Free Radical Scavenging and Anticollagenase Properties of Sargachromanol I from *Myagropsis myagroides*

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The present study investigated the free radical scavenging activity and antiwrinkle properties of methanol extract, hexane, chloroform, ethyl acetate, and butanol partition fractions of *Myagropsis myagroides*. The methanol extract showed higher free radical scavenging (65.46 % at 0.5 mg/ml) and collagenase inhibitory activity (29.64 % at 1000 µg/ml) than the water extract (16.68 % at 0.5 mg/ml and <5 % at 1000 µg/ml). The hexane fraction exhibited strong free radical scavenging activity (96.36 % at 0.5 mg/ml) and collagenase inhibitory activity (IC_{50} of 712.21 µg/ml). The hexane fraction was subjected to silica gel column chromatography, Sephadex LH-20 column chromatography, octadecyl silica Sepak cartridge, and preparative high performance liquid chromatography. Sargachromaol I was isolated, which showed strong free radical scavenging activity (95.82 % at 0.1 mg/ml) and collagenase inhibitory activity (IC_{50} value of 54.01 µg/ml). The potential value in preventing skin aging could be confirmed through effects of extract from *Myagropsis myagroides* on free radical scavenging activity and collagenase inhibitory properties.

Key words: *Myagropsis myagroides*, sargachromanol I, collagenase inhibitory activity, free radical scavenging activity

The process of skin aging is divided into intrinsic and extrinsic aging. Intrinsic skin aging is caused by physiological changes in the elasticity of the skin over time. Extrinsic skin aging occurs predominately because of chronic exposure to sunlight (photoaging)[1]. UV exposure causes physical changes to the skin via alterations to the connective tissue, resulting from the accumulation of reactive oxygen species (ROS)[2], which cause lipid peroxidation and damage to cellular contents and enzymes[3].

Collagen accounts for about 90 % of the extracellular matrix in human dermis. Type I collagen accounts for more than 80 % of the total collagen, and changes in its structure have been considered as a major cause of skin aging and wrinkle formation[4]. Collagenases belong to the family of matrix metalloproteinases (MMPs), the transmembrane zinc endopeptidase enzymes. These play important roles in many processes including tissue modification, tissue homeostasis and repair after wounding by digesting collagen and elastin fibers[5]. However, excessive activation due to photoaging and chronic aging leads to alterations in gelatin, collagen and elastin composition, resulting in wrinkles, laxity, sagging and a rough appearance of the human skin[6,7]. Thus, agents that inhibit collagenase activity might have beneficial effects in maintaining healthy skin by preventing dermal matrix alteration.

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Myagropsis myagroides of the family Sargassaceae, Phaeophyta, grows in the East Asian coast subtidal zone. Studies on *M. myagroides* revealed mainly antiinflammatory[8], antihypertensive[9], anticoagulant[10] and hepatoprotective activities[11]. Sagachromanols (1~16) were isolated from the brown alga *Sargassum siliquastrum* as novel bioactive substances[12]. In a previous study[13], sargachromanol I was isolated from *M. myagroides*, which inhibited amylase activity. In this study, *in vitro* anticollagenase and free radical scavenging activities of *M. myagroides* extracts were investigated, particularly the hexane fraction, and the isolated active compound sargachromanol I.

M. myagroides was collected from the subtidal zone at Song-Jung, Busan, Korea, lyophilized and finely powdered, extracted 3 times with methanol for 24 h at room temperature and filtered. The combined filtrate was concentrated on a rotary evaporator (RE 200, Yamato Co., Tokyo, Japan) at 37° to yield ~5.7 % (based on the weight of the dried *M. myagroides*) of extract.

