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Elastase, Collagenase, and Tyrosinase Inhibition Activity of Palm Oil (*Elaeis Guineensis*) Leaf Extract

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ABSTRACT

The decline in skin function due to aging is influenced by endogenous and exogenous factors, especially UV exposure, which causes photoaging. Photoaging damages collagen and elastin through increased Reactive Oxygen Species (ROS) and activation of metalloprotease enzymes. This has led to the development of anti-aging cosmetics made from natural ingredients, including palm leaves, which have antioxidant activity. However, there is no comprehensive research on the potential of palm oil leaves as an anti-aging ingredient, particularly in inhibiting elastase, tyrosinase, and collagenase, and in topical anti-aging cream formulations. This study aims to evaluate the anti-aging activities of palm oil leaf extract, including antioxidant, anti-elastase, anti-collagenase, and anti-tyrosinase activities. Palm oil leaves were extracted using two different methods: ultrasonication and maceration. Each resulting extract was then concentrated using a rotary evaporator to obtain a thick extract. These extracts were subsequently subjected to phytochemical screening, antioxidant activity assays, and enzyme inhibition assays against elastase, collagenase, and tyrosinase. The results showed that palm oil leaf extract was positive for alkaloids, flavonoids, steroids, tannins, saponins, and coumarins. The ultrasonicated extract at a concentration of 1000 ppm showed the highest antioxidant activity with 64.38% *scavenging*. The ultrasonicated extract's anti-tyrosinase, anti-elastase, and anti-collagenase activities were 44.7%, 24.01%, and 10.34%, respectively. GC-MS analysis identified significant bioactive compounds, including Cyclohexasiloxane and Pluchidiol. These results suggest that palm oil leaf extract has potential as an effective anti-aging base material due to its antioxidant activity and inhibition of aging enzymes. It can also be formulated in topical anti-aging creams.

Keywords: antioxidant; collagenase; elastase; palm oil leaves; tyrosinase

INTRODUCTION

Skin is a vital organ that serves as the body's primary barrier against external aggressions, including infection and injury, and has an essential physiological role in maintaining homeostasis. However, as we age, skin function declines significantly, driven by external factors such as ultraviolet (UV) light, pollution, and stress, leading to a decrease in the thickness and number of skin cells. Skin aging can be divided into two categories: endogenous aging, which occurs naturally, and exogenous aging, which results from environmental exposures such as UV rays, infrared (IR) rays, smoking, and an unhealthy lifestyle (Lee et al., 2021). Photoaging, caused by UV rays, damages collagen and elastin in the dermis, changing the structure of elastic fibers and collagen, thereby reducing elasticity and causing skin sagging (Thring et al., 2009). In addition, UV exposure can increase the production of reactive oxygen species (ROS) and lipid peroxides. While ROS are necessary for normal metabolism, overproduction can lead to oxidative stress, damaging cellular components such as DNA,

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proteins, lipids, and carbohydrates. Oxidative stress also contributes to increased expression of matrix-degrading enzymes, such as matrix metalloproteinases (MMPs) and elastase, which can damage collagen and elastin, leading to wrinkles and sagging skin (Jiratchayamaethasakul et al., 2020). UV exposure can also cause skin darkening. Skin darkening is when the skin overproduces melanin, a pigment that determines skin and hair color to varying degrees. In addition, increased melanin synthesis can cause localized pigmentation or dark spots on parts of the face. Melanin synthesis involves tyrosinase as an essential enzyme that catalyzes the oxidation of tyrosine to dopaquinone. At certain pH levels, the process proceeds spontaneously, converting dopaquinone into dopa and dopachrome, forming melanin (Rahmi et al., 2021). Anti-aging products, typically in topical cosmetics, are designed to treat or relieve symptoms of photoaging. Antioxidants, both synthetic and natural, are often used in these formulations. However, synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) can cause severe side effects, including liver damage and carcinogenic properties (Jannah & Aznam, 2022). Market data shows that the global value of anti-aging products is projected to reach USD 191.7 billion by 2019. However, concerns about the safety and effectiveness of these products are increasing with reports of associated side effects. As an alternative, natural bioactive compounds are increasingly being explored as potential ingredients in anti-aging products. Research has shifted to natural products with hypoallergenic properties and significant potential in addressing skin aging in recent years (Yasin et al., 2017). Oil palm (*Elaeis guineensis* Jacq.) is one of Indonesia's leading vegetable oil sources, with the plantation area increasing from 8,248,328 hectares in 2009 to 8,430,026 hectares in 2010. Besides being used in the food, cosmetics, and biodiesel industries, oil palm waste is also used as fuel and organic fertilizer (Dianto et al., 2017). Although palm leaves have been used in various applications, their potential as an anti-aging drug candidate remains largely unexplored, particularly regarding antioxidant activity and effects on the enzymes elastase, collagenase, and tyrosinase. Therefore, this study aims to investigate the phytochemical composition, antioxidant activity, and inhibitory effects of the leaf extract of palm oil (Elaeis guineensis) on elastase, collagenase, and tyrosinase.

