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Antioxidant and anti-elastase activity of Tampui (*Baccaurea macrocarpa*) and Ketapong (*Terminalia badamia*) barks and leaves

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ABSTRACT: Elastase is a protease that is involved in the breakdown of elastin in the dermis. Likewise, free radicals can cause elastin damage. Inhibition of elastase enzyme activity and scavenging of free radicals can prevent premature aging of the skin, especially wrinkles. The current study was to investigate the antioxidant and elastase inhibition properties of the leaves and bark of the Tampui (*Baccaurea macrocarpa* L.) and Ketapong (*Terminalia badamia* L.) plants, which may be employed as an anti-aging ingredient in cosmetics and nutraceuticals. *B. macrocarpa* and *T. badamia*'s leaves and bark were extracted using reflux and 96% ethanol. Anti-elastase was examined using N-succ-(Ala)3-nitroanilide as a substrate, and the DPPH reagent was used to test the antioxidants' activity. Phytochemical screening indicated the presence of flavonoids, quinone, tannins, and steroids. Antioxidant activity of *B. macrocarpa* leaves (BML), *B. macrocarpa* bark (BMB), *T. badamia* leaves (TBL), and *T. badamia* bark (TBB) had IC_{50} values were 15.09±0.51; 22.89±1.51; 36.43±1.94; 39.23±1.76 ppm, respectively. The anti-elastase activity of BML, BMB, TBL, and TBB had IC_{50} values were 48.86±2.29; 52.03±9.54; 44.42±4.53; 40.16±1.32 ppm, respectively. The ethanol extract of B. macrocarpa and T. badamia's leaves and bark exhibits anti-elastase and antioxidant action, proposing that it may be used as an anti-aging agent.

KEYWORDS: Anti-elastase; anti-oxidant; elastin; wrinkles.

INTRODUCTION

Intrinsic aging is associated with the natural process of aging, whereas extrinsic aging is associated with external factors that affect the aging process (e.g., air pollution, UV radiation, and pathogenic microorganisms). Photoaging is most likely the primary cause of reactive oxygen species (ROS) production. ROS can affect the skin in three primary ways. They can modify the lipids in cellular membranes, thereby influencing cell structure and regulating the transport of nutrients and other molecules; they can genetically alter the cells, potentially leading to disease or increasing susceptibility to premature aging of the skin. The expression of functional collagen and elastin proteins in healthy tissues may be changed [1-2].

Elastin is a functional protein molecule that is elastic and is located within the component of connective tissue. Elastin was in charge of the elastic properties of body tissues. Damaged elastin would cause the tissue to lose its elasticity. Elastase was an enzyme that was responsible for breaking down elastin in the skin dermis. Inhibition of elastase enzyme activity could prevent premature aging of the skin, especially wrinkles. Antioxidants were compounds that could slow down or prevent the oxidation process that could produce free radicals. Therefore, it could overcome the premature aging process [3-7].

The trend of "back to nature" has made cosmetic companies look for sources of natural products to be the main ingredients in their dosage forms. Natural products from plants are one of the substances that are rich in benefits and could be used as cosmetic ingredients. These materials were leaves, roots, fruit, seeds, rhizomes, stems, tubers, fruit skins, and flowers. One of the cosmetics that were in great demand in skin care anti-aging [8-12].

One plant of the genus Terminalia is T. *chebula* Retz. has a mechanism of action against elastase, hyaluronidase, and MMP-2 enzyme inhibitors. T. *chebula* plants have MMP-2 inhibition 1.37 times stronger than ascorbic acid on fibroblasts and anti-elastase with significant induction of collagen expression type II [5]. The other genus Terminalia was T. badamia (TB) or Ketapong, it was easily found in Indonesia and was a

