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The Effect of *Muntingia calabura* L. Leaves Methanolic Extract in Increasing of Collagen Production

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Abstract. Skin elasticity loss is the certain process that happen along with the age. Dermis fibroblast cells decrease the amount of collagen secretion which make a wrinkled appearance. *Muntingia calabura* L. has a high phenolic content that helps in decreasing oxidative stress thus intended increasing collagen production. This study aimed to evaluate the effect of *M. calabura* leaves methanolic extract (MLME) in increasing the amount of collagen production in 3T3 fibroblast cells culture. Tests were performed using the alumunium chloride ($AlCl_3$) colorimetry assay and ELISA assay. The value of total flavonoid content (TFC) obtained through the $AlCl_3$ colorimetry assay is 584.147 ± 20.299 mg QE/100 gram dry weight, while testing for the amount of collagen secreted with ELISA assay showed that MLME statistically had significant effect ($p < 0.05$) in increasing procollagen-1-propeptide N-terminal (P1NP) of 3T3 fibroblast cells culture based on dose-dependent manner.

INTRODUCTION

Aging is the process that occurs over the time which can lead to loss of a skin elasticity and bring a wrinkled appearance [1]. Decreasing of collagen production in the skin is one of the most causes that affect the thinning process of the connection between the epidermis and dermis therefore contributes to the strenght and viscoelasticity of the skin tissue [2,3]. The decrease of collagen in the skin occurs as a result of oxidative stress which is continuously accured in the cell. Hence the appearing of wrinkle is the certain process that surely happen even so lifestyle makes everyone to have the desire to always look younger [3]. Anti-aging agents play an important role in fulfilling everyone's desire, so the search for new source of anti-aging agent continuously to be done. Phenols, flavonoids, alkaloids, and tocopherols always to be used in formulation of the anti-aging agent due to their ability to suppress free radicals thus help in elevating collagen production [4,5,6].

Muntingia calabura L. is the member of *Muntingiaceae* family that has high adaptability and is found in almost all tropical regions [7]. *M. calabura* has high phytochemical content including phenols, flavonoids, tannins, steroids, and triterpenes compounds, however phenols and flavonoids are the highest secondary metabolites contained in the *M. calabura*'s parts, especially leaves extract [8,9,10,11]. The number of phenols and flavonoids in *M.calabura* leaves extract causes *M.calabura* to have high antioxidant and anti-inflammatory activity [10,11,13]. Previous study had shown that *M. calabura* leaves methanolic extract has an ability to be able to accelerate the healing process of gastric lesions through its free radical scavenging activity [11].

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Previously, we reported the presentation that *M.calabura* not only suppresses the production of reactive oxygen species (ROS) in gastrointestinal tissue cells, but also be able to suppress free radicals in 3T3 fibroblasts cell culture thus decreased oxidative stress [13]. However, the ability of *M.calabura* leaves methanolic extract in increasing the amount of collagen production in 3T3 fibroblast was unknown. We then continued the study to evaluate the effect of *M. calabura* leaves methanolic extract (MLME) in increasing the amount of procollagen type I production in 3T3 fibroblast cells culture.

MATERIAL AND METHODS

Preparation and Extraction

Collection of *M. calabura* leaves was conducted in October – November 2017 from Wonorejo, Central Java, Indonesia. Fresh *M. calabura* leaves were air-drying for about two weeks and grinded into a fine powder. Leaves powder (100 g) was soaked in MeOH at a ratio of 1:20 (w/v) for 72 hours then filtered using a filter paper [9,10,11]. The filtered mixture was pooled together and evaporated using a rotary evaporator at 40 °C to obtain *M. calabura* leaves metanolic extract (MLME).

Determination of Total Flavonoid Content

Determination of total flavonoid content (TFC) was performed using alumunium chloride ($AlCl_3$) colorimetric assay [14,15]. A total of 500 µL of MLME (500 µg mL^{-1}) were mixed with 1500 µL of methanol analytic and 100 µL of $NaNO_2$ 5%. The mixture was incubated for 5 minuted at room temperature (27 ± 2ºC) then added 100 µl of $AlCl_3$ 10% and allowed to stand it for 6 minutes. The mixture was added 2 mL of NaOH 1 M and distilled water until reached a volume of 10 mL. The mixture was left to stand for 15 minutes. Absorbance was measured using a UV-Vis spectrophotometer at 510 nm. The absorbance measuring results were calibrated to the standard curve obtained from the absorbance reading of quercetin with a concentrations of 20, 40, 60, 80, and 100 µg mL^{-1}.

Cell Culture and Treatment

3T3 fibroblast cell line were cultured in a complete medium consisting of DMEM (Gibco®) and FBS (Gibco®). 3T3 fibroblast secondary subculture (density 5 x 10^3) then distributed into 96 well-plate and incubated at 37°C in a CO_2 incubator for 24 hours. Culture medium were discarded and replaced with a complete medium that had been mixed with the sample then incubated overnight. Variations in sample concentration used were 5, 10 and 20 µg mL^{-1} based on our previous study [13]. The previous medium was replaced with a new culture medium for subsequent incubation at 37 °C in the CO2 incubator for 48 hours.

