Investigating the Effect of Cape Gooseberry (*Physalis peruviana*) Extract on Osteogenic Differentiation

Nitchakan Srithon[1], Piyanee Ratanachamnong[2], and Kamolchanok Ngamkham[1,*]

[1] Biological Engineering Program, King Mongkut's University of Technology Thonburi, Bangkok, Thailand
[2] Department of Pharmacology, Faculty of Science, Mahidol University, Nakorn Pathom, Thailand
Email: nitcha20469@gmail.com (N.S); piyanee.rat@mahidol.ac.th (P.R.); kamolchanok.nga@mail.kmutt.ac.th (K.N.)
*Corresponding author

***Abstract*—Bone defects are a major health issue, affecting millions of patients and reducing their quality of life. The alternative regenerative approaches for bone healing and formation are required due to the limitations of bone graft, a gold standard treatment, which include immune rejection, pathogen transmission, and limited donor supply. One approach that is being explored is to identify factors or natural products that can induce osteogenic differentiation, an important process involved in bone repair. In this study, the potential of methanolic extract of cape gooseberry (*Physalis peruviana*) on the induction of osteogenic differentiation was investigated. Total phenolic and flavonoid contents of cape gooseberry crude extract were found to be 1.874 mg QE/g and 0.037 mg GAE/g respectively. The cytotoxicity of cape gooseberry extract at the concentration of 10, 25, 50, and 100 µg/ml were tested by MTT assay. The results showed that at the concentration of 10, 25, and 50 µg/ml, the extract was not toxic to the cells and the highest cell viability was observed at concentrations of 10 and 25 µg/ml. Osteogenic differentiation was assessed using two osteogenic assays, Alkaline Phosphatase (ALP) activity and alizarin red staining. The results indicated that cape gooseberry extract can induce the activity of ALP enzyme and the deposition of calcium in the matrix as quantified from alizarin red staining at all time points (7, 14, and 21 days). The ALP induction was significantly higher than both control and differentiation (osteogenic induction) groups. The calcium deposition was significantly increased when the cells were treated with cape gooseberry extract. Furthermore, it was discovered that 25 µg/ml was the extract's most effective concentration. According to all of the findings, cape gooseberry shows promise in promoting osteogenic differentiation, making it a potential candidate for bone tissue regeneration applications.**

***Keywords*—cape gooseberry, *Physalis peruviana*, osteogenic differentiation**

I. Introduction

Bones are vital components of the human body, providing structural support, protecting organs, and facilitating movement. Despite their strength and resilience, bones can fail due to various factors such as falls or accidents can cause fractures when the force applied to the bone exceeds its strength. Osteoporosis is another bone defect commonly seen in older adults, that leads to weak bones due to loss of density and deterioration of bone tissue. Additionally, nutritional deficiencies, aging, and genetic factors can also affect bone strength and increase the risk of fractures. The most common regenerative approach for bone repair is the utilization of autologous bone grafts [1]. However, this option is frequently constrained by limited availability and significant donor-site morbidity.

Osteogenic differentiation, the process by which undifferentiated cells transform into specialized bone-forming cells called osteoblasts, is essential for bone repair and plays a central role in healing fractures and injuries. After a bone injury, the inflammatory response brings signaling molecules and drives its osteogenic differentiation. Differentiated osteoblasts produce bone matrix proteins, such as collagen and facilitate mineral deposition, forming new bone during the repair [2].

Many herbs and plant extracts have been used in traditional medicine for centuries due to their potential therapeutic properties. These natural compounds often contain a variety of bioactive molecules such as polyphenols, flavonoids, terpenes, and alkaloids. They represent a promising alternative due to their easy accessibility and lower cost compared to synthetic counterparts. However, the mechanism of action of these herbal ingredients still requires further study. Currently, there are many natural bioactive compounds that have been reported to promote osteogenic differentiation, e.g. astragalin, naringin, gallic acid etc. [3–5] As a result, scientists are still investigating potential plant extracts that may promote osteogenic differentiation.

