J. Cosmet. Sci., 71, 377–383 (November/December 2020)

Antibacterial Activity of Senkyunolide A Isolated from *Cnidium Officinale* Extract

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Accepted for publication May 8, 2020.

Synopsis

In this study, we investigated the antibacterial and anti-inflammatory properties of *Cnidium officinale* hexane (COH) extract and senkyunolide A (SA). The antibacterial activities were measured using the paper disk diffusion method and minimum inhibitory concentration (MIC) against *Propionibacterium acnes* and *Malassezia furfur*. COH extract showed antibacterial activity at a concentration of 50 mg ml^{-1}. The MICs of COH and SA were determined using the broth microdilution method. COH was found to be active on all the bacteria tested ($10 \leq MIC \leq 20$ mg ml^{-1}). SA showed antibacterial activity against *P. acnes*. The anti-inflammatory properties were determined using a pancreatic lipase inhibition activity method, lipoxygenase inhibition activity, and inhibition of nitric oxide production activity. COH and SA inhibited the production of nitric oxide by up to 50 µg ml^{-1} in a dose-dependent manner. COH and SA possess antibacterial and anti-inflammatory activities. They could be used as antibacterial ingredients in various industries.

INTRODUCTION

The human skin, which is the largest organ of the body, is composed of a variety of microbial genera associated with skin diseases, including *Staphylococcus*, *Propionibacterium*, and *Malassezia*.

Acne and dandruff are associated with hyperkeratosis, elevated levels of skin sebum, and the growth of *Propionibacterium acnes* and *Malassezia furfur*. *P. acnes* have been recognized as pus-forming bacteria that trigger inflammation in acne, whereas *M. furfur* cause dandruff in people who have overactive sebaceous glands (1). *P. acnes* and *M. furfur*, as a usual inhabitant of the human skin, play an important role in skin disease development, related to the regulation of numerous enzymatic activities. These include lipases, proteases, hyaluronidases, and chemotactic factors (2). Among these enzymes, lipase has been recognized as one of the major factors involved in skin diseases such as acne, dandruff, and skin itching. Moreover, lipases promote inflammation by inducing IL-6 production and

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oxidative stress. Bacteria such as *P. acnes* and *M. furfur* act as immune stimulators through the production of proinflammatory cytokines, which are involved in development of the inflammatory process.

P. acnes is an aerotolerant, anaerobic, gram-positive rod-shaped bacterium related to various skin conditions. It is commonly found on the skin (in pores and hair follicles) and plays an important role in acne development.

M. furfur is a lipophilic fungus that affects the hair and causes dandruff. Dandruff is generally characterized by the presence of flakes on the scalp and hair and is often accompanied by itching. The scalp represents a unique environment, with thick terminal hair, large numbers of sweat and sebaceous glands, and high relative humidity, creating favorable conditions for microbial colonization (3,4). Acnes and dandruff are predicated by three major factors: colonization, sebum production, and individual predisposition.

Plants' secondary metabolites present in herbal drugs, cosmetic ingredients, and food are useful in the prevention and treatment of many diseases (5). *Cnidium officinale* belongs to the family Umbelliferae, and its roots have medicinal properties. In Asia, it has been traditionally used as an herbal treatment and is used to treat headaches, abdominal pain, and blood circulation. Senkyunolide A is a known major antioxidant constituent in *C. officinale.* In the present study, we investigated the antibacterial and anti-inflammatory properties of *C. officinale* hexane (COH) extract and senkyunolide A (SA) against *P. acnes* and *M. furfur.*

MATERIALS AND METHODS

PLANT MATERIALS AND EXTRACTION

C. officinale root was collected from local market, and *C. officinale* roots were dried and ground to powder. The powder was extracted for 5 d using 70% ethanol, and then the extract was filtered. The crude extract was sequentially partitioned with *n*-hexane (Hex). The extract and Hex fractions were stored in tightly sealed collection bottles at −20°C until further analysis.

SA

SA (CFN99594, ChemFaces, Wuhan, China) was analyzed by high-performance liquid chromatography (HPLC) to determine its purity. The mobile phase for HPLC consisted of acetonitrile (A) and water (B), using an optimized gradient elution of 50% B for 0–10 min and 50–5% B for 16–30 min. The flow rate was 1 ml min^{-1}. The detection wavelength was maintained at 278 nm.

