World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 4
Page: 457-465

ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY OF ALOE VERA (*ALOE BARBADENSIS* MILLER) RAW GEL

Dong-Myong Kim[1,5*†], Yeo-Jin Lee[2], Chae-Yun Yang[3], Seoh-Yeon Hwang[4], Hyung-Kon Lee[1], Yong-Seong Kwon[1], Yeon-Mea Choi[5], Jung-Sung Park[6] and Hyun-Uk Oh[6]

[1]R&D Center, KJMBIO Ltd, 17 Saimdang-ro, Seocho-gu, Seoul 06649, Korea.
[2]Dept. of Chemical & Biomolecular Engineering, KAIST, Daejeon 34141, Korea.
[3]Dept. of Biomedical Engineering, UNIST, Ulsan 44919, Korea.
[4]Dept. of Biological Sciences, KAIST, Daejeon 34141, Korea.
[5]Kim Jeong Moon Aloe Ltd, 15 Saimdang-ro, Seocho-gu, Seoul 06649, Korea.
[6]Clinical Pharmacology Center, Chonbuk National University Hospital, Jeonju 54907, Korea.
†: contributed equally

Article Received: 13 July 2024 | | ***Article Revised: 02 August 2024*** | | ***Article Accepted: 25 August 2024***

***Corresponding Author: Dong-Myong Kim**
R&D Center, KJMBIO Ltd, 17 Saimdang-ro, Seocho-gu, Seoul 06649, Korea.
DOI: https://doi.org/10.5281/zenodo.13626281

ABSTRACT

Aloe barbadensis miller raw gel (KJM) was extracted and purified to homogeneity and then identified as a single band with a molecular weight greater than 250 kDa by toluidine blue staining on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel treated with 2-mercaptoethanol. The purified KJM was shown to inhibit ACE in a dose-dependent manner (28.7–59.8% ACE inhibition by 102.46–409.84 µg/mL KJM, respectively) using (N-(3-[2-furyl]acryloyl)-Phe-Gly-Gly) (FAPGG) as a substrate by spectrophotometry. The concentration of KJM required for 50% inhibition (IC_{50}) of ACE activity was 256.2 µg/mL, whereas that of captopril was 0.00781 µM (0.0095 nmole). Commercially available polysaccharide pectin (102.46–307.38 µg/mL) did not exhibit any inhibitory activity against ACE. Detection of FAPGG and FAP using fluorescent silica TLC or C 18 reversed-phase HPLC showed that KJM inhibited ACE. KJM exhibited mixed inhibition of ACE, and the Michaelis constant was 0.33 mM in the presence of KJM. Consumption of Aloe barbadensis Miller raw gel may be beneficial for human blood and vascular health.

KEYWORDS: Aloe vera, Raw gel, Mucopolysaccharide, Angiotensin converting enzyme (ACE), HPLC, (N-(3-[2-furyl]acryloyl]-Phe-Gly-Gly) (FAPGG), TLC.

INTRODUCTION

Several risk factors are associated with stroke, including age, gender, elevated cholesterol, smoking, alcohol, excessive weight, race, family history, and hypertension.[1] Although some of these risk factors cannot be modified, one factor that can be controlled and has the greatest impact on the etiology of stroke is high blood pressure.[2] Hypertension is considered to be the central factor in stroke with approximately 33% of deaths due to stroke attributed to untreated high

blood pressure.[1] Several classes of pharmacological agents have been used in the treatment of hypertension.[1] One class of antihypertensive drugs, known as angiotensin I converting enzyme (ACE) inhibitors (ACEI, i.e. a peptidase inhibitor), are associated with a low rate of adverse side-effects and are the preferred class of anti-hypertensive agents when treating patients with concurrent secondary diseases.[3] ACE (peptidyldipeptide hydrolyase EC 3.4.15.1) is a dipeptide-liberating exopeptidase classically associated with the renin-angiotensin system regulating peripheral blood pressure.[4] ACE removes a dipeptide from the C-terminus of angiotensin I to form angiotensin II, a very hypertensive compound. Several endogenous peptides, such as enkephalins, b-endorphin, and substance P, were reported to be competitive substrates and inhibitors of ACE.[4] Several food-derived peptides also inhibited ACE, including a-lactalbumin and b-lactoglobulin,[4] casein,[5] zein,[6] and gelatin.[7,10,11-14] Several antioxidant peptides (reduced glutathione and carnosine-related peptides)[9] and synthetic peptides also exhibited ACEI activities (Chen et al., 2003).[10]

