IJPST
Indonesian Journal of Pharmaceutical Science and Technology

Research Article
Vol. 13 No. 1 (2026), 157-165
https://doi.org/10.24198/69450

Potential of avocado seed ethanol extract (*Persea americana* Mill. var Miki) as an anti-aging

Syadza Alya[1,2], Kartini Hasballah[3*], Fitria Fitria[4], Essy Harnelly[5], Nirwana L. Sary[6]
[1]Master's Program in Biomedical Science, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[2]Faculty of Medicine, Universitas Almuslim, Bireuen, Indonesia
[3]Department of Pharmacology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[4]Department of Dermatology and Venereology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia
[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[6]Department of Physiology, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Abstract
Skin aging, characterized by decreased skin elasticity, is a global concern closely related to elastin degradation caused by oxidative stress and elastase activity. Natural ingredients rich in antioxidants are increasingly explored as potential anti-aging agents. Avocado (*Persea americana*) seeds contain various bioactive compounds that may protect elastin and slow the skin aging process. This study employed in silico and in vitro approaches to evaluate the antioxidant and anti-elastase activities of ethanol extract of avocado seeds from Bener Meriah, Aceh. Extraction was performed using maceration with 70% ethanol, followed by phytochemical screening. Antioxidant activity was assessed using the DPPH method. Anti-elastase activity was evaluated in silico through molecular docking against porcine pancreatic elastase (PPE) and in vitro using the SANA substrate and PPE enzyme. The avocado seed ethanol extract showed strong antioxidant activity with an IC_{50} value of 47.31 ppm. It also demonstrated potent anti-elastase activity with an IC_{50} value of 6.980 ppm. Molecular docking revealed that cholestan-3-ol, 2-methylene-, (3β,5α)- had a binding affinity of −8.0 kcal/mol, comparable to the control. Overall, the ethanol extract of avocado seeds exhibits very strong antioxidant and significant anti-elastase activities both in silico and in vitro, indicating its potential as a natural anti-aging agent.

Keywords: Anti-aging, in silico, in vitro, *Persea americana* Mill, porcine pancreatic elastase.

Potensi ekstrak etanol biji alpukat (*Persea americana* Mill. var Miki) sebagai agen anti-penuaan

Abstrak
Penuaan kulit merupakan proses biologis yang ditandai dengan penurunan elastisitas akibat degradasi elastin dan peningkatan stres oksidatif. Elastase dan radikal bebas berperan penting dalam mempercepat proses tersebut. Pemanfaatan bahan alam yang kaya antioksidan, seperti biji alpukat (*Persea americana*), menjadi alternatif potensial sebagai agen anti-aging alami. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dan anti-elastase ekstrak etanol biji alpukat menggunakan pendekatan *in silico* dan *in vitro*. Biji alpukat yang berasal dari Bener Meriah, Aceh, diekstraksi menggunakan metode maserasi dengan pelarut etanol 70%. Skrining fitokimia dilakukan untuk mengidentifikasi senyawa bioaktif. Aktivitas antioksidan diuji menggunakan metode DPPH. Aktivitas anti-elastase dianalisis secara in silico melalui molecular docking terhadap enzim porcine pancreatic elastase (PPE) serta secara in vitro menggunakan substrat SANA dan enzim PPE. Ekstrak etanol biji alpukat menunjukkan aktivitas antioksidan dengan nilai IC_{50} sebesar 47,31 ppm dan aktivitas penghambatan elastase dengan nilai IC_{50} sebesar 6,980 ppm. Analisis in silico mengidentifikasi senyawa cholestan-3-ol, 2-methylene-, (3β,5α)- dengan afinitas ikatan −8,0 kcal/mol terhadap enzim PPE, mendekati nilai kontrol. Secara keseluruhan, ekstrak etanol biji alpukat memiliki aktivitas antioksidan dan anti-elastase yang baik berdasarkan uji in silico dan in vitro, sehingga berpotensi dikembangkan sebagai bahan alami pendukung anti-aging.

Kata Kunci: Anti-penuaan, elastase pankreas babi, *in silico, in vitro, Persea americana* Mill.

Article History:
Submitted 28th January 2026
Revised 9th February 2026
Accepted 10th February 2026
Published

*Corresponding author:
kartini.hasballah@usk.ac.id

Citation:
Alya S., Hasballah, K., Fitria, F., Harnelly, E., Sary, N.L. Potential of avocado seed ethanol extract (*Persea americana* Mill. var Miki) as an anti-aging. Indonesian Journal of Pharmaceutical Science and Technology. 2026: 13 (1), 157-165.