Cape gooseberry is scientifically named *Physalis peruviana*, also known by various names such as goldenberry, Peruvian groundcherry, or pichuberry. It is a small, round fruit enclosed in a papery husk. Cape gooseberries are a good source of vitamins A, C, and B-complex, as well as minerals such as iron and phosphorus [6]. The fruit contains various antioxidants,

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including polyphenols and carotenoids, which have health benefits. The medicinal uses of this fruit have been confirmed in cancer, leukemia, diabetes, ulcers, malaria, asthma, hepatitis, dermatitis, rheumatism, and several other diseases [7, 8]. Moreover, its antioxidant and anti-inflammatory properties can reduce oxidative stress on membrane lipids in astrocytic cells, potentially by lowering intracellular ROS levels, suggesting its potential as a future drug for treating neurodegenerative diseases [8]. Cape gooseberry is rich in phytochemicals and could potentially have a positive effect on osteogenic differentiation since it contains apigenin, gallic acid, naringin, and kaempferol which are compounds that have been shown to be beneficial in osteogenic differentiation in the past [4, 5, 9, 10]. Therefore, cape gooseberry might be able to support osteogenic differentiation and enhance the activity of osteoblasts through the induction of gene expression related to differentiation and ossification. However, the application of cape gooseberry extract in osteogenic differentiation has not been documented. Hence, the aim of this study is to verify the effect of cape gooseberry extract on promoting and enhancing osteogenic differentiation.

II. Materials and Methods

A. Preparation of Cape Gooseberry Extract

Fresh cape gooseberry (*Physalis peruviana*) fruits were obtained from the Royal Project Foundation's Doi Kham shop. The fruits were carefully cleaned and chosen for the largest size. The extraction process adhered to the methodology outlined by Roy and colleagues (2019) [11]. To create a powdered sample, the fruits were mixed, placed in an ice-water bath, and vacuum-dried for 72 hours at −52ºC. With sporadic shaking, the resultant powder was soaked in 500 milliliters of methanol (MeOH). After filtering the mixture through Whatman No. 1 filter paper, a rotary evaporator was used to obtain the concentrated extract by removing the methanol. The extract was stored at -20ºC for use in further experiments.

B. Quantification of Total Flavonoid Content (TFC)

Total flavonoid content was measured using a colorimetric assay. Quercetin standard solutions were prepared ranging from 0 to 250 µg/mL in 96% ethanol using two-fold dilutions. In a microplate, 50 µl of the extract (1 mg/ml) or standard solution was mixed with 10 µL of 10% aluminum chloride solution. Then, 150 µL of 96% ethanol was added to each well. After thorough mixing, the reaction mixture was incubated in the dark at room temperature for 40 mins. The absorbance was then measured at 415 nm using a microplate reader. Total flavonoid content was quantified and expressed as milligrams of Quercetin Equivalents (QE) per gram of crude extract (n = 4) [12].

C. Quantification of Total Phenolic Content (TPC)

Total phenolic content of the extract was determined using the Folin-Ciocalteu assay. Gallic acid standard solutions were prepared ranging from 0 to 250 µg/mL in 70% ethanol using two-fold dilutions. In a microplate, 25 µL of a 1 mg/mL extract was mixed with 100 µL of a Folin-Ciocalteu reagent. The mixture was allowed to stand for 4 mins, then 75 µL of sodium carbonate solution (100 g/L) was added shaken for 1 minute. After 2 hours at room temperature, the absorbance was measured at 765 nm using a microplate reader. Total phenolic content was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of extract (n = 4) [12].

D. Cell Culture and Cell Seeding

MC3T3-E1 pre-osteoblast cells were cultured in alpha-modified Minimal Essential Medium (α-MEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotic-antimycotic. Pre-osteoblasts were incubated at 37°C in a humidified atmosphere of a 5% CO_2 incubator and the culture medium was changed every 3–4 days. When the cells reached 80-85% confluence, they were sub-cultured. Old culture medium was removed, and the cells were washed with PBS once. Trypsin-EDTA solution (2.5%) was added to the culture flask to cover the adherent cells and incubated at 37°C for 5 mins. New complete growth medium was then added to the flask and mixed with the detached cells. The cell suspension was collected into a tube and centrifuged at 1,500 rpm for 5 mins. The supernatant was removed, and 1–2 mL of medium was added to resuspend the cell pellet. The cells were then counted and prepared for the experiments or aliquoted to a new flask for further expansion. The osteoinductive medium was composed of alpha-modified Minimal Essential Medium (α-MEM) supplemented with 10% Fetal Bovine Serum (FBS) and an antibiotic-antimycotic solution, along with 50 µg/mL ascorbic acid, 10 nM dexamethasone, and 5 mM β-glycerophosphate.