Aloe vera species is a succulent plant belonging to the family *Asphodelaceae*, native to Egypt, Greece, West Africa, Southeast Asia, and the Caribbean.[11-14] In oriental medicine, Aloe is known as a medicinal herb because the dried whole leaves are often used as traditional medicine. The anthraquinone component, a storage polysaccharide of Aloe raw gel, has been shown to exhibit carbonic anhydrase and trypsin inhibitor activities,[15] dehydratase and monohydratase reductase activities,[9] and antioxidant activities.[11-14] Aloe raw gel contains mucilage, which has been reported as acemannan-D-isomer mucopolysaccharide.[16,17] Recently, KimJungMoon Aloe Jeju Aloe vera raw gel (KJM) was reported to exhibit antioxidant activity.[11-14,18] In this study, we report for the first time that purified KJM exhibits a novel dose-dependent ACE inhibitory activity. Captopril was used as a positive control, and commercial pectin was used as a negative control. KJM showed mixed inhibition on ACE, and the Michaelis constant in the presence of KJM was also determined.

MATERIALS AND METHODS

1. Materials

Tris, electrophoretic reagents, and silica gel 60 F254 were purchased from E. Merck Inc. (Darmstadt, Germany); captopril was purchased from Calbiochem Co. (CA, USA); Seeblue prestained markers for SDS-PAGE including myosin (250 kDa), BSA (98 kDa), glutamic dehydrogenase (64 kDa), alcohol dehydrogenase (50 kDa), carbonic anhydrase (36 kDa), myoglobin (30 kDa), and lysozyme (16 kDa) were from Invitrogen (Groningen, The Netherlands); FAPGG, ACE (I unit, rabbit lung), nonylamine, pectin (from citrus fruit, degree of esterification 94%), oluidine blue, coomassie brilliant blue R-250, and other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2. KJM extraction and purification

Fresh *Aloe barbadensis* Miller leaves were directly donated by KimJeongMoon Aloe Jeju Agricultural Factory. After washing and peeling, the aloe raw gel was cut into strips for KJM (KimJungMoon Aloe Jeju Aloe vera raw gel) extraction and purification according to the method.[11-14,18] 1 kg of Aloe raw gel was homogenized with 4 L of 50 mM Tris-HCl buffer (pH 8.3) containing 1% vitamin C. After centrifugation at 14,000 g for 30 min, the supernatant was saved and isopropanol was added to a final concentration of 70%. The solution was stirred rapidly and left overnight at 4ºC. The sediment was filtered, dehydrated with 100% isopropanol, and washed with acetone. After drying in an oven at 40ºC, the crude KJM was ground and collected for further purification by SDS and heating procedures. 1.0 g of

crude KJM powder was dissolved in 200 mL of deionized water and kept warm in a water bath at 50ºC. 40 mL of 5% SDS solution (dissolved in 45% ethanol) was added to the crude KJM solution and kept at 50ºC with gentle stirring for 20 min, and then stirred for 2 h at room temperature. The solution was then placed in an ice bath to lower the temperature and precipitate the SDS-protein complex. After centrifugation at 14,000 g for 30 min at 0ºC, the supernatant was retained and the SDS-protein complex was precipitated with isopropanol as mentioned above. After drying in an oven at 40ºC, the semi-purified KJM was ground, dissolved, and heated in boiling water for 20 min. After centrifugation at 14,000 g for 30 minutes at 0ºC, the supernatant was collected and isopropanol was added to a final concentration of 70%. The purified KJM was filtered, dehydrated, rinsed with acetone, dried, and collected for further use.

