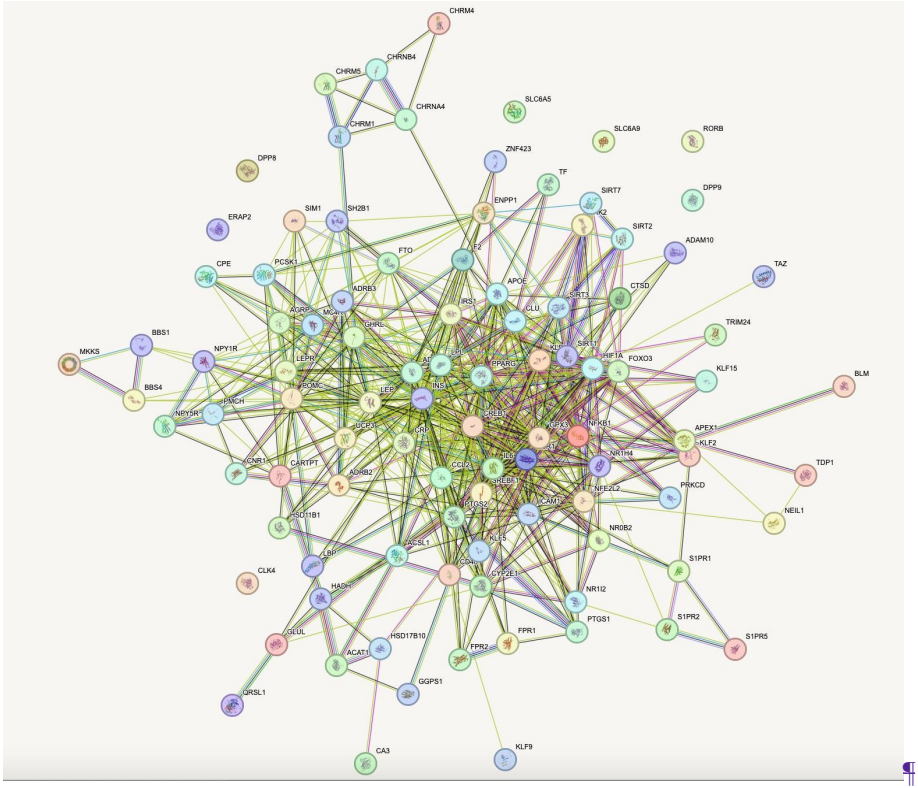

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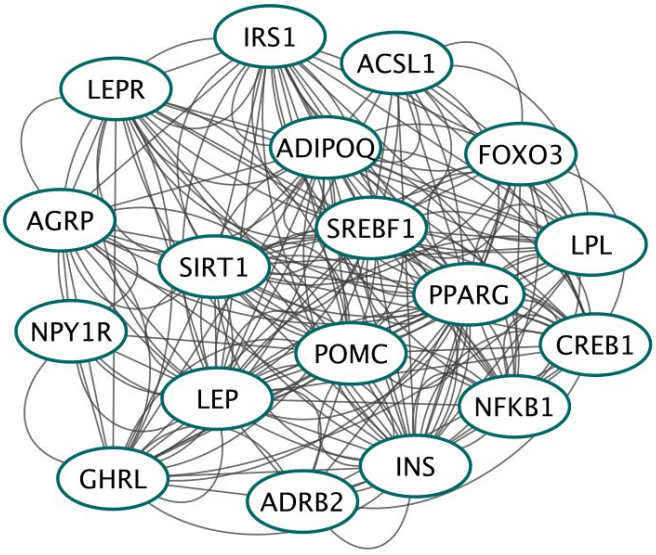

Figure 45. Protein Interaction Network of Metabolism-Related Genes

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. ¶

~~Protein--protein interaction (PPI) networks constructed in STRING (A) and Cytoscape (B).~~