Vol. 13 No. 1 (2026), 157-165

1. Introduction

Aging is a complex biological process characterized by oxidative stress, increased elastase activity, and reduced collagen and elastin synthesis, which together impair skin structure and function. These changes manifest as loss of elasticity, wrinkles, pigmentation, and other visible signs of aging, which affect quality of life and highlight the need for effective interventions. With the growing elderly population, research has increasingly focused on natural compounds as potential anti-aging agents to counteract oxidative damage and enzymatic degradation in the skin.[1,2]

Aging results from intrinsic and extrinsic factors. Intrinsic aging is driven by internal physiological changes, including reduced production of collagen and elastin fibers.[3] Extrinsic aging, on the other hand, arises from environmental factors such as UV exposure and pollution, which increase the production of reactive oxygen species (ROS) in the dermis layer4. ROS accumulation damages DNA, proteins, and lipids, thereby activating signaling pathways such as MAPK and NF-κB. This activation induces matrix metalloproteinases (MMPs), enzymes that degrade extracellular matrix (ECM) components, particularly elastin, a key protein that provides skin elasticity.[4–6]

As elastin synthesis declines with age and environmental damage accumulates, skin loses its elasticity, becoming saggy and wrinkled.[4] Efforts to counteract these effects often involve antioxidants, which combat oxidative stress by neutralizing ROS.[7] Natural compounds rich in antioxidants, such as tannins, phenols, and alkaloids, have been widely studied for their anti-aging properties. Plant-based extracts, including green tea polyphenols (*Camelia sinensis*) and persimmon leaf extracts (*Diospyros kaki*), have demonstrated potential in inhibiting elastase, a key enzyme in elastin degradation.[8]

Indonesia is rich in natural resources with significant antioxidant potential, including avocado (Persea genus), a fruit known for its nutritional benefits. Despite its high phenolic and antioxidant content, the seed, a byproduct of avocado processing, remains underutilized.[9-13]

Research suggests that avocado seeds contain phenolic compounds that can scavene free radicals, inhibit elastase and collagenase activity, and increase elastin and collagen levels.[7,14] Given these findings, this study aims to evaluate the potential of ethanol extract from avocado seeds as an antioxidant and anti-elastase agent through both in silico and in vitro approaches to support skin aging prevention and treatment.

2. Methods

This study was experimental laboratory research using in silico and in vitro approaches with the ethanol extract of avocado seeds. The preparation of extracts, phytochemical analysis, antioxidant activity tests, and in vitro anti-elastase assays were conducted at the Biomedical and Integrated Laboratories, Faculty of Medicine, Universitas Syiah Kuala (USK). Herbarium identification was performed at the Microbiology Laboratory, the Biology Department, Faculty of Mathematics and Natural Sciences, USK. The research was carried out between October and December 2024.

The sample used was an ethanol extract of avocado seeds, which was examined for antioxidant and anti-elastase activities. The independent variable was the ethanol extract of avocado seeds, while the dependent variables were antioxidant activity (%IC_{50}), anti-elastase activity in silico, and anti-elastase activity in vitro (%IC_{50}). Controlled confounding variables included seed harvesting methods, evaporation temperature, and storage duration of the ethanol extract, whereas the uncontrolled confounding variable was the blender temperature.

2.1. Equipment and Materials

The equipment used included a blender, funnel, micropipettes (50-1000 µL), rotary vacuum evaporator, vortex, spatula, tips, analytical balance, pH meter, aluminum foil, test tubes, shaker, 96-well microplate, and TECAN Multimode Reader. Software such as PyRx, Autodock Vina, and PyMOL was used for in silico studies.

The materials included avocado seeds from plantations in Bener Meriah and various chemicals such as distilled water, analytical-grade methanol, 96% ethanol, phytochemical test kits, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, hydrochloric acid, 25% acetic acid, oleanolic acid, N-succinyl-ala-ala-ala-p-nitroaniline (SANA), porcine pancreatic elastase (PPE), DMSO, and Trizma base.

2.2. Data Collection

Data collected included absorbance readings from antioxidant activity tests and GC-MS analysis. Predicted data from in silico analysis included the ability of ligands (compounds in the extract) to bind with the PPE enzyme receptor to form stable complexes. Binding affinity and inhibition constants were assessed. Additionally, absorbance data from in vitro elastase assays were also obtained.

2.3. Procedures

2.3.1. Sample Identification

Avocado seeds were obtained from Selamat Rejo village, Bandar subdistrict, Bener Meriah Regency (coordinates: 4°45'00.5"N 96°57'09.0"E). The seeds, along with other plant parts, were identified at the Biology Department, FMIPA, USK (No. 904/UN11.1.8.4/TA.00.03/2024).

2.3.2. Extraction

The seeds were cleaned, sliced, and dried at room temperature. Once dry, they were ground into a powder and macerated in 70% ethanol at a 1:10 (w/v) ratio. The extract was filtered, re-macerated, and evaporated using a rotary evaporator, then concentrated at 60°C in an oven. The extraction yield was calculated using the formula:

2.3.3. Phytochemical Analysis

Qualitative phytochemical tests were conducted for flavonoids, tannins, polyphenols, saponins, quinones, triterpenoids, steroids, and alkaloids using specific reagents and observation of color changes.

2.3.4. Antioxidant Activity Test

The DPPH method was used to measure antioxidant activity using UV-Vis spectrophotometry at 517 nm. Stock solutions of extract and ascorbic acid were prepared, and different concentrations (6.25–100 ppm for extract and 3–15 ppm for ascorbic acid) were tested.