MATERIALS AND METHODS

Materials

The materials used were: Distilled water, Dragendorf solution, Ascorbic acid, DPPH, FALGPA (Sigma Aldrich), Tricine buffer, Tyrosinase from mushroom (Sigma Aldrich), L-DOPA (Sigma Aldrich), Elastase from porcine pancreas (Sigma Aldrich), Collagenase from Clostridium histolyticum (Sigma Aldrich), N-Succinyl-Ala-Ala-Ala-p-nitroanilide (Sigma Aldrich), Kojid acid (Sigma Aldrich), Phosphate buffer, Tris HCl, Sulfuric acid, Chloroform, 96% alcohol, 70% alcohol, 96% alcohol, Dimethyl sulfoxide (Merck), Phosphate buffer, 3,4-dihydroxy-L-phenylalanine (Sigma Aldrich), Epigallocatechin gallate (Sigma Aldrich).

Sample Extraction

Palm oil leaves (5000 grams) were cleaned, dried in a drying cabinet for 3 days, and pulverized into powder. Extraction was carried out using maceration and ultrasonication methods. The palm oil leaf powder was extracted with distilled water for 5 days, with daily stirring, and then stored in a closed container. Afterward, the solution was filtered through filter paper, and the filtrate was concentrated on a rotary evaporator until the solvent had evaporated. For the ultrasonication method, palm oil leaf powder was dissolved in distilled water (1:10 ratio) and shaken for 12 h at 180 rpm. The mixture was then ultrasonicated for 40 minutes at 20 kHz and 60% amplitude, thereby facilitating cell wall disruption and enhancing extraction efficiency. The result was centrifuged at 4000 rpm for 10 min to separate the extract from the solid components, then concentrated with a rotary evaporator (Farahani, 2021).

Qualitative Phytochemical Test

Phytochemical tests were conducted to detect the presence of alkaloids, flavonoids, triterpenoids, steroids, tannins, saponins, carotenoids, and coumarins in palm oil leaf extracts, according to the standard methods described. The test was carried out by adding specific test reagents to the extract and observing color changes or precipitate formation as indicators of target compounds. Screening assays for these major phytochemicals were conducted utilizing established qualitative methodologies.

Alkaloid test

Identification was performed via a Dragendorff solution. The procedure for preparing a Dragendorff solution is as follows: (i) Solution I:

0.5 g bismuth (III) nitrate, 6 mL acetic acid, and 24 mL distilled water. (ii) Solution II: 12 g of potassium iodide combined with 30 mL of purified water. (iii) Solution I combined with Solution II in a 1 mL to 1 mL ratio. To 1 mL of the combined solution, add 2 mL of acetic acid and 10 mL of distilled water; the solution is then prepared for use. Five milliliters of mushroom extracts were added, followed by two milliliters of KCl, and subsequently one milliliter of Dragendorff solution. A color shift of the solution to orange or red signifies the presence of alkaloids in the extract.

Flavonoid test

Add several drops of dilute sodium hydroxide (1% NaOH) to 1 mL of extract. The emergence of a distinct yellow color in the extract solution, which subsequently becomes colorless upon the introduction of dilute acid (1% HCl), signifies the presence of flavonoids.

Triterpenoid and steroid test

Add 1 mL of extract dissolved in acetone to a test tube; then add 10 drops of anhydrous acetic acid, followed by 2 drops of concentrated sulfuric acid. Shake the test tube and allow it to sit for several minutes. A color change indicates the reaction outcome: red or purple signifies a positive result for triterpenoids, while green or blue indicates a positive result for steroids.

Tannin test

The procedure involved adding 10 mL of the extract solution to a test tube, then adding a 1% $Pb(CH_3COO)_2$ solution to it. Tannins are indicated by the formation of a yellow precipitate during the reaction.

Saponin test

Add 1 mL of extract dissolved in acetone into a test tube, followed by the addition of 10 mL of heated water. The solution is cooled and agitated for 10 seconds; if a foam forms that persists for approximately 10 seconds, reaches a height of 1-10 cm, and remains intact upon the addition of one drop of 2N HCl, it indicates the presence of saponin.

Carotenoid test

One milliliter of extract was mixed with five milliliters of chloroform in a test tube, agitated, and filtered; then, 85% sulfuric acid was added. The formation of a blue color on the surface signifies the presence of carotenoids.

Coumarin test

One milliliter of extract is combined with several drops of sodium hydroxide, followed by the addition of alcohol. The formation of a yellow color signifies the presence of coumarin (Herawati et al., 2021; Kopon et al., 2020).