3. Mucopolysaccharide and Proteoglycan Staining on SDS-PAGE Gels

Eighty µL samples were mixed with 20 µL sample buffer, namely 60 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 25% glycerol and 0.1% bromophenol blue with 2-mercaptoethanol, heated in boiling water for 5 min, cooled to ambient temperature, and subjected to electrophoresis according to the method.[19] This discontinuous gel system contained 2.5 cm, 4% stacking gel, and 4.5 cm, 15% separating gel. Coomassie brilliant blue R-250 was used for mucopolysaccharide staining.[20] The 0.2% toluidine blue solution was used for proteoglycan staining.[21]

4. Determination of ACE Inhibitory Activity of KJM by Spectrophotometry

The ACE inhibitory activity was measured according to the method[22] with some modifications. The total 1.22 mL contained 20 µL (20 mU) of commercial ACE (1 U/mL, rabbit lung, Sigma Chem. Co.), 200 µL of different amounts of KJM (125, 250, 375 and 500 µg) or commercial pectin (degree of esterification, 94%, 125, 250 and 375 µg), and 1 mL, 0.5 mM N-(3-[2-furyl]acryloyl]-Phe-Gly-Gly) [FAPGG, dissolved in 50 mM Tris-HCl buffer (pH 7.5) containing 0.3 M NaCl]. The decreased absorbance readings at 345 nm ($\Delta A_{inhibitor}$) were recorded over 5 min at room temperature. Deionized water was used instead of sample solution for blank reading (ΔA_{blank}). The captopril (MW is 217.3) was used as a positive control for ACE inhibition (0.75, 1.89, 3.77, 5.66, 7.54, 18.8, and 75.4 nM). The ACE activity was expressed as DA 345 nm and the ACE inhibition (%) was calculated as followed: $[1-(\Delta A_{inhibitor} + \Delta A_{control})] \times 100\%$. IC50 was defined as the concentration of samples required to inhibit 50% of ACE activity under these conditions.

5. Determination of ACE Inhibitory Activity of KJM by TLC

The ACE inhibitory activity of KJM was determined by TLC method.[22] The reactions of commercial ACE with purified KJM or commercial pectin were done according to the methods[23] with some modifications. Each 100 µL sample (250 µg) was premixed with 15 mU ACE for one min and then 200 µL of 0.5 mM FAPGG was added and reacted at room temperature for 10 min. The 800 µL methanol was added to stop the reaction. In the blank experiment only FAPGG was used; in the control experiment, ACE reacted with FAPGG under the same conditions. Each reaction mixture was dried under reduced pressure, redissolved with 400 µL methanol and 50 µL of the solution were spotted on a silica gel 60 F254 by CAMAG Linomat IV spray-on technique (CAMAG, Switzerland). The FAPGG and FAP (product of an ACE-catalyzed hydrolysis reaction) were separated by TLC with a developing system of water saturated 1-butanol : acetic acid : water, 4:1:1 (V/V/V) and detected under UV light.[22]

6. Determination of the Kinetic Properties of ACE Inhibition by KJM

The kinetic properties of ACE (20 mU) without or with purified KJM (250 µg) in total volume of 1.22 mL were determined using different concentrations of FAPGG as substrates (0.1 mM to 0.5 mM). The Km (without KJM) and

Km' (with KJM) were calculated from Lineweaver-Burk plots, where Km´ was the Michaelis constant in the presence of 204.92 µg/mL KJM.

RESULTS AND DISCUSSION

1. Extraction and Purification of KJM

The crude KJM was obtained by isopropanol precipitation. KJM was purified by SDS treatment in the first step, during which mucopolysaccharides and proteins could be bound and then removed by centrifugation.[18,23] The average recovery was 52.0 ± 0.28% by SDS treatment in two independent trials. This semipurified KJM was further purified by heating in boiling water for 20 min in the second step. The recovery of the second treatment was 92.4 ± 0.52%. The total recovery of purified KJM after two purification steps was about 48% starting from crude samples. Figure 1 shows the proteoglycan (Figure 1A), mucopolysaccharide and protein (Figure 1B) stainings of YTM on SDS-PAGE gels after 2-mercaptoethanol treatment. Lanes 1 and 2 represent crude and purified KJM, respectively. The mucilage was stainable with toluidine blue dyes, and it proved to be a proteoglycan with molecular mass larger than 250 kDa on a SDS-PAGE gel (Figure 1A, indicated by an arrow). However, the crude mucilage (lane 1, Figure 1B) was found to contain proteins with molecular mass around 32 kDa, which did not appear in the purified YTM (lane 2, Figure 1B).