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multifunctional plant. In Nigerian folklore, the leaves and bark of TB were used to alleviate hypertension, while in Taiwan, it was used to treat hepatitis and prevent hepatoma. It was also effective in treating inflammatory bowel disease, oxidative stress, immunological dysfunction, getting over depression, and anti-aging, according to a previous study. The hydrophilic extract from TB has the potential to be used in anti-aging cosmetics because it enhances procollagen type I production by inhibiting matrix metalloproteinase (PMM) -1, -3, and -9 activity. Human skin fibroblasts exposed to UVB are protected against photoaging by the hydrophilic leaf extract of the TB. At a concentration of 1 mg/mL, the methanol, hydrophilic leaf extract, and its hydrolysate of TB had a collagenase inhibition rate that was greater than 100%. The extract could improve the expression of type I procollagen protein and decrease the expression of MMP-1, MMP-9, and MMP-3 proteins at concentrations of 25 and 50 g/mL, respectively [13-15].

According to earlier studies, B. macrocarpa (BM) was a typical plant of East Kalimantan, Indonesia, with fascinating biological activity. Its edible fruits, known as "Tampui," were a source of extra nutrients and were one of the typical plants of the region. Secondary metabolites found in the Tampui (BM) bark included alkaloid, phenolic, flavonoid, triterpenoid, and steroid. This secondary metabolite was known to have antioxidant properties [16-17]. As of yet, no information has been revealed about the anti-elastase activity of BM and TB.

This article studied the antioxidant and anti-elastase activity from the leaves and bark of Indonesian indigenous plants Tampui (Baccaurea macrocarpa L.) and Ketapong (Terminalia badamia L.). The selection of the plants was based on previous literature studies that had similar chemotaxonomy and had anti-elastase and strong antioxidant activity.

▪ MATERIALS AND METHODS

Plant material

The material used for this research was a dry powder of leaves and bark of Tampui (B. macrocarpa L.) and Ketapong (T. badamia L.) obtained from Bogor, West Java, Indonesia. The plants were determined at the Botanical Gardens Conservation Centre, Bogor, West Java, Indonesia with No 381/IPH/1.01/If.07/II/2021.

Chemical and reagent

Amyl alcohol, ammonium, aquadest, anhydrous acetate acid, chloride acid, H2SO4, Fe (III) chloride hexahydrate, ethanol, petroleum ether, gelatin, chloroform, methanol, sodium acetate, sodium hydroxide, magnesium powder, Dragendorff reagent, Mayer reagent, Stiasny reagent, were obtained from Q-lab Universitas Pancasila. Buffer Trizma, porcine pancreatic elastase (PPE.C.3.4.2136), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Epigallocatechin gallate (EGCG), N-Succ-(Ala)3-p-nitroanilide (SANA) were obtained from Sigma-Aldrich, Inc.

Extraction from BM and TB of leaves and bark

Each part of the plant has been dried and mashed using a blender. Then the dry powder was refluxed with 96% ethanol and filtered. The residue was refluxed again. The liquid extract was concentrated using a rotavapor and evaporated above a water bath to remove the remaining solvent to produce a solvent-free, viscous extract.

Phytochemical screening

All extracts were tested qualitatively for the presence of various phytochemical constituents namely alkaloid, flavonoid, saponin, tannin, and triterpenoid steroid using standard protocols.

Antioxidant activity tests

The DPPH powder was weighed at approximately 4 mg, dissolved with pro-analytical methanol in a 25 mL volumetric flask, and then placed in a dark-colored bottle. The ethanol extract with various concentrations of each was carefully weighed at 10.0 mg, then dissolved with methanol in a 5.0 mL volumetric flask to volume; so that the main solution of 2000 ppm was obtained. Five serial concentrations were prepared with methanol up to 5.0mL. The solution was used in the measurement of antioxidant activity. The preparation of the test solution was carried out in triple. Vitamin C was used as a reference standard.

