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The Antioxidant Activity in Extracts of *Symphyocladia latiuscula*

Hye-jin PARK, Jae-sue CHOI and Hae-young CHUNG*

Dept. of Food and Life Science, Pukyong National University, Pusan 608-737, Korea

**College of Pharmacy, Pusan National University. Pusan 609-735, Korea*

The antioxidant activity of *Symphyocladia latiuscula* was determined by measuring lipid peroxide produced when a mouse liver homogenate was exposed to the air at 37℃, using 2-thiobarbituric acid (TBA) and radical scavenging effect on 1,1 - diphenyl - 2 - picrylhydrazyl (DPPH) radical, and free radical generation inhibition by ACF_2 (Hepatocyte). The methanol extract of *S. latiuscula* showed high antioxidant activity. And the methanol extract was fractionated with several solvents. With regard their fractions, the antioxidant activity were in the order of dichloromethane > hexane > butanol > ethyl acetate > water fraction. The dichloromethane fraction showed the strongest radical scavenging activity (50% inhibitory concentration[IC_{50}]=3.14 μg/mℓ), and strong inhibitory effect on the lipid peroxidation of the mouse liver homogenate, which was compared with L-ascorbic acid, inhibition effect was stronger than L-ascorbic acid. The methanol extract of *S. latiuscula* and its dichlromethane soluble fraction also inhibited over 50% at concentration of 0.2 mg/mℓ and 0.1 mg/mℓ on free radical generation of hepatocyte (AC_2F). While the water fraction was inactive in all the assay for antioxidant activity.

Key words: *Symphyocladia latiuscula*, marine algae, antioxidant activity.

Introduction

Antioxidants, inhibitors of lipid peroxidation, are important not only for food protection but also for the defence of living cells against oxidative damage. The toxic and otherwise unfavorable effects of synthesized food antioxidant have been widely noticed. Nevertheless, phenolic antioxidants such as butylated hydroxy toluen (BHT) and butylated hydroxy anisole (BHA) have been used extensively as food antioxidants because of their excellent results and low cost. When slightly larger doses (50 mg/kg/day) of these phenolic antioxidants were administered to rodents and monkeys, however, certain pathological effects have been observed (Branen, 1975). The demand for the development of alternative natural antioxidants has, therefore, been increased. Many investigators have found different types of antioxidants in various kinds of plant (Larson, 1988).

Phenolic substances are widely distributed in nature, particularly in the plant kingdom. Their main occurrence has been reported from a number of plants and animals. Among marine plants, brown and red algae have provided a host of phenolic and other secondary metabolites, which is a reflection of the fact that these plants have been favorite research targets of marine natural products chemists in recent years. We have been interested in different kinds of seaweed as a new source of natural antioxidants, not only because many marine algae such as green and brown algae are commonly used as foods but also because these algae are abundant in Korea. Although the antioxidant activity of seaweed extracts and their substituted phenols and polyphenols has been known for some time (Kurata and Amiya, 1975), the antioxidant activity of *Symphyocladia latiuscula* has not yet been investigated.

We previously tested the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical using methanol extracts of 19 seaweeds to discover some new effective natural antioxidants and reported that four kinds of extracts exhibited a strong antioxidant activity (Choi et al., 1993). Of these four seaweeds, *S. latiuscula* had the strongest effect and was therefore used in the present study.

In this paper the antioxidant activity of *S. latiuscula* a red algae, was demonstrated by measuring lipid peroxide produced when a mouse liver homogenate was exposed to air at 37℃, using 2-thiobarbituric acid (TBA) and radical scavenging effect on 1,1 - diphenyl-

2 - picrylhydrazyl (DPPH) radical, and free radical generation inhibition by AcF_2 (Hepatocyte).

Materials and Methods

Algae material

S. latiuscula was collected at Chungsapo, Pusan in January, 1998. It was identified by the botanist Prof. K. W. Nam and a voucher specimen is now deposited in the author's laboratory (J. S. Choi). *S. latiuscula* was washed fresh water and shade dried at room temperature.