E. MTT Assay

The cells were seeded in a 96-well plate at a concentration of 5×10^3 cells/well and incubated for 24 hours. Then, different concentrations of cape gooseberry extract (10, 25, 50, and 100 µg/mL) were incubated with the cells for 1 and 3 days. After incubation, the medium was replaced and 50 µL of the filtered MTT reagent (5 mg/mL in PBS) was added to each well. The plate was incubated for 2 hours at 37°C. After incubation, MTT-containing medium was removed and DMSO was added to each well to elute the formazan crystals. Then, the absorbance was measured at 540 nm.

F. ALP Activity Assay

Alkaline phosphatase is an enzyme expressed by osteoblasts during osteogenic differentiation. After being treated with osteoinductive medium and various concentrations of the extracts from cape gooseberry for 7, 14, and 21 days, the culture medium was removed from the cells followed by PBS washing on the samples. The cells were lysed in lysis buffer and supernatants were collected. ALP activity was measured colorimetrically using p-Nitrophenyl Phosphate (pNPP) as a substrate. 75 µL of substrate and 25 µL of sample were added to 96-well plate and incubated at 37°C to allow the reaction to occur for 30 mins. After this, 100 µL of 1N NaOH was added to

each well to stop the reaction. The absorbance was measured at 405 nm. ALP activity was normalized by the amount of total protein, which were measured using BCA assay kit.

G. Alizarin Red S Staining

Alizarin Red S staining was used to assess calcium deposition in the matrix. MC3T3-E1 cells were stained with Alizarin Red S (ARS) on day 7, 14, and 21 after culture. At each time point, the culture medium was removed from each well and washed once with PBS. After the cells were fixed with 4% formaldehyde and washed three times with dH_2O, they were stained with 2% Alizarin Red S solution for 10 mins. Then, the samples were rinsed with water until the solution became clear. For quantitative analysis, each sample was eluted with 200 µL 10% acetic acid for 30 mins under shaking condition and heated to 85°C for 10 mins, then cooled down at 4°C. After centrifugation at 14,000 g for 15 mins and the pH of the supernatant was neutralized using 75 µL of 10% ammonium hydroxide. 50 µL of each sample was transferred into a 96-well plate to measure the absorbance at 405 nm [13].

H. Data Analysis

Data are expressed as the mean ± standard deviation. The significance difference was evaluated by one-way ANOVA along with post hoc Tukey's multiple comparison test, where n = 4, mean ± SD, $p < 0.05$ (*), $p < 0.01$ (**), $p \leq 0.001$ (***).

III. Result and Discussions

A. Quantification of Total Flavonoid and Phenolic Contents

Flavonoids are a diverse group of polyphenolic compounds with antioxidant properties found in various plants. In this study, the obtained results indicate a flavonoid content of 1.874 mg QE/g, surpassing the value reported in the earlier research (Table I). The ethanolic extract of *P. peruviana* fruit was previously investigated in a study, revealing a determined flavonoid content of 1.48 mg QE/g fresh weigh [14].

The total phenolic content of cape gooseberry extract was determined using the Folin-Ciocalteau reagent and reported in terms of the mg of Gallic Acid Equivalent (GAE) / g of the extract. The results in this study reveal a phenolic content of 0.037 mg GAE/g, indicate a higher concentration of phenolic compounds in the crude extract of cape gooseberry compared to the previously reported value of 0.02624 mg GAE/g fresh weigh [6].

The different amount of total flavonoid and phenolic content could arise from several factors such as extraction methods, differences in the plant material used, such as its geographical origin, ripeness, cultivation practices, harvest time or storage conditions. Variations in environmental factors like soil type, climate, and altitude also affect the synthesis of secondary metabolites, including phenolic compounds [15, 16].

TABLE I. The Flavonoid and Phenolic Content

Plants	Family	Part investigated	Total flavonoid (mg QE/g)	Total phenolic (µg GAE/mg)
Physalis peruviana	Solanaceae	Fruit	1.874	0.037

B. MTT Assay

For this experiment, MC3T3-E1 cells were treated with different concentrations of cape gooseberry extract. The results were collected at 1 and 3 days. At all time points, cape gooseberry extract exhibited cell viability levels above 70%, suggesting that it is not toxic to cells [17].