Antioxidant Activity

The antioxidant activity of palm oil leaf extract was determined using DPPH. A standard ascorbic acid solution was prepared by dissolving 5 mg of ascorbic acid powder in methanol to a volume of 50 mL to obtain an ascorbic acid solution with a concentration of 1000 ppm. The sample solution was made by dissolving 1 gram of palm oil leaf extract with methanol in a 1000 mL volumetric flask (1000 ppm). The sample solution was diluted into ten concentration variations (100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm, and 1000 ppm). Each standard or sample solution was pipetted, and 0.1 mM DPPH solution was added at a 1:3 ratio. The mixture was placed in a 96-well microplate and stirred until homogeneous. All samples were incubated in the dark at room temperature for 30 minutes, then counted using a microplate reader. The measurement was done 3 times in repetition. The following formula calculated the percentage of DPPH radical scavenged:

$$\%\text{scavenging} = \left[\frac{(A0-A1)}{A0} \right] \times 100\%$$

Where A0 is the absorbance of the control (DPPH solution), and A1 is the absorbance of the palm oil leaf extract or standard. The scavenging percentage is then plotted against the ascorbic acid standard for comparison (Sukweenadhi et al., 2020).

Anti-Elastase Activity Assay

All enzyme inhibition assays (anti-elastase, anti-collagenase, and anti-tyrosinase) were conducted using palm oil leaf extract at a concentration of 1000 ppm, which was selected based on its highest antioxidant activity observed in the DPPH assay.

An anti-elastase activity assay was performed based on the modified method of Thring et al. (2009) (Thring et al., 2009). The assay was performed in 0.2 mM Tris-HCl buffer (pH 8.0) using porcine pancreatic elastase (PE) dissolved in distilled water to achieve a stock solution of 7.5 units/mL. N-Succinyl-Ala-Ala-Ala-Ala-p-nitroanilide (AAAPVN) substrate was dissolved in DMF to a concentration of 0.5 mM, and epigallocatechin gallate (EGCG) at 0.14 mg/mL was used as a positive control. For each assay, 40 µL of palm oil leaf test extract (1000 ppm) was incubated with Tris-HCl buffer, elastase (1 unit), and AAAPVN substrate in a total volume of 160 µL. After 10 minutes of incubation, the substrate was added, and the mixture was incubated again for 20 min at

room temperature. The absorbance was then measured at 410 nm wavelength using a microplate reader. The percentage of enzyme inhibition was calculated using the formula % inhibition = ((A0 - A1) / A0) x 100%, where A0 is the absorbance of the negative control and A1 is the absorbance of the sample.

Anti-Collagenase Activity Assay

The collagenase inhibitory activity test procedure was performed by adding 20 µL of palm oil leaf extract, 20 µL of collagenase enzyme (5 mg/mL) from *Clostridium histolyticum*, and 20 µL of 50 mM Tricine buffer (pH 7.5 with 40 mM NaCl and 10 mM $CaCl_2$) into a 96-well microplate. Next, 40 µL of FALGPA substrate (0.5 mM) was added to start the reaction, with a total reaction volume of 60 µL. The reaction was incubated for 20 min, and the absorbance was measured at 410 nm after 30 min of incubation. Ascorbic acid 1000 ppm was used as a positive control to validate the experiment, while the blank contained the sample and Tricine buffer without enzyme to account for any non-enzymatic reactions. The percentage of enzyme inhibition was calculated by the formula: % inhibition = ((A0 - A1) / A0) x 100%, where A0 is the absorbance of the negative control, and A1 is the absorbance of the sample (Jiratchayamaethasakul et al., 2020).

Anti-Tyrosinase Activity Assay

Tyrosinase inhibitor activity assay was performed in phosphate buffer (pH 6.8). Tyrosinase enzyme (31 units/mL) was mixed with 40 µL of palm oil leaf extract (1000 ppm) and 40 µL of L-DOPA substrate (2.5 mM) in a final volume of 160 µL. This mixture was incubated for 1 minute; then, the substrate was added to start the reaction. Kojic acid (1000 ppm) was used as a positive control, while the blank contained the sample and phosphate buffer without enzyme. The absorbance was measured at 475 nm after a 10 min incubation using a microplate reader. The percentage of inhibition was calculated using the formula % inhibition = [(A - B) / A] x 100%, with A as the absorbance of the negative control and B as the absorbance of the sample (Priani et al., 2021).

Statistical Analysis

All enzymatic inhibition data (anti-elastase, anti-collagenase, and anti-tyrosinase activities) were expressed as mean ± standard deviation (SD) from three independent experiments (n = 3). The data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among the treatment groups (ultrasonicated extract, macerated extract, and positive control). Prior to ANOVA, Levene's test was used to assess homogeneity of variances; if the data met the assumption of homogeneity ($p > 0.05$), a Bonferroni post hoc test was performed. If the data violated the assumption of homogeneity ($p < 0.05$), a Games-Howell post hoc test was used. A significance level of $P < 0.001$ was considered statistically significant. Superscript letters (a, b, c) were used in figures to indicate significant differences between groups, while asterisks (*) denote overall statistical significance.

