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Research article

Functional evaluation of saclipins A and B derived from the edible cyanobacterium *Aphanothece sacrum*: New bioactivities for anti-wrinkle and anti-hypertension

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Abstract: Saclipin A and saclipin B are bioactive oxylipin compounds derived from an edible cyanobacterium, *Aphanothece sacrum*. Saclipins have been shown to have potent anti-aging properties, including antioxidative, antiglycative, anti-wrinkle, and skin-whitening activities. In this study, we demonstrate that the anti-collagenase and anti-hyaluronidase activities of saclipins are additional anti-wrinkle properties of these compounds. Furthermore, we reveal that saclipins exert angiotensin-converting enzyme (ACE) inhibitory activities, which can mitigate hypertension. These results suggest that saclipins are promising natural products with applications not only for skincare products but also as medical supplements.

Keywords: saclipin; cyanobacteria; *Aphanothece sacrum*; angiotensin-converting enzyme inhibition; collagenase inhibition; hyaluronidase inhibition

1. Introduction

Aphanothece sacrum (Suringar) Okada (hereafter *A. sacrum*) is an edible cyanobacterium endemic to Japan. This organism was originally reported as *Phylloderma sacrum* by Suringar in 1872 [1,2]. Subsequently, Okada reclassified this cyanobacterium as the genus *Aphanothece* [2,3]. *Aphanothece sacrum* forms clumps that can exceed 10 cm in length. In Japan, the vernacular name for *A. sacrum* is Suizenji-nori, and it has been used as a food source for more than 300 years [2,4]. The only known natural habitat of *A. sacrum* is the Kogane River in Asakura City, Fukuoka Prefecture [5].

The *A. sacrum* samples used in this study were obtained from Endokanagawa-Do, which operates an aquaculture business on the Kogane River. Recently, novel ultraviolet (UV)-absorbing compounds, saclipin A and saclipin B, were discovered in *A. sacrum* [6]. Saclipin A and saclipin B, which are *cis-trans* isomers, show absorption maxima at 316 and 319 nm, respectively, and molar extinction coefficients of 26,454 and 30,555 M^{-1} cm^{-1}, respectively [6]. Functional assessment revealed that saclipins exhibit antioxidative and antiglycative activities in addition to their UV-absorbing properties [6]; therefore, saclipins are thought to be promising ingredients for cosmetic applications. This is because the progression of skin aging caused by UV radiation stress is closely related to the generation of reactive oxygen species (ROS) and the glycation of proteins found in the skin [7]. In addition to extrinsic skin aging caused by external environmental stress, predominantly UV exposure, intrinsic skin aging is known to be associated with programmed aging and cellular aging caused by endogenous oxidative stress and cellular damage [8]. It has been reported that the accumulation of ROS can indirectly activate the dermal enzymes collagenase and elastase, which degrade collagen and elastin, respectively [9]. Collagen, which is the most abundant protein in the human dermis, maintains the tensile strength of skin, while elastin is a protein that is a component of elastic fibers, which support collagen fibers [9]. Therefore, the activation of elastase and collagenase accelerates skin aging through the formation of wrinkles and sagging skin as collagen and elastin are broken down [10].

Another important substance found in the skin is hyaluronic acid, which is abundant in the dermis and epidermis and contributes to the smoothness of skin, helping to reduce wrinkles due to its excellent water retention properties [9]. It is important to maintain the hyaluronic acid content of the skin to help keep the skin looking youthful, but hyaluronic acid levels decrease with age, and it is also broken down by hyaluronidase [11]. We recently found that saclipins possess bioactivities, including elastase inhibition and the promotion of collagen and hyaluronic acid production in human fibroblasts [12]. These properties of saclipins may contribute to maintaining skin firmness and preventing wrinkles. These advantageous activities, together with the high stability of saclipins under light irradiation and heat treatment, suggest that saclipins have potential in formulations for skincare cosmetics and oral supplements [12]. In this brief report, we describe the additional bioactivities of saclipins that have anti-wrinkle effects. Furthermore, we show that saclipins exhibit useful anti-hypertensive effects when taken orally.

