

Anti-Elastase, Antioxidant, Total Phenolic and Total Flavonoid Content of Wuru Ketek (*Myrica javanica* Reinw. ex Bl.) from Tangkuban Perahu, West Java - Indonesia

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ABSTRACT

Introduction: The presence of elastase and ROS can cause skin aging, phenolic compounds and flavonoids can be used to inhibit elastase activity and as an antioxidant. **Objective:** This research aimed to evaluate the anti-elastase, antioxidant activities, TPC and TFC of extracts from *Myrica javanica*. **Methods:** In this study, the leaves, stems and fruit of *Myrica javanica* were macerated with 96% ethanol. The extracts obtained were analysed for anti-elastase and antioxidant activities. It was also evaluated for TPC and TFC. **Result:** IC50 anti-elastase on leaves extract (LE), stems extract (SE), and fruits extract (FE) respectively showed 64.71 ppm, 197.49 ppm, and no activity. The anti-elastase result of three extracts are lower if compared with Myricetine (9.54 ppm). SE showed highest DPPH and TPC value (IC50=16.36 µg/mL; 307.00 mgGAE/g dry weight) and LE showed highest FRAP and TFC value (421.68 Mol/gram; 15.80 mgQE/g dry weight). **Conclusion:** In summary, anti-elastase and antioxidant activity are influenced by differences in the content of compounds in the extract.

Key words: Anti-elastase, Antioxidant, TPC, TFC, *Myrica javanica*, Antiaging.

INTRODUCTION

Aging is a common process by which humans gradually lose skin elasticity and collagen fibers. Over the age of 20 years, aging manifestations appear as collagen content per unit area begins to decrease, there is a 1% reduction in collagen content per unit area of the skin each year.¹ Skin aging is determined by a consolidation of intrinsic factors (genetics, cellular metabolism, and hormones) and extrinsic (continual light risk, pollution, ionizing radiation, chemicals, poisons). The subject of most of the research on antiaging and efforts for aesthetic-antiaging strategies related to the skin are the three main structural components of the dermis, namely collagen, elastin, and Glycosaminoglycans (GAGs).²

Skin elasticity and firmness are obtained in the presence of collagen and elastin in the skin layers. Collagen and elastin are broken down by the presence of MMP-1 and elastase enzymes which are increased due to excessive sun exposure. The absorbed UV light causes an increase in oxygen radical species (ROS) and induces oxidative stress. A high amount of ROS increases elastase activity. Expanded elastase enzyme activity triggers different diseases such as rheumatoid arthritis, cystic fibrosis, chronic respiratory diseases, psoriasis, deferred wound healing and premature skin aging with wrinkle formation. Therefore, antioxidants and anti-elastase can potentially be cosmetic in the fight against skin aging.^{3,4}

Plants from the genus *Myrica* such as *Myrica esculenta*, *Myrica faya*, and *Myrica gale* have antioxidant activities because they contain

secondary metabolites such as polyphenols (such as phenolic acids and flavonoids),⁵⁻⁷ and several research results show that polyphenol compounds and flavonoids have strong activity in inhibiting the activity of enzymes and antioxidants.⁸⁻¹⁰ Some compounds contained in the genus *Myrica* reported to have elastase inhibitory activity including myricetin, quersetin.¹¹

Based on this, the *Myrica javanica* plant, which is also a plant from of the Myricaceae family might possible to have pharmacological activities that are no less as potential as the other *Myrica* genus, especially for its antiaging activities, as well as their use in making cosmetic preparations in the development of antiaging cosmetics.

The purpose of this study was to determine the potential anti-elastase and antioxidant of *Myrica javanica* and analysed the TPC and their TFC.

MATERIAL AND METHODS

Reagents

Elastase from porcine pancreas (Sigma, SLBV 9311), N-Succinyl-Ala-Ala-Ala-p-nitroanilide (SANA) (Sigma, SLBR 7591V), Folin Ciocalteu Reagent (FCR) (Sigma), Trizma Base (Sigma, T1503), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma). Sodium carbonate, aluminium chloride, ethanol 96%, methanol, ferric chloride hexahydrate, acetic acid, hydrochloride acid (HCl), dimethyl sulfoxide (DMSO) (Merck, Germany).

Plant materials

The leaves, stems, and fruit of the *Myrica javanica* plant were obtained from the Tangkuban Perahu

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forest in the Bandung area, West Java. Plants are determined by the School of Life Science and Technology, Bandung Institute of Technology (ITB) (Approval No. 3911/I1.CO2.2/PL/2019).