MICROORGANISMS AND CULTURE

P. acnes (ATCC 6919, American Type Culture Collection, Manassas, VA) was incubated in a reinforced clostridial medium (RCM) for 48 h at 37°C, under anaerobic conditions in a jar with a gas pack. *M. furfur* (ATCC 14521) was incubated in modified Leeming–Notman broth (mLNB) for 96 h at 27°C.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

PAPER DISK DIFFUSION METHOD

The antibacterial activities of the samples were evaluated using a disc diffusion assay. In brief, Advantec paper discs of 8 mm diameter were impregnated with 50 µL of the solution impregnating ethanol extract and fractions at a concentration of 50 mg ml^{-1} and evaporated at room temperature for 24 h (6).

Then 1 ml of bacteria (10^7 cfu ml^{-1}) incubated for 24 h was dispensed into petri dishes, and the medium was poured and solidified. And then discs of COH and SA were placed on the RCM (*P. acnes*) and mLNB agar plates (*M. furfur*). The antibacterial activity was evaluated by measuring the zones of inhibition on the agar plate. All experiments were carried out in triplicate.

MINIMUM INHIBITORY CONCENTRATION (MIC)

The MICs were determined using the broth dilution method recommended by the Tenover (7). The cultures were prepared from 48-h and 96-h broth cultures of *P. acnes* and *M. furfur*, respectively. A 15-ml conical tube was prepared by dispensing 3 ml of broth media, sample (concentration ranging from 1.00 to 20.00 mg ml^{-1}), and an appropriate amount of cell suspension (10^7 cfu 3 ml) into each tube. The tubes were incubated at 37°C for 48 h and 27°C for 96 h for *P. acnes* and *M. furfur*, respectively. At the end of the incubation period, the tubes were evaluated for the presence or absence of growth. All experiments were performed in triplicate.

DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY

PANCREATIC LIPASE INHIBITION ACTIVITY

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitrophenyl laurate (p-NPL) as a substrate. The method used for measuring the PPL activity was modified from that previously described by Zheng et al. (8) and Bustanji et al. (9). The reaction mixture consisted of 50 mM Tris-HCl buffer (930 µl, 150 mM NaCl, 1 mM EDTA, and 10 mM MOPS, pH 7.4), PPL 5 mg ml^{-1} (30 µl), and 5 mM p-NPL (20 µl). The reaction was started by adding p-NPL as a substrate, all in a final volume of 1,000 µL. After incubation at 35°C for 15 min, the amount of p-nitrophenol released during the reaction was measured at 405 nm using a UV-visible spectrophotometer. All experiments were carried out in triplicate.

LIPOXYGENASE INHIBITION ACTIVITY

Lipoxygenase (soybean) enzyme activity was measured according to the method of Lyckander and Malterud (10) with a minor modification. Tris-HCl buffer (2,800 µl, pH 9.0) was added to 100 µl of sample at different concentrations, nordihydroguaiaretic acid

(NDGA), and enzyme (100 µl, 500 U ml^{-1} in Tris-HCl buffer). Samples and NDGA were added as dimethyl sulphoxide solutions. After incubation of the test solution for 5 min, 100 µl of linoleic acid was added, and the change in the absorbance of the solution was measured after 60 s at 234 nm.

CELL VIABILITY AND DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY

The cell viability assay was carried out by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). RAW 264.7 cells were treated with various doses of *S. baicalensis* extract (1, 10, 100, 500 µg ml^{-1}). To confirm the anti-inflammatory properties, we examined the inhibitory effects on the production of lipopolysaccharide (LPS)-induced NO in RAW 264.7 cells. NO levels were measured by the Griess reaction. After cells (5×10^5 cells ml^{-1}) were stimulated in 24 wells for 24 h, 100 µl of each cultured medium was mixed with the same volume of Griess reagent (1% sulfanilamide/0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride/2.5% H_3PO_4). NO concentration was determined by measuring the absorbance at 540 nm with a Vmax 96-well microplate spectrophotometer. The NO_2 concentration was calculated with reference to a standard curve of $NaNO_2$ generated by known concentrations. All experiments were carried out in triplicate.

