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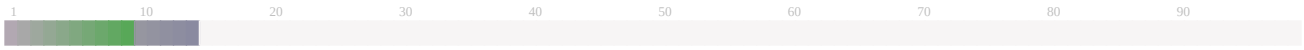

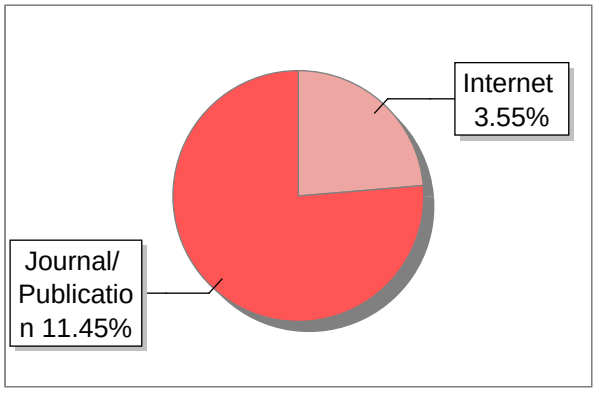

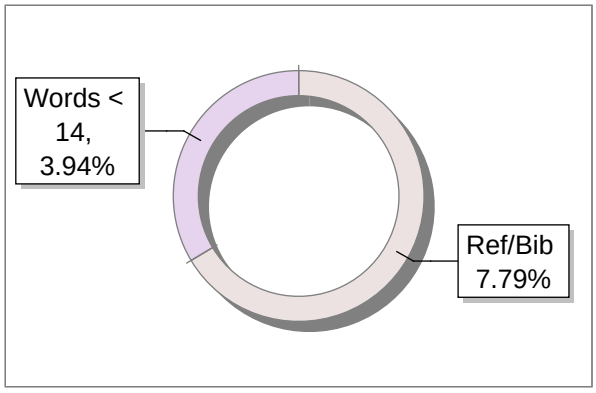

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The Effect of Methanol Concentration on the Extraction of Moringa Leaf (*Moringa oleifera*) and Papaya Fruit (*Carica papaya*) on Elastase and Hyaluronidase Installing Activities

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Abstract

BACKGROUND OF THE STUDY: Β-carotene, flavonoids, and phenolic compounds found in methanol extracts of Moringa leaves and papaya fruit have high antioxidant activity so they can be used for anti-aging. The concentration of solvent is a factor that will affect the levels of active ingredients in methanol extract. This study aims to determine the effect of the concentration of the methanol solvent derived from the extraction of Moringa leaves and papaya fruit on the inhibitory activity of the elastase and hyaluronidase enzymes.

AIM OF THE STUDY: This study aims to know the best extracts of methanol 50, 70, and 96% of Moringa leaf (*Moringa oleifera*) and papaya fruit (*Carica papaya*) as anti-aging agents through inhibition of elastase and hyaluronidase enzymes.

METHODOLOGY: In this study, variations in the concentration of methanol of 50%, 70%, and 96% were used for the extraction of Moringa leaves and papaya fruit. The extract was obtained by maceration method which was then tested for the inhibition of the enzyme activity of elastase and hyaluronidase using enzyme-linked immunosorbent assay.

RESULTS: The IC50 of the elastase enzyme inhibitory activity test on Moringa leaf extract with 50%, 70%, and 96% methanol solvents respectively = 9453.38; 6604.70; and 12,346.44 μg/mL, while the yield of papaya extract 5995.31; 9046.25; and 11,571.54 μg/mL. In addition, the test results showed that the inhibitory activity of the hyaluronidase enzyme showed the Moringa leaf extract with 50%, 70%, and 96% solvents $IC_{50,}$ respectively = 2944.53; 1028.36; and 3001.83 μg/mL, while the yield of methanol extract of papaya fruit is 982.67; 2982.96; and 3530.18 μg/mL.

CONCLUSION: Based on the test results, it can be concluded that the most effective solvent concentration as an inhibition of the enzyme elastase and hyaluronidase methanolic extract of Moringa leaves is 70% methanol solvent, while papaya fruit methanol extract which is effective in inhibiting the enzymes elastase and hyaluronidase is 50% methanol solvent.

Introduction

Skin aging is a natural process caused by environmental factors such as sunlight or chronic UV radiation. Frequent exposure to sunlight or UV radiation will accelerate physical changes in the skin and connective tissue through the formation of lipid peroxides, cell content, and reactive oxygen species (ROS). When exposure to the skin is frequent, it can cause a loss of elasticity in the skin and the formation of wrinkles, uneven pigmentation, skin cancer, and melanoma. The aging process occurs because collagen, elastin, and hyaluronic acid (HA) decrease which causes a loss of strength and flexibility in the skin. This process is also caused by an increase in the activity of the enzymes collagen, elastase, and hyaluronidase [1].