Each solution was kept for 30 minutes at 37 °C protected from light. Measurement of the absorption at 516.5 nm using a UV-Vis Spectrophotometer and conducted three times. The percentage of scavenging activity could be calculated by the formula:

where:

$$Q = \frac{A_0 - A_1}{A_0} \text{ X } 100\%$$

Q: Percentage of inhibition (%)
A0: Control absorption (solvent + DPPH)
A1: Absorption of test solution (solvent + DPPH + sample)

In-vitro anti-elastase activity

At room temperature for 15 min, absorbance at 410 nm was used to measure the produced p-nitroaniline. Then, 800 mL of Tris buffer (0.2 M, pH 8.0), 100 mL of elastase enzyme, 100 mL of 0.8 mM SANA, and its serial concentrations of the extract in Tris-HCl buffer were combined to produce the mixture. When the substrate is added, the reaction has already been preincubated with the enzyme for 20 min at 25 °C. A UV-Vis Spectrophotometer was used to detect absorbance at 410 nm [18]. The inhibitory activity against elastase was determined using this formula:

$$\text{Inhibition (\%)} = \frac{(A - B)}{A} \times 100$$

Where A, was the control absorbance at 410 nm, and B was the sample absorbance at 410 nm.

▪ RESULTS AND DISCUSSION

Phytochemical screening

In the TB plant both leaves and bark obtained positive results for alkaloids, while in BM leaves and bark the results were negative. In all plants, positive results were obtained for flavonoids, quinones, tannins, and steroids. The positive results of flavonoids in all plants were due to the polar nature of the flavonoids which were easily extracted using 96% ethanol which also had polar properties. Differences in screening results for each sample could be influenced by where the plant grows, soil nutrients, and the cultivation process of each plant. The results of the phytochemical screening of BM and TB leaves and bark extract are shown in (Table 1).

Botanical substances from various chemical classes, such as polyphenols, monoterpenes, flavonoids, organosulfides, and indoles have mechanisms of action include anti-inflammatory and immune response stimulation, detoxification, antioxidant modulation, and gene expression alteration. Flavonoids originate in nature as glycosides, sometimes referred to as bioflavonoids. Flavonoid glycosides consist of a flavonoid (aglycone) and a hydrocarbon molecule. The primary activity of flavonoids on the skin is related to their antioxidant capabilities. The majority of flavonoids containing phenol have a comparatively enhanced reduction potential and form as resonance-stabilized anion radicals. The scavenging activity of flavonoids is significantly affected by their structural and physicochemical characteristics (e.g., logP). Flavonoids inhibited ROS production and skin aging. The antiradical activity of flavonoids is linked to their ability to absorb UV light across a broad spectrum, with maximum absorption in the far ultraviolet B (250–280 nm) and A (350–385 nm) ranges. A wide variety of flavonoids exhibit a significant affinity for protein structures [1].

Table 1. Phytochemical constituents of BM and TB extracts.

Phytochemical	BML	BMB	TBL	TBB
Alkaloids	-	-	+	+
Flavonoids	+	+	+	+
Saponin	+	+	+	-
Tannin	+	+	+	+
Quinone	+	+	+	+
Steroid/triterpenoid	+	+	+	+
Essential oil	+	-	+	+
Coumarin	+	-	+	-

Note: (+): Presence; (-): Absence

Antioxidant activity tests

The phytochemical components, which directly function as free radical scavengers, hydrogen donors, and peroxide scavengers, can be used to explain the DPPH scavenging activity [19]. Based on Figure 1, Antioxidant activity was measured using UV-Vis Spectrophotometry. It was found that the antioxidant activity of BML had the strongest activity showing the IC^{50} value at a concentration of 15.10±0.51 ppm, BMB was 22.89±1.51 ppm, TBL was 36.43±1.94 ppm, and TBB was 39.23±1.76 ppm. Differences in antioxidant activity could be caused by differences in phenolic compounds such as flavonoids which were substances with the potential as antioxidants [13], [16]. Our results are all extracts showed good antioxidant activity as also mentioned in other studies.