2. Preparation of purified saclipins

For the assays, we prepared purified saclipins A and B as described in [12]. Briefly, dried *A. sacrum* (10 g), purchased from Endokanagawa-Do (Fukuoka, Japan), was pulverized using a food processor (IFM-C20G, Iwatani, Tokyo, Japan). The resulting powder was mixed with 50 mL of methanol and sonicated using a Model UR-200P sonicator (TOMY SEIKO, Tokyo, Japan). The

suspension was centrifuged at 2,330g for 15 min at 4°C, and the resulting supernatant was transferred to another tube and then dried in a VC-36R centrifugal concentrator (Taitec, Saitama, Japan). The dried materials were dissolved in 5 ml of acetonitrile/formic acid/water (55/0.1/44.9, v/v/v) and then passed through a 0.22 μm-pore-size filter. The samples were subjected to preparative HPLC with an Inertsil ODS-3 column (10 μm; 250 × 20 mm) connected to a guard column (50 × 20 mm) containing the same packing materials. The mobile phase was acetonitrile/formic acid/water (55/0.1/44.9, v/v/v), and the flow rate was 10.0 ml/min. The volume of samples injected was 5 ml. Saclipins were detected at 320 nm. In this preparative HPLC, saclipins A and B were eluted as the same fraction, and this fraction was lyophilized. All purification steps were performed in the dark, and the resulting sample contained saclipins A and B in a ratio of 0.92:0.08, which was used as purified saclipin A (Figure 1). For the preparation of purified saclipin B, purified saclipin A was dissolved in ethanol and then exposed to 6,000 lux light irradiation for 24 h to isomerize saclipin A to saclipin B. The resulting sample contained saclipins A and B in a ratio of approximately 0.09:0.91, which was used as purified saclipin B (Figure 1). The purified saclipins were analyzed by HPLC analysis as reported in [6], with some modifications. In brief, a polymeric ODS column (Inertsil ODS-P; 3 μm, 150 × 4.6 mm) connected to a guard column (3 μm, 30 × 4.6 mm) was used as the stationary phase, and a mixture of acetic acid/acetonitrile/water (0.5:50:49.5, v/v/v) was used as the mobile phase. The mobile phase flow rate was maintained at 0.5 mL/min, and the column temperature was maintained at 40°C. The volume of samples injected was 10 μl. The quantification of saclipins was performed by peak area integration at 320 nm.

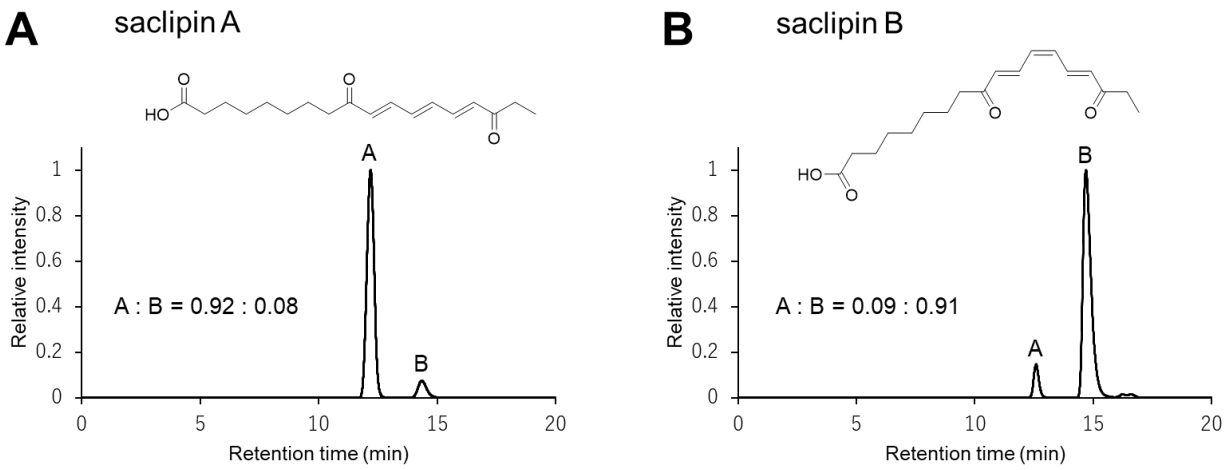

Figure 1. Chemical structures and HPLC chromatograms of saclipin A-rich (A) and saclipin B-rich (B) materials used for the evaluation of their bioactivities.

