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Anti-Elastase Potential in Vitro of Koro Komak (Lablab Purpureus) Extract as Anti-Aging Protection

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ABSTRACT

The aging process leads to skin changes such as wrinkles and loss of elasticity, often accelerated by premature aging caused by factors like UV radiation and reactive oxygen species (ROS). *Elastase*, an enzyme responsible for degrading *elastin* in the skin's extracellular matrix, plays a critical role in this process. This study aimed to investigate the anti-*elastase* potential of *koro komak* (*Lablab purpureus*) extract, a plant rich in polyphenolic compounds, through in vitro testing. A quasi-experimental design was employed, using different concentrations of the extract compared to ascorbic acid as a positive control. Phytochemical screening confirmed the presence of flavonoids, phenols, and tannins. The extract exhibited moderate anti-*elastase* activity with an IC_{50} value of 128 µg/mL, and at 200 µg/mL concentration, it inhibited *elastase* enzyme activity by 65.90%. These findings suggest that *koro komak* extract has promising potential as a natural anti-aging agent by protecting skin elasticity through *elastase* inhibition.

Keywords: premature skin aging, elastase inhibition, koro komak extract

INTRODUCTION

The aging process is a dynamic phenomenon that represents one of the main skin concerns for everyone. This process leads to various skin changes such as wrinkles, pigmentation, dryness, dullness, and roughness (Atmaja et al., 2012; Sundaram et al., 2018). Aging can occur prematurely or at a rate inconsistent with one's biological age, a condition referred to as premature skin aging (Ahmad et al., 2018). Premature aging is characterized by a decline in skin elasticity caused by the degradation of *elastin* by the enzyme *elastase* in the extracellular matrix (Natanael et al., 2021).

Elastase is a type of serine protease enzyme that can damage all components of the extracellular matrix in the skin's connective tissue, including collagen, fibronectin, and *elastin*. In the skin, *elastase* is secreted by neutrophils and is influenced by both intrinsic and extrinsic factors that can increase the production of reactive oxygen species (ROS) (Ahmad et al., 2018). Intrinsic factors include genetic predisposition, hormonal changes, and enzymatic processes within the body, while extrinsic factors include UV radiation exposure, air pollution, cosmetic use, and cigarette smoke. UV radiation and ROS can lead to excessive *elastase* activation, resulting in increased hydrolysis of the skin's connective tissue matrix proteins, particularly *elastin*. This activity leads to the loss of skin elasticity and accelerates the visible signs of premature aging (Chiocchio et al., 2018).

Premature aging caused by UV radiation is known as photoaging. The prevalence of photoaging has increased over the past few decades. A study conducted in Australia by Green reported that 72% of men and 42% of women under the age of 30 showed signs of photoaging. Individuals living in geographic areas with frequent sun exposure are at higher risk of UVB

radiation exposure, making them more susceptible to premature aging (Ahmad et al., 2018). Therefore, efforts are necessary to prevent and address premature aging, including the use of natural compounds that act as anti-*elastase* agents (Wilkerson, 2018).

Inhibiting *elastase* activity can protect *elastin* from degradation due to UV radiation and ROS, thereby preventing damage to the extracellular matrix (Desmiaty et al., 2020). The use of anti-*elastase* agents helps prevent the loss of skin elasticity (Ambarwati et al., 2019).

Many natural ingredients possess anti-*elastase* activity and are commonly used in anti-aging products. Most of these natural ingredients are rich in antioxidant compounds such as polyphenols, particularly flavonoids, phenols, and tannins. Polyphenols are secondary metabolites of various plant species, and research has shown that they exhibit antioxidant, anti-hyaluronidase, anti-collagenase, anti-tyrosinase, and anti-*elastase* activities, making them highly effective in slowing the process of premature aging (Natanael et al., 2020).