The methanol extract (85 g) was sequentially partitioned into hexane, chloroform, ethyl acetate and butanol. The active hexane fraction (13 g) was chromatographed on a silica gel column (70-230 mesh; Merck Art, Darmstadt, Germany; 5×10 cm) and successively eluted with a stepwise gradient of chloroform/methanol (100:1, 50:1, 20:1, 5:1, 1:1 v/v). The fraction eluted with 50:1 v/v chloroform/methanol was subsequently subjected to Sephadex LH-20 column chromatography (Amersham Pharmacia Biotech AB, Uppsala, Sweden; 2.5×90 cm column; chloroform:methanol, 1:1 v/v) and two fractions obtained (fraction 1 and 2) were further subjected to silica gel thin-layer chromatography (No. 5744, Merck, Darmstadt, Germany). Fraction 2 was separated into 5 fractions (fraction 3 to 7) using an octadecyl silica (ODS) Sepak cartridge (SPE C18 10 g, Grace, IL, USA; 60-90 % methanol). The active fraction 4 was successively subjected to Sephadex LH-20 column chromatography (methanol) and an ODS Sepak cartridge (50-60 % methanol). Each fraction was separated into six fractions (fractions 4-1 to 4-6) using ODS high-performance liquid chromatography (HPLC; ODS column i.d. 4.6×150 mm; 50-100 % aqueous methanol gradient, 1 ml/min). Finally, the fraction 4-2 was purified using preparative HPLC (Cosmosil 5C18-MS II; 10×150 mm column, Nacalai Tesque, Kyoto, Japan; 70 % aqueous methanol, 9 ml/min) to isolate sargachromanol I (52 mg). Sargachromanol I was identified using nuclear magnetic resonance (NMR). The following signals were obtained, ^{1}H NMR (600 MHz, $CDCl_3$) 6.37 (1H, d, 1.4 Hz), 5.27 (1H, s), 6.47 (1H, d, 2.8 Hz), 5.11 (1H, br t, 6.5 Hz), 4.87 (1H, dd, 9.7, 3.4 Hz), 3.91 (1H, d, 3.4 Hz), 4.97 (1H, br d, 9.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) 75, 31.3, 22.4, 121.1, 112.6, 147.9, 115.6, 127.1, 39.5, 22.1, 124.8, 134.3, 39.4, 25.2, 33.4, 41.2, 214.6, 74.3, 120.9, 140, 25.9, 18.6, 16, 15.6, 24, 16.

Collagenase inhibitory activity was evaluated according to the method of Wunsch and Heindrich[14] with slight modification. Briefly, calcium chloride (4 mM) dissolved in 0.1 M Tris-HCl buffer (pH 7.5) was used as the final buffer, and substrate 4-phenylazo-benzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg (0.3 mg/ml) was dissolved in the buffer. Then 250 µl of the substrate solution and 100 µl of sample were combined in a tube. *Clostridium histolyticum* collagenase (Sigma-Aldrich Co. St. Louis, MO, USA) was dissolved in the final buffer at a concentration of 0.2 mg/ml, and 150 µl was added to the substrate-sample mixture. After incubation at room temperature for 20 min, the reaction was stopped by adding 6 % citric acid. The reaction mixture was separated by adding ethyl acetate. The absorbance of the supernatant was measured at 320 nm. Epigallocatechin gallate (EGCG) was used as the positive control. Percent inhibition was calculated using the following Eqn., collagenase inhibition activity (%) = (Abs of control–Abs of sample)/Abs of control)×100.

The DPPH radical scavenging assay was performed according to the method described by Blois[15] with a slight modification, which briefly was, 0.5 ml of sample was mixed with 0.5 ml of 0.2 M DPPH solution in methanol. The mixture was then left to stand for 30 min at room temperature in the dark. Butylated hydrozytoluene (BHT) was used as a positive control and the negative control was methanol and 0.2 M DPPH in methanol but without sample. The absorbance of the resulting solution was measured at 517 nm and DPPH radical scavenging activity was calculated using the following Eqn., DPPH radical scavenging activity (%) = (1–(Abs sample/Abs negative control)×100.

The data were analysed using one-way analysis of variance with paired t-test and Duncan's multiple range test. Values are presented as the mean±standard deviation. Probability values (p) of less than 0.05 were considered to be significant.