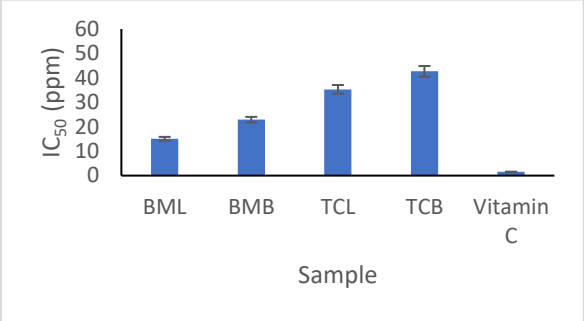

Figure 1: Antioxidant activity of leaves and bark of BM and TB. All values are expressed as mean ± SD, n=3.

The results based on the literature research, show that the BM as a Phyllantaceae family contained a total phenol value of 60.04±0.53 mg Gallic Acid Equivalent/g, total flavonoids, and tannins 44.68±0.67 mg Catechin Equivalent/g, 1.23±0.20 mg C3-Glycosides of Delphinidin and cyanidin / 100 g and 0.81±0.14 mg SM/g, respectively. Methanol extract from the bark of BM could be categorized as an active extract with an IC^{50} value of 11.15 ppm as antioxidant activity [17].

The degree of antioxidant qualities depends on the type of chemical entity and the structure that it is contained in, in addition to the amount of phenolic or flavonoid content. Because of the existence of additional bioactive chemicals, a substance with a low overall amount of phenols and flavonoids may nonetheless have an antioxidant effect. Substances with these properties could prevent aging of the skin associated with oxidative damage.

This recent study supported the findings of previous research by testing antioxidant activity using DPPH as a reagent and vitamin C as a standard. Vitamin C or ascorbic acid is one type of secondary antioxidant compound that may rule out free radicals with the lowest concentration. This assay was carried out on samples with an operating time of 25-30 minutes with 3 repetitions (3 series). DPPH would change color from purple slowly to yellow, which indicated DPPH was hydrolyzed from 1,1-diphenyl-2-picrylhydrazine to 1,2-diphenyl-2-picrylhydrazyl.

In-vitro anti-elastase activity tests

Elastase is a protease that can demolish skin intercellular fiber, called elastin. The skin's depletion of two structural fibers leads to a lack of strength and elasticity, which is responsible for the production of aging and wrinkles. According to the literature, the Phyllantaceae family was investigated for anti-elastase and anti-collagenase. Phyllantus emblica fruit extract has been reported that anti-collagenase and anti-elastase inhibition exhibit low IC^{50} values which were 95.97 µg/mL, 89.32 µg/mL, and 387.85 for MMP-1, MMP-2, and elastase inhibition respectively. The results showed that the number of extracts required for MMP-2 inhibition was lower than for MMP-1. The inhibitor of PMM activity is useful because it prevents collagenase from rupturing the X-gly bond of collagen and the Pro-X-Gly-Pro chain of synthetic peptides, thus inhibiting the degeneration of collagen. X is an amino acid where the amino-terminal is obstructed. P. emblica demonstrated a powerful antioxidant effect, but it was not a good source for elastase inhibitors. In agreement with Wittenauer al., the extract of amla containing gallic acid as the main component had low inhibiting properties of the elastase enzyme [20-21].

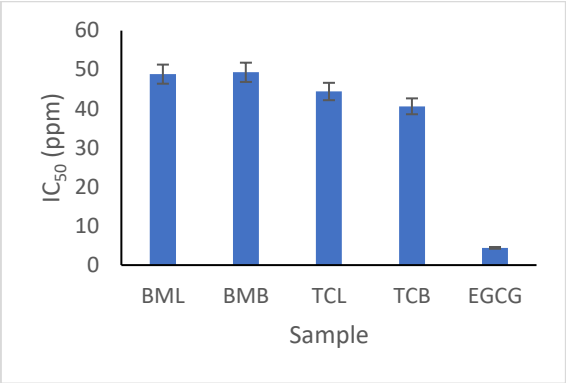

Figure 2: Anti-elastase activity of leaves and bark of BM and TB. All values are expressed as mean ± SD, n=3.