Determination of Procollagen-1-Propeptide N-terminal

Supernatant of the cell culture was subjected to the determination of procollagen-1-propeptide N-terminal (P1NP) using an ELISA assay. The assay was performed according to the Elabscience® Mouse Procollagen-1 N-Terminal Propeptide ELISA Kit's protocol.

Data Analysis

The data of procollagen-1-propeptide N terminal was analysed using IBM SPSS Statistic 23. One-way analysis of variance (ANOVA) and Duncan's multiple range test were used to evaluate the significance ($p < 0.05$) between samples and controls.

RESULT AND DISCUSSION

Determination of Total Flavonoid Content

The total flavonoid was expressed as quercetin equivalent (QE) per 100 gram dry weight. The value obtained of TFC is 584,147 ± 20,299 mg QE/100 g dry weight. Compared to our previous presentation which reported that MLME has a high phenolic contents [13], the value of TFC in this study is considered as a high. The high content of flavonoids in MLME is responsible for the MLME's bioactivity. Flavonoid which is belonging to the phenol compounds has an activity to scavenge free radical because of the presence of hydroxyl groups (-OH) that is capable to work as an electron donors for free radical compounds [2,16,17]. This result is strongly related to the antioxidant ability of *M. calabura* leaves extract in the previous studies [8,9,11,13].

Determination of Procollagen-1-Propeptide N-terminal

MLME treatment resulted in a increased of procollagen-1-propeptide N-terminal (P1NP) amount in the 3T3 fibroblast cell culture. The results shows statistically significant ($p < 0.05$) based on dose dependent manner (**FIGURE 1**). **FIGURE 1** shows that the highest increase of P1NP occurred at a concentration of 20 µg mL^{-1} which indicates that the higher the concentration of the extract given to the cell culture is able to increase the amount of P1NP better, according to this study.

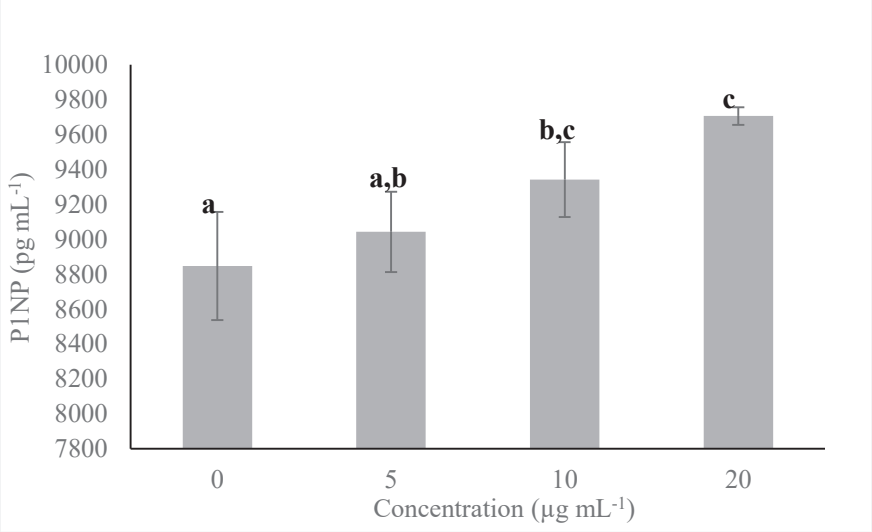

FIGURE 1. Effect of *M. calabura* leaves methanolic extract (MLME) on the procollagen-1-propeptide N-terminal (P1NP) production in 3T3 fibroblasts cell culture. Cells were treated with MLME in DMSO with various concentrations (0-20 µg mL^{-1}) and P1NP was measured by ELISA assay. The result expressed in a bar which shows significantly difference based on one-way ANOVA ($p < 0.05$) followed by Duncan's multiple range test.

Increasing of P1NP after administration of MLME is related to its flavonoid content contained in each consentrations. The highest number of P1NP in the concentration of 20 µg mL^{-1} is considered as an impact of its phytochemical contents that affected its bioactivity. The higher concentration of MLME given, the higher bioactivity of flavonoids that can affect P1NP secretion. These ability related to the antioxidant activity of MLME which reported in our previous presentation that can suppress oxidative stress in 3T3 fibroblasts cell culture [13].

The result indicates that MLME is potential to be used as an inducer for collagen synthesis, eventhough the process of collagen synthesis under MLME's administration is still unknown and can not be explained through this study.

Nevertheless other studies showed that flavonoids and polyphenols can promote collagen synthesis and inhibit degradation of human dermal fibroblasts [6,18,19]. Flavonoid had been reported to be able to activate smad2 and smad3 protein which affect the expression of Col1a2 and Col3a1 genes, thereby accelerating wound healing and slowing down skin-aging process for dermatological and cosmetic purpose [18,19].