From our previous report[11-14,18,25] the Aloe vera raw gel storage mucopolysaccharide, protein exhibited molecular mass of 32 kDa after 2-mercaptoethanol treatment. The 32 kDa mucopolysaccharide and protein in crude KJM (lane 1, Figure 1B) could be dioscorins, and the purified KJM did not contain them.

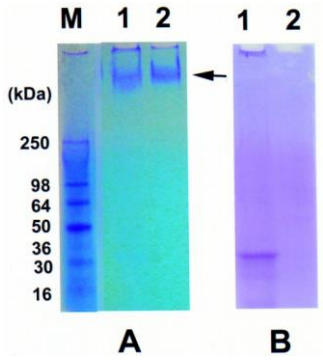

Figure 1: The proteoglycan (A) and protein (B) staining of crude (lane 1) and purified (lane 2) Aloe vera raw gel on SDS-PAGE gels after 2-mercaptoethanol treatment. The gel system contained 2.5 cm, 4% stacking gel and 4.5 cm, 15% separating gel. M indicates the Seeblue prestained markers of SDS-PAGE. Aloe vera raw gel of 25 µg was loaded in each well. Arrow indicates the position of the Aloe vera raw gel.

2. Inhibitory Activity of KJM by Spectrophotometry

The purified KJM was used for determinations of ACE inhibitory activity. Using FAPGG as a substrate, the concentrations of hydrolyzed product (FAP) were monitored by the decrease of the absorbance at 345 nm,[22] found to be proportional to the hydrolysis time. Figure 2A shows the effects of purified KJM and commercial pectin on ACE activity. Figure 2B shows the effects of captopril (0.75, 1.89, 3.77, 5.66, 7.54, 18.8, and 75.4 nM) on ACE activity. In the absence of purified KJM, the ΔA/min of ACE activity was -0.0087, meaning the change in absorbance ($\Delta A_{control}$) was 0.044 after 5 min. However, in the presence of different amounts of purified KJM, the ΔA/min was changed to -0.0062, -0.0046, -0.0042, and -0.0035 for 102.46, 204.92, 307.38, and 409.84 µg/mL, respectively. The ACE inhibition (%) was calculated according to the equation $[1-(\Delta A_{inhibitor} + \Delta A_{control})] \times 100\%$, as shown in Figure 2A.

Purified KJM was found to show dose-dependent ACE inhibitory activities (102.46 to 409.84 µg/mL, respectively, 28.7 to 59.8% ACE inhibition). The IC_{50} of KJM in inhibiting ACE activity was 256.2 µg/mL. Captopril was 7.81 nM (Figure 2B), close to the value (7 nM) reported.[26] Captopril (3-mercapto-2-methylpropanoyl-1-proline) was the first ACE inhibitor designed and marketed for treating hypertension and was an analogue of dipeptide of Ala-Pro.[1] However, the commercial polysaccharide pectin (102.46, 204.92, and 307.38 µg/mL) showed no inhibitory activity against ACE (Figure 2A). Pectins are principal constituents of the middle lamella in the cell wall. It seems that we cannot explain the inhibition of ACE by KJM as an interference of enzyme-substrate interaction by macromolecules. Both pectin and purified KJM were macromolecules; however, only the purified KJM showed special dose-dependent ACE inhibitory activity. In the literature, the mucopolysacchrides and protein hydrolysates were used as resources for purification of peptides as ACE inhibitors,[4-8,10-14] and the KJM was the first reported Aloe vera raw gel to exhibit ACE inhibition. The KJM was reported to be an acemannan-protein complex.[11-14,18] Whether the ACE inhibitory activity of KJM was due to mucopolysacchrides, acemannan, or protein portion, or the acemannan-protein complex must be investigated further. From calculations, the IC50 of KJM in inhibiting ACE activity was 256.2 µg/mL, less than that of the synthetic peptides α-lactorphin (YGLF, 322.7 µg/mL) and β-lactotensin (HIRL, 507.4 µg/mL).[4,11-14,18] Several identified peptide fragments[26] also exhibited much higher IC_{50} values than that of purified KJM; for example, LAHKAL of α-lactalbumin hydrolysates, 406 µg/mL; GLDIQK of β-lactoglobulin hydrolysates, 391 µg/mL; and VAGTWY of β-lactoglobulin hydrolysates, 1171 µg/mL.