GC-MS Spectrometry Analysis

Samples (1 µL) of palm oil leaf extract were injected into the GC-MS system (Agilent 7890B/5977A) using an HP-5MS capillary column, with helium as the carrier gas at a 1.0 mL/min flow rate. The temperature program was set from 60°C to 280°C with a gradual heating. After separation in the column, the detected compounds were ionized using the Electron Ionization (EI) method, generating mass spectra for identification. Chromatogram and mass spectrum data were used to determine the chemical composition of the extract by matching them against the spectrum library. Positive control and blank samples were used to ensure the accuracy of the results.

RESULTS AND DISCUSSION

Phytochemical Test

The results of the phytochemical tests showed that the palm oil leaf extract obtained through ultrasonication and maceration methods contained various groups of bioactive compounds. Out of the seven tests conducted, six yielded positive results: alkaloids (indicated by the formation of brown precipitate), flavonoids (pale yellow solution), steroids (bluish-green solution), tannins (blackish-green solution), saponins (stable foam with a height of approximately 2 cm), and coumarins (yellow solution). Only the carotenoid test showed a negative result, identified by the absence of significant changes other than the brown color on the surface of the solution. A comparative analysis of the phytochemical screening results revealed that both ultrasonication and maceration extracts contained the same six classes of bioactive compounds: alkaloids, flavonoids, steroids, tannins, saponins, and coumarins. No qualitative differences were observed between the two extraction methods in terms of the types of secondary metabolites

detected. However, the color intensity of positive reactions was more pronounced in the ultrasonicated extract, suggesting a higher concentration of these phytochemicals, consistent with the enhanced extraction efficiency of ultrasonication. These results indicate the diversity of potential phytochemical compounds in palm oil leaf extract, except for carotenoid content. The phytochemical test is an initial step in identifying the bioactive compound content in plants, which is essential for therapeutic potential. Based on the test results on palm oil leaf extract, alkaloids, flavonoids, steroids, tannins, saponins, and coumarins were detected, while carotenoids were not. This is consistent with previous studies indicating that carotenoids are more commonly found in other parts of the oil palm, such as the fruit and crude palm oil (Puteh et al., 2022). Non-detection of carotenoids in leaf extracts could be due to natural variations in compound content in different plant parts (Santos et al., 2015).

Antioxidant Activity

Using ascorbic acid as a standard, palm oil leaf extracts by ultrasonication and maceration methods were tested for antioxidant activity. The results showed that the extract obtained by ultrasonication had a higher scavenging percentage than that obtained by maceration at various concentrations. In the ultrasonication method, a concentration of 1000 ppm resulted in the highest antioxidant activity (64.38%), while in the maceration method, the highest activity was achieved at a concentration of 900 ppm (50.60%).

The ultrasonication method uses ultrasonic waves to create cavitation bubbles in the solvent, which collapse and generate intense local heat and pressure. This disrupts the plant cell walls, enhancing the release of intracellular compounds, including polyphenols and flavonoids, which contribute to antioxidant activity (Elnour et al., 2022). Maceration involves soaking plant material in a solvent for an extended period, relying on passive diffusion to extract compounds. This method is less efficient in breaking down cell walls, leading to lower yields of bioactive compounds (Debiasi et al., 2021).

Palm oil leaf extracts by ultrasonication and maceration methods were tested for antioxidant activity using ascorbic acid as a standard. The results showed that the ultrasonication extract had a higher scavenging percentage than the maceration method at various concentrations. In the ultrasonication method, a concentration of 1000 ppm resulted in the highest antioxidant activity (64.38%), while in the maceration method, the highest activity was achieved at a concentration of 900 ppm (50.60%). The study shows that ultrasonication is more effective in extracting antioxidant compounds than maceration, which is consistent with the results of other studies (Irfan et al., 2022) reported that the ultrasonication method resulted in higher polyphenol content and antioxidant activity in lemongrass leaf extract, while Akinmoladun et al. (2022) found similar effects on Chrysophyllum albidum extract. Research by Styani et al. (2022) and (Hrebień-Filisińska & Tokarczyk, 2023) reported that the ultrasonication method produces extracts with high phenolic content, which correlates with antioxidant activity. These findings are supported by improved extraction of bioactive compounds from various plants using ultrasonication. Ultrasonication is considered superior as the process improves mass transfer, breaks cells, and preserves the structure of bioactive compounds. Furthermore, this method promotes cell wall rupture. It increases tissue permeability, facilitating solvent diffusion and mass transfer, thereby enabling more effective and efficient extraction of biologically active substances from natural products than maceration (Staicu et al., 2023).