Table 24 presents a network analysis for several obesity-related genes. PPARγ, LEP, and INS have high Betweenness Centrality values, ~~indicating they play~~showing an

important role in connecting various components within the network. These genes also have high Closeness Centrality values, suggesting ~~they are closely connected~~a close connection to other genes in the network. Meanwhile, NF-κB1 has a high Clustering Coefficient, ~~indicating it is involved~~showing a significant role in the formation of interconnected gene groups. ~~Additionally,~~ PPARγ, LEP, and INS also have high Degree, ~~meaning they are connected~~suggesting connectedness to many other genes in the network~~, and~~. NF-κB1 has a high Topological Coefficient, ~~indicating it has~~showing many connections with other highly connected genes.

Table 24. Topological parameters of 8 interconnected targets ~~of~~ 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

Figure 5~~6~~ shows the molecular function and KEGG Pathway analysis for the genes ~~involved in the~~associated with obesity process. This analysis can provide information about the molecular functions and metabolic pathways associated with ~~these~~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.¶

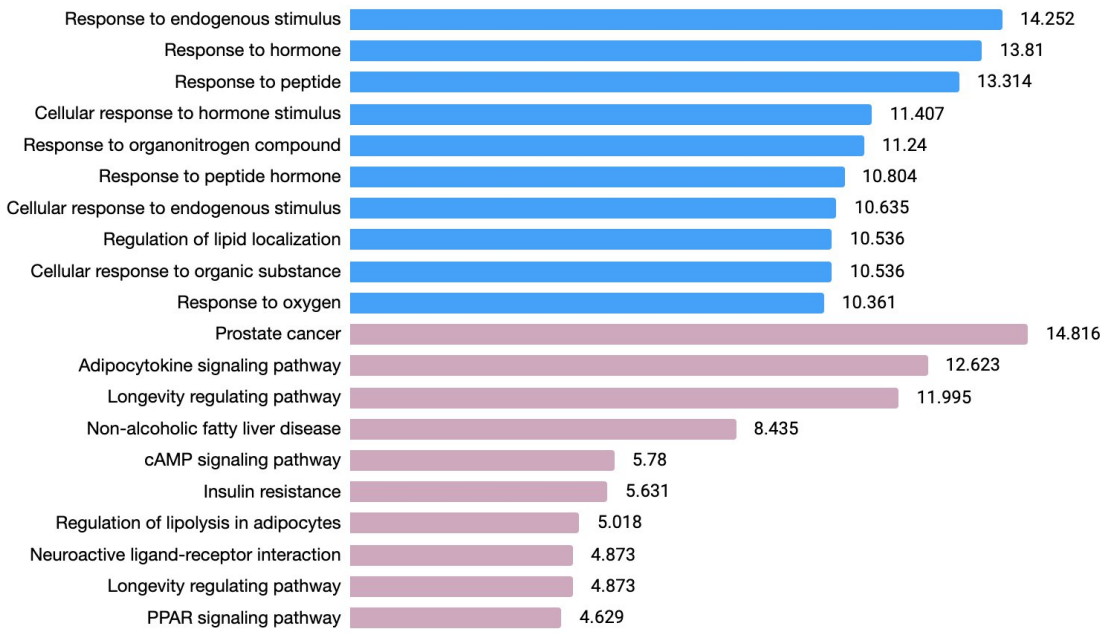

Figure 5.~~6~~: Molecular Function and KEGG ~~pathway~~Pathway Analysis.Enrichment analysis of gene sets highlighting biological processes (blue bars) and pathways (pink bars) related to the inhibition of adipogenesis by Rosella extract. ¶