Koro komak (*Lablab purpureus*) is a low-cost, adaptable plant widely consumed in East Java. However, it remains underutilized and relatively unknown due to its seasonal growth (Diniyah et al., 2020; Kurnianingsih et al., 2021). *Koro komak* contains bioactive secondary metabolites such as saponins, flavonoids, steroids, alkaloids, coumarins, carbohydrates, terpenoids, tannins, oxalates, phytates, cyanogenic glycosides, and free phenolics in its crude extract (Adebis, 2017). Studies have shown that ethanol extracts of *koro komak* possess antihyperglycemic and antihypercholesterolemic effects in hypercholesterolemic diabetic hamsters. Additionally, it can protect cells from UV radiation through its strong antioxidant activity. However, to date, no research has investigated the potential of polyphenols in *koro komak* as anti-*elastase* agents (Wardani et al., 2015; Diniyah et al., 2020).

Based on the above explanation, it is necessary to conduct research on the anti-*elastase* potential of *koro komak* extract through in vitro testing for its anti-aging properties. The use of extracts in this study aims to maximize the isolation of the active polyphenol compounds in *koro komak*. The researchers hope that *koro komak* can become a promising alternative for the prevention and development of therapies for premature skin aging.

METHOD

This research was a quasi-experimental study using a post-test only non-equivalent control group design to analyze the potential of *koro komak* (*Lablab purpureus*) powder extract as an anti-aging agent through the measurement of elastase enzyme inhibition activity. The study was conducted using an experimental method with sample groups consisting of *koro komak* (*Lablab purpureus*) extract at varying concentrations and a positive control in the form of ascorbic acid. The research stages included equipment sterilization, preparation of *koro komak* (*Lablab purpureus*) extract, test sample preparation, control preparation, phytochemical testing, and elastase enzyme inhibition testing.

RESULTS AND DISCUSSION

In this study, 100 grams of koro komak were used to become 12.76 grams of dried extract. The yield value of 70% ethanol coarse extract of koro komak seeds in this study was 12.8%. The manufacture of koro komak extract is carried out by taking 2000 µg of dried extract and dissolving it into 2% DMSO. The preparation is diluted with the same solvent into

concentrations of 200, 100, 50, 25, and 12.5 µg/mL. Furthermore, there is a positive control as a comparison, namely ascorbic acid. Ascorbic acid is weighed and dissolved until a solution with a concentration of 500 µg/mL is obtained, then ascorbic acid is diluted to concentrations of 200, 100, 50, 25, and 12.5 µg/mL

Phytochemical Screening Test of Koro Komak Extract

In this study, the identification of secondary compounds contained in the sample was carried out using a phytochemical screening test of the tubular method based on Gopinath (2021). Koro komak extract shows positive results on flavonoids, phenols, and tannins. The results of the phytochemical screening of koro komak extract are presented in Table 1.

Table 1. Results of phytochemical screening test of koro komak extract

		Discoloration		
Active compounds	**Reagents**	**Back**	**End**	**Result**
Flavonoids	H2SO4	Clear yellow	Orange	+
Phenol	FeCl3	Clear yellow	Black	+
Tannins	gelatin solution	Clear yellow	White deposits	+

Results *Anti-elastane Activity*

The results of inhibition *of anti-elastase* enzymes from each extract showed higher activity as concentration increased. The activity of *the anti-elastase enzyme* of koro komak extract (EKK) can be seen in Table 2 and Figure 1.

Table 2. Percentage of *anti-elastase* activity of koro komak extract

EKK Concentration	**Inhibition of *the enzyme elastase***
(µg/mL)	*(%)*
200	65,90%
100	50,40%
50	28,33%
25	16,72%
12,5	13,87%