2.3.5. In Silico Anti-Elastase Assay

Ligands from GC-MS results were prepared using PyRx and AutoDock Vina. The PPE receptor was downloaded from the NCBI database and processed in PyMOL. Molecular docking was conducted, and binding affinity values (ΔG) were analyzed.

2.3.6. In Vitro Anti-Elastase Assay

Assays were performed using PPE enzyme and SANA substrate in a reaction mixture with Trizma-HCl buffer (pH 8.0). Absorbance was measured at 405 nm using a microplate reader. Oleanolic acid served as a positive control.

2.4. Data Analysis

Data were analyzed descriptively, and means were compared. Normality tests (Shapiro–Wilk) were conducted to determine the use of parametric or non-parametric methods. Linear regression analysis was performed to evaluate relationships between extract concentrations and activities (antioxidant and in vitro anti-elastase) and average binding affinity to determine which ligands have significantly anti elastase activities. Statistical analyses were carried out using IBM SPSS Statistics version 26.0 and GraphPad Prism version 9.0 for regression and graphical representation, with p-value < 0.05 was considered statistically significant.

3. Results

3.1. Ethanol Extract of Avocado Seeds

The research began with collecting avocados from Selamat Rejo Village, Bandar Sub-district, Bener Meriah District. A total of 10 kg of avocados was obtained. The fruits were peeled, and the seeds were collected. The seeds were cut into pieces and dried indoors, avoiding direct sunlight, for five days. After drying, the avocado seeds were blended into simplicia, weighing 250 grams. The 250 grams of simplicia were extracted with 70% ethanol, yielding 122.072 grams of extract. The choice of 70% ethanol was based on previous studies [15,16], which showed that it had higher antioxidant activity than 50% and 96% ethanol. The

Table 1. Phytochemical screening of avocado seed ethanol extract

Phytochemical Screening	Results
Flavonoids	+
Polyphenols	+
Saponins	+
Quinones	+
Triterpenoids	-
Steroids	+
Tannins	+
Alkaloids	
- Mayer	+
-Dragadorf	+
- Wagner	+

Table 2. Chemical composition of avocado seed extract

Peak	R. Time	Compound Name	Chemical Formula	SI	Peak Area (%)
1	29.16	3,7,11,Trimethyl-8,10- dodecedienylacetate	$C_{17}H_{30}O_2$	766	4,41
2	36.44	2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)furan	$C_{21}H_{34}O$	895	15,97
3	39.46	E-11-Methyl-12-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	710	12,96
4	39.54	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	707	19,61
5	40.32	7,11-Hexadecadienal	$C_{16}H_{28}O$	736	9,8
6	40.93	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2pentylcyclo-propyl)methyl]cyclopropyl]methy l]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	750	2,34
7	41.15	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	709	19,47
8	41.76	7,11-Hexadecadienal	$C_{16}H_{28}O$	738	9,56
9	41.95	Formic acid, 3,7,11-trimethyl-1,6,10dodecatrien-3-yl Ester	$C_{16}H_{26}O_2$	702	3,48
10	42.25	Cholestan-3-ol, 2-methylene-, (3ß,5a)-	$C_{28}H_{48}O$	717	1,37
11	42.33	1-Heptatriacotanol	$C_{37}H_{76}O$	717	1,05

percentage yield for the avocado seed ethanol extract was 48.83%.

3.2. Phytochemical Test

The phytochemical analysis showed that the ethanol extract of avocado seeds contained flavonoids, polyphenols, saponins, quinones, steroids, and alkaloids (Table 1). However, triterpenoids were not detected due to the use of 70% ethanol, a polar solvent that predominantly extracts polar secondary metabolites. Non-polar compounds, such as triterpenoids, were not effectively extracted under these conditions.

Solvents with higher polarity will preferentially extract polar secondary metabolites such as flavonoids, polyphenols, tannins, saponins, quinones, and alkaloids. Conversely, triterpenoids, being nonpolar compounds, will not be extracted efficiently by 70% ethanol.[17]

3.3. GC/MS Test

The chemical composition of avocado seed extract is shown in Table 2. The GC-MS results in this study showed that avocado seed extract contained several components, with the highest components being 12-Methyl-E,E-2,13-octadecadien-1-ol (19.61%), followed by 12-Methyl-E,E-2,13-octadecadien-1-ol (19.47%), and 2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl) furan (15.97%).

3.4. Antioxidant Test Using the DPPH Method

The results of the DPPH-based antioxidant activity test of avocado seed ethanol extract at concentrations of 6.25, 12.5, 25, 50, and 100 ppm. The ethanol extract of avocado seeds showed strong antioxidant activity. Absorbance values at concentrations of 6.25 ppm, 12.5 ppm, and 25 ppm were close to the control (Table 3). The calculated IC_{50} value of the extract was 47.31 ppm, indicating very strong antioxidant activity (IC_{50} < 50 ppm is considered very strong). For comparison, the IC_{50} value of ascorbic acid (vitamin C) was measured at 7.26, confirming its strong antioxidant potential.