Elastin is a highly elastic protein found in connective tissues including skin, lungs, and arteries and helps in maintaining the configuration of tissues after stretching. When the skin is frequently exposed to UV light, it will cause oxidative damage so that the skin cannot regulate elastase, a serine protease that hydrolyzes the dermal elastic fiber network. This process can cause a decrease in skin elasticity and linearity of dermal elastic fibers, as well as causing wrinkles and sagging of the skin [2].

HA, also called hyaluronan, is widely distributed in the extracellular matrix of soft connective tissues including skin, umbilical cord, and synovial fluid. HA has the unique ability to retain 6000 mL of water in 1 mg HA and has an important role in reducing wrinkles, healing wounds, and keeping skin smooth and hydrated. The hyaluronidase enzyme breaks down HA into small oligosaccharide molecules by

cleaving the N-acetylglucosamine bond through the elimination process. By catalyzing the hydrolysis of HA, hyaluronidase decreases the viscosity of body fluids and increases the permeability of connective tissue. Hyaluronidase inhibitors are effective regulatory agents, which maintain a balance between HA anabolism and catabolism, and this keeps the skin moisturized and smooth [2].

Antioxidant compounds are substances that the body needs to neutralize free radicals and prevent the damage caused by free radicals to normal cells, proteins, and fats. This compound has a molecular structure that can donate electrons to free radical molecules without being disturbed at all by its function and can break the chain reaction of free radicals [1]. Natural antioxidant compounds are generally in the form of Vitamin C, Vitamin E, carotenoids, phenolic compounds, and polyphenols which can be in the form of flavonoids, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids that can counteract free radicals. This is because the -OH group and the double bond (>C = C<) possessed by the compounds above are able to donate electrons to free radicals so that three free radicals can be inhibited. When free radicals are inhibited, the increase in the elastase enzyme that can accelerate the aging process in the skin can be inhibited so that elastin which plays an important role in the elasticity process of the skin can carry out its duties normally and can prevent the aging process. In addition, when free radicals occur, the inhibition of the increase in the hyaluronidase enzyme which can accelerate the aging process of the skin can also be inhibited so that HA which plays an important role in the skin's moisture process can carry out its duties by maintaining a balance between anabolism and catabolism of HA so that the skin remains soft and smooth [3], [4], [5].

The development of antioxidants as inhibitors of elastase and hyaluronidase enzymes from nature is very necessary because Indonesia has abundant natural resources. One of the plants around us that can be used to inhibit elastase and hyaluronidase enzymes is Moringa leaf (*Moringa oleifera*) and papaya fruit (*Carica papaya*). Moringa leaves contain beta-carotene and Vitamin C, where beta-carotene is one of the agents in plants that function as anti-aging while papaya fruit (*C. papaya*) contains tocopherol, ascorbic acid (Vitamin C), beta-carotene, and flavonoids which are one of the agents in plants that function as anti-aging [3].

The antioxidant activity of Moringa leaf extract using the DPPH test method showed an IC_{50} value of 18.54 g/mL. This is to Jun (2006), antioxidants with IC_{50} values <50 g/mL have very active (very strong) antioxidant intensity. Moringa leaf (*M. oleifera*) can be used as a natural antioxidant by preventing damage caused by free radicals with IC_{50} values ranging from 5.72 to 42.56 g/mL. In addition, research conducted by Wulandari (2018), antioxidant activity, inhibition of tyrosinase and collagenase enzymes, and determination of the SPF value for sunscreen activity from methanol extract of *M. oleifera* leaves, showed that the concentration of the most effective solvent as an antioxidant, inhibition of the enzyme tyrosinase and collagenase, as well as sunscreen is 70% methanol solvent [1], [3].

While papaya fruit (*C. papaya*), antioxidant activity of methanol extract of papaya (*C. papaya*) showed IC_{50} values of 276.20 g/mL and 314.2 g/L. In addition, research conducted by Mayawati *et al.* (2012) on the effectiveness of antioxidants from the methanolic extract of papaya (*C. papaya*) which was formulated into cream preparations and showed the percentage of inhibition obtained to ward off the largest free radicals, namely, 81.17% with an IC_{50} of 99.8599 ppm. While the research conducted by Putri (2018), the anti-aging activity and tyrosinase inhibition of papaya fruit methanol extract with various solvent concentrations showed the optimal solvent to extract antioxidant compounds maximally and was able to provide tyrosinase, sunscreen, anticancer and collagenase inhibition activities was methanol 50% [3].