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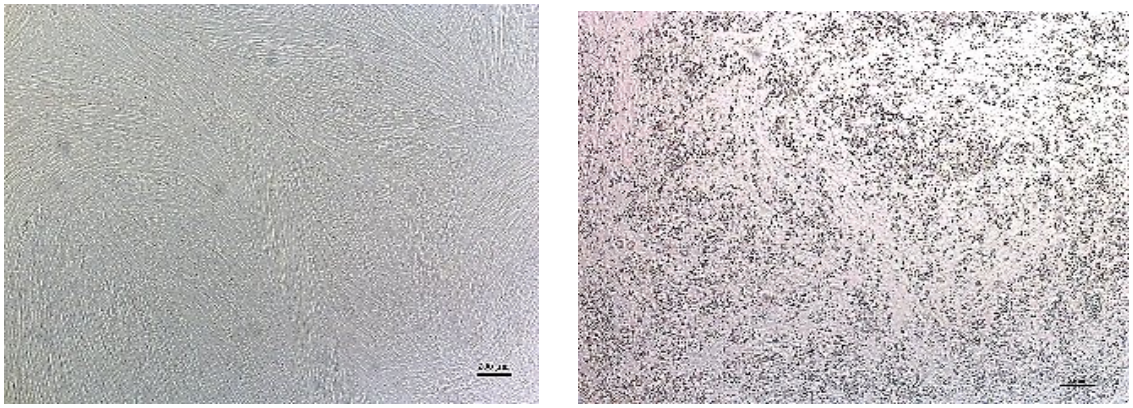
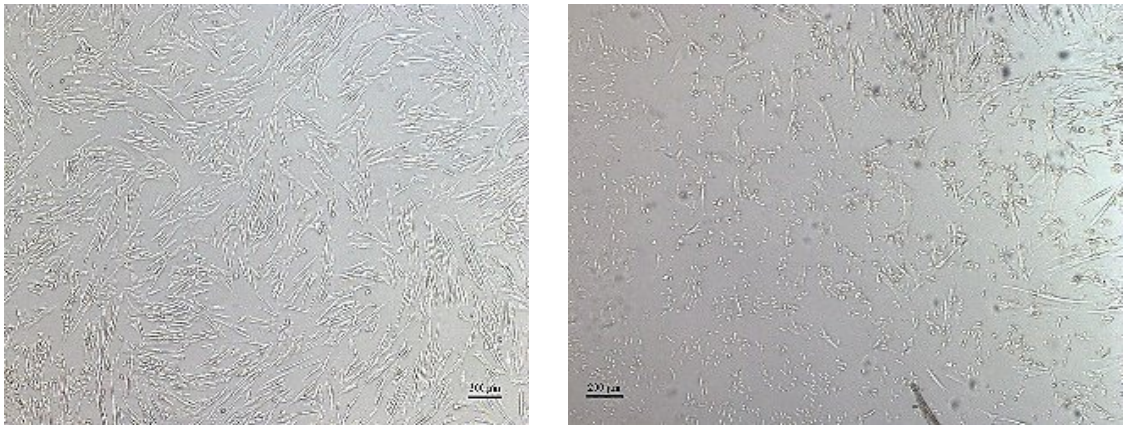
In Vitro Analysis

~~In~~As shown in Figure 6, there are observable differences in the morphology between the positive control (A) and the treatment (C and D). Image A ~~displays~~shows a higher level of cell density differentiation. Conversely, C ~~exhibits~~has a lower cell density compared to A.