3. Evaluation of anti-wrinkle effects of saclipins

To evaluate the anti-wrinkle effects of saclipins, we investigated their collagenase inhibitory activities and hyaluronidase inhibitory activities. The accumulation of collagen and hyaluronic acid in dermal fibroblasts is important for maintaining skin firmness and elasticity [13]. Collagenase is a well-known proteolytic enzyme that breaks down collagen and is associated with skin aging [14]. We previously reported that collagenase inhibitory activity was not detectable in extracts containing saclipins at concentrations of less than 31.25 μM [15], but this activity was observed at the higher

concentrations used in the present study. As shown in Figure 2A, both saclipins A and B showed collagenase inhibitory activities with an IC_{50} value of <400 μM. Their IC_{50} values are comparable to those of mycosporine-like amino acids (MAAs), which are known to be important UV-absorbing compounds in cyanobacteria [16-18]. Interestingly, when comparing saclipins A and B, saclipin A exhibited greater activity than saclipin B. At a concentration of 9.8 mM, there was no notable difference in inhibitory activity between saclipins A and B, but at the lower concentration of 0.4 mM, saclipin B inhibited 67% while saclipin A inhibited 85%. This result indicates that lower concentrations of saclipin A exert a greater inhibitory effect on collagenase. Considering that saclipins can inhibit collagen glycation [6], these findings suggest that saclipins can not only inhibit collagen degradation but also protect its function and thus could play an effective role in skin anti-aging. Saclipins also show inhibitory activity against hyaluronidase, which degrades hyaluronic acid [19] (Figure 2B). Saclipins A and B showed similar activity in inhibiting hyaluronidase, both showing IC_{50} values of <400 μM. These results, together with the previously identified activities, strongly suggest that saclipins are promising compounds that have anti-aging effects. These advantageous activities have great potential for the development of functional skincare products.

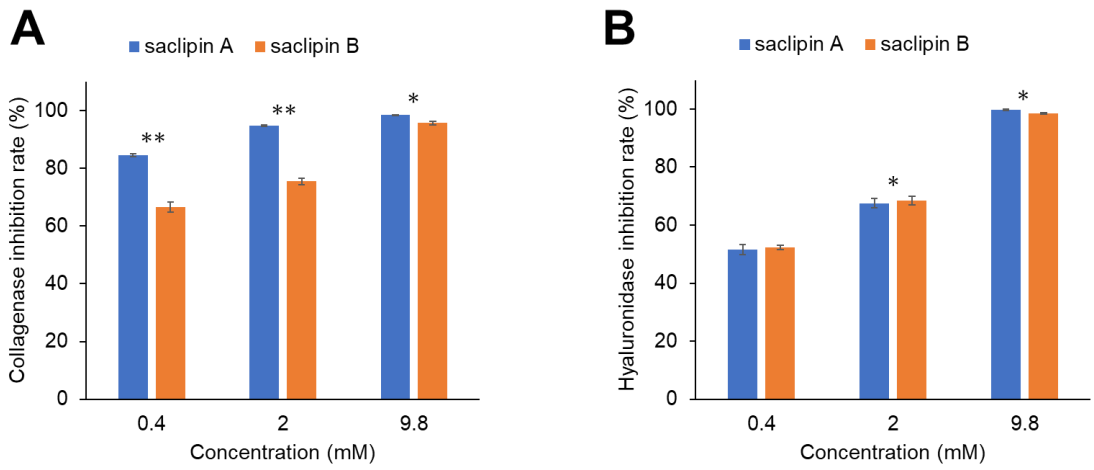

Figure 2. Inhibitory effects of saclipins on collagenase and hyaluronidase. (A) Anti-collagenase activity of saclipins. (B) Anti-hyaluronidase activity of saclipins. The error bars show standard deviations across three different tests. Asterisks indicate a statistically significant difference between saclipin A and saclipin B at that concentration (*$p < 0.05$, **$p < 0.01$, unpaired *t*-test).

The evaluations of collagenase inhibitory activities and hyaluronidase inhibitory activities were performed by Kanagawa Institute of Industrial Science and Technology (KISTEC, Kanagawa, Japan). For the collagenase inhibition assay, collagenase was dissolved at 0.02 mg/mL in 50 mM Tris buffer (pH 7.3) to prepare the enzyme solution. To prepare the substrate solution, MOCAc-Pro-Leu-Gly-Leu-A_2pr (Dnp)-Ala-Arg-NH_2 was dissolved at 1 mM in DMSO and diluted 200-fold with 50 mM Tris buffer (pH 7.3) immediately before use. Measurements were performed using a 96-well microplate. Aliquots of sample solution (50 μL) and enzyme solution (100 μL) were added and incubated at 37°C for 10 minutes. Then, 50 μL of substrate solution was added and the mixture was stirred. After incubation at 37 °C for 60 minutes, the fluorescence intensity (excitation wavelength 320 nm, emission