Extraction and fractionation

The dried material (580 g) was extracted with methanol three times, and the solvent was removed under reduced pressure to a dark brown semisolid (148 g). Successive partitioning yielded hexane (14.15 g), dichloromethane (23.00 g), ethyl acetate (11.20 g), *n*-butanol (36.80 g) and water soluble fractions (53.56 g), respectively (Fig. 1.).

DPPH radical scavenging effect

The DPPH radical scavenging effect was carried out according to the method first employed by Blois (Blois, 1958). Four milliliters of MeOH solution of varying sample concentrations was added to 1.0 ㎖ DPPH methanol solution (1.5×10^{-4}M). After standing at room temperature for 30 min, the absorbance of this solution was determined at 520 nm using a spectrophotometer and remaining DPPH was calculated (Yamasaki et al., 1994; Yoshida et al., 1989). The results were calculated by taking the mean of all triplicate values.

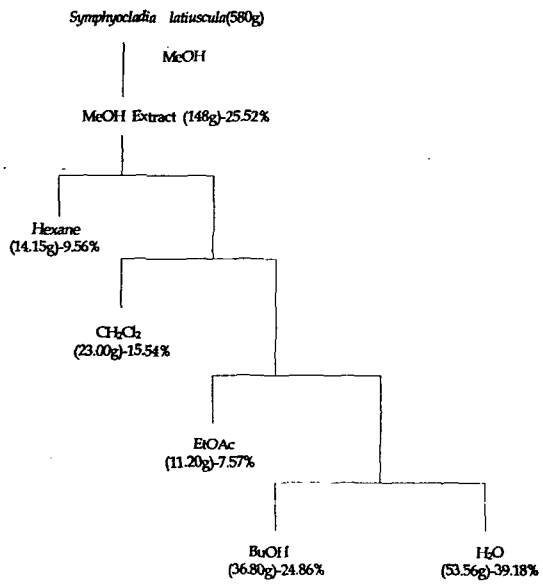

Fig. 1. Extraction and Fractionation of *Symphyocladia latiuscula*.

TBA assay

The antioxidant activity was determined by measuring lipid peroxides using TBA (Hiroshi et al., 1979). 0.3 ㎖ of mouse liver homogenate was mixed with 0.3 ㎖ of aqueous 8.1% sodium dodecyl sulfate (SDS) and 0.1 ㎖ of sample/or distilled water in a test tube. The mixtures were incubated at 37℃ for 8 hrs in a water bath. 1.5 ㎖ of acetic acid and 1 ㎖ of 1.2% TBA solution were added. The test solutions were boiled at 100℃ for 30 min and then cooled to room temperature. The solutions were centrifuged at 2,500 rpm for 15 min and the absorbance of the upper layer was measured at 532 nm and calculated to TBA value per g liver weight (Han et al. 1994). The results were calculated by taking the mean of all triplicate values.

Assay for the free radical generation

Liver cells were incubated for 24 hrs in serum free media in a CO_2 incubator at 37℃ until confluent, and the cells were transferred to multiwell plates with about 10^5 cells/well and cultured with or without a suspension of methanol extract of *S. latiuscula* and their solvent soluble fractions, then incubated with 12.5 μM DCFH-DA (2',7'-dichlorofluorescin diacetate) at 37℃ for 30 min. Fluorescence was monitored on a spectrofluorometer, with excitation wavelength at 460 nm, and emission wave length at 530 nm (Label and Bondy, 1990).

Results and Discussion

The radical scavening effect of the methanol extract and their fractions of *S. latiuscula* on DPPH radical

Active oxygen species such as superoxide radicals, hydrogen peroxide and hydrogen radicals have been recognized as the principle agent responsible for the deterioration of polyunsaturated fatty acids or lipid containing food when exposed to air (Slater et al., 1987). The DPPH stable radical loses its characteristic purple color when supplied with electrons or hydrogen ions.

The capacity of the tested substances to donate electrons can be estimated from the degree of their loss of color (Park et al. 1991). The DPPH radical scavenging effect for the methanol extract and their fractions are shown in Table 1. The radical scavenging effect for the methanol extract and all fractions obtained from the methanol extract was observed in all cases except for the water fraction; the effect of this was dependent on their concentration. The radical scavenging effects of the dichloromethane and hexane fraction were stronger than that of others. IC_{50} of these fractions were 3.14 μg/mℓ and 8.84 μg/mℓ, respectively. The results suggest that the methanol extract, dichloromethane, and hexane fraction of *S. latiuscula* are effective radical scavengers. Especially, radical scaveging activity of dichloromethane fraction was more potent than that of BHT.