Based on the results of the previous studies obtained, the solvent concentration affects the concentration of the active substance which, in turn, affects the activity as an antioxidant, anti-collagenase, anti-tyrosinase, and SPF. This is because the concentration of the solvent affects the process of withdrawing the compounds contained in Moringa leaves and papaya fruit. Therefore, in this study, the results of the analysis will be evaluated by testing the inhibition of elastase and hyaluronidase enzymes with various concentrations of methanol as extraction solvent, namely, 50%, 70%, and 96%. This research is expected to show the best concentration of methanol solvent between Moringa leaf extract (*M. oleifera*) and papaya fruit (*C. papaya* L.) as inhibition of elastase and hyaluronidase enzymes.

Methods

Tool

The tools used in this study were analytical balance (Scout Pro), analytical balance (Mettler Toledo), water bath (Memmert), rotary evaporator (Buchi), oven (Memmert), pH meter (Omega), vortex (Maxi Mix), 96-well microtiter plate, enzyme-linked immunosorbent assay (ELISA) reader (BioTek), micropipette (Acura), volume pipette (Iwaki), arlogy glass (Iwaki), test tube (Iwaki), measuring flask (Iwaki), measuring cup (Iwaki), beaker (Iwaki), dropper (Iwaki), and funnel (Iwaki).

Ingredient

In this study, chemicals were used, including aqua DM, DMSO, absolute methanol (Merck), Moringa leaves (Beringharjo Yogyakarta Market), papaya fruit (Pundong Fruits Agro Papaya Gardens Yogyakarta), DQ elastin (EnzChek), ×10 reaction buffer (EnzChek), elastase from pig pancreas (EnzChek), N-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (EnzChek), HA 20 mg (Krishgen), standard hyaluronidase 10 mg (Krishgen), HSE buffer 30 mL (Krishgen), and 10 mL stop solution (Krishgen).

Simplicia Moringa (M. oleifera)

Sample preparation was carried out, namely, the samples were separated from the leaves and twigs, then dried using an oven with a temperature of 60°C and a drying time of 5 h. The dried Moringa leaves are then ground in a blender to a powder to facilitate the extraction process. Dried Moringa leaf powder is packed in plastic bags and stored in the freezer before the extraction process is carried out [6].

Papaya simplicia (C. papaya L.)

Material preparation is done by choosing undamaged papaya fruit. The papaya fruit is peeled and seeds removed, cleaned with running water, the flesh is taken, after which it is dried in the oven. Furthermore, the dried fruit flesh is crushed using a blender until smooth to facilitate the extraction process [7].

Making Moringa leaf methanol extract (M. oleifera)

Extraction of bioactive compounds from *M. oleifera* leaves was carried out to obtain phenolic compounds and high antioxidant activity, so maceration method was used. The maceration method was carried out with a ratio of 1:40. The preparation of the extract was first carried out by weighing 10 g of dry sample powder and then extracted using methanol solvent, each of which had a concentration of 50%, 70%, and 96% as much as 400 mL. The sample powder that has been weighed is immersed in the solvent for 72 h at room temperature. After that, it was filtered with filter paper and a vacuum pump, the extract obtained was evaporated using a rotary evaporator at a temperature of 70°C until the solvent evaporated and a thick extract was obtained [6], [8].

Making papaya methanol extract (C. papaya L.)

In this study, the papaya fruit used was young papaya that had been dried and powdered. Papaya fruit powder was then macerated using 50%, 70%, and 96% methanol in a ratio of 1:10 and allowed to stand for 72 h tightly closed at room temperature and protected from light while stirring occasionally. After 72 h, the Maserati was filtered with flannel cloth, then the result was filtered again using a Buchner funnel and stored in a glass jar. Next, repeat the same process twice at each concentration of methanol to obtain Maserati II and Maserati III. The three macerates were then combined and evaporated using a rotary evaporator at a temperature of 70°C until a thick extract was formed. The thick extract was then weighed and the yield was calculated [2], [7].

Elastase enzyme inhibitory activity test

Reagent preparation

First of all 1.0 mg/mL stock solution of DQ elastin substrate was added 1.0 mL of deionized water (dH_2O) each directly to the vial containing DQ elastin substrate. Then stir until dissolved. The reconstituted DQ elastin can be stored for approximately 1 week at 4°C, protected from light, with the addition of sodium azide to a final concentration of 2 mM. For longer storage, divide into aliquots and freeze at –20°C. The next step is to dilute 6 mL of ×10 reaction buffer in 54 mL of dH_2O. A volume of 60 mL ×1 reaction buffer is sufficient for at least 200 tests, each containing a volume of 200 L. Next, 100 g/mL of the DQ elastin substrate was taken and the DQ elastin stock solution was diluted ten times in ×1 reaction buffer. A volume of 50 L will be used for each reaction volume of 200 L. A stock solution of 100 U/mL was prepared by dissolving the contents of the vial (Elastase) in 0.5 mL dH_2O. The reconstituted elastase can be frozen in aliquots and stored at –20°C for at least 6 months without significant loss of activity. A 10 mg/mL stock solution of the elastase inhibitor, N-methoxysuccinyl-Ala-Ala Pro-Val-chloromethyl ketone, by adding 50 L of anhydrous dimethylsulfoxide directly to the inhibitor vial (N-methoxysuccinyl-Ala-Ala-Pro-Val chloromethyl ketone). This solution can be stored frozen at –20°C for at least 3 months [2], [4], [9].