Extraction

50 gram of *Myrica javanica* leaves, stems and fruit of each plant was extracted by 3 times maceration method using 96% ethanol (500ml), then stirred and allowed to stand for 24 hours. The extracts were concentrated with a rotary vacuum evaporator and dried over a water bath, stored in a closed container.

Anti-elastase assay

Anti-Elastase assay was determined according to the method of Wittenauer, *et al.* with modifications.¹² Pre-incubation of 20 µl extract sample in DMSO solution, 130 µL buffered Tris-HCl pH 8.0 with PE (Porcine Elastase) 25 µl for 15 minutes at 25°C and the reaction was started with the addition of SANA 20 µl substrate for 15 minutes on 96-well microplate (NUNC). Extracts and substrate solutions without enzymes are used as control. The absorbance was measured at 401 nm using a microplate reader. The inhibitory effect of the sample on anti-elastase activity was calculated as:

$$\text{Percent Inhibition (\%)} = [(A-B)/A] \times 100\%$$

A = absorbance control

B = absorbance sample

After obtaining the percentage of inhibition of each concentration, then the equation $y = bx+a$ was determined by calculating a linear regression curve where x is the concentration (µg/mL) and y is the percentage of inhibition (%). Anti-elastase activity is expressed by 50% Inhibition Concentration (IC50), which is a sample concentration that can reduce enzyme elastase by 50%. Myricetine was used as positive control.

Antioxidant assay

DPPH assay

DPPH free radical scavenging activities of tested extracts were determined based on a protocol modified from Bobo-Garcia, *et al.* A total of 0.3 mM DPPH solution in absolute methanol was available. Samples with different concentrations in absolute methanol were evenly mixed with methanol DPPH solution to purchase a total of 200 µL. After a 30 min incubation period in room temperature and the dark condition, the absorbance was detected at 517 nm by using the microplate reader. Quercetin was used as the positive control.

IC50 antioxidant

IC50 values were calculated based on the presentation of inhibition of DPPH radicals from each concentration of the sample solution with the formula:

$$\% \text{ inhibition} = (\text{Absorbance sample}/\text{Absorbance control}) \times 100\%$$

After obtaining the percentage of inhibition of each concentration, then the equation $y = bx+a$ was determined by calculating a linear regression curve where x is the concentration (µg/mL) and y is the percentage of inhibition (%). Antioxidant activity is expressed by 50% Inhibition Concentration (IC50), which is a sample concentration that can reduce DPPH radical by 50%.

FRAP assay

The FRAP radical method (Tomasina, *et al.*) was a little modified in order to estimate the antioxidant effect of the samples. The FRAP reagent was ready freshly by mixing 10 mL of acetate buffer 300 mM, 1 mL of ferric chloride hexahydrate 20 mM dissolved in distilled water, and 1 mL of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) 10 mM dissolved

in HCl 40 mM. 20 µL sample were mixed with 280 µL FRAP reagent then incubate for 30 min at 37°C. The absorbance was measured at 593 nm with microplate reader.

Total phenolic content (TPC) assay

An amount of 20 µl of methanol extract was added 100 µl of FCR solution shaken for 60 seconds in a 96-well microplate (NUNC). Incubated at room temperature for 5 minutes and then added 80 µl sodium carbonate solution. Shake again for 1 minute and incubate at room temperature for 2 hours. Uptake was measured on a microplate reader at 720 nm. Control solution is made in the same way replaces the extract with distilled water. Calculation of total polyphenol content by means of a conversion on the calibration curve of gallic acid solution 4-24 µg/mL.

Total flavonoid content (TFC) assay

A total of 20 µl of methanol extract was added 20 µL of aluminium chloride solution and 20 µL of sodium acetate solution and 180 µL of distilled water in 96-NUNC well microplate. The mixture was incubated at room temperature for 30 minutes and measured the absorption with microplate reader at 415 nm. Calibration solution curves used quercetin in methanol (4, 6, 8, 10, 12, and 14 µg/mL) were used to calculate total flavonoid contents.

Statistical analysis

All statistics analyses were achieved using SPSS 24.0 for Windows. The data was analysed by Pearson's Correlation. It is technique for investigating the relationship between two quantitative and finding association between two variables.

RESULTS

Elastase assay

Elastase is the one of the main components of extracellular matrix and thus the main to loosening of skin resulting in skin aging. The observations showed anti-elastase activities in some parts extract of *Myrica javanica*. Anti-elastase activity of the *Myrica javanica* was found to be highest in LE, SE showed the second highest anti-elastase activity while FE did not show any anti-elastase activity. Elastase inhibition results for the three extracts are below when compared with myricetine. The results are shown in (Figure 1).