In this study, the anti-elastase activity showed that all extracts were good with an IC^{50} value below 60 ppm. Some polyphenols are supposed to interact with elastase because of their molecular nature. EGCG has been used as a standard of anti-elastase activity. Wittenauer has proven dose-dependent inhibiting properties of EGCG [18]. The best anti-elastase potential could be observed in the comparison between extracts, given the lower the IC^{50} value, the higher the inhibitory activity against elastase. In this test, the enzyme used would break down the elastin substrate. The different results could be caused by differences in the phenolic compounds extracted in the extraction process, and where the plants grew. The anti-elastase activity results are shown in Figure 2. TBB showed the best anti-elastase activity among the extract with IC^{50} was 40.63 ppm. This study explained that extracts with the strongest antioxidant activity uncertainty had great potential for anti-elastase activity. Further research was needed to prove the relationship between antioxidants and anti-elastase.

▪ CONCLUSION

This research confirms that the ethanol extract of BML, BMB, TBL, and TBB had a promising antioxidant and anti-elastase potency, thus it could be used as a potential anti-wrinkle ingredient in the formulation of skincare. The further research would be better to conduct anti-wrinkle test and standardization of the extract before formulation.

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REFERENCES

[1] H. T. Hoang, J. Y. Moon, and Y. C. Lee, "Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives," Dec. 01, 2021, *MDPI*. doi: 10.3390/cosmetics8040106.

[2] C. Mathen, G. Soman, S. Peter, R. Thergaonkar, and M. Teredesai, "Evaluation of anti-elastase and antioxidant activity in antiaging formulations containing terminalia extracts," *International Journal of Herbal Medicine*, vol. 2, no. 2, pp. 95–99, 2014.

[3] G. D. Liyanaarachchi, J. K. R. R. Samarasekera, K. R. R. Mahanama, and K. D. P. Hemalal, "Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals," *Ind Crops Prod*, vol. 111, no. August 2017, pp. 597–605, 2018, doi: 10.1016/j.indcrop.2017.11.019.

[4] Y. Desmiaty, F. C. Saputri, M. Hanafi, R. Prastiwi, and B. Elya, "Anti-Elastase, Anti-Tyrosinase, And Anti-Oxidant of Rubus fraxinifolius Stem Methanolic Extract," *Pharmacognosy Journal*, vol. 12, no. 2, pp. 271–275, 2020.

[5] A. I. Charles Dorni, A. Amalraj, S. Gopi, K. Varma, and S. N. Anjana, "Novel cosmeceuticals from plants—An industry guided review," *J Appl Res Med Aromat Plants*, vol. 7, no. June 2016, pp. 1–26, 2017, doi: 10.1016/j.jarmap.2017.05.003.

[6] Z. N. Azwanida, O. E. Jonathan, and H. Melanie-Jaynes, "Antioxidant, anti-collagenase, anti-elastase and anti-tyrosinase activities of an aqueous cosmos caudatus kunth (Asteraceae) leaf extract," *Tropical Journal of Natural Product Research*, vol. 4, no. 12, pp. 1124–1130, 2020, doi: 10.26538/TJNPR/V4I12.15.

[7] H. R. Rabbani, D. A. Purwanto, and I. Isnaeni, "Effect of Guava Powder Addition on Epigallocatechin Gallate (EGCG) Content of Green Tea and Its Antioxidant Activity," *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, vol. 6, no. 2, p. 85, 2020, doi: 10.20473/jfiki.v6i22019.85-90.

[8] I. Binic, V. Lazarevic, M. Ljubenovic, J. Mojsa, and D. Sokolovic, "Skin ageing: Natural weapons and strategies," *Evidence-based Complementary and Alternative Medicine*, vol. 2013, 2013, doi: 10.1155/2013/827248.

[9] A. Argyropoulou *et al.*, "Natural compounds with anti-ageing activity.," *Nat Prod Rep*, vol. 30, no. 11, pp. 1412–37, 2013, doi: 10.1039/c3np70031c.