RESULT

ANTIBACTERIAL ACTIVITY

The disc diffusion assay and broth dilution method were used to determine the antibacterial activity and the MIC of the COH against two bacterial strains. COH exhibited antibacterial activities (MIC 15.33 ± 2.11–42.33 ± 2.11 mg ml^{-1}) against *P. acnes* and *M. furfur* (Table 1).

PANCREATIC LIPASE INHIBITION ACTIVITY

Lipase is glycerol ester hydrolase (E.C. 3.1.1.) that acts on acylglycerols to liberate fatty acids and glycerol. Several lipases produced by microbial pathogens play an important role in skin diseases (11). The bacterial lipase is an important factor in the pathogenesis of skin diseases such as acne, dandruff, and atopic dermatitis. They can induce severe inflammatory reactions (12). The lipase inhibition activity results are shown in Figure 1

Table I
Antibacterial Activity of COH Fraction and Senkyunolide A against Test Bacteria

	Paper disk test	Bacteria (clear zone size, mm)		Bacteria (MIC, mg ml^{-1})	
Sample	Concentration (mg ml^{-1})	*P. acnes*	*M. furfur*	*P. acnes*	*M. furfur*
COH	50	14.6	13.3	15.33 ± 2.11	>30
SA	50	23.5	ND	5.30 ± 0.47	ND

ND: not detected.

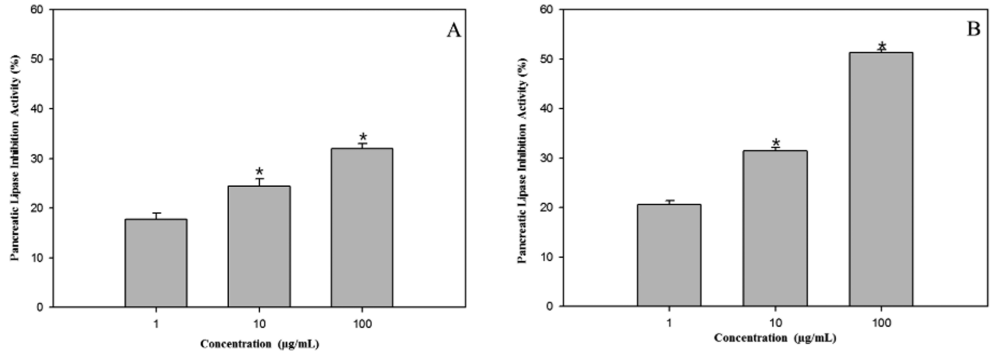

Figure 1. Effects of COH fraction and SA on pancreatic lipase inhibition activity. Data are presented as mean ± standard deviation of three independent experiments. *p value < 0.05. (A) COH and (B) SA.

as compared with a known lipase inhibitor, orlistat (data not shown, 100 μg ml^{-1}, 45.94%). Overall, COH (100 μg ml^{-1}, 31.94%) and SA (10 μg ml^{-1}, 51.36%) had lipase inhibition properties.

LIPOXYGENASE INHIBITION ACTIVITY

The inflammatory pathways involve lipoxygenases (LOXs) that catalyze the addition of molecular oxygen to fatty acids such as arachidonic acid to produce unstable hydroperoxyeicosatetraenoic acids (hyperoxides) (13). Therefore, the *in vitro* inhibition of lipoxygenase represents a good model for the screening of anti-inflammatory activity in plants.

Antilipoxygenase activity was also measured as inhibition of the peroxidation of linoleic acid to hydroperoxy linoleic acid, a reaction which is catalyzed by lipoxygenase. LOX inhibition activity results are shown in Figure 2, compared with a known lipase inhibitor, NDGA (data not shown, 100 μg ml^{-1}, 37.88% ± 2.12%). The COH inhibited LOX activity at lower concentrations (10 μg ml^{-1}). Overall, COH (100 μg ml^{-1}, 31.94%) and SA (10 μg ml^{-1}, 51.36%) had the lipase inhibition properties.

