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Bromophenols as antioxidants and tyrosinase inhibitors

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Introduction

Oxidative metabolism creates free radicals which cause many diseases in human body [1]. Synthetic antioxidants are commonly used to prevent this problem but they have toxicity problems [2]. In addition free radicals may induce α-melanocyte stimulating hormone resulting hyperpigmentation [3]. Therefore, there is strong interest of naturally occurring antioxidant and tyrosinase inhibitor. Bromophenols are commonly found in red algae of the family Rhodomelaceace. Bromophenols have displayed wide range of functionalities including radical scavenging [4] and enzyme inhibition [5]. This study has led to the purification of seven known bromophenol monomers from the Rhodomelaceae algae.

Materials and methods

Neorhodomela aculeata, *Odonthalia corymbifera*, and *Symphyocladia latiuscula* were collected at the coast of Hakodate city, Japan in 2015 and 2013. *N. aculeata* was extracted with acetone while *O. corymbifera* and *S. latiuscula* was with 95% MeOH. Each extract was concentrated and successively partitioned. Each EtOAc-soluble fraction was further fractionated by various chromatographic techniques. Purity of isolated compounds was accomplished by RP-18 HPLC using 70% aqueous MeOH as eluent. Structural elucidation was done by NMR and MS data. Radical scavenging (DPPH & ABTS), metal-reducing (CUPRAC & FRAP) and copper-chelating assays [6-10] were employed for assessment of antioxidant activity. Mushroom tyrosinase inhibition was examined by colorimetric method using tyrosine as a substrate [11].

Results and discussion

Seven known bromophenol monomers were identified after comparison of reported spectroscopic data [5, 12-14]. All compounds showed radical scavenging activity except monophenolic compounds **4** and **5** (Table 1). This type of bromophenols would be required catechol structure for radical scavenging activity. Although compound **3** showed the most potent radical scavenger among them, the activity was similar to the positive control catechol.

Importance of bromine substitution has been reported for radical scavenging activity [15, 16]. Disappointedly bromination is not influenced on radical scavenging activity in this study.

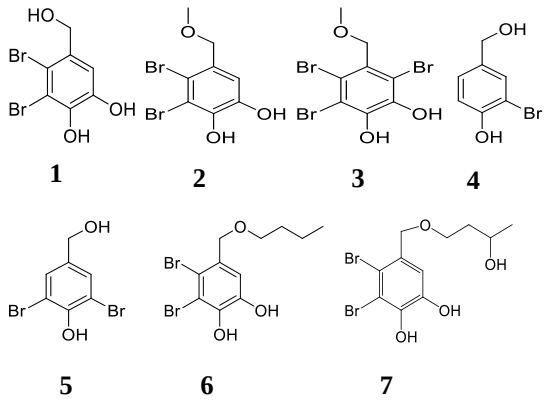

Fig. 1. Bromophenols examined in this study.

Table 1. Radical scavenging activity[a]

Compound	DPPH EC_{50}[b] (μM)	ABTS EC_{50}[b] (μM)
1	21.0±0.0	14.3±0.1
2	25.0±0.1	10.0±0.1
3	18.5±0.1	7.02±0.2
4	>500	>500
5	>500	>500
6	35.6±0.2	12.8±0.1
7	36.0±0.1	14.5±0.1
BHA	34.0±0.1	10.4±0.2
Catechol	16.9±0.1	7.3±0.0

[a] All values are represented as mean ± standard error (n=3).
[b] The half maximal effect concentration.

Compound **3** showed almost identical activity with catechol. While bromine substitution decreases radical scavenging activity, substitution of three bulky bromine atoms on a benzene ring like compound **3** may lead to increasing donation of phenolic hydrogens.

Only compounds **1** and **2** were assessed for metal-reducing, metal-chelating and tyrosinase inhibitory activities. Both compounds displayed higher Cu^{2+}-reducing property compared to positive controls while the compounds showed slightly weaker chelation potency (Table 2). However the compounds exhibited lower Fe^{3+}-reducing property compared to the controls. These results suggested that compounds **1** and **2** were good reductants for copper. Compound **2** exhibited higher Cu^{2+}- and Fe^{3+}-reducing, and Cu^{2+}-chelating activities than compound **1**. The methoxy substitutionin compound **2** would lead to increase reducing and chelating potency [15].

Table 2. Various activity[a] of compounds **1** and **2**

Compound	CUPRAC $EC_{A0.50}$[b] (μM)	FRAP $EC_{A0.50}$[b] (μM)	Cu^{2+} Chelation EC_{50}[c] (μM)	Tyrosinase Inhibition IC_{50}[d] (μM)
1	11.1±0.1	14.5±0.1	46.6±0.1	67.0±0.0
2	9.3±0.1	12.9±0.1	41.9±0.1	96.8±0.1
BHA	16.0±0.1	8.3±0.1		
Catechol	25.4±0.1	9.1±0.1		
EDTA			31.6±0.1	
Kojic acid				35.0±0.0

[a] All values are represented as mean ± standard error (n=3).
[b] The effective concentration for absorbance of 0.50.
[c] The half maximal effective concentration.
[d] The half maximal inhibitory concentration.

In tyrosinase inhibition assay, compounds **1** and **2** showed moderate inhibition compared with positive control kojic acid. Tyrosinase is a copper-containing enzyme [17]. Although the compounds are good Cu^{2+} reductants, they are not good tyrosinase inhibitors. Additionally, methoxy compound **2** exhibited lower inhibitory potency than compound **1**. This type of compounds may act a binder to protein rather than a reductant of copper. Alternatively, the compounds may show hard access property to the enzyme active site.

Conclusions

Algal bromophenols were screened for antioxidant and tyrosinase inhibition activity. Compounds **1** and **2** exhibited higher Cu^{2+}-reducing activity than positive controls. However inhibitory activity of the compounds was moderate against tyrosinase, a Cu-containing enzyme.

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