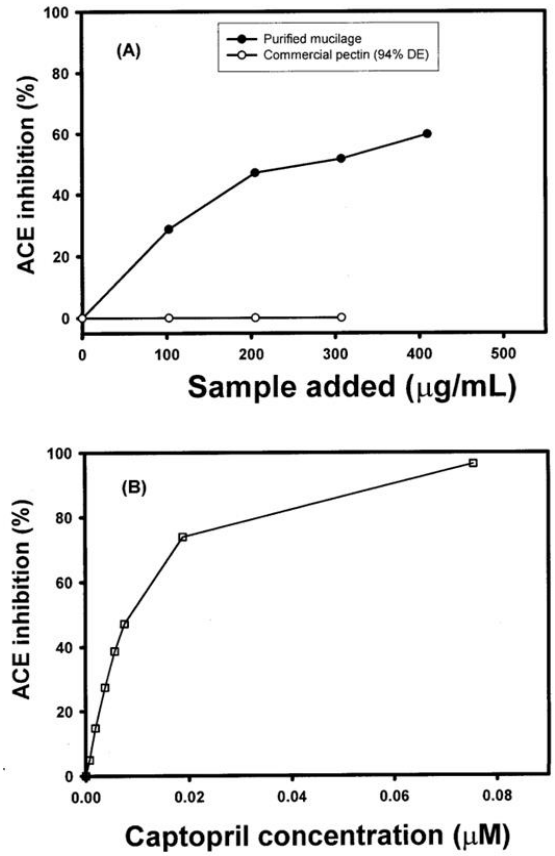

Figure 2: The effects of purified KJM, pectin, and captopril on ACE activity determined by spectrophotometry. (A) Purified KJM (102.46, 204.92, 307.38, and 409.84 µg/mL) or commercial pectin (102.46, 204.92, and 307.38 µg/mL); (B) Captopril (0.75, 1.89, 3.77, 5.66, 7.54, 18.8, and 75.4 nM). The inhibition of ACE (%) was calculated according to the equation [1-($\Delta A_{inhibitor} \div \Delta A_{control}$)] × 100%.

3. Determinations of ACE Inhibitory Activity of KJM by TLC

The FAPGG and FAP (product of ACE catalyzed hydrolysis reaction) were separated by TLC using water saturated 1-butanol : acetic acid : water, 4:1:1 (V/V/V) as developing solvents according to the methods.[22] Figure 3 shows the qualitative results of TLC chromatograms of a silica gel 60 F254 for the effects of commercial pectin and purified KJM (250 µg, lanes 3 and 4, respectively) on 15 mU ACE. Lane 1 is a blank test (FAPGG only); lane 2 is a control test (ACE reacted with FAPGG to produce FAP). Compared to the control test (lane 2), purified KJM (lane 4) was found to inhibit ACE activity, resulting in smaller amounts of produced FAP observed under UV light. However, similar amounts of FAP were found between control test (lane 2) and commercial pectin (lane 3). The results demonstrated again that purified KJM exhibited ACE inhibitory activity.

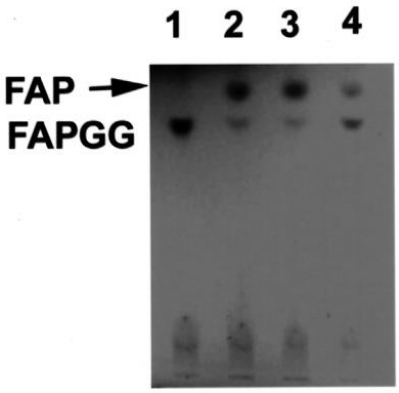

Figure 3: The TLC chromatograms of a silica gel 60 F254 for the effects of Aloe vera raw gel or commercial pectin on ACE activity. Lane 1, blank test (FAPGG only); lane 2, control test (ACE reacted with FAPGG to produce FAP); lane 3, 250 µg commercial pectin added; lane 4, 250 µg Aloe vera raw gel added. Each solution was dried under reduced pressure and redissolved with 400 µL methanol. Each 50 µL was spotted on a silica gel 60 F254 by CAMAG Linomat IV spray-on technique (CAMAG, Switzerland). The FAPGG and FAP were separated by water saturated 1-butanol: acetic acid: water, 4:1:1 (V/V/V). Arrow indicated the position of FAP.