The DPPH radical scavenging activities of hexane, chloroform, ethyl acetate, butanol, and water fractions

are shown in Table 1. The hexane, chloroform, and ethyl acetate fractions showed high free radical scavenging activities. The hexane fraction produced 96.36, 94.18, and 52.77 % scavenging at 500, 100, and 50 µg/ml, respectively, and the chloroform fraction, 95.93, 90.25 and 60.36 %, respectively. Finally, the free radical scavenging activity of the ethyl acetate fraction was 94.59, 94.50, and 73.18 %, respectively. However, the butanol and water fractions exhibited lower free radical scavenging activities compared to the methanol extract. Although the chloroform and ethyl acetate fractions exhibited higher activities than the hexane fraction, their yields were lower than that of the hexane fraction. Therefore, the hexane fraction was selected for further fractionated. It was subjected to silica gel chromatography. The active chloroform:methanol (50:1, v/v) fraction was further purified by a series of techniques such as Sephadex LH-20 column chromatography, ODS Sepak cartridge chromatography, and preparative HPLC. Ultimately, an active compound, sargachromanol I was isolated from the hexane fraction (fig. 1).

Sargachromanol I had a DPPH radical scavenging activity of 91.26 % at 5 µg/ml (Table 2). Its activity was approximately seven times higher than that of BHT, the positive control. Jang *et al*.[12] reported that sargachromanol I from *Sargassum siliquastrum* exerted free radical scavenging activity of 83.20 % at 100 µg/ml, and sargachromanol I previously reported to exhibit high antioxidant activity. However, compared to results of previous studies, results of the current study showed significantly higher free radical scavenging activity for sargachromanol I. It has been found that free radicals can oxidize nucleic acids, proteins, lipids and DNA that lead to tissue damage and cause degenerative diseases[16]. Thus free radical scavenging assay provides an important indicator of antioxidant activity for the effective use of *M. myagroides*.

Collagenase inhibitory activity of the crude *M. myagroides* methanol extract was 29.6 and 16.17 % at 1 and 0.5 mg/ml, respectively (Table 3). The hexane and chloroform fractions exhibited higher inhibitory activities compared to the methanol extract. In particular, the IC_{50} value of hexane fraction was 712 µg/ml. Sargachromanol I inhibited collagenase with an IC_{50} of 54.01 µg/ml, which was similar to that of the positive control EGCG (IC_{50}, 40.62 µg/ml, Table 4). A number of collagenase inhibitors isolated from higher plants were described in the literature such as flavonoids[17,18], catechins[19], polyphenols[20,21], and steroids[22]. Chromenes are related to tocotrienols and are usually found in brown algae, *Sargassum* sp.[23,24]. Tocotrienols are hydrophobic molecules that were shown to reduce plasma cholesterol levels and other lipid- and non-lipid-related risk factors of cardiovascular disease[23]. Moreover, the phenolic hydroxyl group on the chromanol ring might react with free radicals. During the process, an inactive tocopheroxyl free radical is formed, which is converted to tocopheryl quinine[25,26].

Collagen is a major component of many of the skin's constituents and is degraded by MMPs. MMPs are categorized as zinc-dependent endopeptidases that are

TABLE 1: DPPH RADICAL SCAVENGING EFFECT OF DIFFERENT FRACTIONS FROM *MYAGROPSIS MYAGROIDES* METHANOL EXTRACT

	DPPH radical scavenging effect (%)		
	500 µg/ml	100 µg/ml	50 µg/ml
Hexane	96.36 ± 0.62^{Aa1}	94.18 ± 0.14^{Ba}	52.77 ± 0.39^{Cc}
Chloroform	95.93 ± 0.82^{Aab}	90.25 ± 1.85^{Bb}	60.36 ± 1.11^{Cb}
Ethyl acetate	94.59 ± 0.46^{Ab}	94.50 ± 0.72^{Aa}	73.18 ± 0.78^{Ba}
Butanol	14.06 ± 0.82^{Ae}	5.14 ± 0.41^{Be}	=[2]
Water	30.99 ± 0.48^{Aa}	5.82 ± 0.55^{Be}	=
Methanol	65.46 ± 0.20^{Ad}	16.35 ± 0.59^{Bb}	=
BHT	95.79 ± 0.06^{Aa}	71.62 ± 0.81^{Bc}	48.03 ± 0.75^{Cd}

