COVER LETTER FOR SUBMISSION OF MANUSCRIPT

June 9, 2019

Dear Editor,

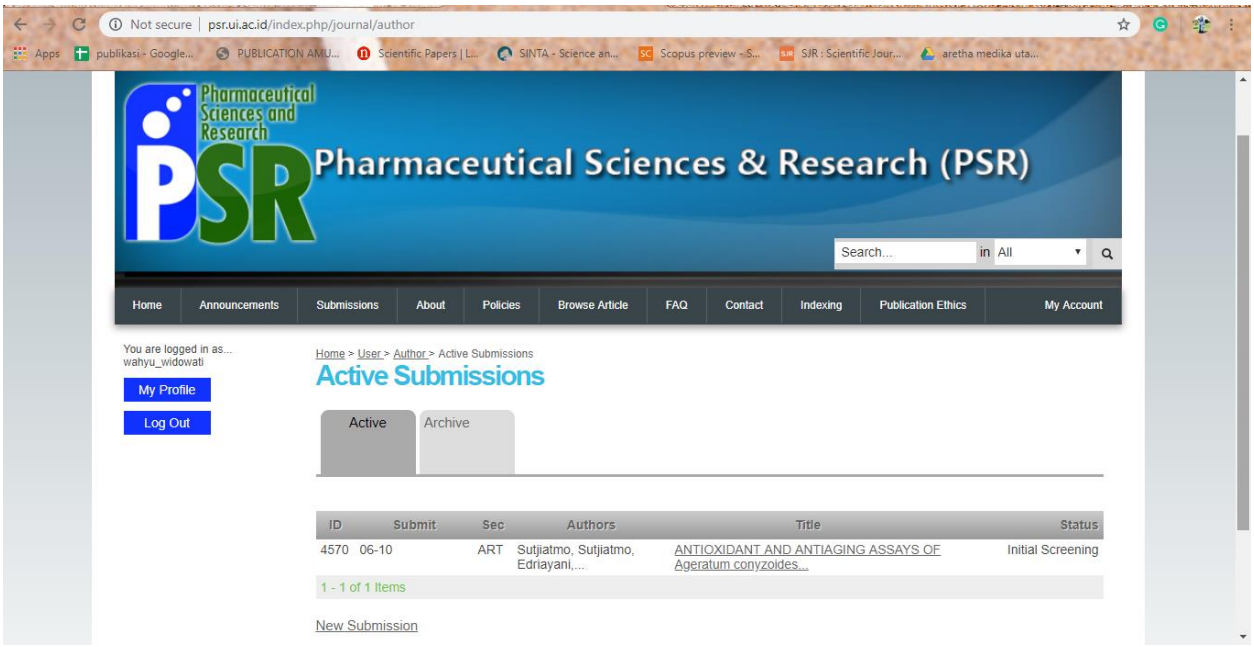
I am pleased to submit an original research article and I am enclosing here with a manuscript entitled "**Antioxidant and Antiaging Assays of *Ageratum conyzoides* (L.) Ethanol Extract Compared to Quercetin**" submitted to *Pharmaceutical Sciences & Research* for possible evaluation.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have directly approved the final version manuscript. We also no have conflicts of interest in this study.

Thank you for your consideration.

Sincerely,

Dr. Wahyu Widowati, M.Si
Faculty of Medicine, Maranatha Christian University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ANTIOXIDANT AND ANTIAGING ASSAYS OF Ageratum conyzoides...
Initial Screening
1 - 1 of 1 Items
New Submission

Antioxidant and antiaging assays of *Ageratum conyzoides* (L.) Ethanol extract compared to quercetin

GRAMMATICAL ERRORS
There are many grammatical errors to be rectified.

Singular vs plural problems, for example:
- …tissu**es** to repair or replace **it**self ..

Word-form problems, for example:
- Aging is caused by free radical **oxidative** that …. (adjective?)
- The plants were **determination** at … (noun?)

