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Compositional Difference of Phenolic Compounds between Two Seaweeds, *Halimeda* spp.

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Abstract: *Halimeda macroloba* and *Halimeda opuntia* were collected at their growing district (Ishigaki island, Okinawa Pref., Japan), in the same season. Polyphenolic and related phenolic compounds were extracted from two *Halimeda*, and analyzed by high performance liquid chromatography. Polyphenolic compounds (catechin, epicatechin, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, rutin, quercitrin, hesperidin, myricetin, morin, luteolin, quercetin, apigenin, kaempferol, baicalein) and related phenolic compounds (caffeic acid and catechol) were determined. The composition of polyphenolic and related phenolic compounds was different between two *Halimeda*. The extremely large amount (28,000μg/g dry matter) of epigallocatechin was found in *H. macroloba*. Caffeic acid and hesperidin were found only in *H. macroloba*. Catechol was detected in *H. macroloba* 5 times as much as catechol in *H. opuntia*. Myricetin and morin were found in *H. macroloba* approximately double amounts from *H. opuntia*. Other polyphenolic compounds tested in this study were not found from both *Halimeda*.

Keywords: *polyphenolic compounds, Halimeda, HPLC analysis*

Introduction

Polyphenolic compounds occur ubiquitously in foods of plant origin. Since polyphenolic compounds have several hydroxyl (OH) groups, it was expected to express radical scavenging effect[1,2] or sometimes to have prooxidant effect as a source of reactive oxygen species. [3,4] Polyphenolic compounds may have beneficial health effects because of their antioxidant properties and their inhibitory role in various stages of tumour development in animal studies. There are many studies to report polyphenolic composition in teas[5-8], wines[8-10], cacaos[11,12], fruits and vegetables[13-17]. There might be some high flavonoid/catechin concentration in seaweeds which were not used for our life in comparison to the land plant. Previously we reported the contents of catechins and their related compounds in seaweeds,[18] there were two *Caurelpa*, but they did not have similarity in catechin composition. In this report, there are two *Halimeda*; one is Hiroha-sabotengusa (*H. macroloba*), the other is Sabotengusa (*H. opuntia*) in Japanese. They are living in the subtropical area, but are not used as a food like *Caurelpa*. *Halimeda* is living in the seashore, and easy to collect even by children. Therefore we tried to analyze the contents for efficient utilization. In spite of the similar shape and living area each other, no one reported the differences of their contents. Thus, the purpose of this research was to note the difference of polyphenolic composition in two *Halimeda*.

Materials and Methods

Seaweed samples

Green algae *Halimeda macroloba* and *Halimeda opuntia* were collected at their growing district, Ishigaki, Okinawa Prefecture, and transported under refrigeration. After washing with tap water and wiping with a paper towel, they were minced by a food cutter (MK-K75, Matsushita Electric Co., Osaka, Japan), and stored at -20°C until use.

Chemicals

Authentic catechin, epicatechin, epigallocatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, rutin, caffeic acid, catechol, hesperidin, quercitrin, myricetin, morin, luteolin, quercetin, apigenin, kaempferol, and baicalein were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were commercially available.

Extraction and Analysis of Catechins

Catechins were extracted according to the method for tea catechin[7] as mentioned in the previous paper.[18] The extracts were kept at -80°C (freezer, Sanyo MDF-392) until analysis. All samples were extracted and analyzed in triplicate.

Catechins were determined by a high-performance liquid chromatograph modified from the methods of Suematsu et al.[6] and Terada et al.[5] Catechins were

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separated by an ODS column (Inertsil ODS-2, 5μm, 250 mm x 4.6 mm ID, GL Science Inc., Tokyo, Japan) in a column oven at 40°C fitted with a guard column (10 mm × 4.0mm ID) using acetonitrile/ethyl acetate/0.1% phosphoric acid (85/20/895, v/v/v) as a mobile phase, flow rate at 1 ml/min, and analyzed at 280 nm with a spectrophotometric detector SPD-6A UV (Shimadzu Co., Kyoto, Japan). Standard solutions for all catechins were prepared in methanol. Catechin concentrations in the seaweeds were calculated using standard curve at the range of 0-50 μg/ml. A peak of each catechin was identified by retention time.

Extraction, Hydrolysis and Analysis of Flavonoids

Total flavonoids were extracted according to the method of Hertog et al.[19] as follows. Each minced fresh seaweed sample, 5g, was homogenized with 40 ml of 75% methanol with 2g/l TBHQ (*t*-butylhydroquinone) using a mixer (Ultra-Turrax T-25 Janke & Kunkel, GmbH Co. Staufen, Germany) at 5,000-10,000 rpm for 60 s. Ten mililiters of 6M hydrochloric acid was added and carefully mixed. The homogenate was refluxed at 90°C for 2 hours, and final concentration of hydrochrolic acid was approximately 1.2 N. After cooling, the supernatant was filtered through an Advantec filter paper No. 101 (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and transferred to a volumetlic flask with methanol. After replacing air with nitrogen gas to inhibit decomposition of flavonoids, the extracts were kept at -80°C until analysis. The effect of hydrolysis on seaweed samples by methanol-HCl mentioned above was compared with that of methanol extract. All samples were extracted and analyzed in triplicate.