wavelength 405 nm) was measured. Collagenase inhibitory activity was calculated based on the change in fluorescence intensity. For the hyaluronidase inhibition assay, the enzyme solution was prepared by dissolving bovine hyaluronidase at 4 mg/mL in 0.1 M acetate buffer (pH 4.0). The substrate solution was prepared by dissolving potassium hyaluronate at 0.8 mg/mL in acetate buffer (pH 4.0). The enzyme activation solution was prepared by dissolving Compound 48/80 at 0.5 mg/mL in acetate buffer (pH 4.0). The color-development stock solution was prepared by dissolving *p*-dimethylaminobenzaldehyde at 0.1 g/mL in a mixture of hydrochloric acid and acetic acid, which was diluted 10-fold with acetic acid just before use. The reaction was carried out in a microtube. Aliquots of sample solution (50 μL) and enzyme solution (25 μL) were added and incubated at 37℃ for 20 minutes. Next, 50 μL of enzyme activation solution was added and incubated at 37°C for 20 minutes. Then, 125 μL of substrate solution was added and incubated at 37℃ for 40 minutes. The reaction was then stopped by adding 50 μL of 0.4 N sodium hydroxide solution, then 50 μL of 0.8 M borate buffer (pH 9.0) was added. The mixture was heated in a heat block at 105℃ for 5 minutes. It was then thoroughly cooled on ice, and 50 μL of the mixture was transferred to a 96-well microplate. Color-developing solution (200 μL) was added, and the mixture was incubated at 37℃ for 30 minutes, after which the absorbance at 585 nm was measured. Hyaluronidase inhibitory activity was calculated based on the change in absorbance.

4. Evaluation of anti-hypertensive property of saclipins

Due to their ability to absorb UV radiation, saclipins have been evaluated for their biological activity in terms of anti-aging of the skin. As *A. sacrum* is also used as a food product, it is important to evaluate the function of saclipins as oral drugs or supplements. In this study, we evaluated the angiotensin-converting enzyme (ACE) inhibitory effect of saclipins to determine whether they have anti-hypertensive properties. ACE is known to convert angiotensin I to the active vasoconstrictor angiotensin II, a compound that triggers increased blood pressure by inducing vasoconstriction and the release of aldosterone [20]. ACE inhibitors suppress the formation of angiotensin II, thereby preventing an increase in blood pressure. As shown in Figure 3, both saclipins A and B notably inhibited ACE activity. Saclipin A exhibited a greater inhibitory effect than saclipin B. The IC_{50} values of saclipins A and B were 0.5 and 1.2 mM, respectively. These results indicate that saclipins have potential as anti-hypertensive agents and that *A. sacrum* itself may be a suitable food ingredient for modulating blood pressure. The molecular mechanism underlying the ACE inhibitory effects of saclipins is currently unknown and would be an interesting topic for future research. Most of the currently available ACE inhibitors are short peptides and their derivatives, and their activities are based on antagonism at the three active sites of ACE that recognize angiotensin I [21]. The interaction of ACE inhibitors with the zinc ion present in the active center of ACE is also important [21]. It has been reported that the ketone groups of ketone-containing ACE inhibitors bind to the zinc ion in ACE in the form of a hydrated geminal diolate [22]; therefore, it is possible that the two ketones in saclipins can bind to the zinc ion in ACE. The difference in inhibitory activity between saclipins A and B is thought to be due to differences in their conformations, such as the formation of hydrogen bonds due to the smaller distance between the two ketone groups in saclipin B than in saclipin A, as we discussed previously [6].

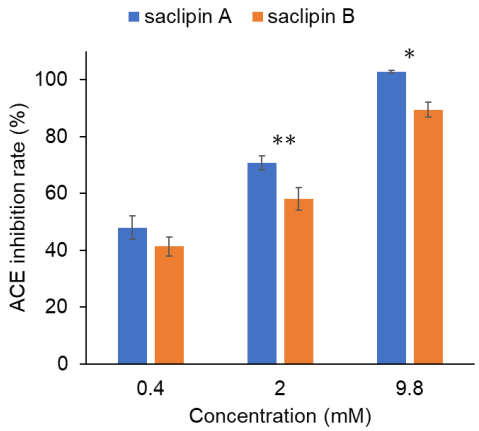

Figure 3. Inhibitory effects of saclipins on ACE. The error bars show standard deviations for three separate assays. Asterisks indicate a statistically significant difference between saclipin A and saclipin B at that concentration (*$p < 0.05$, **$p < 0.01$, unpaired *t*-test).

The evaluations were performed by KISTEC. ACE inhibitory activity was calculated using ACE Kit-WST (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's protocol. This kit is based on a method that detects the absorbance at 450 nm of the enzyme reaction product of 3-hydroxybutyric acid generated from 3-hydroxybutyryl-Gly-Gly-Gly, which is used as a substrate for ACE [23,24].