Table I. Antioxidant activity of palm oil leaf extract

No	Concentration	% Scavenging	
		Ultrasonication	Maceration
1	100 ppm	51.33±0.56	19.60±1.02
2	200 ppm	53.6±0.57	36.21±0.57
3	300 ppm	57.2±0.58	14.37±0.43
4	400 ppm	58.42±0.72	28.40±0.63
5	500 ppm	56.96±0,5	28.83±1
6	600 ppm	57.67±1.12	46.50±0.57
7	700 ppm	52.34±0.5	48.35±0.72
8	800 ppm	61.13±0.54	49.35±0.57
9	900 ppm	62.53±0.51	50.60±0.55
10	1000 ppm	64.38±0.42	46.30±0.36
11	Ascorbic acid	70.13±0.89	70.13±0.55

The phytochemical content of a plant significantly influences its antioxidant activity, as demonstrated by various studies. Phytochemicals, such as phenolics and flavonoids, are bioactive compounds that contribute to the antioxidant properties of plants. These compounds can neutralize free radicals, thereby reducing oxidative stress and potentially preventing various diseases.

Several studies have shown a strong correlation between the total phenolic and flavonoid content and antioxidant activity. For instance, in wild grape root extracts, the ethyl acetate: methanol fraction exhibited the highest phenolic and flavonoid content, which corresponded to its superior antioxidant activity, except in the ABTS assay (Srihanam & Siripipatthana, 2019). Similarly, in herbal supplements, a strong correlation was observed between phenolic and flavonoid contents and antioxidant capacity measured by ORAC MR5 assays (Villaruz et al., 2023)

Anti-elastase activity

Based on measurements at a wavelength of 410 nm, the results showed that the extract obtained by ultrasonication had an elastase inhibitory activity of 24.01%, while the maceration method was 23.26%. For comparison, EGCG was used as a positive control and showed elastase inhibition activity of 11.51% (Figure 1).

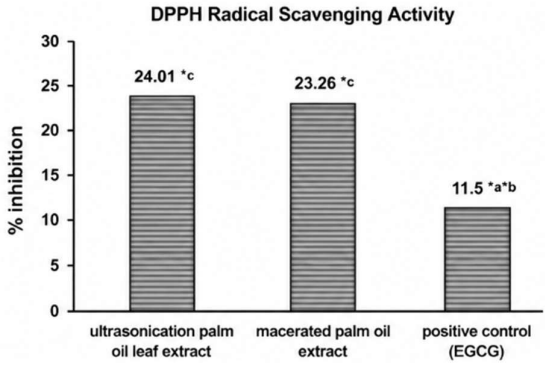


Figure 1. Activity of anti-elastase.

The average percentage inhibition values for each treatment are as follows: oil palm leaf extract using the ultrasonication method (24.01%), oil palm leaf extract using the maceration method (23.26%), and the positive control EGCG (11.5%). The asterisk (*) indicates a statistically significant difference at the P<0.001 confidence level. The letter *c* indicates no significant difference between the treatments using ultrasonication and maceration. The letters *a* and *b* indicate that the positive control (EGCG) differs significantly from both types of oil palm leaf extracts.

Elastase is a protease that breaks down elastin in the skin, leading to decreased elasticity and skin aging. Elastase inhibition can help prevent skin aging. The results showed that palm oil leaf extract, especially when ultrasonicated, has greater potential as an elastase inhibitor than the positive control, EGCG. Plant extracts contain polyphenolic compounds, flavonoids, and terpenes, which contribute to their anti-aging effects (Ambarwati et al., 2019; Thring et al., 2009). Previous research supports these findings, with compounds from plant extracts shown to inhibit enzymes that damage elastin, such as collagenase and elastase, thus suggesting potential as anti-aging agents (Henderson et al., 2020; Jiratchayamaethasakul et al., 2020). Plant extracts have shown potential in inhibiting elastase activity, sometimes surpassing the effectiveness of epigallocatechin gallate (EGCG), a well-known elastase inhibitor. While EGCG is effective, certain plant extracts, such as those from Artocarpus elasticus and Baccharis trimera, exhibit comparable or superior elastase-inhibitory activity. The specific IC_{50} values and the presence of multiple active compounds in these extracts contribute to their enhanced efficacy (Ban et al., 2020)

Anti-collagenase activity

Anti-collagenase activity testing was conducted on palm oil leaf extract using sonication and maceration methods. Measurement results using a microplate reader at 410 nm showed that the ultrasonicated palm oil leaf extract had an average absorbance (A0) of 0.058 and a % inhibition of 10.34%. In comparison, the macerated extract showed a lower % inhibition of 1.72%. The positive control using ascorbic acid showed % inhibition of 8.62% (Figure 2).