The lipid peroxidation of a mouse liver as a function of the incubation time

Mouse liver homogenate was incubated at 37℃, and the auto-oxidation of the lipid as a function of the incubation time is shown in Fig. 2. There was an increase in the TBA value as a function of the incubation time, and TBA value rapidly increased after 8 hr. Because of this rapid increase, the antioxidant activity of the methanol extract and their fractions were measured for values under 8 hrs of the incubation time.

Table 1. The radical scavenging effect of the methanol extract and their fractions of *Symphyocladia latiuscula* on 1,1-dihenyl-2-picrylhydrazyl (DPPH) radical

Fractions	IC_{50} (μg/mℓ)*
MeOH extract	9.03
Hexane fraction	8.84
CH_2Cl_2 fraction	3.14
EtOAc fraction	15.44
BuOH fraction	9.41
H_2O fraction	> 120
L-ascorbic acid	1.22
BHA	1.06
BHT	3.21
α-Tocopherol	1.28

* Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations, obtained by interpolation of concentration-inhibiton curve.

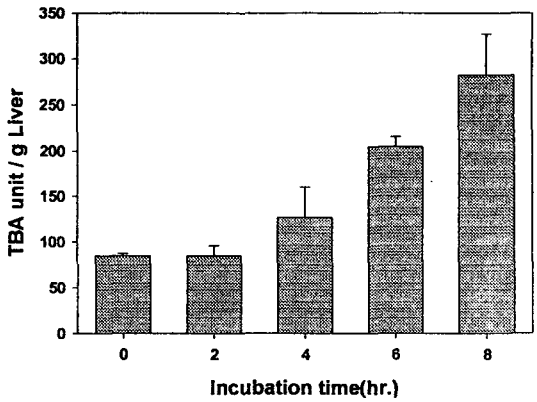

Fig. 2. Auto-oxidation of lipid in mouse liver homogenate as a function of incubation time.

Table 2. The antioxdative effect of various fractions obtained from the methanol extract of *Symphyocladia latiuscula* on lipid peroxidation of liver homogenate

Fractions	IC_{50} (mg/mℓ)
MeOH extract	7.92
Hexane fraction	3.53
CH_2Cl_2 fraction	3.97
EtOAc fraction	8.92
BuOH fraction	5.86
H_2O fraction	> 100
L-ascorbic acid	> 100

* TBA values were measured after incubation for 8 hr.

The antioxidative effect of the methanol extract of *S. latiuscula* and their various solvent fractions on lipid peroxidation of a mouse liver homogenate

Membrane lipids are abundant in unsaturated fatty acids. These unsaturated molecules are most susceptible to oxidative processes. It is well established that lipid peroxidation is one of the reaction set into motion as a consequence of free radicals in cells and tissues. The one-electron reduction products of O_2, superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxy radical (OH^-) actively participate in the initiation of lipid peroxidation (Mayumi et al., 1993). As shown in Table 2, methanol extract and their various solvent fractions of *S. latiuscula* on lipid peroxidation showed high antioxidant activities. With the previously noted exception of the water fraction, the addition of each fraction inhibited the lipid peroxidation significantly. The hexane and dichloromethane fractions showed greater activity than the others in terms of antioxidant

activity, and inhibited the lipid peroxidation at a concentration of 1 mg/mℓ by 72% and 56%, respectively.

The antioxidative effect of the methanol extract of *S. latiuscula* and their various solvent fractions on free radical generation by AC_2F (Hepatocyte)

Recently, 2',7'-dichlorofluorescin diacetate (DCFH-DA) has been used as a probe of reactive oxygen species (ROS) such as O_2 and H_2O_2 etc. Liposoluble DCHF-DA becomes water-soluble dichlorofluorescin (DCHF) by mitochondrial esterase or hydrolysis, then oxygenized to dichlorofluorescein (DCF) which has strong fluorescence. Therefore, this method is useful to measure changes of ROS (Label et al., 1990). The effect of methanol extract and their various solvent fractions on free radical generation is shown in Table 3. The dichloromethane fraction showed greater inhibitory action than other fractions. Compared with control, the dichloromethane and hexane fractions reduced free radical generation to 63% and 52%, respectively.