Incorrect word choice, for example:
- Ethanol filtrate was filtered, and **wastes** were remacerated…
- The **pasta** form is obtained by **condensation** using ...
- Flavonoid **identification**, alkaloid **identification**, etc… (in material and method)

Active-passive problems, for example:
- Measurement of 2,2-diphenyl 1-pichylhydazy (DPPH) scavenger **used** for antioxidant assay, and inhibitory of elastase and collagenase activities **used** for antiaging assay.
- **Add** 100mL of hot water, simmer for 5 minutes then filter.

Other typos, for example:
- Ses**k**uiterpenoids
- 1 **g**ram of sample… *(beginning of sentence)*

Incorrect chemical name:
- **2,2-diphenyl 1-pichylhydazy** (DPPH)

TITLE
Is “compared to quercetin” really necessary to be included in the title? Because it makes the results less meaningful since the activity is lower compared to quercetin.

INTRODUCTION
This part should be emphasized on “why this research is important”. Research question(s) and speciific purpose(s) of the research should be clearly stated.

MATERIAL AND METHODS
Methods should refer to the primary article of the standard method. For example, it is very unlikely that Widowati *et all* are the inventors of phytochemical sreening and DPPH assay methods used in this research.

RESULTS
Numeric data should be written in appropriate significant figure.

DISCUSSION
The discussion should be focused on the new findings obtained, comparing them to previous results of other authors etc. Rather than comparing to quercetin, it might be useful to compare with the activity of other plant extracts containing similar components. Also, it could be better if the authors

knows the quercetin content of the extract (by quantitative analysis or from literature) while using pure quercetin for the comparing standard.

REFFERENCES

It would be better if the author select only the closely related articles for refferences.

REVIEWER'S OPINION

The authors have publishable data, however the article needs rather big revision to make it acceptable for publication. It is advisable for the authors to have the article read by a native speaker before re-submission.

Abstract: There is a mention of "EEB", is it the same with "BEE"? If yes, please use the same acronym. If no, please give the long word for "EEB".

Yes, it's the same. I have changed it to BEE.

Introduction: Are "bandotan" in paragraph 4 and "babandotan" in paragraph 5, same with "babadotan"? If yes, please use the same term. In addition, please explain the research objectives.

Yes, it's the same too. I have changed it to babadotan and add the research objectives.

Materials and Method: Please make the methods in passive sentences. Please give the correct references, such as from the primary sources, not from a secondary paper that refer to other paper. In addition, there is a mention of "EEHB" in the "Collagenase Inhibitory Activity Assay", is it the same with "BEE"? If yes, please use the same acronym. If no, please give the long word for "EEHB".

I have changed it to passive sentences. For the methods we used, we used the modified methods by the cited journals, so it is different from the primary sources.

EEHB is the same with BEE. I have changed it to BEE.

Results: There are mentions of "EEHB" in the "Phytochemical Screening of BEE" and "Collagenase Inhibitory Activity Assay". Is "EEHB" the same with "BEE"? If yes, please use the same acronym. If no, please give the long word for "EEHB".

Yes it is the same. I have changed it.

Discussion: Please also compare the results with other plant extracts containing similar components. Please give explanation why the results are important or useful.

I have added the compared results with other plant.

English: There are many grammatical errors. Some of the sentences are confusing, such as there is no subject, predicate, or object, etc. Please correct them. It is recommended that the article is proofread by a native English speaker.

I have already corrected all the grammatical errors.

Overall: The data are publishable, but poorly written. It needs major revision. It can be published when the English has been corrected.

Thank you for the comment, we have already corrected all the grammatical errors in the entire manuscript and made revisions according to reviewer's comment.