Testing of elastase enzyme inhibitory activity

N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone solution was pipetted 50 L as an elastase inhibitor, EMDK 50%, 70%, 96%, and elastase inhibitory activity test of papaya fruit methanol extract (EMBP) 50%, 70%, 96% as sample in each well, add 50 L of DQ elastin solution with a concentration of 100 g/mL and pre-incubate for 5 min. Then, add 100 L of elastase enzyme at a concentration of 0.1 U/mL. Pipette up and down to mix and start the reaction. Incubate the samples at room temperature and protected from light for 60 min. Measure fluorescence using an ELISA rider with a wavelength of 505/515 nm (EnzChek® Elastase Assay Kit (E-12056), 2001) [2], [4], [9].

Hyaluronidase enzyme inhibition activity test

Reagent preparation

HA as much as 10 mg was dissolved in 10 mL HSE buffer. Standard hyaluronidase (HASE) 500 U/mL 10 mg dissolved in 10 mg HSE buffer [2].

Hyaluronidase enzyme inhibitory activity testing

The first step is a pipette of 60 L HASE, 60 L EMDK 50%, 70%, 96%, and 60 L EMBP 50%, 70%, 96%, respectively, into well A. Add 80 L HSE buffer. Pipette up and down to mix. Incubation at 37°C for 15 min. In the second stage, 100 L pipette from well A and put into well B. Add 100 L HA. Incubation at 37°C for 45 min. Then the third step, pipette 75 L from well B into well C. Add 75 L stop solution. Incubate at room temperature for 10 min. Measure the absorbance at a wavelength of 550 nm using a spectrophotometer ELISA (Hyaluronidase Enzymatic Assay Kit Krishgen Biosystems ver 3.0., 2018) [2].

Data analysis

Determination of elastase enzyme inhibition

Determination of the percent value of enzyme inhibition aims to determine the ability of Moringa leaf extract and papaya fruit to inhibit enzymes which have the property of being able to decompose enzymes produced naturally by the skin to maintain skin elasticity and provide flexibility and moisture to the skin. To calculate the % inhibition of the elastase enzyme, follow the following equation:

$$\text{Inhibition (\%)} = (1\text{-}A/B) \times 100$$

Note: A is the fluorescence of the sample (extracts of Moringa leaves and papaya fruit) and B is the fluorescence of elastase inhibitors (N-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone).

Determination of hyaluronidase enzyme inhibition

The absorbance obtained from the methanol extract of Moringa leaves and papaya fruit was compared with the inhibitor activity. The calculation of the percentage of hyaluronidase enzyme activity can be used the following formula:

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

Note: A = Sample Absorbance, B = Control Absorbance (tannic acid)

The results of the percent inhibition of methanol extract of Moringa leaf and papaya fruit were calculated IC_{50} value using linear regression equation. The smaller the IC50, the greater the antioxidant activity. IC_{50} is defined as the effective concentration of the substance in the methanol extract which can reduce the absorbance by 50% compared to the control absorbance. To find out the significant differences, the IC_{50} values of each concentration of methanol extract and IC_{50} were examined for differences using the ANOVA test.

Statistic test

The data obtained from the elastase and hyaluronidase enzyme inhibition tests, the primary data obtained from the absorbance of each comparison solution, made with a calibration curve and obtained a linear regression equation. The test data for the inhibition of elastase and hyaluronidase enzymes were obtained by entering into the linear regression equation y = bx + a, which was obtained from the calibration curve of each comparison solution, where y was the absorbance and x was the concentration or concentration of the compound. The test data for inhibition of elastase and hyaluronidase enzymes were analyzed by hypothesis testing, namely, normality test and homogeneity test. If the data from the normality and homogeneity test results show that the results are normally distributed and homogeneous, then proceed with the parametric test using one-way ANOVA statistical analysis. However, if it is not homogeneous or normally distributed, then it is continued with a non-parametric test using Kruskal–Wallis statistical analysis using SPSS.

Results and Discussion

Elastase enzyme inhibitory activity test

The elastase enzyme inhibitory activity was tested using an ELIZA spectrophotometer to measure fluorescence. The absorption obtained (fluorescence) was used to determine how much activity the methanol extract of Moringa leaves or papaya fruit had in inhibiting the DQ elastin reaction. Determination of elastase inhibitor activity was carried out by reacting the enzyme (elastase) with the substrate (DQ Elastin) along with the inhibitor (N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone) and, respectively, 50%, 70% methanol extract, and 96% Moringa leaf or papaya fruit where this reaction is an enzymatic reaction between the substrate and elastase to form elastin [2], [4], [10].

