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# *Anti-Aging Potential and Quercetin Determination of Melastoma malabathricum L. Leaves Extract*

***Thia Amalia[1]****

[1]*Departemen of Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia*

**email:* thiaamalia@ub.ac.id

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*A B S T R A C T*

**Background:** *Melastoma malabathricum* L. is a wild shrub that traditionally used in South-East Asia as wound healing. This plant extracts contain phenolic, flavonoid and tannin. The leaves extract also has many pharmacological activity however the anti-aging activity of the leaves extract has not been studied. **Methods**: The leaves of *M. malabathricum* were macerated and refluxed using chloroform and ethanol consecutively using. Quercetin content of the leaves extract was determined by HPLC. Anti-aging activity was evaluated by anti-elastase, and anti collagenase inhibitor activity of the leaves extract was assessedby a fluorometric method. **Results:** The Yield Percentage of the *M. malabathricum* leaves extract was 16.53% w/w containing 9.2 mg/g quercetin.. The leaves extract of *M. malabathricum* L. possed anti-elastase and anti collagenase activity with $IC_{50}$ 80.39±2.36 ppm and 63.3±3.32 ppm, respectively. **Conclusions:** *M. Malabathricum* leaves extract was promising as a raw material for anti-aging cosmetic

**Keywords:** *Melastoma malabathricum* L.; anti-aging; anti-elastase; anti collagenase; quercetin

## INTRODUCTION

*Melastoma malabathricum* L. (Melastomataceae) is a wild shrub that widely grows in South-East Asia. It is traditionally used as herbal medicine in Malaysia, India, China, and Indonesia. All parts of this plant has been used as ethnomedicine to treat diarrhea, dysentery, toothache, and stomachach. The roots are commonly used to alleviate pain from mouth ulcers in children, while the stems are frequently employed to treat various skin diseases. Powdered leaves and roots can be used to accelerate the healing of wounds or chickenpox . The leaves are traditionally used to treat wounds, acne, and dark spots on the skin (1). Dayak Tribe in Kalimantan, Indonesia, also used the leaves as wound healing(2).

Ethanol extract of *M. malabathricum* leaves has demonstrated wound healing and antiviral activities against herpes simplex virus type I. The leaf extract significantly helps fasten wound healing by reducing bleeding time, improving scar tissue formation, and diminishing acne (3,4). Additionally, the leaf extracts exhibit pharmacological activities such as antibacterial (3,5), antiproliverative(6), antioxidant(7), and antiinflammatory(6,8). The powdered leaves demonstratedastringent properties that contribute on dysentery treatment, relieve hemorrhoid pain, and heal wounds (6). Phytochemical analysis reveals that the leaves contain flavonoids, triterpenes, tannins, saponins, steroids, glycosides, and phenolic compounds (1). Flavonoids, triterpenes, and tannins contribute to the anti-inflammatory activity of the leaves (3,4). Notably, the leaf extract contains quercetin, quercitrin, and kaempferol-3-O-(2',6'-di-O-p-trans-coumaroyl)-β-glucoside (3,4).

Despite its broad pharmacological activity, there is still a lack of studies about an anti-aging activity of the leaves extract. *M. malabathricum* leave has potent wound healing properties which improve collagenation, enhances epithelization and promotes wound closure(5). High flavonoid and tannins, including ellagitannin and kaempferol-3-O-β-D-glucoside (astragalin), were responsible for the wound healing activity(3,4). The bioactive compounds of the leaves extract also increase the proliferation rates of human skin fibroblast(4). The previous study stated that glucoside also stimulated collagen formation in human skin fibroblast(5). Moreover, Ellagic acid derivative showed inhibitor activity on elastase release of human neutrophil(9). A mixture of extracts from three melastoma species (*M. malabathricum, M. decemfidum,* and *M. hirta*) has been tested for anti-elastase and anti-collagenase activities (10).

* *Corresponding* author: Thia Amalia, *Departemen of Pharmacy, Faculty of Medicine, Universitas Brawijaya, Malang, East Java*, Indonesia. Email: thiaamalia@ub.ac.id

Therefore, This study aims to evaluates the anti-aging activity of *M. malabathricum* leaves extract through *in vitro* anti-elastase and anti collagenase assay.

## MATERIALS AND METHODS

### Materials

Quercetin (Sigma Aldrich, Singapore), Phosphatidylcholine (Phospholipon 90G and P 30 Lipoid ®, Germany), Neutrophil Elastase Inhibitor Assay Kit (Abcam, UK), Collagenase Inhibitor Assay Kit (Abcam, UK), Carbomer (Carbopol Ultrez 30), female *Sprague Dawley* rats (Agricultural, IPB, Indonesia). The use of animal in this study was approved by ethics committee from Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia (No. 0103/UN2.F1/ETIK/2018). All solvent used in this study was an analytical grade or higher.

### Methods

- **Plant Materials and Extract Preparation**

The leaves of *M. malabathricum* were collected from the hill in Samboja, Kutai Kertanegara, East Kalimantan, Indonesia and authenticated in Indonesian Science Institution (Lembaga Ilmu Pengetahuan Indonesia, LIPI. The leaves were air dried under a black cloth, then powdered using a grinder. The leaves powder was stored in an airtight container protected from light until further use.