Note: In order to make the tracking easier, please use red font for all the changes in the revision

Original Article

Pharmaceutical Sciences and Research (PSR), x(x), 2020, xx -xx

Antioxidant and Antiaging Assays of *Ageratum conyzoides* (L.) Ethanolic Extract

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ARTICLE HISTORY

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ABSTRACT

Background: The declining ability of tissues to repair or replace themselves and maintain their structure and normal function is called aging. Aging is caused by free radical oxidative that can induce the activation of elastase and collagenase. *Ageratum conyzoides* (L.) or known as Babadotan is a medicinal plant that has been reported to be a good source of antioxidants that also inhibit aging process. **Research objective:** The purpose of this research is to evaluate the antioxidant and antiaging properties of Babadotan Ethanolic Extract (BEE). **Method**: Franswoth method was used to determine the phytochemical assay of BEE. Measurement of 2,2-diphenyl 1-picrylhydrazyl (DPPH) scavenger was used for antioxidant assay, and inhibitory of elastase and collagenase activities was used for antiaging assay. **Results:** The analysis of phytochemical exhibited the presence of flavonoids, alkaloids, tannins, polyphenols, steroids and triterpenoids, monoterpenoids and terpenoids, and the absence of saponin. BEE has lower activity on DPPH scavenging activity (IC_{50} = 80.7 µg/mL) than quercetin (IC_{50} = 3.25 µg/mL). BEE exhibited lower elastase and collagenase inhibitory activity (IC_{50} = 45.35 ± 2.2 µg/mL and 55.07 ± 1.1 µg/mL, respectively) compared to quercetin (IC_{50} = 11.64 ± 0.67 µg/mL and 19.91 ± 0.46 µg/mL). Overall, BEE possesses antioxidant and antiaging activities, although the activities are lower than those of quercetin.

Keywords : *antioxidant; antiaging; babadotan; quercetin*; *collagenase*

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INTRODUCTION

The aging process is a physiological process that will occur in all living things, including all organs and skin. Naturally, every human being wants to look young, but the aging process will happen slowly and the skin is one of the body's tissues that directly shows the aging process. Although aging is something that must happen, the effort to prevent it never subsides. Various ways can be done to keep the skin healthy and young (Adam, 2005). Collagen and elastin are biomaterials that make up the dermis layer which plays an important role in the body, such as to repair and rebuild connective tissue (Widowati et al., 2018).

Under normal conditions, the skin produces enzymes such as elastase. Reactive oxygen species (ROS) or excessive exposure to ultraviolet (UV) light will accelerate the activation process of the elastase enzyme that can degrade elastin. Elastin is a major component of connective tissue elastic fibers. Elastic fibers on the skin, together with collagen fibers, form a tissue under the epidermis. Activation of the elastase enzyme will attack all major connective tissue matrix proteins, including elastin, which will lead to shrinkage of the skin (Kim et al., 2007; Mukherjee et al., 2011).

Antioxidant treatment may be useful for protecting the skin from aging (Taniguchi, 2012). Bioactive compounds as antioxidants from the plant have been widely studied to prevent aging. Various plants have been known to have activities as antioxidants that can prevent aging such as green tea, grape seeds, ginger (Cai et al., 2004), *Oryza sativa* (Widowati et al., 2016), *Hibiscus sabdariffa* (Widowati et al., 2017), and *Jasminum sambac* (Widowati et al., 2018).

Ageratum conyzoides or known as Babadotan is a plant that is widely grown in Indonesia and has been used since ancient times in the field of medicine. Babadotan plants are traditionally used for wound healing. According to a study, the ethanol extract of babadotan leaves with a concentration of 3%, 5%, and 7% can accelerate the process of wound healing and density of collagen fibers (Afrianti et al., 2014).

Ethanol extract of babadotan leaves with a concentration of 15% can accelerate the growth of collagen fibers, where the density of collagen fibers occurs on the 11th day of treatment (Afrianti et al., 2014). Babadotan plants also have antioxidant activities that can inhibit aging. The study stated that babadotan plants had antioxidant activity of free radical scavenging with IC_{50} value of 46.01 ± 2.23 µg/mL (Nasrin, 2013).

The active compounds contained in babadotan are flavonoids, alkaloids, chromenes, benzofuran, and pentenoids. The highest content of active compounds in babadotan is flavonoids, especially quercetin. Quercetin, a flavonol compound, has antioxidant activity and is widely explored for therapeutic use of babadotan (Galati et al., 2008; Tambunan et al., 2017). Other studies say that quercetin has anti-collagenase activity (Bougeois et al., 2016). Therefore, this study aims to examine the antioxidant and antiaging activity of babadotan ethanolic extract (BEE).