4. Determinations of ACE Inhibitory Activity of KJM by HPLC

The FAPGG and FAP were separated isocratically with a mobile phase consisting of a mixture of 0.02 M nonylamine (adjusted to pH 2.4 with phosphoric acid): acetonitrile, 67.5:32.5 (V/V) according to the methods.[23] Figure 4 shows the HPLC chromatograms of Lichrospher 100 RP-18 endcapped column for the inhibitory activity of Aloe vera raw gel or commercial pectin on ACE. (A) is a blank test (FAPGG only with a retention time of 7.16 min); (B) is a control test (ACE reacted with FAPGG to produce FAP, with a retention time of 13.57 min); (C) is 250 µg commercial pectin; (D) is 250 µg Aloe vera raw gel against 15 mU ACE. Compared with the control test (Figure 4B), it was found that purified KJM (Figure 4D, 250 µg) inhibited ACE activity since the FAP area was reduced to about 52.8% of the original value (control test, Figure 4B). However, the addition of pectin (250 µg, Figure 4C) showed no effect on FAP production compared with control (Figure 4B). This meant that purified KJM did have inhibitory activity against ACE. By using different methods, including spectrophotometry, TLC and HPLC for the determination of ACE assay, it was confirmed that purified KJM exhibited ACE inhibitory activity.

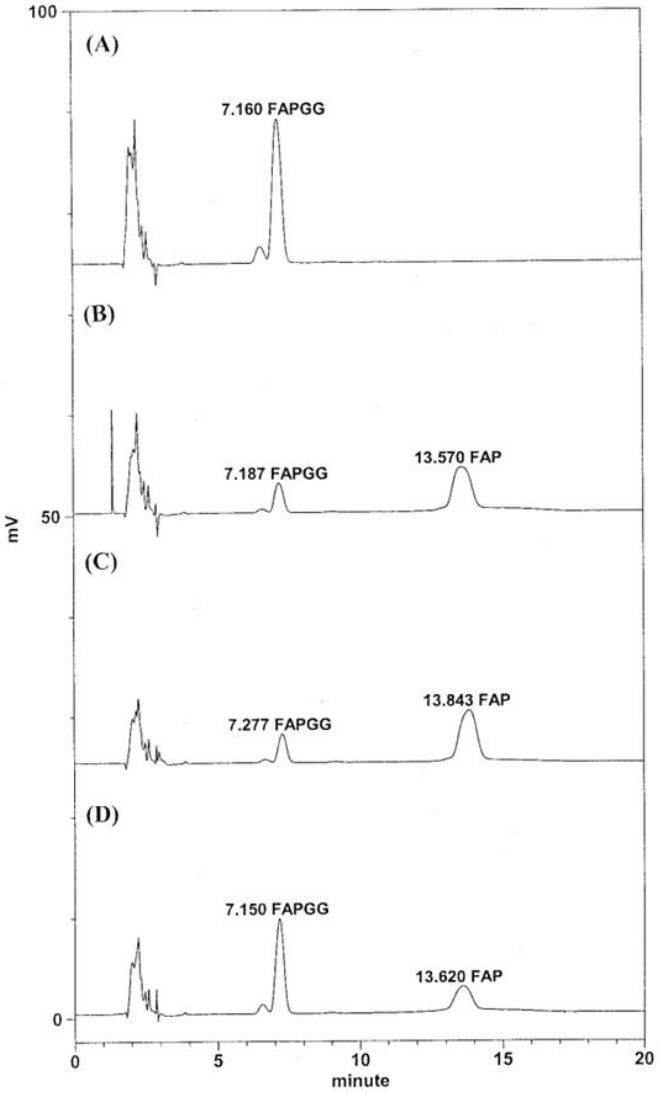

Figure 4: The HPLC chromatograms of Lichrospher 100 RP-18 endcapped column for the effects of Aloe vera raw gel or commercial pectin on ACE inhibitory activity. (A) blank test (FAPGG only with a retention time of 7.16 min); (B) control test (ACE reacted with FAPGG to produce FAP which had a retention time of 13.57 min); (C) 250 µg commercial pectin; (D) 250 µg Aloe vera raw gel against 15 mU ACE. The mobile phase consisted of a mixture of 0.02 M nonylamine (adjusted to pH 2.4 with phosphoric acid): acetonitrile, 67.5:32.5 (V/V). The flow rate was 1 mL/min; the injection volume was 100 µL; and the eluted fractions were detected at 345 nm.