CONCLUSIONS

Our main result indicated that *Muntingia calabura* L. is potential to be used as a new source for anti-aging agent because of its ability to increase the amount of P1NP in a dose-dependent manner. However, further research related to specific compounds and the process of collagen synthesis of *Muntingia calabura* L. extract in skin tissue needs to be evaluated.

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REFERENCES

1. Panwar, P., Butler, G. S., Jamroz, A., Azizi, P., Overall, C. M., Bromme, D. Aging-associated modifications of collagen affect its degradation by matrix metalloproteinases. Matrix Biol. **65**, 30-44 (2017).
2. Datta, H. S., Mitra, S. K., paramesh, R. and Patwardhan, B. . Theories and management of aging: modern and ayurveda Perspective. Evid.-Based Complementary Altern. Med. 2011, 1-6 (2011).
3. Binic, I., Lazarevic, V., Ljubenovic, M., Mojsa, J., and Sokolovic, D. Skin ageing: natural weapons & strategies. Evid.-Based Complementary Altern. Med. 2013, 1-10 (2013).
4. Makpol, S., Jam., F. A., Yusof, Y. A. M. and Ngah, W. Z. W. Modulation of collagen synthesis and its gene expression in human skin fibroblasts by tocotrienol-rich fraction. Arch Med Sci **7 (5),** 889-895 (2011).
5. Lee, H. Y., Ghimeray, A. K., Yim, J. H., Chang, M. S. 2012. Antioxidant, collagen synthesis activity in vitro and clinical test on anti-wrinkle activity of formulated cream containing *Veronica officinalis* extract. JCDSA **5**, 45-51 (2012).
6. Kang, C.H., Rhie, S.J., and Kim, Y.C. Antioxidant and skin aging effect of marigorld methanol extract. Toxicol. Res. **34 (1),** 31-39 (2018).
7. Lim, T. K. *Muntingia calabura*. *Edible Medicinal and Non-Medicinal Plants* 3 (Springer Netherlands, 2016), pp. 486-492.
8. Zakaria, Z. A., Mohamed, A. M., Jamil. N. S. M., Rofiee, M. S., Hussain, M. K., Sulaiman, M. R., Teh, L. K. and Salleh, M. Z. In vitro antiproliferative and antioxidant activities of the extracts of *Muntingia calabura* leaves. Am. J. Chin. Med**. 39 (1),** 183-200 (2011).
9. Zakaria, Z. A., Sani, M. H. M., Cheema, M. S., Kader, A. A., Teh, L. K., Salleh, M. Z. Antinociceptive activity of methanolic extract of *Muntingia calabura* leaves: further elucidation of the possible mechanisms. BMC Compl Altern Med. **14 (63),** 1-12 (2014).
10. Zakaria, Z. A., Balan, T., Suppalan, V., Ahmad, S. & Jamaludin, F. Mechanism(s) of action involved in the gastroprotective activity of *Muntingia calabura*. J Ethnopharm **151**, 1184–1193 (2014).
11. Balan T, Sani MHM, Ahmad SHM, Suppalah V, Mohtarrudin N, Zakaria ZA. Antioxidant and anti-inflammatory activities contribute to the prophylactic effect of semi-purified fractions obtained from the crude methanol extract of Muntingia calabura leaves against gastric ulceration in rats. J Ethnopharmacol **164**, 1–15 (2015).
12. Mahmood ND, Nasir NLM, Rofiee MS, Tohid SFM, Ching SM, Teh LK, Salleh MZ, Zakaria ZA. *Muntingia calabura*: A review of its traditional uses, chemical properties, and pharmacological observations. Pharm Biol **52 (12),** 1598-1623 (2014).
13. Rahmawati, A. N., Astirin, O. P., Pangastuti, A. Intracellular antioxidant activity of *Muntingia calabura* leaves methanolic extract. Nusantara Bioscie. **10 (3),** 210-214 (2018).
14. Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. G. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. **10 (3),** 178-182 (2002).

15. Baba, S. A. and Malik, S. A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci **9**, 449-454 (2015).
16. Rahman, K. Studies on Free Radicals, Antioxidants, and Co-Factors. Clin Interv Aging **2 (2):** 219-236 (2007) .
17. Bosch R, Philips N, Suarez-Perez JA, Juarranz A, Devmurari A, Chalensouk-Khaosaat J, Gonzalez S. Mechanisms of photoaging and cutaneous photocarcinogenesis, and photoprotective strategies with phytochemicals. Antioxidants **4**, 248-268 (2015).
18. Zhang, Y., Wang, J., Cheng, X., Yi, B., Zhang, X., Li, Q. Apigenin induced dermal collagen synthesis via smad2/3 signaling pathway. European Journal of Histochemistry **59**, 98-106 (2015).
19. Stipcevic, T., Piljac, J. and Berghe, D. V. Effect of different flavonoids on collagen synthesis in human fibroblasts. Plant Food Hum Nurt. **61(1),** 27-32 (2006).