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DPPH RADICAL SCAVENGING AND HYDROXYL RADICALS (OH) ACTIVITY EFFECT OF *GELIDIUM AMANSII* FOR NOODLES

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ABSTRACT

The present study was investigated plant extracts as sources of natural antioxidants and to examine whether red algae, *Gelidium amansii* having significant1- diphenyl 2-picrylhyorazyl (DPPH) activity and hydroxyl radicals (OH) activity. DPPH scavenging activity of ethanol extract of *G. amansii* was evaluated at 0.1 mg/ml was 13.7% and 69.3% at 8.0 mg/ml. After boiling 5 minutes, the DPPH scavenging activity of instant noodles with *G. amansii* without *G. amansii* were 5.8% and 66.0%, respectively. OH scavenging activity of extracts of *G. amansii* was evaluated at 0.1 mg/ml was 15.2% and 72.1% at 8.0 mg/ml. The antioxidant activity of instant noodles with *G. amansii* and 3.5% NaCl after 10 boiling minutes was 60% for the OH radical. When the L-Ascorbic acid used as a control, extract of *G. amansii* was 82.4% effect on the activation of DPPH and 83.5% effect on the activation of OH. The DPPH inhibitory activity of *G. amansii* (IC_{50} = 3.26 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 1.0 ug/ml). The OH inhibitory activity of *G. amansii* (IC_{50} = 4.75 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 1.0 ug/ml). The overall values of OH activity of stem were higher than those of DPPH and there were show a statistically significant difference ($p > 0.05$).

KEYWORDS: 1, 1- diphenyl 2-picrylhyorazyl (DPPH), *Gelidium amansii*, hydroxyl radicals (OH), noodle.

INTRODUCTION

Antioxidants came to public attention in the 1990s, when scientists began to understand that free radical damage was involved in the early stages of artery-clogging atherosclerosis and may contribute to cancer, vision loss, and a host of other chronic conditions. In the health-conscious societies of today, the word “antioxidant” has become synonymous with words such as healthy, anti-aging and even cancer prevention. Some studies showed that people with low intakes of antioxidant-rich fruits and vegetables were at greater risk for developing these chronic conditions than were people who ate plenty of these fruits and vegetables. Clinical trials began testing the impact of single substances, especially beta-carotene and vitamin E, as weapons against heart disease, cancer, and the like.

There is great number of methods for determination of antioxidant capacity of foods and beverages based on different principles: Hydroxyl radical ('OH), The 1, 1-diphenyl 2-picrylhyorazyl (DPPH), Total Radical-trapping Antioxidant Power (TRAP) and so on. The DPPH method is rapid, simple, convenient, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods and beverages.[1,2]

Antioxidant foods and ingredients are an important component of the food industry. Noodles are a staple food in many cultures made from unleavened dough which is stretched, extruded, or rolled flat and cut into one of a variety of shapes. Noodles have been a staple food in many parts of the world for at least 2,000 years, though whether the modern version of the stringy pasta was first invented by the Chinese, Italians, or Arabs is debatable. The origin of noodles has been disputed, but the evidence heavily favors its origin in China.[3] One of the major concerns with instant noodle is that it can produce oxidised fat and oil if it is not managed properly during the manufacturing process. This is of concern if the cooking oil is not maintained at the proper temperature or the oil is not changed as often as necessary. When food remains in your digestive tract for such a long time, it will also impact nutrient absorption, but, in the case of processed ramen noodles, there isn't much nutrition to be had. Instead, there is a long list of additives, including the toxic preservative tertiary-butyl hydroquinone (TBHQ).

Seaweeds are considered to be a rich source of antioxidants.[4-6] Algal biomass and algae-derived compounds have a very wide range of potential applications for human nutrition and health products. Some algae are considered as rich sources of natural

antioxidants.[7] The main substances with potential economic impact in food science, pharmaceutical industry and public health, biosynthesized by algae, are polyunsaturated fatty acids, sterols, minerals, polysaccharides, terpenoids, proteins and halogenated compounds.[8]

Gelidium amansii Lamouroux is an economically important species of red algae in the family Gelidiaceae. The species commonly found and harvested in the shallow coast (3 to 10 meters or 10 to 33 feet of depth below the water) of many East Asian countries including North and South Korea, China, Japan, Singapore, and northeast Taiwan.[9] This algae is used to make agar, whose components are the polysaccharide agarose and agaropectin, from the large amount of algin which is located in the algae's cell wall, as well it is sometimes served as part of a salad, puddings, jams, and other culinary dishes in producing regions.[10]

The purpose of the present study is to evaluate *G. amansii* extracts as sources of natural antioxidants for DPPH and OH radical to examine whether the extractions of *G. amansii* are not losing significant OH activity during cooking noodles.

