SHORT REPORT

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Bromophenols from the Marine Red Alga *Symphyocladia latiuscula* and Their Radical Scavenging Activity

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Abstract: A new (**1**) and five known (**2-6**) bromophenols were isolated from the organic extract of the marine red alga *Symphyocladia latiuscula*. Their structures were assigned by interpretation of NMR and MS data, and these compounds were determined as 2,3,5′-tribromo-3′,4,4′,5-tetrahydroxy-diphenylmethane (**1**), 2-methoxy-3-bromo-5-hydroxymethylphenol (**2**), 5-(2,3-dihydroxybenzyl)-3,4-dibromobenzene-1,2-diol (**3**), 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol(**4**), methyl 2-(3,5-dibromo-4-hydroxybenyl) acetate (**5**), and 3,4-dibromo-5-(methoxymethyl)benzene-1,2-diol (**6**). All these compounds were evaluated for DPPH radical scavenging activity, and they displayed potent antioxidant activity with IC_{50} values ranging from 9.6 to 31.5 μM. These compounds also showed moderate ABTS radical scavenging activity with TEAC value ranging from 2.1 to 3.0 mM. The results suggested that these bromophenols or the marine red alga *S. latiuscula* may have potential application in food as natural antioxidants.

Keywords: *Symphyocladia latiuscula;* bromophenols; secondary metabolites; antioxidant activity.

1. Algal Source

S. latiuscula was freshly collected from the Yantai coastal zone of Shandong province, China, in July, 2022. It was identified by Dr. X. Li at the Institute of Oceanology, Chinese Academy of Science. A voucher specimen (No. SL0722) has been deposited in the Yantai Center for Food and Drug Control.

2. Previous Studies

Bromophenols have caused great attention as antioxidants and fresh algae has been used as diets and traditional remedies in Asian countries for a long time. *S. latiuscula* belong to marine red alga of the family Rhodomelaceae, and large amounts of bromophenols have been isolated from this family [1-5]. Numerous studies have shown that most bromophenols possess biological activities such as α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity [6], antimicrobial activity [7], and inactivator of α-glucosidase [2], and so on. Previous studies have reported that a large number of bromophenols with various kinds of biological activity have been isolated from *S. latiuscula* [8,9].

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3. Present Study

In our ongoing investigation toward new and bioactive bromophenols from red alga *S. latiuscula*, one new bromophenol, 2,3,5′-tribromo-3′,4,4′,5-tetrahydroxy-diphenylmethane (**1**), along with five known bromophenols, 2-methoxy-3-bromo-5-hydroxymethylphenol (**2**), 5-(2,3-dihydroxybenzyl)-3,4-dibromobenzene-1,2-diol (**3**), 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)-3,4-dibromo-benzene-1,2-diol (**4**), methyl 2-(3,5-dibromo-4-hydroxybenyl)acetate (**5**), and 3,4-dibromo-5-(methoxymethyl)benzene-1,2-diol (**6**) were isolated and identified. The details of isolation, structural elucidation, and radical scavenging activity of these bromophenols are described.

The air-dried red alga *S. latiuscula* (5.8 kg) was smashed and extracted with CH_2Cl_2 and MeOH (1:1, v/v), after filtration, the organic solvents were removed under reduced pressure to obtain 50.0 g of crude residue. The extract was subjected to silica gel column chromatography (CC) with solvent systems of petroleum ether (PE)/EtOAc (20:1 to 0:1, v/v) and CH_2Cl_2/MeOH (20:1 to 1:1) to afford 11 fractions. Fraction 5 (6.1 g) eluted with PE/EtOAc 1:1 and was further purified by Sephadex LH-20 (MeOH) to yield **5** (2.7 mg). Fraction 6 (5.8 g) eluted with EtOAc and was further purified by CC (PE/EtOAc, 1:1) and Sephadex LH-20 (MeOH) to yield **3** (6.5 mg), **4** (4.2 mg), and **6** (3.1 mg). Fraction 8 (4.7 g), eluted with CH_2Cl_2/MeOH 10:1, was further purified by CC on RP-18 (MeOH/H_2O, 7:3) and Sephadex LH-20 (MeOH) to yield **1** (2.8 mg) and **2** (5.0 mg).