3.5. In Silico Elastase Inhibition Test

The following molecular docking analysis showed the binding affinities of ligands obtained from previous GC-MS analysis for porcine pancreatic elastase (PPE). The 3 most common ligands in avocado seeds

Table 3. The result of the antioxidant test using the DPPH method

Concentration (ppm)	Absorbance		Average
	1	2	
6.25	0.943	0.945	0.944
12.5	0.821	0.769	0.795
25	0.563	0.542	0.553
50	0.264	0.266	0.265
100	0.153	0.162	0.158
Control (Vitamin C)	0.95	0.948	0.949

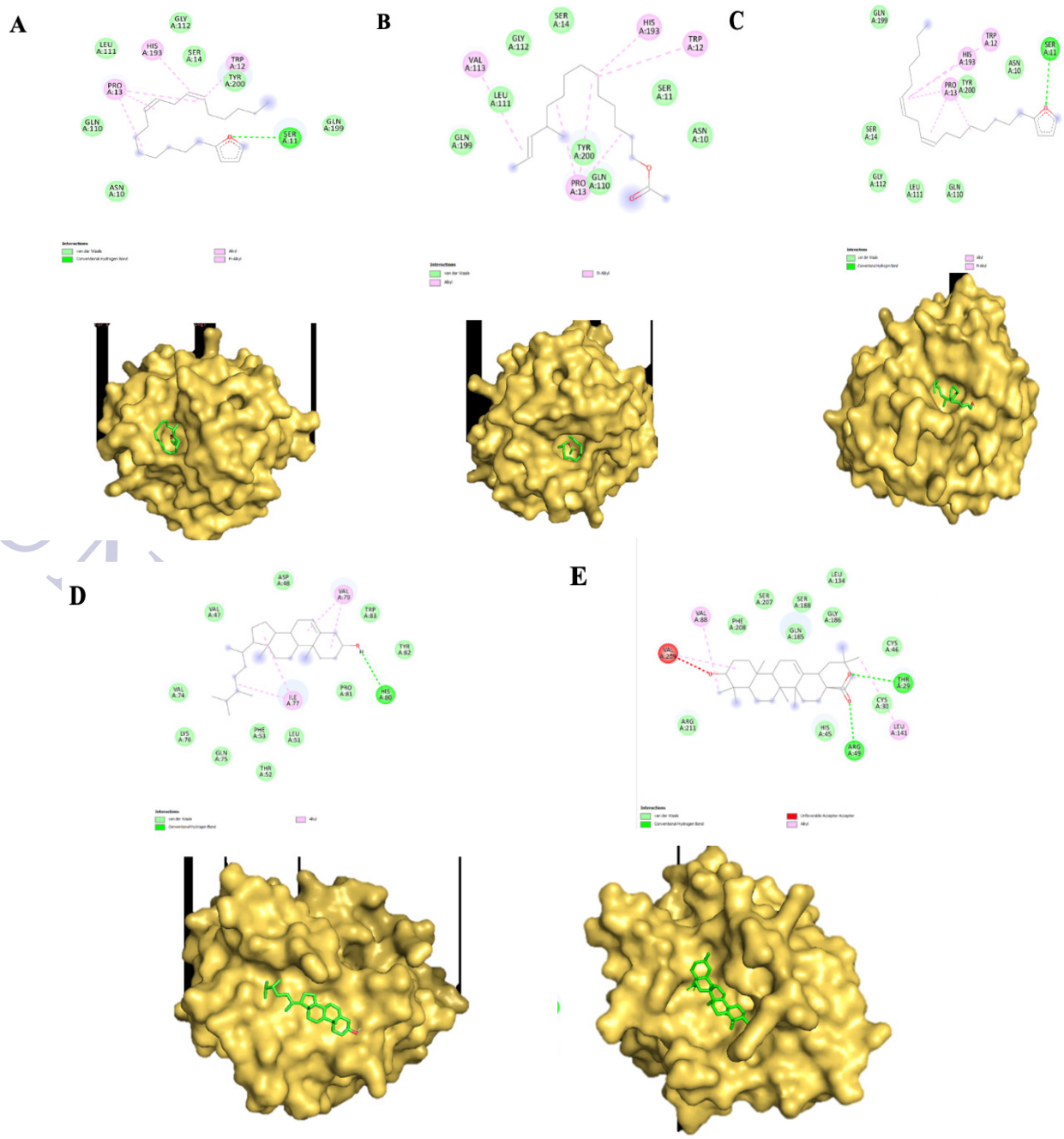

Figure 1. 2D and 3D visualization of (A) 2-((8Z,11Z)-Heptadeca-8,11-dien-1-yl)furan bound to the target protein (PPE), (B) E-11-Methyl-12-tetradecen-1-ol acetate bound to the target protein (PPE), (C) 12-Methyl-E,E-2,13-octadecadien-1-ol bound to the target protein (PPE), (D) Cholestan-3-ol, 2-methylene-, (3ß,5a)- bound to the target protein (PPE), and (E) Oleanolic Acid bound to the target protein (PPE).

ethanol extract have the binding affinity between -4.3 and -4.9 kcal/mol, which is greater than cholestan-3-ol, 2-methylene-, (3ß,5a)- with binding affinity -8.0 kcal/mol, which is nearest to the binding affinity of control (oleanolic acid: -8,1 kcal/mol).