Antioxidant assay

DPPH assay

DPPH radical scavenging activity was determined by using quercetin as standard ($R^2 = 0.9908$) (Figure 2). Researches showed presence of potent DPPH radical scavenging activity in all part of *Myrica javanica* extracts (Table 1).

FRAP assay

The anti-oxidant ability of the *Myrica javanica* extracts from the three different parts to scavenge radical FRAP *in vitro* was analysed by microplate reader. The results are shown in Table 2.

Total phenolic content (TPC) and total flavonoid content (TFC)

TPCs of *Myrica javanica* extracts were determined by using gallic acid as standard ($R^2 = 0.9964$) (Figure 3). It was highest in SE and lowest in fruit extract (Table 3). TFC were determined by using quercetin as standard ($R^2 = 0.997$) (Figure 4). LE showed highest TFC and FE showed lowest TFC (Table 3).

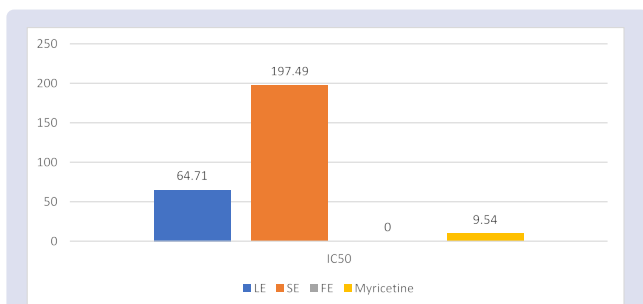


Figure 1: IC50 of some parts extract of *Myrica javanica* and Myricetine as positive control.

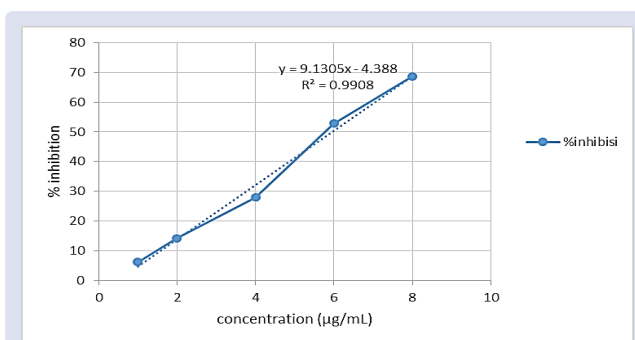


Figure 2: Calibration curve of Quercetin.

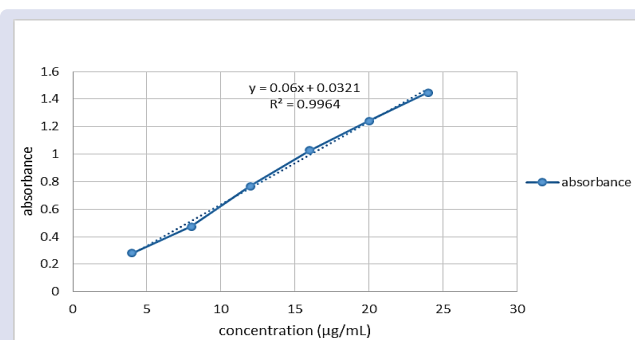


Figure 3: Calibration curve of gallic acid.

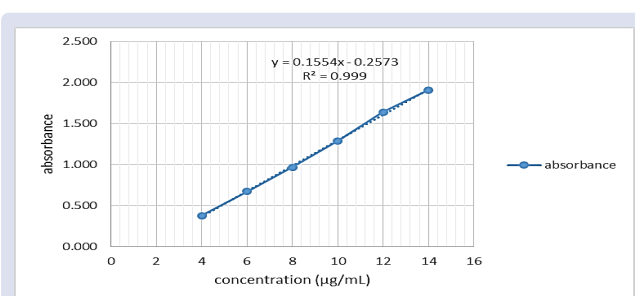


Figure 4: Calibration curve of Quercetin.

Table 1: Antioxidant Activities with DPPH Assay of Some Parts of *Myrica javanica* Extract.

Sample	Equation	R ²	IC50 DPPH (ppm)
Leaf Extract	$y = 2.2892x + 0.03$	0.9993	21.83
Stem Extract	$y = 3.3542x - 4.877$	0.9978	16.36
Fruit Extract	$y = 0.2637x + 13.643$	0.9996	137.87
Quercetin	$y = 9.1305x - 4.388$	0.9908	5.96

Table 2: Result FRAP Assay of *Myrica javanica* extracts indicated by the amounts of ferrous ion (Mol/gram).

Sample	FeEAC (Mol/gram)
Leaves extract	421.68
Stems extract	390.00
Fruit extract	73.04

Table 3: TPC and TFC in the ethanol extracts of *Myrica javanica*.