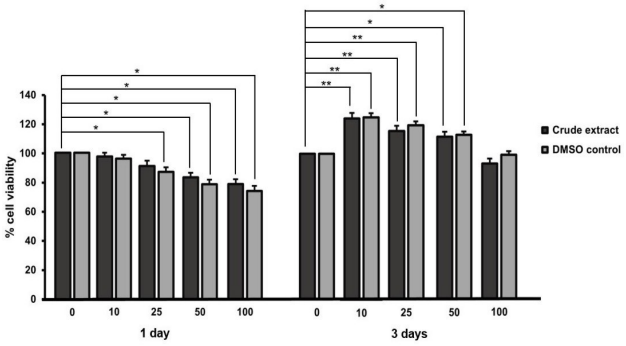

Fig. 1. Cytotoxicity of different concentrations of cape gooseberry extract on the MC3T3-E1 cells. The cells were cultured with 10, 25, 50, and 100 µg/mL extract for 1 and 3 days. All data are presented as the mean ± SD (n = 4).

On day 1, cell viability exhibited a dose-dependent decline, with significant decreases observed at 50 and 100 µg/mL compared to the control, while lower concentrations (10 and 25 µg/mL) maintained viability levels similar to those of the control group. On days 3, treatment with 10 and 25 µg/mL resulted in a significant enhancement of MC3T3-E1 cell viability, followed by 50 µg/mL. In contrast, at a concentration of 100 µg/mL, a significant decrease in cell viability was observed compared to the control (Fig. 1). The increase in cell viability above 100% indicates a stimulatory effect on cell proliferation, which is a promising finding regarding the potential bioactivity of these extracts in promoting osteoblast function and bone health. The observed increase in cell proliferation could be attributed to bioactive compounds present in cape gooseberry extracts, such as polyphenols, flavonoids, or alkaloids. These compounds are known for their antioxidant properties, which may reduce oxidative stress and support cellular growth and repair mechanisms [18]. DMSO control was also performed since the cape gooseberry extract used in the experiment was dissolved in DMSO. The results showed that DMSO at different concentrations did not exhibit a significant difference in cell viability when compared to the extract. The comparison between crude extract-treated and DMSO-treated cells indicates that the viability of crude extract-treated cells remained similar or slightly lower, suggesting that part of the observed effects was due to the solvent [19]. Therefore, the concentrations

of 10 and 25 μg/mL, which have the lowest toxicity were selected for further experiments.

C. ALP Activity

The MC3T3-E1 cells were treated with osteoinductive medium and 10 and 25 μg/mL of extracts for 7, 14, and 21 days. On days 7 and 14, the results indicate that treatment with cape gooseberry extract at 10 and 25 μg/mL, as well as the osteoinduction group (Diff), can enhance ALP activity compared to the control, suggesting a stimulatory effect on osteogenic differentiation. However, a significant increase was observed only at a concentration of 25 μg/mL. On day 21, all cape gooseberry extract conditions resulted in significant increases in ALP activity compared with the control group (Fig. 2).

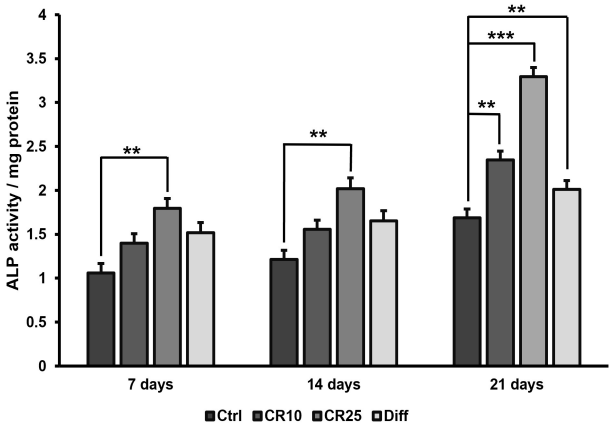

Fig. 2. ALP activity of MC3T3-E1 cells after cultured with cape gooseberry extract at the concentrations of 10 and 25 μg/mL for 7, 14, and 21 days. All data are presented as the mean ± SD (n = 4).