A B

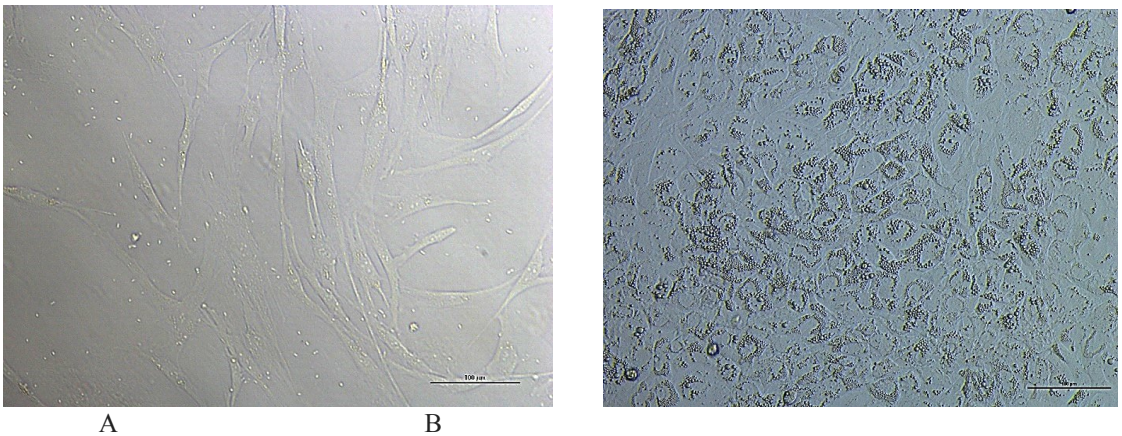
~~B~~



C D ~~D~~

Figure ~~6~~6. (A) Positive control with differentiated medium, (B) Negative control with undifferentiated medium; (C) Differentiated medium with 250 µg/ml rosella extract; (D) Differentiated medium with 350 µg/ml rosella extract. Treatment duration: 14 days. Scale bar represents 100 µm in magnification 4x

In Figure 7 with 20x magnification, the positive control (A) shows the morphology of mature adipocyte cells after ~~the~~ adipogenesis induction process~~; the cells~~. 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, ~~the~~ cells appear larger in size compared to ~~the~~ cells before differentiation into adipocytes, compared to the negative control (B). This ~~indicates~~shows an increase in volume and lipid accumulation within the cytoplasm during the adipocyte maturation process. Meanwhile, ~~the~~ stem cells given adipogenesis induction with 250 µg/ml rosella extract (C) and 350 µg/ml rosella extract appear to have smaller adipocyte morphology and do not develop optimally compared to normal adipocyte cells. ~~They~~Cells have an irregular structure and do not ~~exhibit~~show the typical morphology of mature adipocyte cells. The accumulation of lipid droplets in the ~~cell~~ cytoplasm ~~is~~was also inhibited.



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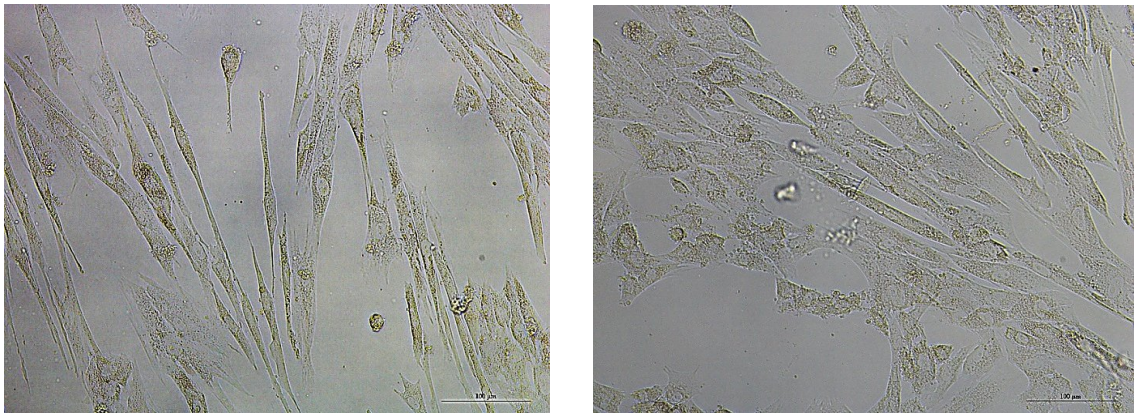