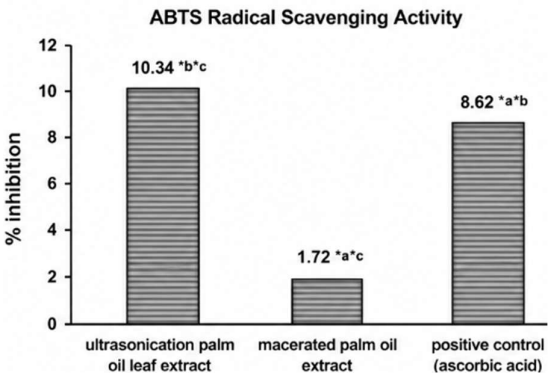


Figure 2. Activity of anti-collagenase.

The graph shows the mean percentage of enzyme inhibition across three treatments: ultrasonicated palm oil leaf extract (10.34%), macerated palm oil extract (1.72%), and the positive control with ascorbic acid (8.62%). The asterisks (*) indicate statistically significant

differences at $P < 0{,}001$. The notation *a*, *b*, and *c* denotes significant differences between the treatment groups. Specifically, there is a significant difference between the ultrasonication and macerated extracts, as well as between each extract and the positive control. These results suggest that the ultrasonication method yields the highest enzyme-inhibitory activity compared with maceration and the positive control.

Collagenase enzymes are metalloproteinases that can decompose molecules such as aggrecan, elastin, fibronectin, gelatin, laminin, and collagen. Therefore, agents that inhibit collagenase activity may have beneficial effects on maintaining skin health by preventing degradation of the skin matrix (Ghimeray et al., 2015). One study reported that total phenolics were an essential element in collagenase inhibitory activity, in addition to flavonoids (Shukri et al., 2021). For instance, *Pueraria candolleivar mirifica* root extract showed inhibitory activity equivalent to the positive control (Chattuwatthana & Okello, 2015). In addition, red pitaya peel extract has been reported to show a very high % inhibition against collagenase, reaching 87.62% (Vijayakumar et al., 2017). The mechanism of collagenase inhibition may involve polyphenolic compounds interacting with Zn ions at the enzyme's active site, thereby blocking the reaction between collagenase and its substrate. Bioactive compounds in plant extracts, such as flavonoids and tannins, can bind to Zn ions and inhibit collagenase activity (Pientaweeratch et al., 2016). These results suggest that palm oil leaf extract has potential as an active ingredient in skincare products that can help prevent collagen degradation and support skin health.

Anti-tyrosinase activity

Anti-tyrosinase activity testing was conducted on palm oil leaf extracts using sonication and maceration methods. Measurement results with a microplate reader at a wavelength of 410 nm showed that the ultrasonicated palm oil leaf extract had an average absorbance of A0 = 0.103 and a % inhibition of 44.17%. In comparison, the macerated extract showed a lower % inhibition of 8.25%. The positive control with ascorbic acid showed the highest % inhibition at 51.94% (Figure 3).

The bar graph displays the mean percentage of enzyme inhibition for three treatment groups: ultrasonication palm oil leaf extract (44.17%), macerated palm oil extract (8.25%), and the positive control using ascorbic acid (51.94%). Asterisks (*) indicate statistically significant differences at $P < 0.001$. The notations *a*, *b*, and *c* show significant differences among the groups. The ultrasonicated extract shows significantly greater inhibition than the macerated extract, while the positive control shows the highest inhibition overall. These findings indicate that both the ultrasonication method and the positive control (ascorbic acid) exhibit stronger enzyme-inhibitory effects than the maceration method.

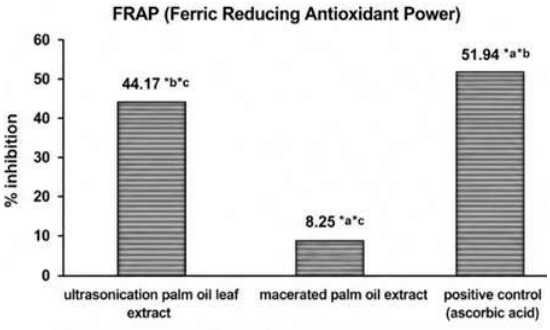


Figure 3. The activity of anti-tyrosinase

Tyrosinase is a multifunctional copper-containing polyphenol oxidase that plays an essential role in melanogenesis, the process that converts L-tyrosine into melanin, the pigment that gives skin, hair, and eyes their color. Although melanin has essential functions, overproduction can lead to dermatological problems such as lentigo, melasma, and age-related hyperpigmentation. Therefore, tyrosinase inhibitors are considered potential skin-whitening agents. Although synthetic tyrosinase inhibitors such as hydroquinone exist, the side effects and toxicity they cause are of grave concern, prompting the search for natural compounds with safer tyrosinase inhibitory effects (Ersoy et al., 2019). Previous studies reported that some of the 50 medicinal plant extracts tested showed anti-tyrosinase activity exceeding 50%, with the highest activity found in *Hypericum laricifolium* (Quispe et al., 2017). In addition to direct inhibitory mechanisms, many plant extracts have strong antioxidant properties. Antioxidants can lower the level of free radicals and reduce tyrosine and DOPA oxidation, which contributes to the overall decrease in melanin production (Klomsakul & Chalopagorn, 2019). Compounds in plant extracts can bind to the active site of tyrosinase, inhibiting the interaction between the enzyme and natural substrates (such as L-tyrosine) in a competitive inhibition mechanism (Attaallah & Amine, 2021).