Generally, algae has been used as a form of folk medicine in the curing of curare, helmintics, gout, eczema and gallstones in Korea. Park *et al.* (1991) demonstrated the presence of two effective natural antioxidant compounds in three edible algae *Laminaria sinclairii*, *Undaria pimmatifida* and *Enteromorpha linza*; these were confirmed to be benzene-derivative substances. In a sense of successive screening tests for antioxidant principles in marine algae, Fujimoto et al. (1980) reported that more than half of them showed this effect. In particular, the chloroform-soluble fractions extracted from several species of brown algae, *Eisenia bicyclis* and *Undaria pinnatifida*, showed excellent antioxidant activities. And they also found that bromophenols which were isolated from a red algae, *Polysiphonia ulceolate*, showed a marked antioxidant activities (Fujimoto et al., 1985). Glombitza et al. (1976) were the first to publish a comparison of free and esterified lanosol in red algae. The results of the their paper suggested to the possibility that the bromophenols and the bromochlorophenols may occur in at least four different forms in the red algae. The algae that contain both bromochlorophenol and tetrabromodiphenymethanes were also reported to contain floridorubin. Whether the report is valid for all red algae containing bromophenols remains to be elucidated.

Table 3. The antioxidative effect of various fractions obtained from the methanol extract of *Symphyocladia latiuscula* on free radical generation of hepatocyte (Ac_2F)

Fractions	Fluorescence intensity/min
MeOH extract[a]	5.90
Hexane fraction[b]	5.75
CH_2Cl_2 fraction[b]	4.40
EtOAc fraction[b]	5.70
BuOH fraction[b]	6.10
H_2O fraction[b]	12.50
Control	11.95

a : 0.2 mg/mℓ, b : 0.1 mg/mℓ

As shown above, extracts of *S. latiuscula* showed high antioxidant activity. Especially hexane and dichloromethane fraction showed highest activity in several kinds of experiment. It means high antioxidant activity of *S. latiuscula* was not only results from scavenger of free radical, but also inhibition of free radical generation. According to the results of papers which dealt with red algae, main active component of *S. latiuscula* would be bromophenols. In order to identify the antioxygenic active principle of *S. latiuscula*, further study is being set up.

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보라우무 (*Symphyocladia latiuscula*) 추출물의 항산화활성

박혜진 · 정해영* · 최재수
부경대학교 식품생명과학과, *부산대학교 약학대학

일본, 따렌 등에 분포되어 있고 우리 나라에서 동해안, 서해안, 남해에서 흔히 볼 수 있는 해조류로서, 조간대의 바위 위에서 서식하는 빨강 검둥이과 홍조류인 보라우무 (*Symphyocladia latiuscula*)를 대상으로 하여 안정한 free radical인 1,1-diphenyl-2-picrylhydrazyl (DPPH)를 사용하여 그 소거능을 측정하고, 또한 생체내의 과산화지질을 평가하는 방법으로 과산화지질 분해산물인 malondialdehyde를 thiobarbituric acid (TBA)로 비색정량하는 방법과 Hepatocyte에 의해 생성되는 free radical 생성량 측정 등의 다양한 방법을 통하여 항산화능을 측정하였다. DPPH radical을 이용한 항산화활성 검색에서 강력한 radical 소거 효과를 나타내었다. 각 용매 분획별로 항산화활성을 살펴본 결과 수층을 제외한 모든 분획들에서 농도 의존적으로 항산화활성이 나타났다. 특히 dichloromethane과 hexane 가용성 분획의 항산화활성이 가장 크게 나타났다. 지질과산화 억제 효과에서도 유사한 결과를 보였으며 dichloromethane 가용성 분획의 항산화활성이 가장 높게 나타났으며 L-ascorbic acid의 항산화활성과 비교하여 더 높은 활성을 나타내었다. Free radical 생성 억제 효과에서도 dichloromethane 가용성 분획의 경우 control과 비교하여 0.1 mg/㎖에서 50% 이상의 저해효과를 나타내었다.