Docking simulations revealed distinct binding affinities among the tested ligands. cholestan-3-ol, 2-methylene, (3ß,5a) exhibited the highest binding affinity compared to all other compounds. In contrast, more abundant ligands demonstrated weaker binding affinities, suggesting less stable inhibition. The strongest candidate, Cholestan-3-ol, 2-methylene-, (3ß,5a), formed a hydrogen bond with Ser11 and hydrophobic contacts with Pro13, Tyr200, His193, and Trp12 within the substrate-binding pocket, with six van der Waals interactions. These interactions indicate a potential for effective inhibition of elastase activity. Oleanolic

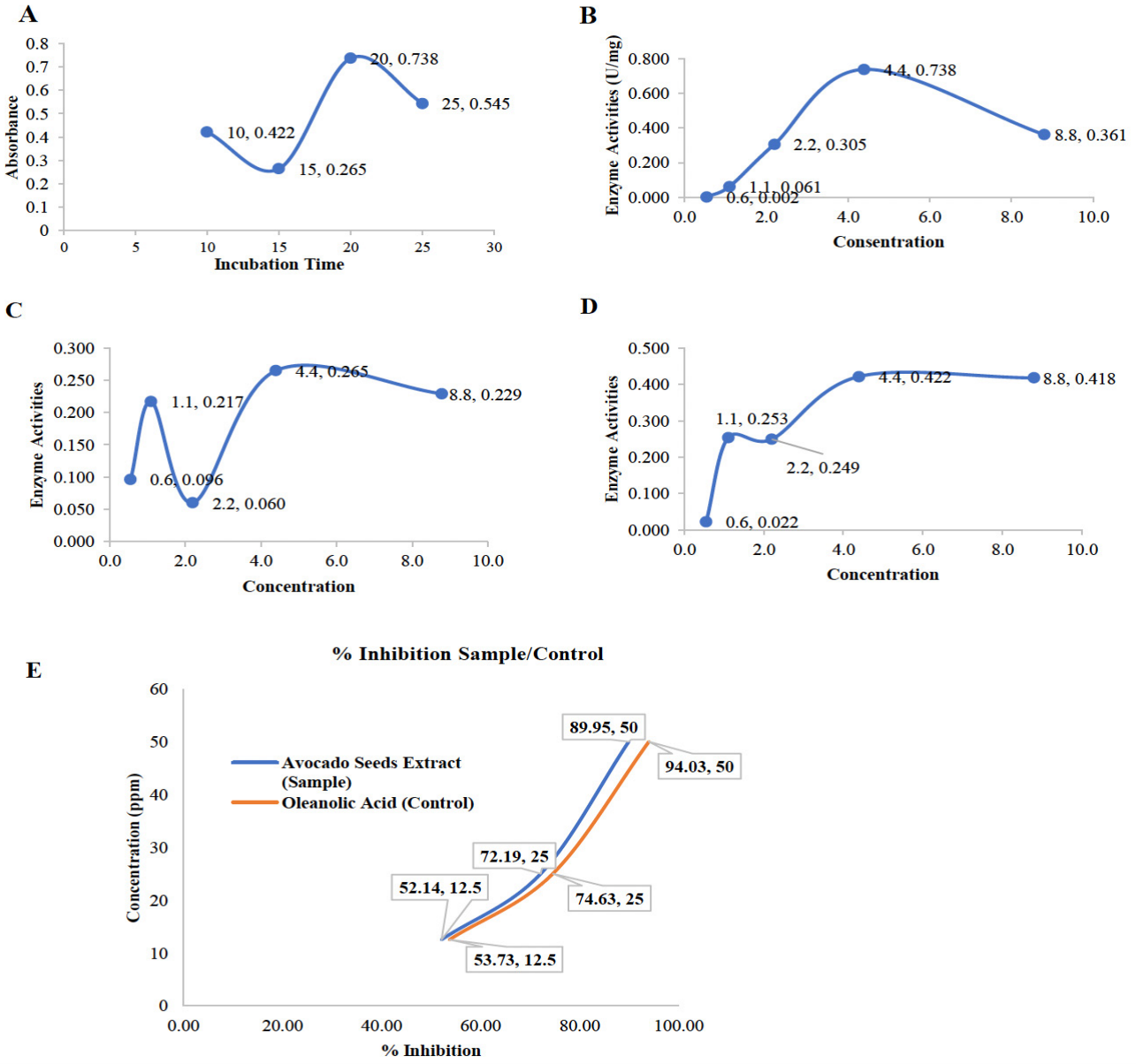

Figure 2. (A) Enzyme-substrate incubation time optimization test chart. A graph of three plates containing 5 concentrations of SANA substrate, with the duration of each plate being (B) 10 minutes, (C) 15 minutes, and (D) 20 minutes. (E) Elastase inhibition by ethanol extract of avocado seeds and oleanolic acid.

acid formed two hydrogen bonds, two hydrophobic interactions, nine van der Waals contacts, and one unfavorable acceptor–acceptor interaction. The ligand 2 ((8Z,11Z) Heptadeca 8, 11 dien 1 yl)furan displayed a greater number of van der Waals interactions compared to the other ligands. Meanwhile, 12 Methyl E,E-2,13-octadecadol formed one hydrogen bond, four hydrophobic interactions, and six van der Waals contacts. The ligand E 11 Methyl 12 tetradecen 1 ol acetate did not form hydrogen bonds but exhibited hydrophobic and van der Waals interactions. Figure 1A-E illustrates the 2D and 3D visualization of these ligands bound to the target protein (PPE).

