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EVALUATION OF NITRIC OXIDE RADICAL SCAVENGING ACTIVITY OF A HEALTH SUPPLEMENT, HERBALONE

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ABSTRACT

Nitric oxide (NO) plays a key role in the production of reactive nitrogen species (RNS). The objectives of this study were to determine antioxidant activities of ethanol extracts of raw materials and product samples were obtained from SNBIO Co. The product name is the HERBALONE. The antioxidant activity of their extracts was measured on the basis of the scavenging activity of nitric oxide radical ($NO^{\cdot}$)-scavenging activity. The assay was measured by the Greiss reagent. NO scavenging activity of product samples at 100.0 mg/ml was 14.3%. Overall, the degree of NO inhibition activity of product samples was not high. NO scavenging activity at 100.0 mg/ml was 74.5%. The antioxidant activity for NO-scavenging found on raw materials. Thus, in order to exhibit a similar NO scavenging function, the content should be increased by about 5.2 (74.5/14.3) to 20.8 (49.8/2.4) times.

KEYWORDS: Nitric oxide radical (NO), product samples, raw materials.

INTRODUCTION

Nitric oxide (NO) and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species.[1] Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide.[2] Nitric oxide (NO) is an omnipresent intercellular messenger in all vertebrates, modulating blood flow, thrombosis, and neural activity.[3] Low concentrations of NO are sufficient in most cases to effect the physiological functions of the radical. NO is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation, and antimicrobial and antitumor activities.[4]

Curry powder, a commercially prepared mixture of spices, is largely a Western creation, dating to the 18th century.[5] Dry curries are cooked with very little liquid which is allowed to evaporate, leaving the other ingredients coated with the spice mixture. Though curry was introduced to Korea in the 1940s, the Indian dish was only popularized decades later. Korean curry, usually served with rice, is characterized by the golden yellow color from turmeric.

The onion (*Allium cepa* L.), also known as the bulb onion or common onion, is a vegetable that is the most widely cultivated species of the genus *Allium*. Onions are rich in two chemical groups that have perceived benefits to human health. These are the flavonoids and the alk(en)yl cysteine sulphoxides (ACSOs).[6]

Saccharina japonica is a marine species of Phaeophyceae (brown algae), a type of kelp or seaweed, that is extensively cultivated on ropes in between the seas of Japan and Korea. It is widely eaten in East Asia. *S. japonica* and that it could be a potential source of natural antioxidants and emulsifiers.[7]

The health benefits of radish leaves are varied ranging from treating diabetes to rheumatism.[8] It contains essential vitamins and minerals and it also acts as a detoxifying agent. It consists of iron, phosphorus, folic acid, calcium and vitamin C that are essential for many bodily functions.

Soybeans are legumes and part of the pea family that is the world's major food crop today. They are also processed into oil, milk, tofu, and soy protein. They are also known as Edamame in China, Korea, and Japan. Soybeans are low in fat and calories and are a rich source of protein, fiber and many other essential vitamin and mineral. Soybeans are also useful in many medical conditions and symptoms. Therefore, in this study, the ethanol extracts of various plants were evaluated with regard to antioxidant properties

MATERIALS AND METHODS

Sample extract

Raw materials and product samples were obtained from

SNBIO Co., Gangseo-Gu, Busan-Ci, the Republic of Korea. The product samples were composed of five kinds of plant species supplied by the SNBIO Company (Table 1). The product name is the HERBALONE which is a health supplement. Dry product sample (100 g) was ground with pestles and liquid nitrogen at –70℃ and homogenized prior to beginning extraction experiments. Dried powder was packed into a Soxhlet apparatus. The samples were blended with 50% ethanol, and then an aliquot of the mixture (100 µL, 200 mg sample / ml 50% ethanol) was further mixed with 100 mM Tris-HCl buffer (400 µL, pH 7.4). The mixture was further stirred with a magnetic bar at 65°C for 12 hours. The sample was treated with ultrasound at room temperature for 2 hours. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA). The mixture was shaken vigorously for one hour at room temperature and left in the dark at room temperature for 20 min. The extract was filtered through Whatman filter paper No. 1. The sample was evaporated to remove solvent under reduced pressure and controlled temperature (55°C) by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber. The extract was dried, weighed (2.6 g) and stored at 4°C in storage vials for experimental use.

Table 1: The composition of products.

Components	%
Curry powder	2
Onion	1
Kelp (*Saccharina japonica* J.E. Areschoug)	1
Dry radish leaves	1
Soybeans	95

Assay of nitric oxide radical scavenging

Nitric oxide radical ($NO^{\cdot}$)-scavenging activity is the nitric oxide radical scavenging assay. The assay was measured by the Greiss reagent as described in a previous study.[2,9] The extracts were prepared from a 100.0 mg/mL ethanol crude extract. These were then serially diluted with distilled water to make concentrations from 1.0 mg/mL to 50.0 mg/mL. These were stored at 4°C for later use. L-Ascorbic acid solutions ranging from 1.0 to 100.0 mg/mL were used to prepare a standard curve. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the ethanol extracts and incubated at 25°C for 180 mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The color tubes contained ethanol extracts at the same concentrations with no sodium nitroprusside. A volume of 150μL of the reaction mixture was transferred to a 96-well plate. The optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 546 nm. Corresponding blank sample was prepared and L-Ascorbic acid was used as reference standard (positive control). The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the ethanol extracts and L-Ascorbic acid were calculated.