MATERIALS AND METHODS

Sample extract

Gelidium amansii was collected from seven randomly selected markets for food purpose in Korea. The red algae were washed, shade dried and then milled into coarse powder by wind mill. The algae were ground with pestles and liquid nitrogen at -70°C and homogenized prior to beginning extraction experiments for the fine powder. The ground powders were dissolved in 1000 ml ethanol and treated with ultrasound at room temperature for three hours. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was further stirred with a magnetic bar at 65°C for 12 hours. Extracted sample was filtered with Whatman filter paper No. 1. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber. The powdered plant material was weighed and a powder sample lyophilized was used in the experiment.

Preparation of noodles

One cup flour, 3.5% teaspoon salt, and one cup extraction solution of *G. amansii* were mixed and dissolve everything together well. The liquid was covered with cloth and let rest for 30 minutes. To get homemade noodles to the desired thickness was by passing the dough through a pasta machine. The dough cut the strips into 0.5 cm-wide strips and 20- to 30-cm lengths.

DPPH free radical

The antioxidant activity of the *C. roseulatum* extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al.[10] with slight modifications. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[16] DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of the previous algae extracts of various concentrations (0.1, 1.0, 2.0, 4.0, and 8.0 mg/ml). DPPH was added to the solutions prepared with algae extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µL of ethanol DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. After 30 min, the optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 517 nm. Absorbance changes are measured at 517 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1.0 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula.

% Inhibition =[1 -OD (DPPH + sample)/OD (DPPH)] x100%.

The 50% inhibition (IC50) is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. A dose response curve was plotted to determine IC50 values.

To determine the IC50 value of the active component, the technique using 96-well microplates was employed.[11]

Hydroxyl radical assay

The scavenging activity for hydroxyl radicals was measured with fenton reaction. Reaction mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4 mL of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.0 mL of algae extract at various concentrations. Different concentrations (0.1, 0.5, 1.0, 2.0, 4.0, and 8.0 mg/ml) of these extracts were examined by using 96-well microplates. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with UV visible spectrometer Shimadzu, UV-1800, Japan. The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula.

Inhibition % = (IA-As)/IA×100

Where IA is the absorbance of the 100% initial and As is

the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

Statistical analysis

All the analysis were carried out in triplicate. The results were expressed as the mean values ± standard deviation (SD). Differences were tested with analysis of variance (ANOVA) followed by multiple comparison test. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0).

IC_{50} is defined as the concentration of inhibitor necessary for 50% inhibition of the enzyme reaction of a maximum scavenging capacity. To determine the IC_{50} value of the active component, the technique using 96-well microplates was employed.[11] Regression analysis by a dose response curve was plotted to determine the IC_{50}values.

RESULTS AND DISCUSSION

The mean contents of crude extracts from *G. amansii* were 262.02 mg/kg in combined with heat and ultrasonic treatment. DPPH scavenging activity of extracts of *G. amansii* was evaluated at 0.1 mg/ml was 13.7% and 69.3% at 8.0 mg/ml (Fig. 1). As the extract concentrations of *G. amansii* increase, the inhibition of DPPH scavenging activity also increase. OH scavenging activity of extracts of *G. amansii* was evaluated at 0.1 mg/ml was 15.2% and 72.1% at 8.0 mg/ml (Fig. 1). The overall values of DPPH activity of stem were higher than those of DPPH and there were show a statistically significant difference ($p > 0.05$). When the L-Ascorbic acid used as a control, extract of *G. amansii* was 82.4% effect on the activation of DPPH and 83.5% effect on the activation of OH (Fig. 2). Antioxidant was measured after boiling for 5 and 10 minutes. The longer the time to boil, the lower the antioxidant function. However, there were show a statistically significant difference on two boiling time ($p > 0.05$). After boiling 5 minutes, the DPPH scavenging activity of instant noodles with *G. amansii* without *G. amansii* were 5.6% and 66.0%, respectively (Fig. 3). After boiling 7.5 and 10 minutes, the DPPH scavenging activity of instant noodles with *G. amansii* were 63.2% and 62.1%, respectively Dough usually uses about 3.5% salt for gluten formation. The dough with salt decreased the antioxidant. The antioxidant activity of instant noodles with *G. amansii* and 3.5% NaCl was 61% for the DPPH radical. The antioxidant activity of instant noodles with *G. amansii* and 3.5% NaCl after 10 boiling minutes was 58.1% for the OH radical (Fig. 4). DPPH radical scavenging activity of callus cultures of miswak (*Salvadora persica*) increased gradually from 1.26- to 1.78-fold when grown on increasing concentrations of NaCl for 30 days.[12] The addition of salt had shown a negative effect on DPPH radical scavenging activity of yam.[13] It mean that salt can be low antioxidant function when cooking noodles. The DPPH inhibitory activity of *G. amansii* (IC_{50} = 3.26 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 1.0 ug/ml) (Fig. 5). The OH inhibitory activity of *G. amansii* (IC_{50} = 4.75 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 1.0 ug/ml) (Fig. 5).