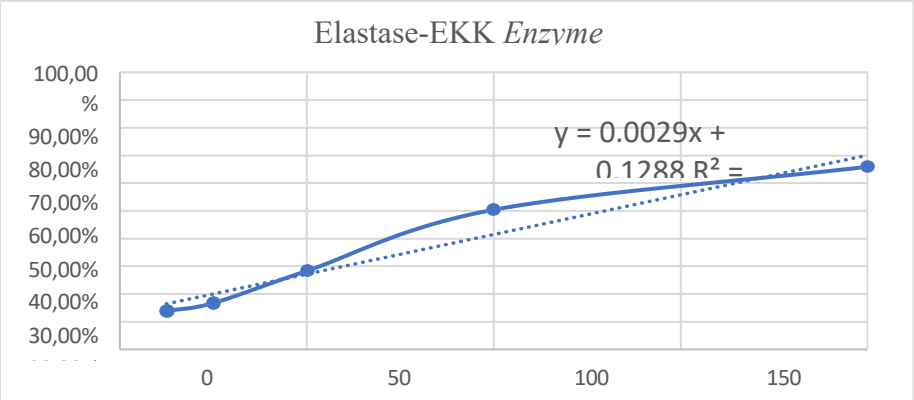

Figure 1. Percentage *of anti-elastase* of koro komak extract

The results showed that the highest *anti-elastase activity* of koro komak extract was at a concentration of 200 µg/mL with an inhibition percentage of 65.90% and the lowest activity was at a concentration of 12.5 µg/mL with an inhibition percentage of 13.87%. The results were then compared with the positive control, namely ascorbic acid (AA) which can be seen in Table 3 and Figure 2.

Table 3. Percentage of *anti-elastase* activity of ascorbic acid

AA concentration (µg/mL)	Inhibition of *the enzyme elastase (%)*
200	90,79%
100	73,43%
50	39,86%
25	23,25%
12,5	15,55%

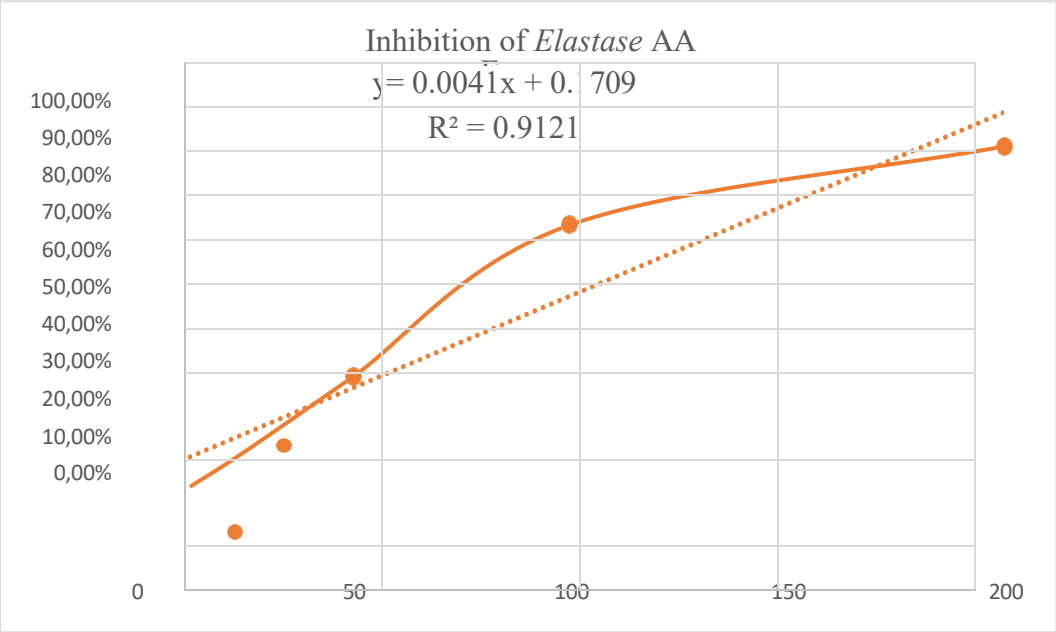

Figure 2. Percentage *of anti-elastase* activity of ascorbic acid

Ascorbic acid showed the highest *anti-elastase* activity at a concentration of 200 µg/mL with a percentage of 90.79% and the lowest activity was at a concentration of 12.5 µg/mL with a percentage of 15.55% shown in Table 3 and Figure 2.

Data Analys

After knowing the inhibition activity of *elastase enzymes* from koro komak extract and ascorbic acid, the next step is data analysis. The method used to analyze the sample data in this study is to use the IC50 value obtained from linear regression analysis. IC Value it is a concentration that can inhibit 50% of *the activity of anti-elastase* enzymes and has properties that are inversely proportional to the inhibition percentage of an enzyme. The smaller the IC50 value, the higher the *anti-elastane* activity (Sawant and Varsha, 2017). The IC50 value of koro

komak (EKK) and ascorbic acid (AA) extracts in this study was obtained from the results of linear regression analysis as shown in Table 4 and Figure 3 below.