Sample	TPC (mg GAE/g Dry Weight)	TFC (mg QE/g Dry Weight)
Leaf Extract	259.40	15.80
Stem Extract	307.00	10.03
Fruit Extract	36.80	4.09

Table 4: Pearson's Correlation value in some extracts of *Myrica javanica* (n=3).

	TPC	TFC
Elastase inhibition (%)	0.683	0.990*
DPPH (IC50)	-0.992*	-0.850
FRAP (%)	0.965*	0.916*

Note: *r > 0.9 has a very strong correlation

DISCUSSION

Based on the results obtained, for anti-elastase activity have a very strong correlation with the TFC values shown in Table 4. That means with the increase in TFC, the percent inhibition of elastase will be even greater. Based on Kanashiro *et al.* (2007), some flavonoid compounds such as quercetin, myricetin can inhibit elastase.¹¹

The antioxidant activity (DPPH Assay) of SE (IC₅₀ = 16.36 µg/mL) and LE (IC₅₀ = 21.83 µg/mL) have strong antioxidant activity (the IC₅₀ value < 10 µg/mL), while FE (IC₅₀ = 137.87 µg/mL) has antioxidant activity that is weak (the IC₅₀ value range from 100-250 µg/mL), if IC₅₀ is above 250 µg/mL then the compound has no antioxidant activity.¹⁵

Likewise, for FRAP results in FE have the lowest percent inhibition among others. This present study is correlated with Spinola *et al.* (2014) that FE from another *Myrica* genus show weaker antioxidant activity compared to LE⁶, but in Li *et al.* (2017) study that FE of *Myrica rubra* have stronger antioxidant activity compared to LE and SE.¹⁶

TPC was showed a very strong relationship with DPPH and FRAP (Table 4). Due to the same principle that utilizes an electron-transfer mechanism, extract with high TPC contribute to express high DPPH scavenging activity.¹⁷

CONCLUSION

Among the test samples, anti-elastase and antioxidant activity are influenced by differences in the content of compounds in the extract. In the elastase inhibition and reduction of Fe ions, the compounds that play a role are the flavonoid group. As for the inhibition of free radicals are a phenolic group. Therefore, it is hoped that further research into a main compound that has the role of inhibiting the antiaging process.

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CONFLICTS OF INTEREST

The authors have not declared any conflict of interest.

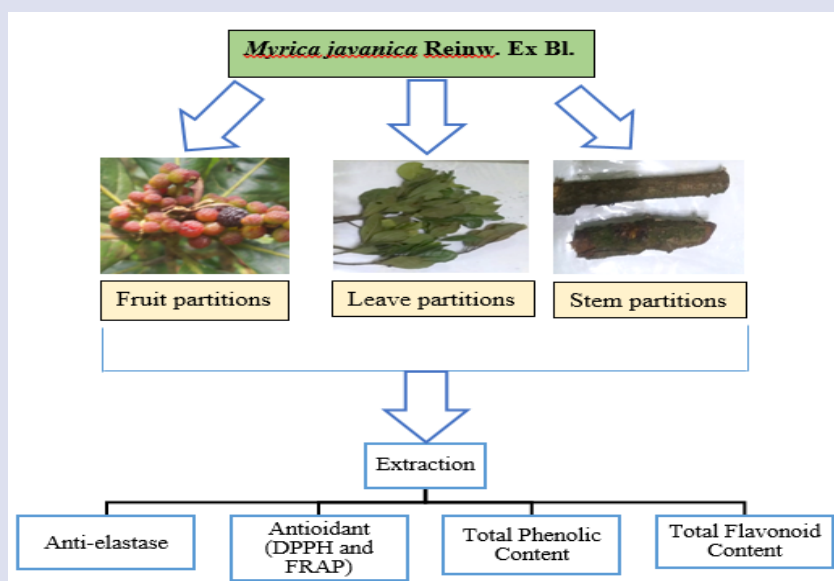
ABBREVIATIONS

ROS: oxygen radical species, **TPC:** total phenolic content, **TFC:** total flavonoid content, **LE:** leaves extract, **SE:** stem extract, **FE:** fruit extract, **DPPH:** 2,2-difenil-1-pikrilhidrazil, **FRAP:** ferric reducing antioxidant power, **TPTZ:** 2,4,6-Tris(2-pyridyl)-s-triazine.

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GRAPHICAL ABSTRACT



SUMMARY

- IC₅₀ anti-elastase on leaves extract (LE), stems extract (SE), and fruits extract (FE) respectively showed 64.71 ppm, 197.49 ppm, and no activity. The anti-elastase result of three extracts are lower if compared with Myricetine (9.54 ppm).
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