C D

Figure 7~~7~~. (A) Positive control with differentiated medium, (B) Negative control with undifferentiated medium; (C) Differentiated medium with 250 µg/ml rosella extract; (D) Differentiated medium with 350 µg/ml rosella extract. Treatment duration: 14 days. Scale bar represents 100 µm in magnification 20x

Based on the data shown in ~~the figure~~Figure 8~~7~~, the negative control treatment had a viability percentage of 99.33%, while the positive control treatment had a viability percentage of 98.67%. ~~The roselle~~Rosella treatment with a concentration of 250 µg/ml had a viability percentage of 96.67%. ~~The roselle~~Rosella treatment with a concentration of 350 µg/ml had a lower viability percentage of 77.33~~%. The roselle 350 µg/ml treatment had a significantly lower viability~~ %compared to the negative control.

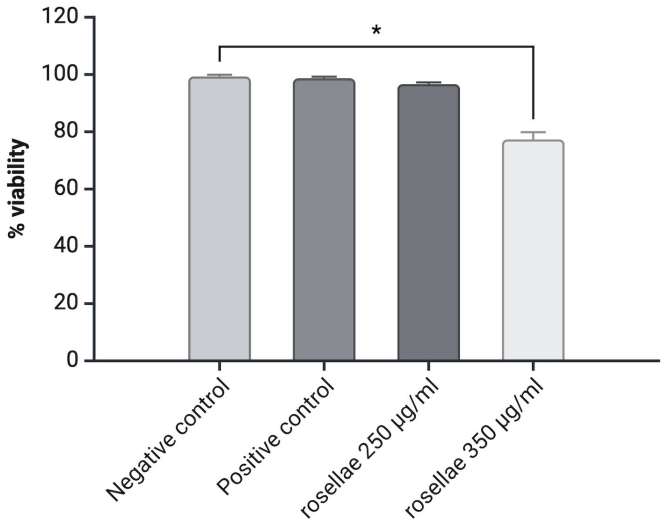

Figure 8~~8~~. Viability of Human Umbilical Stem cells with induction of adipogenesis at various concentrations in 14 days

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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 ~~its~~the ability to inhibit the maturation of fat cells, is still limited~~.~~ (Singab, B., et al., 2024) (Ujianti, I., et al., 2023~~)~~). This ~~research uses~~study used tissue pharmacology to explore and predict the potential active components and underlying mechanisms of rosella in addressing obesity. ~~Furthermore, the study will validate these analyses~~Validation was carried out through in vitro functional tests and ~~examine~~ the morphological changes in stem cells ~~induced~~ to ~~undergo~~induce adipogenesis~~. The study found~~ were examined. GC-MS, which identified Triacetone, Pentadecanol, Phenol, and Propionic Acid in ~~Roselle~~Rosella extract, ~~confirming~~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 ~~highlights~~shows the significance of basic cellular processes~~.~~ (Tomkins, J.E., & Manzoni, C., 2021~~)~~). Inhibiting a protein within the PPI network can disrupt multiple mechanisms. In this study, PPARγ, LEP, INS, and ADIPOQ were found to be the top five targets in the constructed PPI network. CREB1 can interact with PPARγ, INS, ADIPOQ, and SIRT1. Inhibiting CREB in relation to PPARγ has a substantial impact on the process of adipogenesis and adipocyte maturation. CREB1 is typically active in the early stages of adipogenesis, stimulating the expression of PPARγ and C/EBPβ, before PPARγ takes over to complete adipocyte differentiation~~.~~ (Ambele, M.A., et al., 2020~~)~~). When PPARγ is inhibited, the disruption of the transcriptional cascade inhibits the expression of adipocyte-specific genes. ~~As a result, the~~Consequently, cells ~~can be~~are trapped in the preadipocyte stage, leading to the accumulation of partially differentiated cells. Inhibiting PPARγ can also reduce lipogenesis and alter the adipokine secretion profile~~.~~ (Jakab, J., 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 ~~researcha~~a study on the pharmacology of obesity networks~~.~~ (Wang, Y., et al., 2020~~)~~).