Table 4. Linear regression equation and IC50 values of *anti-elastase activity*

Sample	Equation	R2	IC50 (µg/mL)
EKK	y = 0.0029x + 0.1288	0,9425	128
AA	y = 0.0041x + 0.1709	0,9121	80

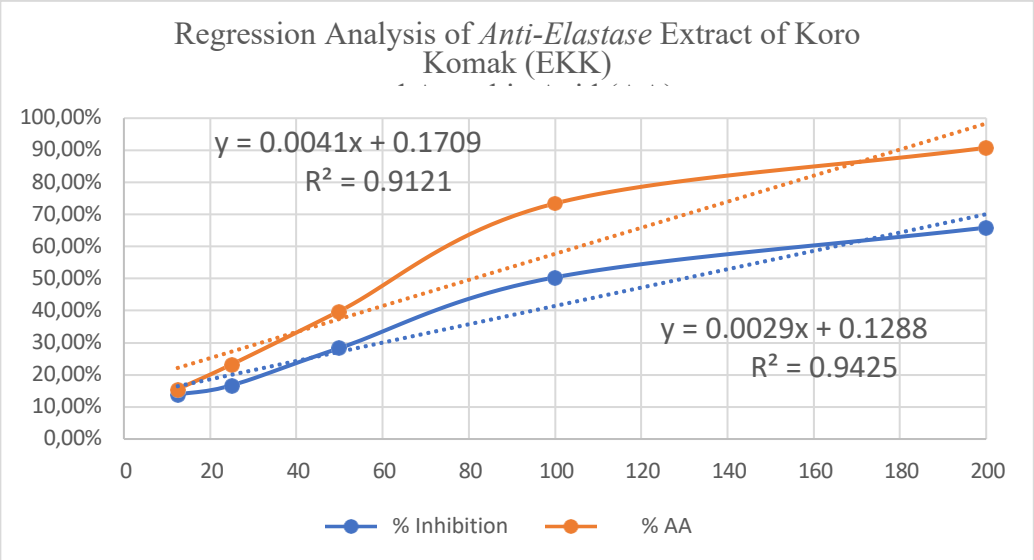

Figure 3. Comparison of the results of *anti-elastase regression* analysis of koro komak (EKK) extract and ascorbic acid (AA).

The reaction between *the elastase enzyme* and the SANA substrate is characterized by the formation of a yellow color that can be measured with a spectrophotometer. The higher the ability of a sample to inhibit *the elasticase* enzyme, the fewer reactions that occur and the clearer the color that forms (Juliana, 2020). The results of *the anti-elastase* activity test are categorized according to the IC50 value shown in Table 5.

Table 5. Categories of activity test results *Anti-Elastase* by IC50

Category	IC50 (µg/mL)
Very powerful	<50
Strong	50-100
Quite powerful	101-150
Weak	150-200

(Source: Sukandar *et al*., 2017)

The yield value of 70% ethanol extract of koro komak in this study was quite high, namely 12.76% compared to the yield of koro kratok extract (Phaseoulus lunatus L) of 10.6% (Nafi et al., 2007). This is because the high drying rate in koro komak flour is caused by the amount of free water in koro kratok which is used for the sprout growth process and then free

water releases in the ovening process. The nature of free water in food is volatile in the drying process (Winarno, 2004). In addition, the protein content in koro komak is higher than in koro kratok. This is supported by the research of Liadi et al. (2019) which showed the result of koro komak protein content of 41.39±1.1 and koro kratok of 10.06±0.6.

The variation in concentration of the prepared koro komak extract samples has gone through phytochemical screening tests and anti-elasticase activity tests. Phytochemical screening tests were carried out qualitatively to determine the presence of secondary compounds from koro komak extract samples before conducting anti-elasticase activity tests. The anti-elastase activity test aims to see the effect of compounds inside koro extract the inhibition of the elastase enzyme was then compared with ascorbic acid and analyzed using linear regression.