5. Conclusions

In this study, we showed for the first time that saclipins possess the physiological activity of suppressing hypertension, in addition to collagenase and hyaluronidase inhibition activity. Since the identification of saclipins from *A. sacrum* was first reported in 2023 [6], various useful properties of saclipins have been discovered, as shown in Table 1. Comparing saclipin A and saclipin B, saclipin A has greater singlet oxygen quenching, anti-elastin glycation, anti-elastase, and anti-ACE activities, while saclipin B has superior UV absorption, ABTS radical-scavenging, and anti-collagen glycation activities. Although saclipin A is quantitatively more abundant than saclipin B in *A. sacrum*, saclipin A is readily photoisomerized to saclipin B upon exposure to light [6]. Therefore, the ability of saclipin B to absorb UV is likely to be useful for applications in topical skin products. One possible application for saclipin B is in skincare creams. Saclipin B is a fat-soluble compound derived from a fatty acid (probably α-linolenic acid), and its application could be differentiated from water-soluble MAAs, which are also UV-absorbing compounds derived from cyanobacteria and have already been commercialized [14]. Conversely, the ACE inhibitory activity of saclipin A is promising for the development of applications involving oral products. By incorporating saclipin A into oral supplements, it is possible that the antioxidant and antiglycation effects of saclipin A may contribute to anti-aging from within the body.

In conclusion, this study has provided new information regarding the functionality of saclipins. This functionality can add value not only to saclipins but also to *A. sacrum*. The natural habitat of *A. sacrum* has been shrinking due to environmental changes, and it is now only found in a small area of the Kyushu region of Japan [5]. It is a rare species that has been designated as a Class IA endangered

species by Japan's Ministry of the Environment [5]. It is anticipated that the knowledge of this organism's added value will lead to conservation activities and revitalization of the aquaculture industry involving *A*. *sacrum*.

Table 1. Biological and pharmacological activities of saclipins.

Characteristics and activities	Saclipin A	Saclipin B	Ref.
UV-absorption properties			
Molar extinction coefficient	26,454 M^{-1} cm^{-1} (316 nm)	30,555 M^{-1} cm^{-1} (319 nm)	[6]
Antioxidant activities			
ABTS radical-scavenging	Weak (IC_{50}: 717 μM)	Weak (IC_{50}: 142 μM)	[6]
Singlet oxygen quenching	Weak (IC_{50}: 1.2 mM)	Weak (IC_{50}: 2.1 mM)	
Antiglycative activities			
Anti-elastin glycation	Yes (IC_{50}: < 4 mM)	Yes (IC_{50}: > 4 mM)	[6]
Anti-collagen glycation	Yes (IC_{50}: 1.9 mM)	Yes (IC_{50}: 0.9 mM)	[6]
Anti-wrinkle activities			
Anti-elastase	Yes (IC_{50}: < 12.5 μM)	Yes (IC_{50}: < 25.0 μM)	[12]
Anti-collagenase	Yes (IC_{50}: < 400 μM)	Yes (IC_{50}: < 400 μM)	This study
Anti-hyaluronidase	Yes (IC_{50}: < 400 μM)	Yes (IC_{50}: < 400 μM)	This study
Collagen production promotion	Yes	Yes	[12]
Hyaluronic acid production promotion	No	Yes	[12]
Skin-whitening effects			
Anti-tyrosinase	Yes	Yes	[12]
Melanin production inhibition	Yes	Yes	[12]
Melanin precursor darkening inhibition	No	No	[12]
Anti-hypertensive activity			
Anti-ACE	Yes (IC_{50}: 0.5 mM)	Yes (IC_{50}: 1.2 mM)	This study

Author contributions

Conceptualization, H.K. and R.W.S.; methodology, Y.U., M.H., R.W.S., and H.K.; investigation, Y.U. and H.K.; Data curation, Y.U., R.W.S., and H.K.; writing-original draft, H.K.; writing – review & editing, Y.U., M.H., and R.W.S.; funding acquisition, H.K. All authors have read and agreed to the published version of the manuscript.

Use of Generative-AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

Rungaroon Waditee-Sirisattha and Hakuto Kageyama are the Guest Editor of special issue "Recent advances in exploring bioactive natural products from cyanobacteria and microalgae" for AIMS Molecular Science. Rungaroon Waditee-Sirisattha and Hakuto Kageyama were not involved in the editorial review and the decision to publish this article. All authors declare no conflicts of interest in this paper.

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