Elastase inhibitory activity test of moringa leaf methanol extract (EMDK)

Moringa leaves contain beta-carotene compounds, total flavonoids, and polyphenols such as quercetin which is thought to have elastase inhibitory

activity. The results of the elastase enzyme inhibition test of Moringa leaf methanol extract (EMDK) are presented in Table 1.

Table 1: EMDK

Sample	Value IC50 ± SD (µg/mL)	CV (%)
EMDK 50%	9453.38 ± 109.90^{A}	1.16
EMDK 70%	6604.70 ± 44.91^{B}	0.67
EMDK 96%	12346.44 ± 54.60^{C}	0.44

Different letters show a significant difference ($p < 0.05$). EMDK: Elastase enzyme inhibition test results Moringa leaf methanol extract.

Based on the results of the analysis, it can be said that the 70% methanol extract was the most optimal in showing the inhibition of the elastase enzyme. Where 70% methanol extract has the highest total phenolic content. Polyphenols have spectrophotometrically exhibited high antioxidant properties through free radical scavengers and were shown to be highly correlated with anti-collagenase and anti-elastase activity. In another study conducted on green tea installs (*Camellia sinensis*), polyphenols such as catechins and epigallocatechin Gabe are inhibitors of collagenase and elastase, subsequently decreasing skin wrinkling [2], [4], [10].

Moringa contains flavonoid compounds of the phenol group which are polar compounds because they have a substituted hydroxyl group or sugar so that it will dissolve in polar solvents such as methanol. So that the more polar the solvent concentration, the higher the flavonoid concentration should be. However, this is in contrast to the results of the study. Based on the results obtained, 70% methanol extract has the best elastase enzyme inhibitory activity compared to 50% methanol extract which is more polar. This is presumably because the flavonoid compounds attracted during the extraction process polyethoxyflavonoid compounds. The ethoxyflavonoid compound is a type of flavonoid compound that has a high solubility in non-polar solvents. So that causes the higher the solvent concentration of the extract (the more non-polar), the higher the total flavonoid content contained [2], [4], [6], [8], [10].

Based on Table 1, the IC_{50} value of the best inhibition of the enzyme elastase in Moringa leaves was found in 70% methanol extract of 6604.70 g/mL. The 70% EMDK had the greatest inhibition, presumably because 70% EMDK contained polyethoxyflavonoid compounds. Research by Wulandari *et al.* (2018) showed that 70% of methanol extract contained the most optimal total phenolic compounds compared to 50% and 96% of methanol solvents. In addition, 70% methanol extract showed the greatest antioxidant activity compared to 50% and 96% methanol extract. Methanol is a strong polar solvent which that considered in most plant secondary metabolite extracts [2], [4], [6], [8], [10].

EMBP

Papaya fruit contains flavonoid compounds of the phenol group which are polar compounds because they have a substituted hydroxyl group sugar, so they will dissolve in polar solvents such as methanol. Based on the research of Putri *et al.* (2018), papaya fruit methanol extract has antioxidant activity with an IC_{50} ranging from 101 to 250 g/mL. However, the results of this study are better than the research conducted by Maisarah *et al.*, 2013, which states that half-ripe papaya fruit gives an IC_{50} value of 430 g/mL, this is probably due to the type of papaya fruit and the method used for testing is different. The results of the EMBP elastase enzyme inhibition test are presented in Table 2.

Table 2: Papaya fruit elastase inhibition enzyme inhibition test results (EMBP)

Sample	Value IC50 ± SD (µg/mL)	CV (%)
EMBP 50%	5995.31 ± 106.42^{A}	1.77
EMBP 70%	9046.25 ± 28.96^{B}	0.32
EMBP 96%	11571.54 ± 50.04^{C}	0.43

Different letters show a significant difference ($p < 0.05$). EMBP: Enzyme inhibition test results

Based on the data results, it can be seen that the greater the concentration of the solvent, the lower the inhibitory activity of the elastase enzyme. This may be due to the active compound in papaya fruit which has more elastase enzyme inhibitory activity, namely, the solvent is polar so based on the results above, papaya fruit extract is more easily extracted in a 50% more polar methanol solvent [2], [4], [6], [7], [10].