Phytochemical Screening Test

Based on Table 1, it is known that through phytochemical screening, koro komak extract is proven to have flavonoid compounds, phenolics, and tannins. A positive result of flavonoids is characterized by a change in color. This change is due to the addition of H2SO4 which aims to reduce the benzopiron core in the flavonoid structure so that flavylium salts are formed which is shown by the change in clear yellow color to orange or orange (Ergina, 2014). The results of phenolic screening on koro komak extract were characterized by a change in clear yellow color towards dark blue to black. The phenolic screening test was qualitatively performed by mixing samples and 5% FeCl3. The reaction of FeCl3 with hydroxyl groups bound to unsaturated carbon in the phenol structure will form a deep green, red, purple, blue, or black color (Harborne, 1987).

The tannin screening test of koro komak extract was shown by the formation of white deposits. The deposits are formed due to the mixing of samples and gelatin solutions. If an extract contains tannin compounds, then the gelatin solution will react with tannins to form protein deposits on gelatin (Harborne, 1987).

Based on phytochemical test screening, koro komak extract (Lablab purpureus) is proven to have polyphenol antioxidant compounds in the form of flavonoids, phenolics, and tannins. This is shown from the positive results in all three phytochemical screening tests. The test proved that koro komak extract has the potential to have anti-elastase properties. The results of this study are supported by previous research that legumes, one of which is koro komak, are rich in phenolics and flavonoids such as gallic acid, chlorogenic acid, p-coumaric acid, myricetin, vanillic acid, quercetin glycosides, genistein, kaempferol, luteolin, daidzein, and tannins (Diniyah et al., 2020). Nuts show the value of absorption capacity high oxygen radicals (ORAC) (66.97 umol TE/g) due to the significant amount of polyphenol compounds, especially flavonoids, present in the seed shell (Aguilera et al., 2010). This finding is in accordance with Diniyah's (2020) research that extracts from koro komak seeds have polyphenols in the form of galleic acid (2.245%), catechins (0.058%), and epicatechin (0.040%) which were tested using high-performance liquid chromatography (HPLC) tests.

Research by Diniyah (2021) showed that the total polyphenol component of ethanol extract was 70% koro komak through quantitative screening, namely 2.245% galic acid, 0.058% catechins, and 0.040% epicatechins. Meanwhile, in the research conducted by Sadh et al. (2017) found that the total polyphenol component of galic acid in ethanol extract was 54%

koro komak 46.78 ± 0.17 μM GAE/g. Research by Lohvina et al. (2021) showed that the polyphenol component in paddy rice plants (Limnophila aromatic) was only 6.25 μM GAE/g. This shows that the polyphenol component in koro komak extract is quite high.

Anti-Elastase Activity

The skin is a part of the human body that plays an important role as a protective barrier for organs from physical, chemical, and biological disorders. The activity of ROS (reactive oxygen species) and the enzyme elastase are known to be factors that can accelerate skin aging (Wilkerson, 2018). The accumulation of ROS after exposure to UV radiation on the skin can activate dermal enzymes, such as collagenase and elastase, which hydrolyze collagen and elastin thereby lowering skin elasticity (Jiratchayamaethasakul et al., 2020). In this study, the inhibition activity of elastase enzyme in koro komak extract samples was tested through spectrophotometry. Based on Table 4.2, it is known that koro komak extract samples are proven to inhibit the enzyme elastase.

The results showed that koro komak extract (Lablab purpureus) had a strong anti-elastase activity (128 μg/mL; IC50 101- 150 μg/mL). Through these results, it can be stated that koro komak extract has the potential to be an anti-elastase but has a lower IC50 value than ascorbic acid (80 μg/mL). This can be due to the presence of secondary metabolites that are strong specific inhibitors of the elastase-enzyme (Natanael et al., 2021). The IC50 value of elastase-enzyme inhibition of koro komak extract was shown to be higher than the IC50 value of elastase-enzyme inhibition of green algae (Chlorella emersonii) and Pueraria candollei (49000 μg/mL and 143 μg/mL) due to the higher polyphenol and antioxidant content of koro komak (Desai and Varsha, 2017 and Chattuwatthana, 2014).