Alkaline Phosphatase (ALP) is an enzyme expressed by osteoblasts during osteogenic differentiation. High ALP activity indicates active osteoblasts and suggests that cells are effectively differentiating toward an osteogenic lineage [20]. The increase in ALP activity might be attributed to the presence of phytochemicals in cape gooseberry extracts, such as antioxidants, flavonoids, and vitamins, which are known to support bone cell metabolism and differentiation. These compounds may influence key signaling pathways, such as BMP/Smad or Wnt/β-catenin, which are critical for osteoblast differentiation and ALP expression [21]. Therefore, further experiments were conducted with 10 and 25 μg/mL concentrations, which showed the greatest increase.

D. Alizarin Red S Staining

For this experiment, MC3T3-E1 cells were treated with 10 and 25 μg/mL of cape gooseberry extract, and results were collected at 7, 14, and 21 days. For the quantitative analysis, alizarin red dye was extracted using acetic acid and neutralized with ammonium hydroxide. The results present the concentrations of ARS-extracted solution from the staining of calcified nodules in MC3T3-E1 cells. On day 7, the highest calcification was observed in the differentiation condition (osteoinductive medium), while no calcification was detected in the other conditions.

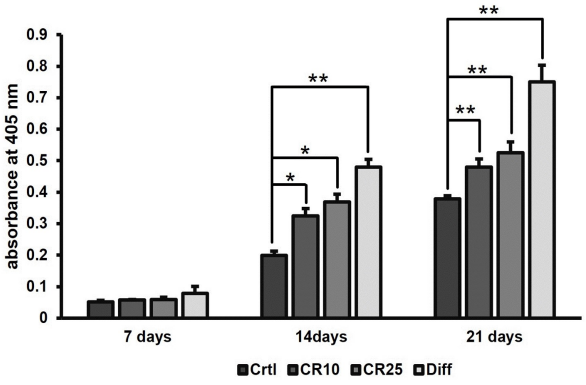

Fig. 3. Alizarin red staining of MC3T3-E1 cells treated with cape gooseberry extract at the concentration of 10 and 25 μg/mL for 7, 14, and 21 days. All data are presented as the mean ± SD (n = 4).

On days 14 and 21, the differentiation condition (Diff) exhibited the highest level of calcification, followed closely by the 25 and 10 μg/mL treatment groups. Both treatment conditions demonstrated significantly higher calcification levels compared to the control (Ctrl), highlighting their potential effectiveness in promoting mineralization (Fig. 3). Our findings indicate that the crude extract of cape gooseberry promotes calcium deposition during bone mineralization. This result may be attributed to the phenolic compounds or flavonoids present in cape gooseberry crude extract, such as naringin, kaempferol, gallic acid [4, 10, 22]. A previous study reported that naringin, a component of cape gooseberry crude extract, enhances mineral accumulation and calcium deposition in a dose-dependent manner when MC3T3-E1 cells are cultured for 14 days with 0, 10, 50, and 100 μg/mL of naringin [4]. Furthermore, it has been reported that MC3T3-E1 cells treated with kaempferol induce the formation of calcified nodules that stain a deep red compared to the control [23]. A previous study determined that gallic acid can increase the number of mineralized nodules after 20 days of treatment [22].

IV. Conclusion

In this study, cape gooseberry (*Physalis peruviana*) fruit was extracted, and the total phenolic and flavonoid contents were quantified, measuring 0.037 mg GAE/g and 1.874 mg QE/g, respectively. The results of the MTT assay revealed that cape gooseberry extracts were not toxic to the cells at concentrations of 10, 25, and 50 μg/mL. The highest viability was observed at 10 and 25 μg/mL. Cape gooseberry extract was also able to induce osteogenic differentiation of MC3T3-E1 cells, as indicated by ALP activity and alizarin red staining. The extracts significantly induced the activity of the ALP enzyme and promoted calcium deposition in the matrix. In addition, the most effective concentration of the extract was found to be 25 μg/mL. Further investigation is necessary to understand the molecular biological mechanisms underlying the effects of cape gooseberry.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NS conducted the research and wrote the paper; PR extracted cape gooseberry; KN initiated the study and wrote part of the paper; all authors had approved the final version.

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