3.6. In Vitro Elastase Inhibition Test

Before conducting the in vitro elastase inhibition test, we need to optimize the enzyme–substrate incubation time and the substrate SANA concentration (Figure 2A). We conclude that 20 minutes is the optimal incubation time (among 10, 15, 20, and 25 minutes) for the enzyme-substrate complex to react.

In the substrate concentration optimization test, 5 concentrations were tested: 8.8, 4.4, 2.2, 1.1, and 0.55 mM. Of the five concentrations, the optimal substrate concentration used was 4.4 mM (Figure 2B-D). After

optimizing the enzyme-substrate incubation time and substrate concentration, we will conduct the in vitro elastase test and present the result as a percentage inhibition (% inhibition). The results of this text are presented in Figure 2E.

The avocado seed ethanol extract demonstrated very strong elastase inhibitory activity, with an IC_{50}value below 50 (6.980 ppm), confirming its potency as an elastase inhibitor. These results show that avocado seed ethanol extract is a candidate elastase inhibitor, similar to oleanolic acid, with an IC_{50} below 50 (5.785 ppm).

4. Discussion

The findings of this study highlight the significant potential of ethanol extracts from avocado (*Persea americana* Mill.) seeds in health and cosmetic applications, focusing on their phytochemical content, antioxidant capacity, and elastase enzyme inhibition. The ethanol extract of avocado seeds contained flavonoids, polyphenols, tannins, saponins, quinones, steroids, and alkaloids. Triterpenoids were absent, likely because the 70% ethanol solvent used is polar and favors the extraction of polar compounds. This aligns with previous findings that avocado seeds are rich in phenolic compounds, contributing to their health benefits.[13]

The choice of 70% ethanol as a solvent is critical because of its intermediate polarity. A 70% ethanol solvent yields high phenolic content because its polarity efficiently dissolves moderately polar phenolics and flavonoids. The water fraction helps break down plant cell walls, while ethanol enhances solubility, making 70% ethanol the most effective balance for extracting antioxidant compounds from avocado seeds. Jubaidah (2024) and Azzahra et al. (2022) demonstrated that 70% ethanol extracts produced high yields of phenolic and flavonoid compounds, comparable to higher concentrations (100%) that are also effective, but less balanced toward polar phenolics, while lower concentrations (50%) dilute extraction efficiency.[18,19]

Using avocado seeds, often discarded as waste, for extracting bioactive compounds promotes sustainable practices. This aligns with the principles of circular bioeconomy and waste valorization in the food and cosmetic industries.[20] The study corroborates findings from previous research on the bioactive potential of avocado seeds. For instance, Riwanti et al. (2018) and More PR (2021) emphasized that 70% ethanol effectively extracts antioxidants from plant materials, which aligns with this study's choice of solvent and results in high phenolic content.[15,16] Additionally, the observed antioxidant capacity supports earlier studies indicating that avocado seeds have a rich phenolic and flavonoid composition, known for their health-promoting properties.[19]

The extract's IC_{50} value of 47.31 ppm indicates strong free radical scavenging activity, suggesting its potential to combat oxidative stress-related conditions. This aligns with the growing interest in natural antioxidants for managing diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases. Moreover, this property is particularly valuable in skincare, where oxidative stress accelerates aging.[21]

Phenolic and flavonoid compounds have been reported to present significant antioxidant properties. These compounds donate hydrogen atoms or electrons to neutralize free radicals, thereby reducing oxidative stress. In addition, it can directly inhibit elastase by binding to its active site or altering its conformation, preventing elastin degradation. Certain phenolic compounds (for epicatechin, catechin, resveratrol, and procyanidin B2) and the flavonoids (kaempferol, quercetin, and myricetin) have been shown to have anti-elastase activity (inhibited elastase activity) that is dose-dependent.[22,23] This dual mechanism (ROS scavenging and enzyme inhibition) explains the strong antioxidant and anti-elastase activities of avocado seed extract.

Molecular docking analysis demonstrated that the 3 most common ligands in avocado seeds ethanol extract have a higher binding affinity than Cholestan-3-ol, 2-methylene-, (3ß,5a)-, which is nearest to the binding affinity of control (oleanolic acid), even though Cholestan-3-ol, 2-methylene-, (3ß,5a)- has the smallest concentration in the GC-MS test. GC-MS concentration reflects the amount of compounds in an extract, while binding affinity predicts how strongly a compound binds to a target protein (elastase). Thus, a compound with low concentration can still show strong binding affinity if its structure fits well into the active site.