5. Determinations of the Kinetic Properties of ACE Inhibition by KJM

The Lineweaver-Burk plots of ACE (20 mU) without or with purified KJM mucilage (204.92 µg/mL) in different concentrations of FAPGG are shown in Figure 5. The results indicated that purified KJM acted as a mixed type inhibitor with respect to the substrate (FAPGG). Without the purified KJM, the calculated Km was 0.255 mM FAPGG for ACE, which was close to the result (0.3 mM) of Holmquist et al. (1979). In the presence of purified YTM (204.92 µg/mL), the calculated Km´ was 0.3304 mM. In conclusion, the KJM exhibited dose-dependent ACE inhibitory activity and acted as a mixed type inhibitor with respect to the substrate (FAPGG). The IC50 of YTM in inhibiting ACE activity was 256.2 µg/mL, less than several peptides acting as ACE inhibitors. It might be beneficial to blood and vascular health when people consume Aloe.

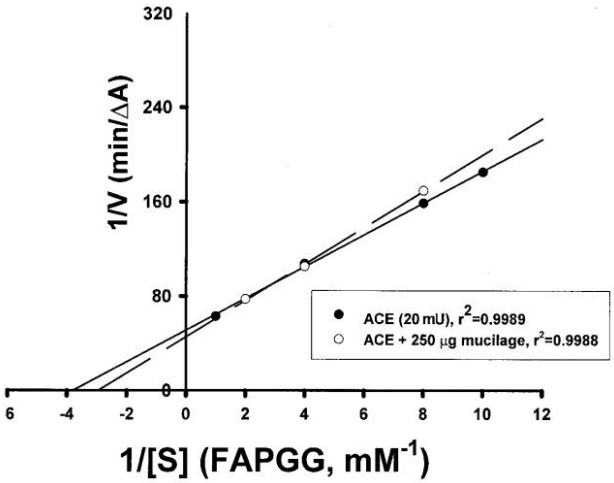

Figure 5: The Lineweaver-Burk plots of ACE (20 mU) without or with purified KJM mucilage (204.92 µg/mL) in different concentrations of FAPGG (0.1 to 0.5 mM).

ACKNOWLEDGEMENT

This research was supported by the project entitled 'Technology Development of Marine Industrial Biomaterials' funded by the Korea Institute of Marine Science & Technology Promotion (Program number: 20100090-8) and the Ministry of SMEs and Startups (MSS, Korea) under Grant the Technology development Program [number S3049433].

REFERENCES

1. Mark KS, Davis TP. Stroke: development, prevention and treatment with peptidase inhibitors. Peptides, 2000; 21: 1965-1973.
2. Dunbabin D. Cost-effective intervention in stroke. Pharmacoeconomics, 1992; 2: 468-499.
3. Fotherby MD, Panayiotou B. Antihypertensive therapy in the prevention of stroke: what, when, and for whom? Drugs, 1999; 58: 663-674.
4. Mullally MM, Meisel H, FitzGerald RJ. Synthetic peptides corresponding to a-lactalbumin and b-lactoglobulin sequences with angiotension-I-converting enzyme inhibitory activity. Biol. Chem., 1996; 377: 259-260.
5. Maruyama SH, Mitachi J, Awaya M, Kurono N, Tomizuka N, Suzuki H. Angiotension I-converting enzyme inhibitory activity of the C-terminal hexapeptide of as1 casein. Agric. Biol. Chem., 1987; 51: 2557-2561.
6. Yano SK, Suzuki H, Funatsu G. Isolation from azein of thermolysin peptides with angiotension I-converting enzyme inhibitory activity. Biosci. Biotechnol. Biochem., 1996; 60: 661-663.
7. Chen TL, Lo YC, Hu WT, Wu MC, Chen ST, Chang HM. Microencapsulation and modification of synthetic peptides of food proteins reduces the blood pressure of spontaneously hypertensive rats. J. Agric. Food Chem., 2003; 51: 1671-1675.
8. Kim SK, Byun HG, Park PJ, Shahidi F. Angiotension I converting enzyme inhibitory peptides purified from bovine skin gelatin hydrolysate. J. Agric. Food Chem., 2001; 49: 2992-2997.
9. Hamid H. Effect of Aloe vera gel coating on antioxidant capacity, antioxidant enzyme activities and decay in raspberry fruit. LWT-Food Sci. Tech., 2015; 60(1): 495-501.
10. Chen TL, Ken KS, Chang HM. Study on the preparation of angiotension-converting enzyme inhibitors (ACEI) from hydrolysates of gelatin. J. Chin. Agric. Chem. Soc., 1999; 37: 546-553.
11. Kim DM, Lee HK, Kwon YS, Choi YM, Kim CO, Cho GJ. Study on the antithrombotic effect of Aloe vera raw gel