MATERIAL AND METHODS

Preparation of BEE

The plants of *A. conyzoides* were collected from Kebun Percobaan Manoko Lembang, Bandung, West Java, Indonesia. The plants were determinated at Tissue Culture Laboratory, Departement of Biology, Padjajaran University, Bandung, West Java, Indonesia. The dried simplicia of *A.conyzoides* (400 g) was sorted, extracted with distilled ethanol 70% by maceration method. The ethanol filtrate was filtered, and the obtained residues were re-macerated in triplicates until colourless filtrate was obtained. Then, the filtrate was dried using a rotary evaporator at 50°C. The yield of BEE was 49.5 g and stored at 20°C. The BEE was used in the experiment. Quercetin was used as a standard compound (Widowati et al., 2016; 2017; 2018).

Qualitative Phytochemical Assay

The phytochemical screening assay of BEE was evaluated by modified Farnsworth method to identify the presence of flavonoids, alkaloids, saponins, polyphenol, tannins, quinon, steroids and triterpenoids, monoterpenoids and sesquiterpenoids as listed below (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020; Farnsworth, 1966).

Flavonoid Identification

Sample of BEE (1 g) was added into a beaker glass. After that, 100 mL of hot water was added to beaker glass, then the mixture was simmered for 5 minutes before being filtered. The obtained filtrate (5 mL) was inserted into a test tube. Magnesium powder [Merck EM105815, USA] and 1 mL 2 N HCl were added to the test tube. The mixture in the test tube was heated before being filtered. After that, amyl alcohol was added to the 5 mL filtrate. A yellow to red colour will be formed if there were flavonoids in the mixture (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Alkaloid Identification

Sample of BEE (1 g) was weighed and inserted into the mortar. Then, the sample was grounded with 5 mL of ammonia. Chloroform 20 mL was added to the mixture. The mixture was filtered and the obtained filtrate was put into a test tube. HCL 2 N was added to the test tube, which was subsequently shaken to form 2 layers. After that, the Dragendorf reagent was added to the test tube. An orange-brown precipitate will form when there are alkaloids in the mixture. (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Saponin Identification

Sample of BEE (1 g) was put into a test tube, 10 mL of water was added, then the test tube was heated. The formed foam was observed. (positive saponin if 1 cm of foam was formed for 5-10 minutes) (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Tannin Identification

Approximately 1 g of sample was placed in the test tube with 2 mL of HCl 2 N [Merck 1003171000], then heated for 30 minutes. The mixture was filtered and amyl alcohol [Merck 10979, USA] was added to the filtrate. The colour purple will be formed if there are tannins in the mixture (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Polyphenol Identification

Sample of BEE (1 g) was heated with water. The mixture was filtered, and 2-3 drops of 1% $FeCl_3$ solution was added to the filtrate. The presence of polyphenols was characterized by the formation of blackish-blue (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Quinon Identification

Briefly 1 g of sample was heated with water, then filtered. The filtrate was added with 2-3 drops of 5% potassium hydroxide solution. The presence of quinones was characterized by the formation of red (Farnsworth, 1966).

Steroid /triterpenoid Identification
Approximately 1 g of sample was placed on the plate, then the sample was soaked with acetic acid for 10-15 minutes. One drop of absolute sulfate (H_2SO_4) [Merck 109073, USA] was added to the sample. Steroids were indicated by green/blue formations, and triterpenoids were indicated by the formation of orange/red sediments (Widowati et al., 2016, 2017, 2018; Siregar et al., 2019; Prahastuti et al., 2020).

Monoterpenoid and Sesquiterpenoid Identification
Briefly 1 g of BEE was added with 20 mL ether, then filtered. The obtained filtrate was evaporated on the evaporating dish to dry. At the residue, 2-3 drops of 10% vanillin reagent in concentrated sulfuric acid was added. The presence of monoterpenoids and sesquiterpenoids was characterized by the formation of colours (Farnsworth, 1966).