Despite its low abundance (1.37% in GC MS), Cholestan 3 ol, 2 methylene , (3β,5α) showed binding affinity closest to the control (oleanolic acid). It formed a hydrogen bond with Ser11 and hydrophobic contacts with Pro13, Tyr200, His193, and Trp12, stabilizing the ligand in the binding pocket. Ser11 is not part of the catalytic triad (Ser195, His57, Asp102) essential for elastase activity, but Pro13, Tyr200, His193, and Trp12 are supportive binding residues that contribute to ligand stabilization and substrate blocking. Interactions with these residues strengthen binding affinity and thereby support the predicted anti-elastase activity of these compounds. Hydrophobic contacts with residues near the substrate-binding

pocket can block or restrict substrate entry, indirectly reducing enzymatic activity.[24] These contacts indicate a potential for effective inhibition of elastase activity, as predicted by in silico docking analysis.

The negative value for the change in binding energy (ΔG) signifies that the binding of the ligand to the receptor is energetically favorable and occurs spontaneously. This suggests that the ligand fits well into the receptor's binding site, resulting in a stable and energetically favorable drug-receptor complex25.

Based on Figure 2, the optimal incubation time for the elastase test is 20 minutes. Some other studies, such as Natanael et al. (2021), Alvarez et al. (2025), and Desmiaty (2020), used an incubation time of 20 minutes. Some other studies use an incubation time of 15 minutes.[26–28] The optimal incubation time is important for forming the enzyme-substrate complex and inducing elastase activity. If the incubation times are shorter or longer, the complex reaction will not be accurate.

Determining the concentration of the SANA substrate is as important as determining the optimal incubation time for the enzyme-substrate complex. The optimal concentration of SANA substrate used was at a concentration of 4.4 mM (seen from the peak point of the graph in Figure 2B-D). This concentration is consistent with the SANA substrate concentration used in the anti-elastase test conducted by Sholikha (2013), who used 4.4 mM.[29] The strong elastase inhibition (IC_{50}<50 ppm) underscores the extract's potential in preventing elastin degradation, a key factor in skin aging. This creates opportunities to incorporate avocado seed extracts into anti-aging formulations, positioning them as sustainable alternatives to synthetic inhibitors.[20]

The ethanol extract of avocado seeds can strongly inhibit elastase (PPE), with an IC_{50} of 6.980 ppm (less than 50 ppm). The IC_{50} value is lower than that of other plants, such as the fruit extract of the Zodia plant, which has anti-elastase activity with an IC50 of 145.67 ppm.[30] In addition, the methanol extract of Rubus rosifulius inhibits elastase activity with an IC50 of 186.13 ppm.[27] Based on this exposure, the ethanol extract of avocado seeds can be a good natural ingredient as an anti-elastase in the future, compared to other natural ingredients.

The elastase inhibition findings also align with studies highlighting the potential of natural extracts in skin health and anti-aging. However, this study uniquely emphasizes avocado seed extracts, contributing to the broader body of research advocating the use of natural inhibitors over synthetic counterparts.[20]

5. Conclusion

The conclusion of this study is that the ethanol extract of avocado seeds has very strong antioxidant activity (IC_{50}<50 ppm). Therefore, this avocado seed ethanol extract also has anti-elastase activity both in silico and in vitro (IC_{50} < 50 ppm). Further research is needed on both animals and humans to develop cosmetics made from natural avocado seeds that can inhibit aging.

Acknowledgement

The authors would like to thank the Directorate of Research, Technology, and Community Services, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, for the research funding provided through the Operational Assistance for State Universities (BOPTN) Program, Research Grant for Fiscal Year 2024 (Grant No. 094/E5/PG.02.00.PL/2024).

The authors also thank the Rector of Universitas Almuslim Bireuen, as well as the Head and staff of the dr. Imal Indra Infection Laboratory and the Biomedical Laboratory, Faculty of Medicine, Universitas Syiah Kuala, for their valuable support and assistance during this study.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Tu Y, Quan T. Oxidative stress and human skin connective tissue aging. Cosmetics. 2016;3:28.
2. Zhang X, Yang X, Liu X, Huang J, Zhang Y, Xu X, et al. Decoding skin aging: the role of KNG1 in collagen and elastic fibre degradation. Mech Ageing Dev. 2023;210:111776.
3. Kerns ML, Chien AL, Kang S. Skin aging. In: Kang S, Amagai M, Bruckner AL, et al., editors. Fitzpatrick's Dermatology. 9th ed. New York (NY): McGraw-Hill Education; 2019.
4. Lee H, Hong Y, Kim M. Structural and functional changes and possible molecular mechanisms in aged skin. Int J Mol Sci. 2021;22(22):12489.
5. Reichheld SE, Muiznieks LD, Lu R, Sharpe S, Keeley FW. Sequence variants of human tropoelastin affecting assembly, structural characteristics and functional properties of polymeric elastin in health and disease. Matrix Biol. 2019;84:68–80.
6. Wei M, He X, Liu N, Deng H. Role of reactive oxygen species in ultraviolet-induced photodamage of the skin. Cell Div. 2024;19(1):1.
7. Petruk G, Di Giudice R, Rigano MM, Monti DM. Antioxidants from plants protect against skin photoaging. Oxid Med Cell Longev. 2018;2018:1454936.
8. Hossain A, Shahidi F. Persimmon leaves: nutritional, pharmaceutical, and industrial potential—A review.