The extraction method was adapted from Savic (2016)(11)and Michel (2014)(12). The leaves powder (350 g) was pre-extracted with chloroform (1:10 w/v, 3x24h) and then air dried to omit chloroform residue. The dried leaves powder was refluxed with ethanol (1:10 w/v, 3x2h) to obtain ethanol extract. The extract was then concentrated with a vacuum rotary evaporator (Buchi Rotavapor R-100, Japan) to obtained dry extract. The dried extract was stored in airtight container and kept in the refrigerator until further use. Yield of extraction was calculated by the formula:

$$Percentage\ yield\ \left(\%\frac{w}{w}\right) = \frac{(weight\ of\ dried\ extract)}{(weight\ of\ sample\ powder)} \times 100$$

- **Determination of quercetin content**

Quercetin content, as a marker constituent, was determined by HPLC method(13). HPLC analysis (Shimadzu LC-20AD, Japan) was performed with reversed phase column (Hypersil Gold C18, 150x4.6 mm, 5 µm diameter particles) and mobile phase consists of acetonitrile:2% acetic acid in water(40:60, v/v). The mobile phase and samples were filtered through a 0.45 µm nylon membrane filter (Whatman, USA) and degassed ultrasonically for 10 min before use. Samples were analyzed with an injection volume of 20 µL, the flow rate of 1 mL/min, and detection at 370 nm using a diode array detector (DAD). Calibration curves of quercetin were established by plotting the areas of peaks against eight concentration of quercetin standards (500-3.90625 µg/mL). This calibration curve was used to determine quercetin concentration of the extract.

- **Anti Elastase activity *in vitro* assay**

Antielastase activity was evaluated fluorometrically as protocol provided in the kit. Briefly, five concentrations of the extracts were prepared as sample tests. A fifty µL diluted Neutrophil Elastase (NE) solution was mixed with a 25 µL sample tests (or assay buffer for enzyme control) in 96-well black microplate (Thermo, USA). The mixture was incubated at 37°C for 5 min, and a 25 µL substrate was mixed to each sample. The kinetics of the enzyme activity was measured fluorometrically (RFU) at Ex/Em 400/505 nm (Glomax ver.3) from 0 min to 30 min. The calculation of percentage inhibition as the following formula.

$$Inhibition\ (\%) = \frac{(RFU_{enzim\ control} - RFU_{sample})}{RFU_{enzim\ control}} \times 100$$

The results were expressed as mean±SD of duplo experiments. Five concentration of extract sample and % inhibition of elastase was plotted for the $IC_{50}$ values using analysis of probit (SPSS Inc.).

- **Anti-Collagenase activity *in vitro* assay**

Anti-collagenase activity was performed fluorometrically according to the protocol provided in the kit. Briefly, five concentrations of extracts in water were prepared as sample tests. A one µL sample test (or assay buffer for enzyme control) was mixed with a 5 µL diluted collagenase and 44 µL assay buffer in 96-well black microplate. The mixture was incubated for 15 minutes at room temperature. A 50 µL substrate was added to each well. The fluorescences (RFU) were measured immediately at Ex/Em 490/520 nm on a microplate reader in kinetic mode for 45 minutes at 37°C protected from light. The relative inhibition was calculated as the following formula:

$$Relative\ Inhibition\ (\%) = \frac{(RFU_{enzim\ control} - RFU_{sample})}{RFU_{enzim\ control}} \times 100$$

The experiment was done in duplo and the results were expressed as mean±SD. The $IC_{50}$ values were calculated from plotting five concentration of the extract samples versus % inhibition of collagenase using analysis of probit (SPSS Inc.).

## 3 Result and Discussion

### Result

**Percentage yield and quercetin content of the *M. malabathricum* leaves extract**

The yield of extraction was 16.53% w/w. Equation of calibration curve of quercetin standard was y = 82094x + 11933,

$R^2$ = 0,9984. The quercetin content of the dried extract was 9.20 ±0.81 mg/g extract.

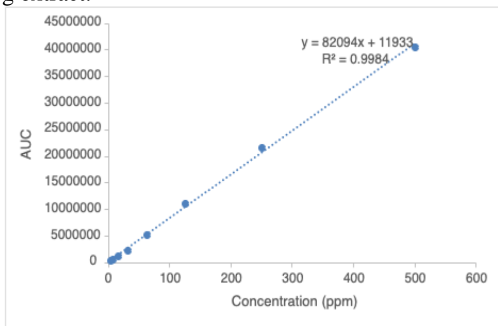
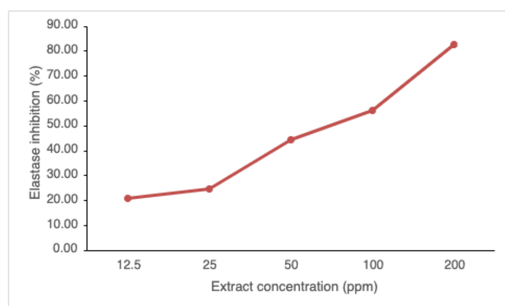



Figure 1. Calibration Curve of Quercetin Standard

**Anti-aging activity of the extract**

Anti-aging activity was determined *in vitro* by elastase inhibitor and collagenase inhibitor activity of the extract. Probit analysis of the sample concentration versus % inhibition activity shown that extract has potent inhibition activity against elastase and collagenase with $IC_{50}$ 80.39±2.36 ppm and 63.29±3.32 ppm, respectively.