2,2-Diphenyl-1-Picrylhydrazil (DPPH) Assay
Method from Widowati et al. (2016) was used for the DPPH assay (Widowati et al., 2016; 2017; 2018). The method is based on the formation of non-radical DPPH-H resulted from the addition of hydrogen from an antioxidant characterized by a reduction in alcoholic DPPH solution (Sohn et al., 2003). Briefly. 50 µl various concentration of samples (BEE, quercetin), was introduced in 96-well microplate followed by addition of 200 µl of 0.077 mmol/L DPPH [Sigma Aldrich D9123, USA] into the well. The mixture was incubated at room temperature for 30 min in the dark. After that, absorbance was measured using a microplate reader [MultiskanTM GO Microplate Spechtrophotometer] at 517 nm. The scavenging activity (%) was calculated as below:

Scavenging Activity (%) = (Ac - As)/Ac x 100

Ac = negative control absorbance (without samples)
As = samples absorbance
The scavenging activity (%) was then continued to be calculated as median inhibitory concentration (IC_{50}) (Widowati et al., 2016; 2017; 2018).

Collagenase Inhibitory Activity Assay
Collagenase inhibitory assay was measured using previously modified method (Thring et al., 2009; Widowati et al., 2016; 2017; 2018; Utami et al., 2018). Mixed solution consisted of 60 µl Tricine buffer (50 mM, pH 8, containing 10 mM $CaCl_2$ and 400 mM NaCl), 10 µl collagenase enzyme from *C. histolyticum* [Sigma C8051] (0.2 U/mL in cold aquadest) and 30 µL of various level of sample (0- 1000 µg/mL for BEE, 0-1000 µM for compounds) (50 µg/mL, 25 µg/mL, 12.50 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 1.56 µg/mL and 0.78 µg/mL) was prepared. Then, the solution was incubated in the orbital shaker for 20 minutes at 37ºC. Then, 20 µL of N-[3-(2-Furyl)acryloyl]-leugly-Pro-Ala (FALGPA) [Sigma F5135] (col 1) substrate was added, then incubated for 20 minutes at 37°C, the absorbance was measured of 345 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific) (Thring et al., 2009; Widowati et al., 2016; 2017; 2018; Utami et al., 2018). The collagenase inhibition activity is calculated using the formula:

Inhibitory Activity (%) = (Ac - As)/Ac x 100

Ac = negative control absorbance (without samples)
As = samples absorbance
The median inhibitory concentration (IC_{50}) of collagenase assay were also calculated.

Elastase Inhibitory Activity Assay
Inhibitory activity of elastase was measured using previously modified method (Thring et al., 2009; Widowati et al., 2016; 2017; 2018; Utami et al., 2018). 10 µL samples (0.78 – 50 µg/mL) was pre-incubated at 25°C for 15 min with 5 µL elastase from porcine pancreas [Sigma 45124] (0.5 mU/mL in cold aquadest) and 135 µL Tris buffer (100 mM, pH 8, Phamacia Biotech 17-1321-01). After preincubation, the mixture was then added with 10 µL N-Sucanyl-Ala-Ala-Ala-p-Nitroanilide substrate [Sigma 54760] (2 mg/mL in Tris buffer), incubated at 25°C for 15 min. Absorbance was measured at 410 nm wavelength (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA).

Inhibitory activity (%) = (Ac-As)/Ac×100

Ac: negative control absorbance (without sample)
As: sample absorbance
The median inhibitory concentration (IC_{50}) of elastase assay was also calculated.

Table 1. The result of qualitative phytochemical screening of BEE

Phytochemical content	BEE
Phenols	+
Flavonoids	+
Triterpenoids/Steroids	+
Saponins	-
Tannins	+
Alkaloids	+
Quinons	+
Monoterpenoids and Sesquiterpenoids	+

+ : detected; - : Not detected

RESULTS

Phytochemical Screening of BEE

Phytochemical screening of BEE was conducted to find out presence of phenols, flavonoids, triterpeniods and steroids, tannins, alkaloids, kuinons, monoterpenoids and sesquiterpenoid. The result of BEE phytochemical screening was showed in Table 1. Phytochemical screening showed that phenols, flavonoids, triterpeniods and steroids, saponins, tannins, alkaloids, kuinons, monoterpenoids and sesquiterpenoid were detected, while saponins were not detected.