Plants. 2023;12(4):937. doi:10.3390/plants12040937.
9. Ifesan BOT, Olorunsola BO, Ifesan BT. Nutritional composition and acceptability of candy from avocado seed (*Persea americana*). Int J Adv Innov Res. 2015;3(6):1630–1634.
10. [Jurnal Tersedia] Nguyen, Van Lam & Nguyen, & Tran, Thi & Bui, Thi & Paxayavong, Souksavanh & Lan, Thi & Tran, Huong. (2022). Bioactive compounds, antioxidant activity and lipid content of various avocado fruits. Carpathian Journal of Food Science and Technology. 10.34302/crpjfst/2022.14.2.4.
11. da Vinha AF, Moreira JMR, Barreira S. Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (*Persea americana* Mill.). J Agric Sci. 2013;5:100.
12. Segovia FJ, Hidalgo GI, Villasante J, Ramis X, Almajano MP. Avocado seed: A comparative study of antioxidant content and capacity in protecting oil models from oxidation. Molecules. 2018;23(10):2421.
13. Bangar SP, Dunno K, Dhull SB, Siroha AK, Changan S, Maqsood S, Rusu AV. Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. Food Chem X. 2022;16:100507.
14. Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The potential of plant phenolics in prevention and therapy of skin disorders. Int J Mol Sci. 2016;17(2):160.
15. Riwanti P, Izazih F, Amaliyah A. Pengaruh perbedaan konsentrasi etanol pada kadar flavonoid total ekstrak etanol 50, 70, dan 96% Sargassum polycystum dari Madura. J Pharm Care Anwar Med. 2020;2(2):82–95.
16. More PR, Arya SS. Intensification of bio-actives extraction from pomegranate peel using pulsed ultrasound: Effect of factors, correlation, optimization and antioxidant bioactivities. Ultrason Sonochem. 2021;72:105423.
17. Sulistyarini I, Sari DA, Wicakson TA. Skrining fitokimia senyawa metabolit sekunder batang buah naga (Hylocereus polyrhizus). J Ilm Cendekia Eksakta. 2020;5(1):56–62.
18. Jubaidah S. Avocado seed extracts (*Persea americana* Mill.) bioactive compounds profile as a source of antioxidants. Indones J Pharm Sci Technol. 2024.
19. Azzahra F, Sari IS, Ashari DN. Penetapan nilai rendemen dan kandungan zat aktif ekstrak biji alpukat (*Persea americana*) berdasarkan perbedaan pelarut ekstraksi. J Farmasi Higea. 2022;14(2):159.
20. Ong ES, Low J, Tan JCW, Foo SY, Leo CH. Valorization of avocado seeds with antioxidant capacity using pressurized hot water extraction. Sci Rep. 2022;12(1):13036.
21. Kupnik K, Primožič M, Kokol V, Knez Ž, Leitgeb M. Enzymatic, antioxidant, and antimicrobial activities of bioactive compounds from avocado (*Persea americana* L.) seeds. Plants (Basel). 2023;12:1201.
22. Pientaweeratch S, Panapisal V, Tansirikongkol A. Antioxidant, anti-collagenase and anti-elastase activities of Phyllanthus emblica, Manilkara zapota and silymarin: an in vitro comparative study for anti-aging applications. Pharm Biol. 2016;54(9):1865–72.
23. Wittenauer J, Mäckle S, Sußmann D, Schweiggert-Weisz U, Carle R. Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity. Fitoterapia. 2015;101:179–87.
24. Pitasi G, Brancale A, Floris S, Fais A, Gitto R, De Luca L. Computational approach to identifying new chemical entities as elastase inhibitors with potential antiaging effects. Int J Mol Sci. 2024;25:11174.
25. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol Rep. 2019;24:e00370.
26. Natanael GI, Simorangkir GF, Purba NP, Mardiana PBT, Amansyah A, Nasution AN. Potensi antioksidan dan anti-elastase ekstrak daun kelor (Moringa oleifera) terhadap antiaging. J Keperawatan Prior. 2021;4(1):69–76.
27. Desmiaty Y, Mulatsari E, Saputri FC, Hanafi M, Prastiwi R, Elya B. Inhibition of pancreatic elastase in silico and in vitro by Rubus rosifolius leaves extract and its constituents. J Pharm Bioallied Sci. 2020;12(3):1–7.
28. Álvarez-Martínez FJ, Herranz-López M, Barrajón-Catalán E, Micol V. Quantifying the modulation of elastase enzyme activity through colorimetric analysis. J Vis Exp. 2025.
29. Sholikha M. Penapisan in silico basis data senyawa tanaman obat di Indonesia dan uji in vitro aktivitas penghambatan elastase daun Melaleuca leucadendron L. [Thesis]. Depok (ID): Universitas Indonesia; 2013.
30. Swandiny GF, Desmiaty Y, Rahmah HU. Antioxidant and anti-aging activities from zodia (Evodia suaveolens J.R. Forst & G. Forst) fruit and leaf extract. J Jamu