In ~~our~~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, J.S., 2023~~)~~). The cAMP activates two key effectors~~:~~ namely protein kinase A (PKA) and the cAMP-directly activated exchange protein (Epac~~).~~) (Valentine, J.M., 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 PPARγ, which are crucial for adipocyte differentiation~~.~~~~(~~ (Benchoula, K., 2021~~)~~). The ~~findings~~results suggest that rosella suppresses the formation of fat cells by inhibiting the activities of two key proteins, CREB and C/EBPβ. This, in turn, leads to decreased expressions of C/EBPα and PPARγ, as well as other genes ~~involved~~implicated in fat cell development. This is consistent with ~~researcha~~a study conducted by Zhang et al., which showed inhibition of CREB activation during adipocyte maturation, although ~~that researchthe~~ study used berberine extract~~.~~~~((~~Zhang, X., et al., 2019~~)~~). Additionally, ~~research by~~ Aranaz et al. ~~indicates~~reported that polyphenols can decrease the expression of PPARγ target genes ~~involved~~implicated in adipogenesis and lipid metabolism in fat cells.

This result was ~~also~~ strengthened in vitro ~~in our~~ study, ~~where the results of our study showed~~showing that the differentiation process of adipocytes from an immature to a mature form can be stopped by administering ~~an~~ extract to stem cells derived from human umbilical cord. This inhibition was ~~seen to be~~ effective at a concentration of 250 µg/ml, while at a concentration of 350 µg/ml there was a ~~lot~~significant level of cell death. ~~The study conducted by~~ Ray et al. found that the administration of 6-shogaol induced cell death in cancer stem cells. ~~While the~~Although extract was different, ~~it is known that~~ 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-κB and PPARγ~~.~~ (Ray, A., 2015~~)~~). Cell viability appears to decrease at a concentration of 350 µg/ml. Further comparison shows that the study by Ray et al. used the compound 6-shagol, ~~which is~~ part of the active phytochemical components, to induce cell death in cancer stem cells. Meanwhile, this study used ~~roselle~~Rosella extract, which likely contains active phytochemical compounds and ~~shows~~caused cell growth inhibition ~~effects~~ at similar concentrations. ~~Although targeting different subjects, both~~Both studies ~~demonstrate~~show cell growth inhibition effects ~~involving~~related to the activation of NF-κB and PPARγ pathways.

According to ~~our~~the in silico analysis, ~~it appears that the roselle~~Rosella extract has the ability to ~~stop the~~inhibit adipogenesis process through the ~~following~~ mechanism~~: Inhibition~~ of inhibiting adipogenesis. Structural-activity analysis on Rosella extract shows potential as ~~an~~ 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, B. S., & Thiyagarajan, G., 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-γ receptors, which ~~are involved~~play a role in adipocyte maturation~~.~~ ~~(~~ (Haque et al., 2023~~)~~). Overall, the analysis results ~~show~~showed that Rosella extract has a promising prospect as a drug or supplement candidate for addressing the problem of obesity.

The ~~findings~~results of this study have interesting implications for the development of ~~roselle~~Rosella extract as a complementary therapy for obesity management. ~~Its~~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 ~~demonstrate its~~show effectiveness and safety. Further development, including formulation optimization and clinical trials, will be required to realize the full therapeutic potential of ~~roselle~~Rosella extract in obesity management.

~~**Conclution**~~¶

~~**Roselle**~~**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 PPARG pathway.~~Rosella extract has shown~~showed ~~promising anti-obesity effects in both computer simulations and lab experiments. The extract appears~~Extract appeared ~~to work by inhibiting the adipogenesis process, which is the formation of fat cells. Key factors in this anti-~~

~~obesity effect are the proteins PPARG, which play a central role in regulating adipogenesis and lipid metabolism.~~¶

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