Table II. Gas Chromatography-Mass Spectrometry analysis result

RT	Name	Molecular Formula	% Area	Phytochemical group	Therapeutic activity
5948	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	846	Alkaloid	Antibacterial
7448	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	1725	Terpenoid	Antibacterial, antioxidant
8348	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	990	Ester	N.A.
8728	3,4-Dihydroxyphenylethanol	$C_8H_{10}O_3$	3399	Polyphenol	Antioxidant
8809	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	1439	Terpenoid	Antibacterial
9224	Acorenone B	$C_{15}H_{24}O$	7023	Sesquiterpene	Anticholinesterase
9617	1-Benzoxirene-2,5-dione, 4-(3oxobutyl)hexahydro-3,3,4-trimethyl	$C_{13}H_{18}O_4$	1015	benzoxirane	Antimicrobes, antifungal
9755	3-Buten-2-one, 4-(4-hydroxy-2,2,6trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	$C_{13}H_{20}O_3$	1265	norisoprenoid	Antioxidant, anti-glucosidase
9836	Pluchidiol	$C_{13}H_{20}O_2$	10000	Sesquiterpene	Antioxidant, Anti-Inflammatory, and Antiproliferative
10286	3-Hydroxy-6-(N,N dimethylamino) methylpyridazine 1 oxide	$C_7H_{11}N_3O_2$	1402	pyridine	Antifungal, antimicrobial

Ultrasonication has demonstrated superior enzyme inhibition activity compared to maceration due to its ability to enhance the extraction efficiency of bioactive compounds. This method leverages the mechanical effects of ultrasound waves, which disrupt cell walls and facilitate the release of intracellular compounds, leading to higher yields of active substances (Aleixandre et al., 2022). Ultrasound-assisted extraction is effective in various applications, including the extraction of antioxidative enzymes from cashew apple bagasse, which can be used in therapeutic applications due to their enhanced activity post-sonication (Fonteles et al., 2022).

GC-MS analysis results

Some of the major compounds identified in palm oil leaf extract, which have potential antibacterial activity (Table II). The content of dodecamethyl cyclohexasiloxane, tetradecamethyl cycloheptasiloxane, and hexadecamethyl cyclooctasiloxane most likely influences this antibacterial activity. Dodecamethyl cyclohexasiloxane, which belongs to the alkaloid group, is known to have antibacterial and antifungal activities (Patil & Rathod, 2014). In a previous study, *Polyathia cinnamomea* plant extract was reported to contain 14.1% dodecamethyl cyclohexasiloxane and showed significant antibacterial activity (Mahmud et al., 2018). The compound tetradecamethylcycloheptasiloxane, also identified in the aqueous extract of ganyong tuber, showed a percentage area of 13.40% with a retention time of 18.805 min. This compound has been shown to inhibit the growth of several bacteria and to exhibit potent antioxidant activity (Savira et al., 2023). Another study confirmed that tetradecamethylcycloheptasiloxane is a macrocyclic organosiloxane that exhibits antibacterial and antioxidant activity (Kumar et al., 2020). In addition, 3,4-Dihydroxyphenylethanol (hydroxytyrosol), which is widely found in olives, is known to have two hydroxyl groups on the phenolic ring that provide strong antibacterial and antioxidant properties (Santarelli et al., 2022). The antibacterial mechanism of hydroxytyrosol involves disruption of the cell membrane and binding to bacterial DNA, which increases membrane permeability and damages its integrity (Wei et al., 2018). Hydroxytyrosol also functions as an antioxidant by preventing free radical production, stimulating antioxidant enzymes, and protecting DNA base modifications (Driss & El-Benna, 2010). Polyphenols present in hydroxytyrosol are thought to exhibit tyrosinase enzyme inhibitory activity through copper binding to the active center of tyrosinase. Molecular simulations have shown that certain polyphenols can directly interact with the binuclear copper ions in tyrosinase, supporting the hypothesis that