*2,3,5′-Tribromo-3′,4,4′,5-tetrahydroxy-diphenylmethane (**1**)*: Colorless oil; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Table 1; HREIMS: *m/z* 465.8042 $[M]^+$ (calcd for $C_{13}H_9{}^{79}Br_3O_4$, 465.8045).

Radical Scavenging Activity Assay (DPPH): These bromophenols were assayed for DPPH free radical scavenging using a method described in the literature [10]. In brief, 2.0 mL aliquot of sample methanolic solution was added into 2.0 mL of 0.16 mM DPPH solution (in MeOH), and mixed for 1 min, then kept at 25 °C for 30 min in the dark, the absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated as follow equation:

$$\text{Scavenging effect (\%)} = [1-(A_{sample}-A_{sample\ bank})/A_{control}] \times 100\%$$

(A_{sample}: absorbance of the test sample; $A_{sample\ bank}$: absorbance of the sample without DPPH solution; $A_{control}$: absorbance of the control)

The halfmaximal inhibitory concentration (IC_{50}) was calculated by a curve of the concentration plotted against the percent inhibition. BHT was used as positive control.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay: The method was carried out according to previously reported method [11]. In brief, the ABTS radical cation was obtained via filtering a solution of ABTS in phophated-buffered saline (PBS) through MnO_2 powder. Then the solution was diluted to an absorbance of 0.7 at 734 nm in 5 mM PBS (pH 7.4), and pre-incubated at 25 °C for 12 h. The tested samples were diluted to gradient concentrations of solutions (2.0, 1.5, 1.0, 0.5, and 0.3 mM) with MeOH. After mixing the sample and ABTS solution and incubation for 6 min, the absorbance value was measured at 730 nm by a UV/Vis spectrophotometer. In addition, Ascorbic acid and Trolox were used as positive control and antioxidant standard respectively. A standard calibration curve was constructed for Trolox at different concentrations of 0, 2, 5, 10, 50, 100, and 150 mM. TEAC values were calculated by the Trolox standard curve and expressed as Trolox equivalents.

Compound **1** was obtained as colourless oil. The EIMS spectrum gave the tribrominated molecular ion peak cluster at *m/z* 466/468/470/472 with a ratio of abundances 1:3:3:1. HREIMS analysis gave the molecular formula $C_{13}H_9Br_3O_4$ (*m/z* 465.8042, calcd for $C_{13}H_9{}^{79}Br_3O_4$, 465.8045). The ^1H NMR spectrum (Table 1) displayed one singlet at δ = 6.61, two doublets at δ = 6.74 and δ = 6.53 assignable to three aromatic protons, and one singlet at δ = 3.87 attributable to methylene proton. The ^{13}C NMR and DEPT

spectra exhibited 13 resonances assignable to one methylene, one tetrasubstituted benzene ring and one pentasubstituted benzene ring with four oxygenated carbons (δ>142). The above spectral data implied that **1** possessed a tribrominated diarylmethane structure with four hydroxyl groups. HMBC correlations from H_2-7 to C-1, C-1′, C-2, C-2′, C-6 and C-6′ confirmed the diarylmethane structure of **1**. The substituted patterns of the aromatic rings were established by HMBC correlations from H-6 to C-2, C-4, and C-5, from H-2′ to C-3′, C-4′, and C-6′, from H-6′ to C-2′, C-4′, and C-5′. Thus, the structure of **1** was determined to be 2,3,5′-tribromo-3′,4,4′,5-tetrahydroxy-diphenylmethane.