2,2 Diphenyl-1-Picrylhydrazil (DPPH) Assay

Antioxidant capacity of BEE and quercetin was determined using DPPH free radical scavenging activity. The method was based on the formation of non-radical DPPH-H resulted from the addition of hydrogen from an antioxidant characterized by a reduction in alcoholic DPPH solution (Sohn et al., 2003). The antioxidant molecule quenched DPPH free radical, which was characterized by the changing of purple colour of DPPH sample to become colourless. The median Inhibitory Concentration (IC_{50}) could be seen in Table 2. As shown in Table 2, the IC_{50} value of BEE was higher (80.7 µg/mL) than quercetin (2.73 µg/mL). These results indicate low scavenging activity of BEE among the sample.

Collagenase Inhibitory Activity Assay

Collagenase activity assay was measured by a spectrophotometric method. Collagenase is the enzyme that degrade collagen, an important component of the skin. The collagenase inhibitory activity can be seen in Figure 1. The IC_{50} value of collagenase inhibitory activity of BEE and quercetin was shown in Table 3. The inhibitory activity of BEE was comparable with quercetin. Figure 1. shows that collagenase activities of BEE (44.01 %) were lower than quercetin (89.73 %). However, as shown in Table 3, IC_{50} value of BEE (55.07±1,1 µg/mL) was higher than quercetin (19.91±0,46 µg/mL). These findings indicate BEE has lower collagenase inhibitory activity if compared to quercetin.

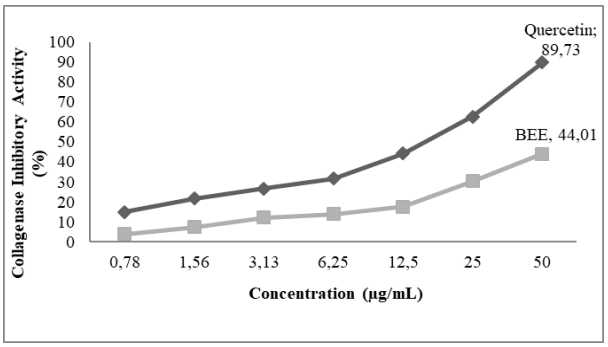

Figure 1. Collagenase inhibitory activity of BEE and quercetin (final concentration: 0.78; 1.56; 3.13; 6.25; 12.5; 25; and 50 µg/mL)

Table 2. The IC_{50} value of DPPH scavenging activity of BEE and quercetin

Samples	Equation	R^2	IC_{50} µg/mL	IC_{50} (µM)
BEE	y = 0.3811x + 19.245	0.996	80.7	-
Quercetin	y = 15.63x + 7.364	0.991	2.73	10.75

*Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated. IC_{50} of BEE was presented in µg/mL, while quercetin were presented in µM and µg/mL.

Table 3. The IC_{50} value of collagenase inhibitory activity of BEE and Quercetin

Samples	Equation	R^2	IC_{50} µg/mL	IC_{50} (µM)
BEE	y = 0.7707x + 7.558	0.9623	55.07±1.1	-
Quercetin	y = 1.4522x + 21.086	0.9696	19.91±0.46	65.93±1.53

*Linear equations, coefficient of regression (R^2) and IC_{50} of each sample were calculated. IC_{50} of BEE was presented in µg/mL, while quercetin were presented in µM and µg/mL.

Table 4. The IC_{50} value of elastase inhibitory activity of BEE and quercetin

Samples	Equation	R^2	IC_{50} µg/mL	IC_{50} (µM)
BEE	y = 0.7914x + 14.105	0.975	45.35 ±2.2	-
Quercetin	y = 1.6216x + 31.454	0.9873	11.44±0.67	37.84±1.4

*Linear equations, coefficient of regression (R2) and IC50 of each sample were calculated. IC_{50} of BEE was presented in µg/mL, while quercetin were presented in µM and µg/mL.