Statistical analysis

The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The results were expressed as the mean ±SD. 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). 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.

Relative inhibitor rate of raw materials and product samples for L-Ascorbic acid was calculated.

RESULTS AND DISCUSSION

Nitric oxide radical (NO)-scavenging activity was measured by the Greiss reagent. NO scavenging activity of product samples from SNBIO was evaluated at 1.0 mg/ml was 2.4% (Table 2). NO scavenging activity at 10.0 mg/ml and at 50.0 mg/ml were 7.2% and 12.0%, respective. NO scavenging activity at 100.0 mg/ml was 14.3%. It is also observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. However, the degree of NO inhibition activity of product samples was not high. There was no significant difference among the three experimental groups ($p < 0.05$).

NO scavenging activity of raw materials was evaluated at 1.0 mg/ml was 49.8% and that NO scavenging activity at 10.0 mg/ml was 54.3%, and that of at 50.0 mg/ml was 66.7%. NO scavenging activity at 100.0 mg/ml was 74.5%. The high antioxidant activity for NO-scavenging found on raw materials. There was no significant difference among the three experimental groups ($p < 0.05$). The all values of NO scavenging activity of raw materials were higher than those of product samples. The all groups for product samples and raw materials were shown a statistically significant difference ($p > 0.05$).

Table 2: The effect of nitric oxide radical scavenging (NO) by product samples from SNBIO at different concentrations.

Concentration (mg/ml)	Measurement (%)			Mean ± Standard deviation
	1	2	3	
1.0	1.556	3.670	1.835	2.354±1.148
10.0	3.891	11.927	5.810	7.209±4.196
50.0	7.393	16.514	12.232	12.046±4.563
100.0	8.560	18.654	15.596	14.270±5.176
F-test	1.936 (p> < 0.05)			

Table 3: The effect of nitric oxide radical scavenging (NO) by raw materials at different volumes.

Volume (ml)	Measurement (%)			Mean ± Standard deviation
	1	2	3	
1.0	48.249	55.657	45.566	49.824±5.227
10.0	52.529	58.410	51.988	54.309±3.562
50.0	65.759	75.841	58.410	66.670±8.751
100.0	75.875	81.346	66.361	74.527±7.583
F-test	1.261 (p> < 0.05)			

When the L-Ascorbic acid used as a control, relative NO scavenging activities of product samples and raw material extracts were 17.8% and 83.5%, respectively (Fig. 1).

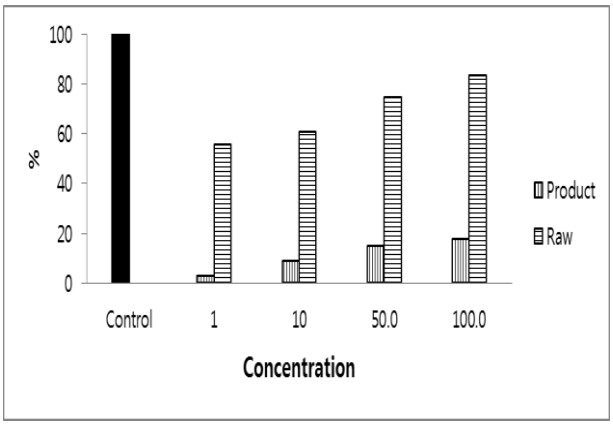

Figure 1: Relative inhibitory effects on NO by raw materials and product samples from SNBIO Co. Control is L-Ascorbic acid.

The search for natural bioactive compounds with potential for the treatment and prevention of human diseases and to meet other needs is currently a key topic in many laboratories and industries.[10] Various extraction techniques have been applied to recover antioxidant compounds from natural and organic sources.[11-12] Solvents such as methanol, ethanol, acetone, ethyl acetate, and their combinations have been used to extract phenolics from coffee or the coffee residues, often with different proportions of water.[13]

In this study, it seems that the oxidative power of the production, HERBALONE is not much stronger than that of the antioxidant capacity of the raw materials. The additive soybean reduced the content of dry matter in contrast to the raw materials which contributed to its increase. The raw materials did not contained soybeans. Many beans were included when making the product (Table 1). From this finding it can be inferred that additives such as non-antioxidants applied brought about a decrease in the total content of the NO scavenging activities. The strong antioxidant activity was not shown in product samples from SNBIO Co. The amounts of NO scavenging activities on production ranged from 2.4% to 12.3% (Table 1). The amounts of NO scavenging activities on raw materials ranged from 49.8% to 74.5% (Table 2). Thus, in order to exhibit a similar NO scavenging function, the content should be increased by about 5.2 (=74.5/14.3) to 20.8 (=49.8/2.4) times. Because the qualitative and quantitative studies of bioactive compounds from plant materials mostly rely on the selection of proper extraction method,[14] the increased understanding about dynamic chemical nature of the diverse bioactive molecules.

CONCLUSION

The strong antioxidant activity was not shown in product samples from SNBIO Co. The high antioxidant activity for NO-scavenging found on raw materials.

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