The overall values of OH activity of stem were higher than those of DPPH and there were show a statistically significant difference ($p > 0.05$). There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs.[14] Many studies have been done to determine antioxidant capacity in seaweeds and some researchers have stated high scavenging activity for red algae species.[15-17] For example, the percentages of DPPH free radical scavenging by *Kappaphycus striatum* extracts ranged between 12.29% and 56.63%.[18] The percentages of DPPH free radical scavenging activity by *Kappaphycus alvarezii* extracts were ranged between 18.34 and 35.63%.[18] The percentage of OH inhibition was obtained by the aqueous extract of *Asparagopsis armata* (68.76%), followed by that of *Boergeseniella thuyoides* (PS) (35.05%), *Pterosiphonia cartilagineum* (28.84%), *Sphaerococcus coronopifolius* (25.88%), *P. complanata* (25.37%), *S. coronopifolius* (24.30%) and *B. thuyoides* (19.13%).[6] The radical scavenging ability of methanolic extract of same genus *Gelidium sesqulpedale* at 25 mg/mL was observed to be 53%.[19] Consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events.[4,20]

We have shown that 8.0 mg/ml weight of ethanol *G. amansii* extract has antioxidants for DPPH. In addition, strong activation of OH enzymes by extract from *G. amansii* makes this red algae an interesting topic for further biological and phytochemical examination.[21-22] Noodles produce oxidizing substances during the digestion process. The extract from *G. amansii* could show antioxidant properties during digestion.

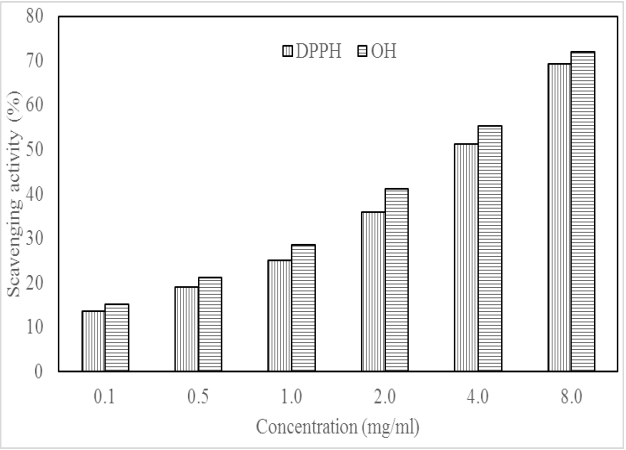

Figure 1: DPPH and OH radical scavenging activity of *Gelidium amansii* at different concentrations.

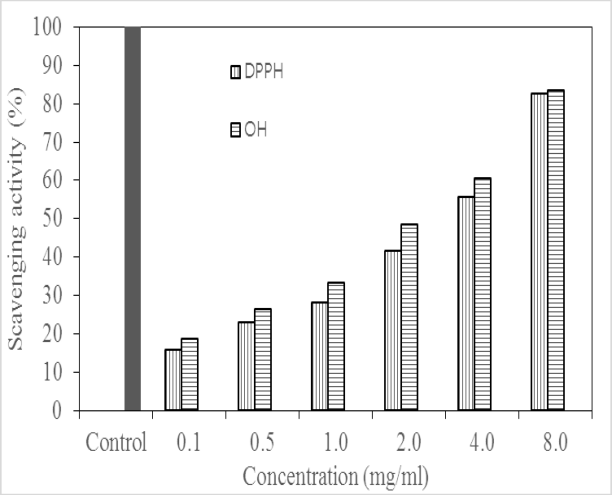

Figure 2: Relative antioxidant values of the *Gelidium amansii* extracts for control group (L-Ascorbic acid).