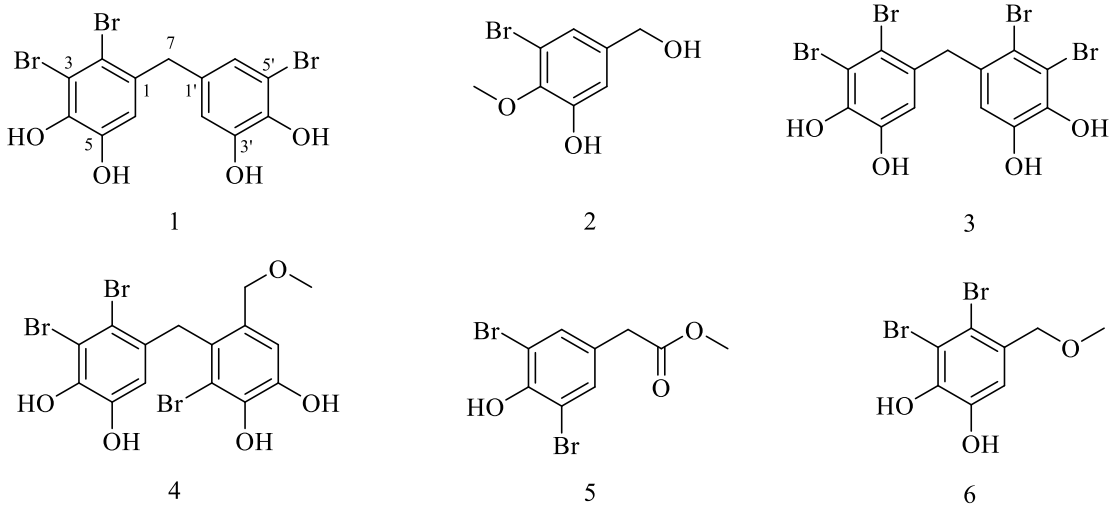

Figure 1. Chemical structures of isolated compounds **1-6**

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (δ in ppm) in MeOD

No	δ_H (*J* in Hz)	δ_C, type
1		133.8[a], C
2		116.7, C
3		114.4, C
4		144.5[b], C
5		146.4[b], C
6	6.61, s	117.1, CH
7	3.87, s	42.7, CH_2
1′		133.9[a], C
2′	6.53, d (1.6)	115.9, CH
3′		147.4, C
4′		142.5, C
5′		110.7, C
6′	6.74, d (1.7)	124.5, CH

[a,b]Data are interchangeable

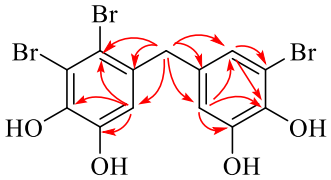

Figure 2. Key HMBC correlations of **1**

Compounds **1-6** were evaluated for radical scavenging activity. The result indicated that all of bromophenols tested displayed remarkable antioxidant activity against DPPH radicals (Table 2). Compound **6** showed the strongest activity with an IC_{50} of 9.6 μM, which was 8-fold potent than that of

the widely known antioxidant BHT (IC_{50} = 85.1 μM), and compounds **1**-**5** also presented high antioxidant activity with $IC_{50} \leq 31.5$ μM. In addition, compounds **1**-**6** showed moderate ABTS radical scavenging activity with TEAC values ranging from 2.1 to 3.0 mM. All these compounds displayed higher antioxidant activity than the ascorbic acid, and compound **3** possessed the best antioxidant activity in the TEAC assay (TEAC value = 3.0 mM).

Table 2. Radical scavenging activity of compounds **1**-**6**

Compound	DPPH radical scavenging activity (IC_{50}, μM)	ABTS radical scavenging activity (TEAC, mM)
1	30.4 ± 0.2	2.8 ± 0.1
2	24.5 ± 0.1	2.1 ± 0.2
3	16.1 ± 0.2	3.0 ± 0.2
4	20.6 ± 0.2	2.7 ± 0.1
5	31.5 ± 0.3	2.3 ± 0.1
6	9.6 ± 0.1	2.8 ± 0.2
BHT	85.1 ± 0.3	
Ascorbic acid		1.0 ± 0.0

Chemical investigation towards the marine red alga *S. latiuscula* resulted in the isolated of one new bromophenol, 2,3,5′-tribromo-3′,4,4′,5-tetrahydroxy-diphenylmethane (**1**), along with five known bromophenols, 2-methoxy-3-bromo-5-hydroxymethylphenol (**2**), 5-(2,3-dihydroxybenzyl)-3,4-dibromobenzene-1,2-diol(**3**), 5-(2-bromo-3,4-dihydroxy-6-(methoxymethyl)benzyl)-3,4-dibromobenzene-1,2-diol(**4**),methyl-2-(3,5-dibromo-4-hydroxybenyl)acetate (**5**), and 3,4-dibromo-5-(methoxymethyl)-benzene-1,2-diol (**6**), increasing the molecular diversity of the species. Outstanding radical scavenging activities of these bromophenols imply that they are key contributors to the antioxidant properties of the red alga *S. latiuscula*. In addition, these bromophenols or the marine red alga may be exploited as food additives or health products in the further, but their toxicity should be evaluated before using as a foodstuff.

Conflicts of Interest: The authors declare no conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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