Elastase Inhibitory Activity Assay

The elastase inhibitory activity of BEE and quercetin was measured. The percentage of elastase inhibitory activity of BEE was shown in Figure 2. The elastase inhibitory activity of the highest concentration of BEE was lower (53.99%) than quercetin (115.36%). The IC_{50} value of elastase inhibitory activity of BEE and quercetin was shown in Table 4. The IC_{50} value of elastase inhibitory of quercetin (11.44 ± 0.67 µg/mL) was lower than BEE (45.35 ± 2.2 µg/mL) The result shows that BEE posseses lower elastase inhibition activity if compared to quercetin.

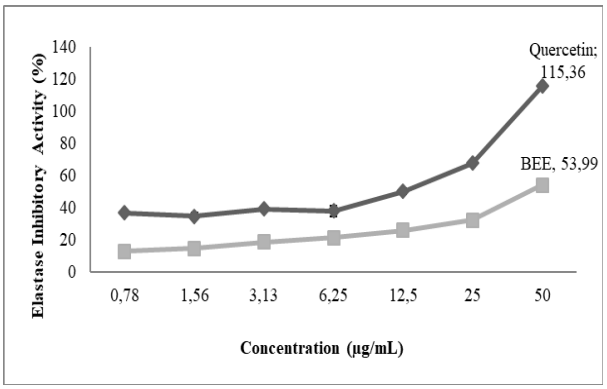

Figure 2. Elastase inhibitory activity of BEE and quercetin (final concentration: 0.78; 1.56; 3.13; 6.25; 12.5; 25; and 50 µg/mL)

DISCUSSION

Phytochemical screening is a qualitative analysis carried out by observing the colour changes. Table 1. showed that the results of phytochemical tests on BEE positively contained flavonoids, alkaloids, tannins, polyphenols, monoterpenesquiterpenes, steroids-triterpenoids, and quinones, while the saponin content in BEE was negative. The previous study of phytochemical content of Babadotan ethanol extract (*Aconyzoides* (L.) L.) showed no saponins (Wuyep et al., 2017). The active compounds contained in babadotan herbs include alkaloids, flavonoids, tannins, glycosides, minerals, steroids, coumarins, chromens, terpenoids, phenols and various other compounds (Amadi et al., 2012).

Babadotan has high flavonoid compounds. Flavonoids are phenolic compounds that have the potential to be antioxidants because they can prevent the development of free radicals in the body while repairing damaged body cells and can prevent aging by inhibiting factors that can accelerate aging. The presence of terpenoids in BBE has the potential to also act as an antioxidant because terpenoids can donate Hydrogen (H) atoms so that they can convert free radicals to reactive. This result was supported by previous research that the major compound of terpenoids in *Thuidium tamariscellum* extract had high antioxidant property by DPPH radical scavenging, $ABTS^{+}$ radical scavenging, hydrogen peroxide radicals scavenging, FRAP assay, and metal chelating activity (Mohanmedas & Kumaraswamy, 2018).

Reactive Oxygen Species (ROS) play an important role in the aging process. Therefore, antioxidants are used for the prevention of aging. Excessive production of ROS and oxidative stress were caused by UV radiation which is absorbed by the skin continuously. Oxidative damage caused other damage too, including modifying proteins and genes that alter the structure and function of proteins (Irshad et al., 2002). Excessive ROS level will result in the activation of collagenase and elastase, which contribute to the skin aging (Mukherjee et al., 2011).

Research on active compounds derived from plants has been carried out and some plants have been found to have anti-collagenase and anti-elastase activity, like white tea, green tea, and cleavers. Anti-colagenase and anti-elastase activities are caused by the presence of various phenolic compounds such as flavonoids derived from these plants (Thring et al., 2009).