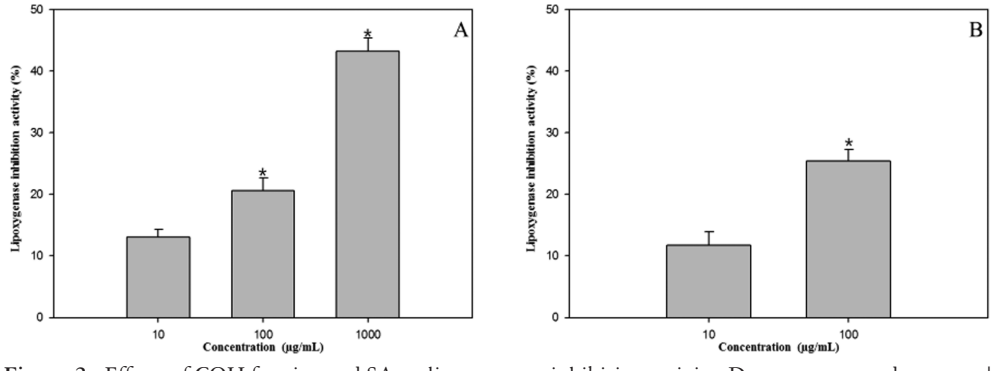

Figure 2. Effects of COH fraction and SA on lipoxygenase inhibition activity. Data are presented as mean ± standard deviation of three independent experiments. *p value < 0.05. (A) COH and (B) SA.

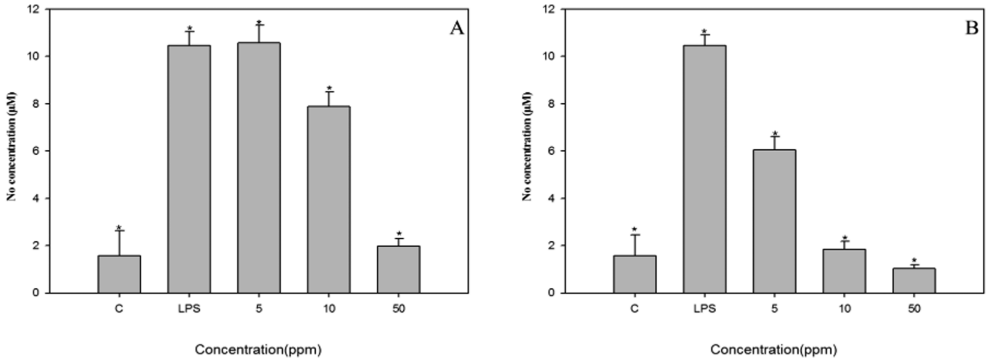

Figure 3. Effects of COH fraction and SA on anti-inflammatory activity. Cells were pretreated with tested samples (various concentrations of COH and SA) for 1 h, followed by treatment with LPS (1 μM) and incubation for 24 h. Data are presented as mean ± standard deviation of three independent experiments. **p* value < 0.05. (A) COH, (B) SA.

ANTI-INFLAMMATION ACTIVITY

To investigate the anti-inflammatory effects of COH and SA, we stimulated the RAW 264.7 cells with LPS, treated cells using various concentrations of COH and SA, and measured the production of inflammatory mediators. Cell survival was measured by MTT assay. COH and SA did not show toxicity up to concentrations of 50 μg ml^{-1}.

As shown in Figure 3, LPS treatment produced high levels of NO. However, COH and SA treatment significantly decreased the production of NO in a dose-dependent manner.

DISCUSSION AND CONCLUSION

Here, we identified the antibacterial and anti-inflammatory activities of *C. officinale* plant extracts, prepared using a 70% ethanol solvent, and then partitioned with Hex. *C. officinale* hexane fraction and senkyunolide A showed antibacterial activity against test bacteria (*P. acne* and *M. furfur*). Some studies have reported that senkyunolide A has cytoprotective and antiproliferative activities and protects against cell injuries (14). NO and cytokines have been implicated as important factors in the inflammatory pathway. *C. officinale* hexane fraction and senkyunolide A decreased the production of NO in a dose-dependent manner. Therefore, these products may be used for treating skin diseases including acne, dandruff, and skin erythema.

In conclusion, *C. officinale* plant extract and senkyunolide A possess antibacterial and anti-inflammatory activities. They could be used as antibacterial and anti-inflammatory ingredients in various industries.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Trade, Industry, and Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT), through the Encouragement Program for the Industries of Economic Cooperation Region (P0002162).

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