extract for wound healing treatment and drug development. ACTA Pharm. Sci., 2024; 8(6): 11-23.

12. Kim DM, Yang CY, Lee YJ, Hwang SH, Lee HK, Kwon YS, Choi YM, Hollestelle T, Bakker ML, Karang A, Naya A, Goodman S. Study on optimization spray drying process conditions for maximum stable granular Aloe vera raw gel powder with a low unstable rate of aloverose. American J Engin. Res., 2024; 13(7): 46-56.
13. Kim DM, Yang CY, Lee YJ, Hwang SH, Lee HK, Kwon YS, Choi YM. . Assessment of genotoxicity and chromosomal stability of Aloe extract complex using an in vitro test. J Biotech. Biochem., 2024; 10(4): 10-19.
14. Kim DM, Hwang SH, Yang CY, Lee YJ, Lee HK, Kwon YS, Choi YM. Micronucleus test in bone marrow cells and oral toxicity study on aloin-quercetin in rats. J Environ. Sci. Toxic. Food Tech., 2024; 18(7): 12-19.
15. Nuray G, Paksoyb ÖI. GC/MS evaluation of the composition of the Aloe vera gel and extract. Food Chem. X., 2024; 23: 101536-101549.
16. Zhang L, Tizard R. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. Immuno Pharmacol., 1996; 35(2): 119-28.
17. Gerardo DSG, Rocío CR, Azucena GH, Jorge LA, Abelardo CM., Acemannan, an Extracted Polysaccharide from Aloe vera: A Literature Review. Natural Product Com., 2014; 9(8): 1217-1221.
18. Kim DM, Yang CY, Lee YJ, Hwang SH, Lee HK, Kwon YS, Choi YM. Human clinical study of Aloe vera raw gel (The Aloe Prime Aloe Gel) on blood lipids and obesity. World J Pharm Sci Res., 2024; 3(4): 238-245.
19. Laemmli UK. . Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970; 227: 680-685.
20. Neuhoff V, Stamm R, Eibl H., Clear background and highly sensitive protein staining with Coomassie blue dyes in polyacrylamide gels: a systematic analysis. Electrophoresis, 1985; 6: 427-448.
21. Carney SL., Proteoglycans, chapter 4. Carbohydrate analysis. In M. F. Chaplin and J.F. Kennedy (eds.), A Practical Approach. IRL Press Limited, 1986; 97-141.
22. Holmquist B, Bunning P., and J.F. Riordan, A continuous spectrophotometric assay for angiotensin converting enzyme. Anal. Biochem, 1979; 95: 540-548.
23. Anzenbacherova E, Anzenbacher P, Macek K, Kvetina J. Determination of enzyme (angiotensin convertase) inhibitors based on enzymatic reaction followed by HPLC. J. Phrmaceut. Biomed. Analys, 2001; 24: 1151-1156.
24. Mariana C, Musuc AM, Popa M, Moreno C. Aloe vera-based hydrogels for wound healing: Properties and therapeutic effects. Gels, 2023; 9(7): 539-249.
25. Marzanna H, Dziedzic K, Górecka D, Anna JG, Gujska E. Aloe vera (L.) Webb. Natural sources of antioxidants-A review. Plant Foods Hum. Nutr., 2019; 74(3): 255–265.
26. Pihlanto-Leppälä A, Rokka T, Korhonen H. Angiotensin I converting enzyme inhibitory peptides derived from bovine milk proteins. Int. Dairy J., 1998; 8: 325-331.