[1]Means in the same row (A-C) and column (a-e) bearing different superscript are significantly different by Duncan's multiple range test ($p<0.05$). [2]Not done

TABLE 2: DPPH RADICAL SCAVENGING EFFECT OF SARGACHROMANOL I FROM HEXANE FRACTION OF *MYAGROPSIS MYAGROIDES*

	DPPH radical scavenging effect (%)		
	100 µg/ml	50 µg/ml	5 µg/ml
Sargachromanol I	95.82±0.13	93.69±0.06	91.26±1.41
BHT	71.62 ± 0.81^{A}	48.03 ± 0.75^{B}	12.55 ± 0.50^{C}

A and B bearing different superscript are significantly different by Duncan's multiple range test ($p<0.05$)

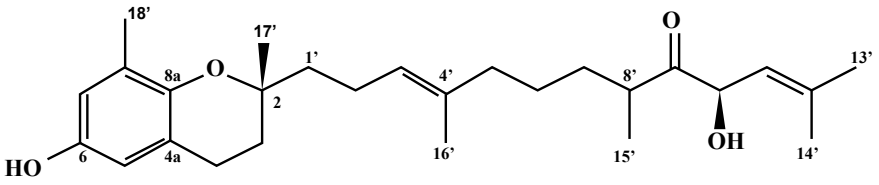

Fig. 1: The structure of sargachromanol I

TABLE 3: COLLAGENASE INHIBITORY ACTIVITY OF DIFFERENT FRACTIONS FROM *MYAGROPSIS MYAGROIDES* METHANOL EXTRACT

	Collagenase inhibitory activity (%)			
	1000 µg/ml	500 µg/ml	50 µg/ml	IC_{50} (µg/ml)
Hexane	70.37 ± 4.83^{Ab1}	48.15 ± 10.48^{Bb}	35.61 ± 04^{C}	712.21±48.93
Chloroform	47.01 ± 4.03^{Ac}	19.37 ± 4.43^{Bc}	3	=
Ethyl acetate	27.92 ± 6.04^{Ad}	-2	=	=
Butanol	8.26 ± 0.00^{Ae}	-	=	=
Water	14.67 ± 0.42^{Ae}	-		=
Methanol	29.64 ± 0.00^{Ad}	16.17 ± 2.12^{Bc}	=	=
EGCG	81.37 ± 3.55^{Aa}	73.91 ± 5.51^{Ba}	61.69±30	40.62±2.83

[1]Means in the same row (A-C) and column (a-e) bearing different superscript in samples are significantly different by Duncan's multiple range test ($p<0.05$), [2]less than 5 %, [3]not done

TABLE 4: COLLAGENASE INHIBITORY ACTIVITY OF SARGACHROMANOL I FROM HEXANE FRACTION OF *MYAGROPSIS MYAGROIDES*

	Collagenase inhibitory activity (%)			IC_{50} (µg/ml)
	500 µg/ml	50 µg/ml	5 µg/ml	
Sargachromanol I	72.09 ± 2.39^{A1}	46.30 ± 0.90^{B}	=[2]	54.01±1.05
EGCG	73.91 ± 5.51^{A}	61.69 ± 4.30^{A}	36.99 ± 4.76^{B}	40.62±2.83

[1]Means in the same row (A-B) bearing different superscripts are significantly different by Duncan's multiple range test ($p<0.05$), [2]not done

secreted as pro-MMPs[27]. Collagenases specifically degrade collagen fibers[28]. It is proposed that the deprotonation of the hydroxyl groups of sargachromanol I might contribute to the blocking of the collagenase active sites by catalytic chelation of the zinc metal ion[29]. This could explain how sargachromanol I might inhibit collagen degradation in the dermis.

In conclusion, the present investigation showed that sargachromanol I was a strong free radical scavenger and inhibited collagen degradation by collagenases. Therefore, these results suggest that sargachromanol I have the potential for preventing skin aging.

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Conflicts of interest:

The authors declare that there is no conflict of interest.

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