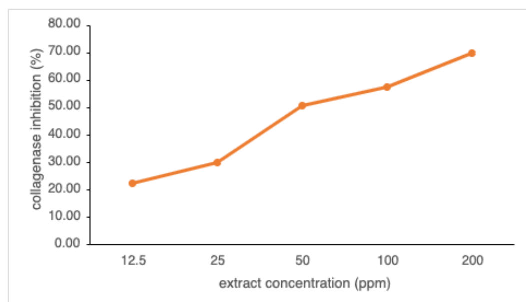




Figure 2.

## Discussion

Extraction method affects the phytoconstituents in the extract. In this study, *M. malabathricum* leaves were pre-extracted with chloroform to removed non-essentials content, such as chlorophyll which can interfere with biological activity and physical appearance of the extract (12). After defatting with chloroform, the dried powder leaves were refluxed with 96% ethanol as this organic solvent is best to extract the marker constituent of the extract, i.e., quercetin (11). Our previous study showed that pre-extraction process with chloroform did not influence the quercetin content of *M. malabathricum* leaves extract (MLE) (14). However, quercetin content of the MLE in this study was higher than the previous study in which the quercetin content of *M. malabathricum* from a different location in Malaysia ranged between 0.1-1.5 mg/g extract (15). The chemical composition of plants can be influenced by environmental factors. Variations in soil properties, nutrient content, and climatic conditions in different growing locations can cause plants of the same species to exhibit differences in their chemical constituents or active compounds (16).

The present study investigated the anti-elastase and anti-collagenase activities of the MLE, highlighting its potential in anti-aging applications. The findings revealed that MLE has the $IC_{50}$ -which is less than 100 ppm for both anti-elastase and anti-collagenase activity. It concludes that MLE exhibits high inhibitory effect on both elastase and collagenase enzymes, which are crucial in skin aging process. Elastase and collagenase are enzymes that degrade elastin and collagen, respectively, contributing to skin elasticity and firmness loss, leading to the formation of wrinkles and sagging skin (17). The inhibition of these enzymes is a strategy for maintaining skin structure and function, thereby reducing the visible signs of aging (18). Another study analyzing anti-elastase and anti-collagenase activity of seaweed extracts used IC50 values within the range of 6.25–100 µg/mL. These ranges demonstrate high potency and are consistent with benchmarks in enzyme inhibition studies (19).

Matrix metalloproteinases (MMPs), including collagenases (e.g., MMP-1, MMP-8) and elastase, play critical roles in the degradation and remodeling of the extracellular matrix (ECM). Collagenases degrade fibrillar collagen into smaller fragments, while elastase targets elastin, a protein essential for maintaining skin elasticity and structural integrity. Under normal physiological conditions, these enzymes are tightly regulated; however, their overexpression contributes to pathological processes such as skin aging (19).

The high anti-elastase and anti-collagenase activity observed in MLE align with its traditional use in wound healing, providing a scientific basis for its effectiveness (2). this activity of MLE is attributed to its high phenolic and flavonoid contents. Phenolic is known to have anti-aging activity against elastase and hyaluronidase (20). Total phenolic content of MLE was range between 145-222 mg GAE/g, supporting its potency (14). Previous studies have shown that flavonoids can inhibit MMPs, including elastase and collagenase. This inhibition occurs through mechanisms such as binding to the active sites of these enzymes and reducing oxidative stress, which regulates MMP expression. By protecting ECM components, flavonoids

demonstrate anti-inflammatory, anti-aging, and therapeutic potential in preventing ECM degradation-related conditions (19). The MLE contained flavonoid compounds e.g., Quercetin, quercitrin, and kaempferol-3-O-(2",6"-di-O-p-trans-coumaroyl)-β-glucoside (3,4). The marker constituent, quercetin, was known to have anti-elastase and anti-collagenase (21–23). Besides that, kaempferol and rutin which is also contained in the extract(15,23,24) have the anti-collagenase activity(21,25).

## 4. Conclusion

In conclusion, the anti-elastase and anti-collagenase activities of *M. malabathricum* leaves extract showed its potential as an effective natural agent for skin aging prevention. The presence of bioactive compounds, such as phenols and flavonoids, supports its traditional use in wound healing. Quercetin, as a flavonol compound, support the mechanism of action of the extract. This study provides a foundation for further exploration of M. malabathricum as a valuable ingredient in anti-aging products.

## 5. CONFLICT OF INTEREST

There is no conflict of interest in this study.

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