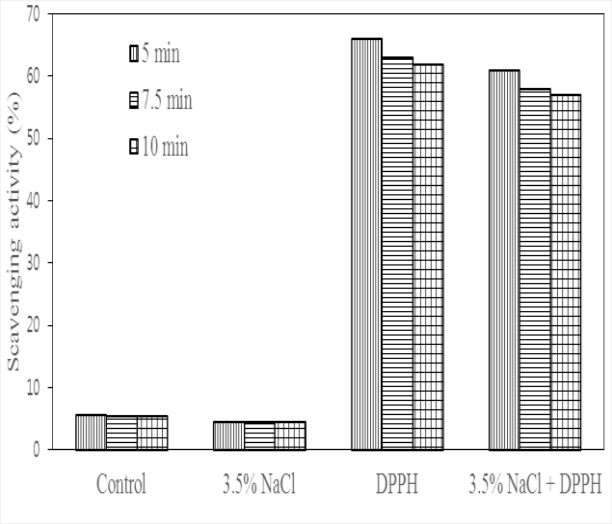

Figure 3: DPPH radical scavenging activity of noodles with and without *Gelidium amansii* after 5, 7.5, and 10 minutes for boiling times.

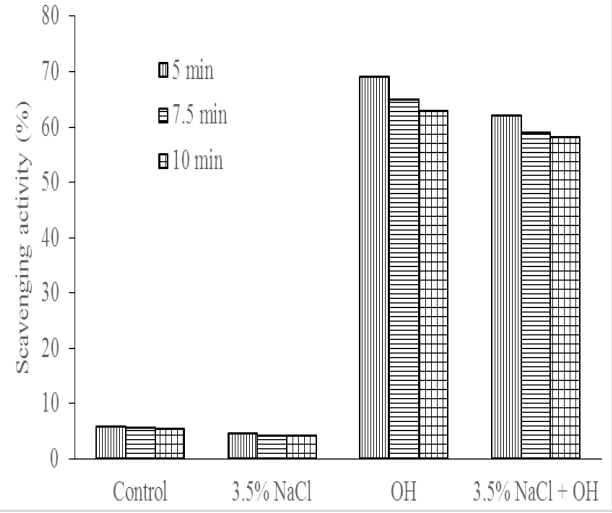

Figure 4: OH radical scavenging activity of noodles with and without *Gelidium amansii* after 5, 7.5, and 10 minutes for boiling times.

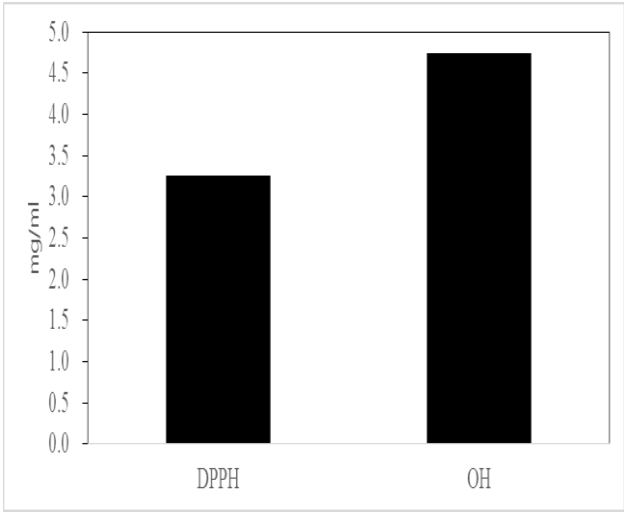

Figure 5: Inhibitory effects {IC_{50} (mg/ml)} on DPPH and OH radical activity of 1.0 M *Gelidium amansii*.

CONCLUSION

The results presented in this study represent the first significant assessment of the antioxidant activity of *G. amansii* for instant cooking noodles. Using the DPPH and OH assay, it was determined that ethanol extract of *G. amansii* tested showed antioxidant activity and the various concentration treatments of red algae were active. Although the longer the boiling temperature, the less the activity decreased, but there was no significant difference.

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