Flavonoids and related phenolic compounds were determined by high-performance liquid chromatograph modified from the methods of Hertog et al.[19] and Vinson et al.[20] Flavonoids and related compounds were separated by a C_{18} column (Nova-Pak C_{18} , 4μm, 150 mm× 3.9 mm ID, Waters Co., Milford, MA, USA) fitted with a guard column (20 mm × 3.9 mm ID), using 25% acetonitrile in 0.025M KH_2PO_4 at pH 2.4 as mobile phase, flow rate at 0.9 ml/min and analyzed by a diode array detector SPD-M10Avp (Shimadzu Co., Kyoto, Japan). Authentic reagents were dissolved in methanol. Flavonoid concentration in the seaweed was calculated using a calibration curve within concentration 0-200μg/ml. Samples were separated and identified by retention time and spectra of each peak.

Results and Discussion

Distribution of the polyphenolic and related compounds in two *Halimeda* are shown in Table 1. Contents of the polyphenolic compounds are expressed as μg of compounds per g of dry weight of seaweeds.

Table 1. Distributions of polyphenolic compounds in *Halimeda* (mean ± SD μg/g dry matter)

Compounds	Hiroha-sabotengusa *Halimeda macroloba*	Sabotengusa *Halimeda opuntia*
Catechin	-	-
Epicatechin	-	-
Epigallocatechin	28,000 ± 6,200	12,700 ± 930
Catechin gallate	-	-
Epicatechin gallate	-	-
Epigallocatechin gallate	-	-
Rutin	-	-
Caffeic acid	84.9 ± 21	-
Catechol	1,880 ± 650	384 ± 130
Quercitrin	-	+
Hesperidin	144 ± 100	+
Myricetin	414 ± 80	147 ± 1.2
Morin	429 ± 4.3	234 ± 7.7
Luteolin	-	-
Quercetin	-	-
Apigenin	-	-
Kaempferol	-	-
Baicalein	-	-

Catechin, epicatechin, catehin gallate, epicatechin gallate, epigallocatechin gallate, rutin, quercitrin, luteolin, quercetin, apigenin, kaempferol, baicalein were not detected from both *Halimeda*. Quercitrin was detected in *H. opuntia*, but it was extremely small amount.

H. macroloba and *H. opuntia* belong to the same species, and live in the same area. Their difference of the shape is quite small, however, composition of polyphenolics was different. *H. macroloba* had 28,000μg of epigallocatechin and 1,880μg of catechol, in contrast, *H. opuntia* had 12,700μg and 384μg of each compound. *H. macroloba* had caffeic acid and hesperidin, but *H. opuntia* did not contain caffeic acid and it had hesperidin in trace amount. In this research, while samples were collected in the same seashore and in the same season, the result showed the difference of polyphenolic composition. Polyphenolic contents of seaweed may have seasonal and local variations. Therefore, further research is required for this field of study.

Terada et al.[5] reported that dried tea leaves contained 20,000μg of epigallocatechin, 25,000μg of epigallocatechin gallate, 18,000μg of epicatechin gallate, and 8,000μg of epicatechin. Tea leaves from Shizuoka Prefecture, Japan, were analyzed by Suematsu et al.[6], and they reported 670μg of epigallocatechin, 220μg of epicatechin, 740μg of epigallocatechin gallate, 140μg of epicatechin gallate, and 35μg of catechin. Concentrations of catechins in tea leaves varied widely according to the reports. *H. macroloba* was found to have a larger amount of epigallocatechin (28,000μg) than tea leaves, but except that, both *Halimeda* had smaller amount of catechins than tea leaves. Epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin were found to be the most important components in terms of antioxidant ability among all the catechins.[19-21] All of the active catechins mentioned above were not found in both *Halimeda*. Polyphenolic and related compounds occupied more than 3% of dry weight *H. macroloba*, and such a large percentage of polyphenolic content was also reported in case of *Scutellaria baicalensis* (one of the herb).[21] *S. baicalensis* contained mainly baicalein, however, *H.macroloba* contained epigallocatechin instead of baicalein. Quercetin, luteolin and apigenin were reported to be "anticarcinogenic" flavonoids,[22] but *Halimeda* did not contain them. There are free polyphenolic compounds and conjugated one. It was reported that some vegetables had more than 50% of conjugated form.[20] From this report, we found the difference of polyphenolic composition of *Halimeda*, but we should find out the difference of the structure of polyphenolic compounds, and make clear the difference of their activity on our health. As Cao et al.[23] mentioned the structure-activity relationships of flavonoids, we are continuing the research on the activities as an antioxidant or a prooxidant of polyphenolic compounds from seaweeds with several oxidation models.

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サボテングサ 2 種のポリフェノール類の含量

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ポリフェノール化合物は、脂質やDNAの酸化に対して影響力を持つとされている。本研究では沖縄で同時期に採取されたヒロハサボテングサならびにサボテングサを試料とし、ポリフェノール化合物16種および関連化合物としてカテコール、カフェイン酸を、ODSカラム、C_{18}カラムとダイオードアレイ検出器を用いたHPLCによって分析した。ヒロハサボテングサにのみカフェイン酸が検出された。ヒロハサボテングサからはサボテングサでごく微量であったヘスペリジンが検出された。ヒロハサボテングサのカテコール含量はサボテングサの5倍ほどであった。エピガロカテキン、ミリセチンとモリンはヒロハサボテングサにおいてサボテングサの約2倍の含量が認められた。このように、類似した海藻2試料から異なる含量のポリフェノールならびに関連化合物が検出された。

キーワード：ポリフェノール化合物、サボテングサ、HPLC-ダイオードアレイ分析