The results of the data analysis showed that for every 1% increase in anti-elastase activity, a concentration of 0.0029 μg/mL was required. The results of the regression correlation value are positive so that it can be said that the anti-elastase activity is directly proportional to the increase in the concentration of koro komak extract. The positive correlation is also shown by the control solution, namely ascorbic acid as per Figure 3.

Based on Table 2, the results of the study prove that koro komak extract has the activity of inhibiting the elasticase enzyme. This is because specific compounds in koro komak extract in the form of polyphenols can inhibit the elastase-enzyme directly or through ROS inhibition. Previous research reported that polyphenols and flavonoids have antioxidant, anti-hyaluronidase, anti-collagenase, anti-tyrosinese, and anti-elastasian activity (Natanael et al., 2020). This is proven by Diniyah et al., (2020) that koro komak extract with 70% ethanol has more polyphenols and flavonoids so that it can inhibit free radicals more strongly compared to koro komak extract with water or 100% ethanol.

The results in Table 2 also show that the highest anti-elastase activity in koro komak extract was at a concentration of 200 μg/mL with an inhibition percentage of 65.90% and the lowest activity was at a concentration of 12.5 μg/mL with an inhibition percentage of 13.87%. This shows that in this study, koro komak extract with a concentration of 200 μg/mL has the highest elastase-inhibition activity compared to the concentration variation below it, but no peak effect has been found. Peak effect is concentration highest to achieve maximum effect (Lipicky, 1994). This study needs to identify peak effects so that we can determine the peak concentration to achieve maximum inhibition of elastase enzymes.

The gradient shown in Figure 1 is of positive value so it can be said that the anti-elastase activity is directly proportional to the increase in the concentration of koro komak extract. This is because polyphenol compounds as elastase inhibitors concentrated in koro komak extract compete directly with the SANA substrate to occupy the active side of the elastase enzyme. This is supported by Vijayakumar's (2017) research that polyphenol compounds carry hydroxyl groups that can react with the elastane enzyme and then form bonds with carboxyl groups on the active side of the elastase-enzyme and change the mechanism of action of the enzyme. As a result, the enzyme elastase becomes inactive and is unable to hydrolyze peptide bonds on elastin. Therefore, the higher the concentration of koro komak extract, the higher the inhibition activity of the elastase enzyme. The results of the study support the hypothesis that koro komak extract (Lablab purpureus) as an anti-aging has anti-elastic potential.

Ascorbic acid was used as a positive control in this study. Based on Table 4 and Table 5, the inhibition activity of elastase enzyme in koro komak extract has a lower value than ascorbic acid. The concentration required by koro komak extract to inhibit the elastane enzyme is higher than the concentration required by ascorbic acid. This shows that the inhibition activity of the elastase-enzyme of koro komak extract is not good enough when compared to ascorbic acid. Ascorbic acid was used as a positive control because ascorbic acid could be a gauge in anti-elastase effectiveness in this study. Ascorbic acid is one of the most potent antioxidants in plants and has been shown to have strong anti-elastase activity as a single molecule compared to other plant extracts that contain various bioactive compounds (Abdelgawad et al., 2019). Previous studies have reported that ascorbic acid has anti-elastase activity with an extraordinarily strong IC50 value (9.47□0.18 μg/mL) (Vijayakumar et al., 2017). Ascorbic acid inhibits most ROS due to the oxidation of ascorbic into mono-dehydroascorbate then to dehydroascorbate and has various functions, such as the maintenance of physiological states in human skin (Shirzad et al., 2018).

CONCLUSION

The study concluded that *koro komak* (*Lablab purpureus*) extract contains polyphenolic compounds such as flavonoids, phenols, and tannins, confirmed through phytochemical screening. The extract demonstrated moderate anti-elastase activity with an IC_{50} value of 128 μg/mL, indicating its potential as an anti-elastase agent in vitro. At a concentration of 200 μg/mL, the extract inhibited elastase enzyme activity by 65.90%, showing its most effective anti-elastase potential. Future research is recommended to explore the extract's anti-elastase effects in vivo, assess its safety and efficacy in clinical settings, and isolate specific active compounds responsible for the activity to optimize its development as a natural anti-aging agent.

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