According to Putri *et al.* (2018), the total flavonoid content is abundant in the methanol extract of papaya fruit which was extracted with 50% methanol as solvent. This is because the active compounds contained in papaya fruit which are anti-aging have the same polarity properties as 50% methanol which contains more water mixture so it will affect increasing the priority of the filtered liquid which can help increase the contact surface area of methanol to attract flavonoid compounds and this causes more flavonoids in papaya fruit such as extracted quercetin (Sudirman *et al.*, 2016). Based on research conducted by Putri *et al.* (2018), it was reported that papaya fruit extracted with 50% methanol contained the highest flavonoid, namely, quercetin. Quercetin is a flavonoid compound of the flavonol group and belongs to the type of polyhydroxy flavonoid containing 5 OH groups so the compound tends to be more polar. In addition to quercetin, the flavonoid compound that is also extracted from 50% methanol is myricetin. Myricetin is a flavonoid compound that has 6 OH groups, making it tend to be polar [2], [4], [6], [7], [10].

Based on the results obtained, it can be concluded that the elastase inhibitory activity of papaya fruit extract methanol is found in 50% methanol solvent where the value of sig. ($p < 0.005$) which can be said to be a significant difference in 50% methanol. This is also to research conducted by Putri *et al.* (2018) which reported that 50% of methanol extract has the active compounds quercetin and myricetin which have anti-aging activity in the skin aging process. Based on the IC_{50} EMDK and EMBP results in Figure 1, the most effective in inhibiting the elastase enzyme in papaya fruit methanol extract with an IC_{50} value of 5995.31 g/mL. It can be concluded that the inhibition of the elastase enzyme can be inhibited by polar solvents,

namely, 50% methanol concentration. This is directly proportional to the research conducted by Putri (2018) which states that 50% methanol solvent has the highest antioxidant activity compared to 70% and 96% methanol extracts because the higher the antioxidant activity of an extract, the formation of ROS in skin tissue can be prevented so that elasticity in the skin can be controlled normally. Furthermore, the data were carried out with way ANOVA statistical test, the results obtained were significantly different between each group, which means that the solvent concentration influenced the inhibition of the elastase enzyme [2], [4], [6], [7], [10].

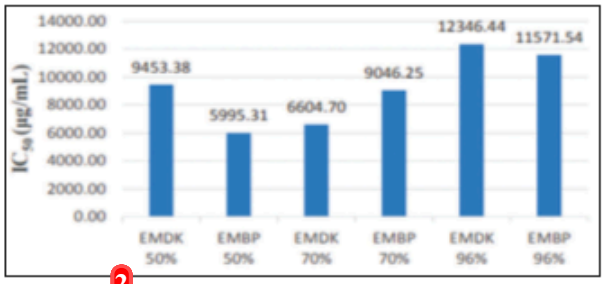

Figure 1: IC_{50} value of Moringa leaf ethanol extract and papaya fruit methanol extract on elastase enzyme test

Furthermore, in this test, the inhibitory activity of the elastase enzyme was determined using EMDK and EMBP with concentrations of 5000, 7500, 10,000, 12,500, and 1500 g/mL. The results of the IC_{50} values of Moringa leaf methanol extract and papaya fruit methanol extract with variations in the concentration of methanol solvent against elastase enzyme inhibition are shown in Figure 1.

Hyaluronidase enzyme inhibitory activity test

The purpose of this test was to determine how much activity the methanol extract of Moringa leaves or papaya fruit had in inhibiting the hyaluronidase enzyme inhibitory activity. This test was carried out using an ELISA spectrometer to measure absorbance. The wavelength used in testing the inhibitory activity of the elastase enzyme was 550 nm. Determination of hyaluronidase inhibitor activity was carried out by reacting the enzyme (hyaluronidase) with standard HA along with the inhibitor (stop solution) and each 50%, 70%, and 96% methanol extract of Moringa leaf or papaya fruit where this reaction is an enzymatic reaction [2].

Hyaluronidase inhibitory activity test of moringa leaf methanol extract (EMDK)

Moringa contains protein, vitamins, B-carotene, amino acids, and phenolic compounds. In addition, Moringa plants are also rich in zeatin, quercetin, sitosterol, caffeoylquinic, and kaempferol compounds (Anwar *et al*., 2007). Moringa leaves have been reported to be a rich source of B-carotene, protein, Vitamin C, calcium, and potassium and act as a good source of natural antioxidants; and thus increase the shelf life of fatty foods due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids [2], [6], [8]. The results of the hyaluronidase enzyme inhibition test results from Moringa leaf methanol extract are presented in Table 3.