[10] C. Veeresham, "Natural products derived from plants as a source of drugs.," *J Adv Pharm Technol Res*, vol. 3, no. 4, pp. 200–1, Oct. 2012, doi: 10.4103/2231-4040.104709.

[11] P. K. Mukherjee, N. Maity, N. K. Nema, and B. K. Sarkar, "Bioactive compounds from natural resources against skin aging," *Phytomedicine*, vol. 19, no. 1, pp. 64–73, 2011, doi: 10.1016/j.phymed.2011.10.003.

[12] R. Tundis, M. R. Loizzo, M. Bonesi, and F. Menichini, "Potential Role of Natural Compounds Against Skin Aging," *Curr Med Chem*, vol. 22, no. 12, pp. 1515–1538, Mar. 2015, doi: 10.2174/0929867322666150227151809.

[13] C. C. Lin, Y. L. Chen, J. M. Lin, and T. Ujiie, "Evaluation of the antioxidant and hepatoprotective activity of terminalia catappa," *American Journal of Chinese Medicine*, vol. 25, no. 2, pp. 153–161, 1997, doi: 10.1142/S0192415X97000172.

[14] H. M. Chiang *et al.*, "Inhibitory effects of Terminalia catappa on UVB-induced photodamage in fibroblast cell line," *Evidence-based Complementary and Alternative Medicine*, vol. 18, no. 1, pp. 1–9, 2018, doi: 10.1186/s12906-018-2308-4.

[15] Y. H. Huang, P. Y. Wu, K. C. Wen, C. Y. Lin, and H. M. Chiang, "Protective effects and mechanisms of Terminalia catappa L. methenolic extract on hydrogen-peroxide-induced oxidative stress in human skin fibroblasts," *BMC Complement Altern Med*, vol. 18, no. 1, pp. 1–9, 2018, doi: 10.1186/s12906-018-2308-4.

[16] M. Fadzelly, A. Bakar, N. E. Ahmad, F. A. Karim, and S. Saib, "Phytochemicals and antioxidative properties of Borneo indigenous liposu (Baccaurea lanceolata) and tampoi (Baccaurea macrocarpa) fruits," *mdpi.com*, vol. 3, pp. 516–525, 2014, doi: 10.3390/antiox3030516.

[17] E. Erwin, W. Pusparohmana, I. Sari, … R. H.-, and undefined 2018, "Phytochemical and antioxidant activity evaluation of the bark of Tampoi (Baccaurea macrocarpa)," *ncbi.nlm.nih.gov*, Accessed: Dec. 09, 2021. [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6915813.1/

[18] J. A. E. Kraunsoe, T. D. W. Claridge, and G. Lowe, "Inhibition of Human Leukocyte and Porcine Pancreatic Elastase by Homologues of Bovine Pancreatic Trypsin Inhibitor †," *Biochemistry*, vol. 2960, no. 95, pp. 9090–9096, 1996.

[19] R. Rungruang, W. Ratanathavorn, N. Boohuad, O. Selamassakul, and N. Kaisangsri, "Antioxidant and anti-aging enzyme activities of bioactive compounds isolated from selected Zingiberaceae plants," *Agriculture and Natural Resources*, vol. 55, no. 1, pp. 153–160, Jan. 2021, doi: 10.34044/J.ANRES.2021.55.1.20.

[20] J. Wittenauer, S. Mäckle, D. Sußmann, U. Schweiggert-Weisz, and R. Carle, "Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity," *Fitoterapia*, vol. 101, pp. 179–187, 2015, doi: 10.1016/j.fitote.2015.01.005.

[21] S. Pientaweeratch, V. Panapisal, and A. Tansirikongkol, "Antioxidant, anti-collagenase and anti-elastase activities of Phyllanthus emblica, Manilkara zapota and silymarin: an in vitro comparative study for anti-aging applications," *Pharm Biol*, vol. 54, no. 9, pp. 1865–1872, 2016, doi: 10.3109/13880209.2015.1133658.