copper binding is a significant factor in their inhibitory activity (Tang et al., 2018). The inhibition of tyrosinase by polyphenols can occur through multiple mechanisms, including competitive and non-competitive inhibition. For instance, flavonoids from Humulus lupulus have been shown to inhibit tyrosinase through competitive mechanisms, which may involve direct interactions with copper ions (Kim et al., 2016). Hydroxytyrosol is structurally similar to other polyphenols that have demonstrated tyrosinase inhibitory activity. While specific studies on hydroxytyrosol's interaction with tyrosinase are limited, its structural characteristics suggest a potential for copper binding and enzyme inhibition (Panzella & Napolitano, 2019). 3,4-Dihydroxyphenylethanol is also thought to be able to inhibit collagenase. 3,4-Dihydroxyphenylethanol is believed to inhibit collagenase by binding to its active site, which prevents the enzyme from interacting with collagen substrates. This binding is facilitated by the compound's phenolic structure, which allows it to form hydrogen bonds and hydrophobic interactions with the enzyme, effectively blocking its activity (Li et al., 2022). Acorenone B, which belongs to the class of terpenoid compounds and is specifically a sesquiterpenoid, also shows potential as an antibacterial agent despite its weak activity (Calva et al., 2017). In this context, the compound 1-Benzoxirene-2,5-dione, 4-(3-oxobutyl) hexahydro-3,3,4-trimethyl, which belongs to the lactone group, has biological activities that include antimicrobial and antifungal properties (Habib et al., 2012) . The most significant bioactive compound in palm oil leaf extract is pluchidiol, known for its antioxidant activity. This activity is essential for combating oxidative stress, contributing to cell damage (Kumar et al., 2011). Pluchidiol, found in *Pluchea dioscoridis*, exhibits various biological activities, including antimicrobial and anticancer effects (El-Ghorab et al., 2015). Pluchidiol is a sesquiterpene compound, and its molecular structure is characterized by a complex arrangement of carbon and hydrogen atoms, typical of terpenoids. Terpenoids, have been studied for their ability to inhibit MMP-1, a collagenase involved in the breakdown of collagen in the extracellular matrix. This inhibition is crucial for anti-aging and therapeutic applications (Yasmeen & Gupta, 2019). The interaction of terpenoids with collagenase involves binding to the enzyme's active site, potentially through hydrogen bonds and van der Waals forces, which can inhibit its activity. This is supported by studies on other terpenoids that show strong binding affinities to MMP-1 (Atienza et al., 2022). Pyridazine-derived compounds such as 3-Hydroxy-6-(N, N-dimethylamino)methylpyridazine 1-oxide show potential as antifungal agents with mechanisms that disrupt fungal cell wall integrity (Mena et al., 2022). Research shows that new thiazolyl-pyridazine-dione derivatives have significant antimicrobial activity, suggesting the potential to be used in developing antibiotic therapy (Abu-Melha et al., 2021). The relationship between the antioxidant activity of plant extracts and the inhibition of enzymes such as elastase, tyrosinase, and collagenase is significant in anti-aging and cosmetic applications. Antioxidants neutralize free radicals, which, if left untreated, can accelerate skin aging by increasing the activity of enzymes that damage skin proteins (Nurrochmad et al., 2018). Other studies have shown that high phenolic and flavonoid content in plant extracts correlates with strong antioxidant activity, which can inhibit enzymes that contribute to skin aging (Eun et al., 2020). In addition, phytochemical content significantly affects the activity of elastase, collagenase, and tyrosinase inhibitors, as demonstrated by various studies on plant extracts. These enzymes are crucial in skin aging processes, and their inhibition is a target for anti-aging and cosmetic products. Phytochemicals, such as polyphenols, flavonoids, and other bioactive compounds, have shown promising inhibitory effects on these enzymes, suggesting their potential use in skincare formulations. Previous studies have reported that *Viburnum mullaha* fruit extract demonstrated significant anti-elastase activity, attributed to its high polyphenol and flavonoid content, which are known for their antioxidant properties (Singh et al., 2017). The acetone extract of honeybush effectively inhibited collagenase, highlighting the role of polyphenols in this activity (Hering et al., 2023). Another study also mentioned that pomegranate and red maple extracts, enriched with phenolics, showed synergistic collagenase inhibition, with combination indices indicating a synergistic effect (Li et al., 2022). The extract of Ajwa date pulp, also demonstrated tyrosinase inhibition, with an IC50 of 59.53 mg/mL, further supporting the role of polyphenols in enzyme inhibition (Alshammari et al., 2023).

CONCLUSION

This study shows that palm oil leaf extract has significant potential as a natural anti-aging

agent. The extract obtained via ultrasonication showed the highest antioxidant activity, neutralizing free radicals and effectively inhibiting the enzymes elastase, collagenase, and tyrosinase. GC-MS analysis identified several bioactive compounds, including Cyclohexasiloxane and Pluchidiol, that possess antioxidant properties and inhibit enzymes associated with skin aging. Thus, palm oil leaf extract can be considered for development in topical skincare products as an effective and safe anti-aging active ingredient. Further research is needed to optimize the formulation and evaluate its effects in vivo to ensure its safety and efficacy in long-term use.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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