Table 3: Hyaluronidase enzyme inhibition test results Moringa leaf methanol extract

Sample	Value IC_{50} ± SD (µg/mL)	CV (%)
Tanic acid	57.53 ± 6.94	12.06
EMDK 50%	2944.53 ± 28.88	0.98
EMDK 70%	1028.36 ± 82.93	8.06
EMDK 96%	3001.83 ± 84.09	2.80

Based on the results obtained in the enzyme inhibition test hyaluronidase EMDK, 70% methanol extract obtained the highest IC50 activity compared to 50% and 96% methanol solvents. This is to research conducted by Wulandari *et al*. (2018) which stated that 70% methanol extract had the highest total phenolic content and antioxidant activity value compared to 50% and 96% methanol extract. This is to the research conducted by Mohammedi and Atik (2001) showing that better phenolic content was found in 70% methanol extract compared to other solvents and is in perfect agreement with the previous finding that aqueous alcohol solvents were effective against *Tamarix aphylla* extraction due to the advantages of a mixture of alcohol and water in adjusting the polarity of the solvent. The IC_{50} results of the hyaluronidase enzyme in Moringa leaf methanol extract are shown in Table 3. Based on the results obtained, 70% methanol extract had the highest inhibitory activity (IC_{50}) which was close to the standard compared to 50% and 96% methanol extract. Whereas 50% methanol extract has more polar properties than 70% methanol extract. This is presumably because the flavonoid compounds attracted during the extraction process are polyethoxyflavonoid compounds where this polyethoxyflavonoid compound is a type of flavonoid compound that has a high solubility in non-polar solvents. This is consistent with the results of other studies which show that extraction with non-polar solvents will increase flavonoid levels [2], [6], [8]

EMBP

The purpose of this test was to determine the ability of papaya fruit methanol extract to inhibit the hyaluronidase enzyme so that the normal amount of HA in the skin is not large or small so that the skin is kept moist because if HA is not normal, it can cause skin aging or skin dryness which can cause skin irritation hyperpigmentation is formed [2], [7]. The results of the EMBP hyaluronidase enzyme inhibition test are presented in Table 4.

Table 4: Results of hyaluronidase enzyme inhibition test results of papaya fruit methanol extract

Sample	Value IC_{50} ± SD (µg/mL)	CV (%)
Asam Tanat	57.53 ± 6.94	12.06
EMBP 50%	982.67 ± 22.43	2.28
EMBP 70%	2982.96 ± 28.17	0.94
EMBP 96%	3530.18 ± 12.23	0.34

EMBP: Elastase inhibitory activity test of papaya fruit methanol extract

Based on the results of the EMBP hyaluronidase enzyme inhibition test in Table 4, it can be concluded that the higher the concentration of the solvent used, the lower the hyaluronidase enzyme inhibition, so it can be stated that 50% methanol extract has the highest polar activity. This is directly proportional to the research conducted by Putri *et al*. (2018), 50% methanol solvent has the highest antioxidant activity compared to 70% and 90% methanol extracts because the higher the antioxidant activity of an extract, the formation of ROS in skin tissue can be prevented so that melanin synthesis can be prevented. This is presumably because 50% methanol extract contains the highest flavonoid compounds Flavonoids can work to inhibit the hyaluronidase enzyme due to the presence of free hydroxyl groups at positions 3 and 5 or at positions 3' and 4' which can form chelates with hyaluronidase. The active site of the enzyme, namely, the copper part will bind to form a complex with the phenol group contained in polyphenol and flavonoid compounds. Based on the results obtained above, it can be concluded that the hyaluronidase enzyme inhibitory activity of papaya fruit extract is found in 50% methanol solvent where the IC50 results are close to the standard compared to 70% and 96% methanol. This is also to research conducted by Putri *et al*. (2018) which reported that 50% of methanol extract has the active compounds quercetin and myricetin which have anti-aging activity in the skin aging process [2], [7].

The results show that based on Figure 2, the most effective in inhibiting the hyaluronidase enzyme between Moringa leaf extract and papaya fruit with varying concentrations, namely, papaya fruit methanol extract with a concentration of 50%. Hence, it can be concluded that the hyaluronidase enzyme can be inhibited by polar solvents. This is directly proportional to the research conducted by Putri (2018) which states that 50% methanol solvent has the highest antioxidant activity compared to 70% and 96% methanol extracts, because the higher the antioxidant activity of an extract, the formation of ROS in skin tissue can be prevented so that melanin synthesis can be prevented. Factors that can increase the occurrence of hyperpigmentation can be reduced. In the hyaluronidase enzyme test, statistical tests were not carried out because the hyaluronidase enzyme test only carried out two replications due to the lack of kits for the hyaluronidase enzyme [2].

Furthermore, in this test, the hyaluronidase enzyme inhibitory activity was determined using EMDK and EMBP with concentrations of 625, 1250, 2500, 5000, and 7500 g/mL and positive control tannic acid with concentrations of 25, 125, 250, 500, and 625 g/mL. The selection of tannic acid as a positive con tool is because it is a flavonoid group that has hyaluronidase inhibitory activity of 94–100%. The results of the IC50 values of Moringa leaf methanol extract and papaya fruit methanol extract with varying concentrations of methanol and tannic acid on the hyaluronidase enzyme are shown in Figure 2.