The anti-elastase activity of BEE showed that BEE could potentially inhibit the elastase enzyme in degrading elastin compared to the negative control group because of the large amount of secondary metabolites present in BEE. The previous research reported that Babadotan has antioxidant activity with DPPH IC_{50} value of 46.01 ± 2.23 µg/mL (Nasrin, 2013). Study on another plant that contains quercetin like Babadotan has been done. The result showed that *Moringa oleifera* has antioxidant activity with a percentage of DPPH IC_{50} value 79 ± 0.28 µg/mL (Alimsyah et al., 2020). According to the results, antioxidant activity of Babadotan was more active than *Moringa oleifera.*

In the anti-elastase test, BEE was compared to quercetin. Quercetin is one of the flavonoid class active substances that is reported to have elastase inhibitory activity (Xu et al., 2009). According to the literature, babadotan extract has high flavonoids content, especially quercetin. Quercetin is a flavonoid compound of the flavonol group that can have antioxidant activity in babadotan. Therefore, quercetin can be chosen as a comparison in this test. The quercetin content in babadotan (*A. conyzoides* (L.) L.) is 209.622 ppm, which was obtained by digestion technique for two hours at 60°C using ethanol as a solvent. (Tambunan et al., 2017; Thring et al., 2009).

Based on the result, BEE can inhibit the elastase but this activity was lower than quercetin. It is because quercetin used in this experiment is pure quercetin, while BEE is a thick extract obtained from the extraction using ethanol

solvents. There are also other secondary metabolites contained in BEE. BEE has anti-elastase activity with IC_{50} value of 45.35 ± 2.2 µg/mL. Even though anti-elastase activity on BEE was lower than quercetin, there's study on another plant that contain quercetin. The study was based on *Moringa oleifera* plant and the anti-elastase activity of *Moringa oleifera* is 6444 ± 314.5 µg/mL (Alimsyah et al., 2020). It shows that the anti-elastase activity of BEE was more active than *Moringa oleifera.*

The anti-collagenase activity assay showed that quercetin had a higher collagenase inhibitory activity compared to BEE because it was suspected that quercetin was a compound that has been proved to have anti-collagenase activity of 79.3 ± 0.1µg/mL, according to the study (Bougeois et al., 2016). A previous research showed that anti-collagenase activity of quercetin is caused by the role of antioxidants (Ozgen et al., 2016). Antioxidants can inhibit free radicals that activated collagenase, so antioxidants can be anti-collagenase. According to a study, flavonoids especially flavonol, can inhibit collagenase with IC_{50} of 286 µM, and quercetin is a flavonoid compound that has anti-collagenase activity (Sin & Kim, 2005).

Based on the result, BEE has anti-collagenase activity with IC_{50} of 55.01 ± 0.1 µg/mL, it is suspected the anti-collagenase activity is caused by the role of flavonoids found in BEE. According to the study, BEE contains flavonoids, especially quercetin. Quercetin found in babadotan is Quercetine-3-O-glycoside and Quercetine-3-O-rutinoside (Tambunan et al., 2017). Study on *Moringa oleifera,* which contains quercetin like babadotan, has anti-collagenase activity of 47.25% (Nurulita et al*.,* 2019). Babadotan has anti-collagenase activity of 44.01%, a little lower than *Moringa oleifera*, but babadotan still can inhibit collagenase.

BEE, which contains quercetin, can prevent auto-oxidation by preventing the formation of peroxide radicals by fast-binding to radical compounds, so these radical compounds do not bind to oxygen. Quercetin binds to peroxide radicals that have been formed and stabilize them, so fast and chain auto-oxidation reactions can be inhibited. Quercetin will bind free radical species, so that it can reduce the reactivity of free radicals and inhibit anti-collagenase activity (Ikawati et al., 2008; Ozgen et al., 2016). In conclusion, BEE possesses antioxidant and antiaging activities that can prevent the aging process.

CONCLUSION

Phytochemical analysis of BEE showed the presence of flavonoids, alkaloids, tannins, polyphenols, quinones, steroids and triterpenoids, monoterpenoids and sesquiterpenoids, and the absence of saponin. BEE has lower activity on DPPH scavenging activity, elastase and collagenase inhibitory activity, if compared to quercetin. Overall, both BEE and quercetin possess antioxidant and antiaging activities.

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