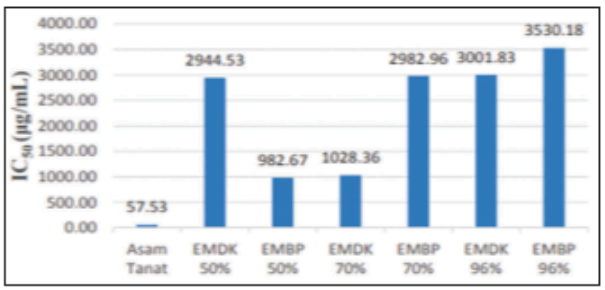

Figure 2: IC_{50} value of Moringa leaf methanol extract and papaya fruit methanol extract on hyaluronidase enzyme test

Optimal extracts and solvents in all test parameters

Based on Table 5 above, it can be concluded that the inhibition test of elastase and hyaluronidase enzymes from the two extracts including Moringa leaf extract and papaya fruit extract which has the best IC_{50} value in the inhibition test of elastase and hyaluronidase enzymes, respectively, is papaya fruit extract of 5995.31 g/mL and 982.67 g/mL. Variations in solvent concentration also greatly affect the extraction of active substances in both extracts, where based on Wulandari's (2018) research on Moringa leaf extract, it is suspected that the active compound that plays a role in inhibiting the elastase enzyme and hyaluronidase enzyme is a polyethoxyflavonoid compound found in 70% methanol which has a value highest IC_{50}. Meanwhile, based on research conducted by Putri (2018) on papaya fruit extract, it is suspected that the active compound extracted with 50% methanol contains the highest flavonoids, namely, quercetin and myricetin which play a role in inhibiting elastase and hyaluronidase enzymes [2], [4], [9], [10].

Table 5: Optimal extract and solvent test results in all parameters

Test parameters	Extract	Test results	Value IC_{50} (µg/mL)
Elastase	Moringa leaf	EMDK 70% > EMDK 50% > EMDK 96%	6604.70 > 9453.38 > 12346.44
	Papaya fruit	EMBP 50% > EMBP 70% > EMBP 96%	5995.31 > 9046.25 > 11571.54
Hyaluronidase	Moringa leaf	EMDK 70% > EMDK 50% > EMDK 96%	1028.36 > 2944.53 > 3001,83
	Papaya fruit	EMBP 50% > EMBP 70% > EMBP 96%	982.67 > 2982.96 > 3530.18

From the explanation above, compounds that play a role in inhibiting elastase and hyaluronidase enzymes, namely, polyethoxyflavonoids, myricetin, and quercetin are compounds that can counteract free radicals. The process of skin aging is caused by free radicals, where when human skin is often exposed to

sunlight or UV rays that can enter the deepest skin structure, namely, dermal fibroblasts, this can trigger an excessive increase in free radicals. In the human body, there are antioxidant compounds that can ward off free radicals, but when human skin is often exposed to excessive sunlight, antioxidant compounds in the body are unable to ward off free radicals that enter continuously and increase. For this reason, antioxidant compounds from outside the body are needed. In this study, the inhibition of elastase and hyaluronidase enzymes in Moringa leaf extract and papaya fruit was carried out to obtain antioxidant compounds such as flavonoids, quercetin, and myricetin which have a free radical inhibitory activity that can donate electrons to free radicals. The activity of free radical can be inhibited [2], [4], [9], [10].

When free radicals in the skin increase, this can trigger a rapid skin aging process because the enzymes that play a role in the aging process increase excessively, including the enzymes collagenase, hyaluronidase, and elastase. For this reason, the presence of antioxidants from outside the body such as flavonoid compounds, myricetin, and quercetin found in Moringa leaf extract and papaya fruit is needed, which has activity in inhibiting free radicals by donating electrons to free radicals. When free radicals are inhibited, elastin which plays an important role in skin elasticity can carry out its duties normally so that the skin aging process can be avoided. In addition, HA which plays an important role in controlling skin moisture can also carry out its duties properly so that the skin aging process can also be avoided [2], [4], [9], [10].

From all the test parameters, the results of the methanol extract of Moringa leaves and papaya fruit extract with various concentrations can be concluded as shown in Table 5.

Conclusion

Based on the results of the tests that have been carried out, it can be concluded:

1. The concentration of solvent that showed the most optimal inhibitory activity of elastase and hyaluronidase enzymes in Moringa leaf (*M. oleifera*) methanol extract was 70% methanol extract.
2. The concentration of the solvent that showed the most optimal inhibitory activity of the elastase and hyaluronidase enzymes in the papaya fruit (*C. papaya* L.) methanol extract was 50% methanol extract.
3. The most optimal extract in showing the inhibitory activity of the elastase and hyaluronidase enzymes was the methanol extract of papaya fruit (*C. papaya* L.) with a concentration of 50% each with IC_{50} values of 5995.31 g/mL and 982.67 g/mL.

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