Preliminary Phytochemical Screening and Antioxidand Activity of *Brassica oleracea* Linn. (Broccoli)

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Abstract

The edible parts of *Brossica oleracea* plant are a rich source of phytochemical compounds which possess antioxidant potential. The present research is concerned with the study of phytochemical constituents and antioxidant activity of broccoli. The preliminary phytochemical investigation of broccoli was found to contain alkaloids, carbohydrates, glycosides, phenolic compounds, reducing sugar and saponins. Starch and tannins were not found in the sample. The antioxidant activities of watery and ethanolic extracts of broccoli were screened by DPPH method. In the screening, ascorbic acid was used as the standard. The concentrations of standard ascorbic acid, watery and ethanolic extracts were 6.25, 12.5, 25, 50 and 100 μg/mL. The IC_{50} values of standard ascorbic acid, watery and ethanolic extract were obtained by the linear regression equations from each graph of percent inhibitions vs. concentrations. From the screening, the IC_{50} values of standard ascorbic acid, watery and ethanolic extract of broccoli were observed as 5.15, 20.6 and 15.25 μg/mL, respectively. Therefore, the antioxidant activity of ethanolic extract is more potent than that of watery extract.

Keywords: Antioxidant activity, Broccoli, DPPH method, IC_{50}, Phytochemical screening

Introduction

Broccoli is an edible green plant in the cabbage family (*Brassicas*) whose large flowering head and stalk are eaten as vegetable. The word *broccoli* comes from the Italian plural of *broccolo*, which means "the flowering crest of a cabbage", and is the diminutive form of *brocco*, meaning "small nail" or "sprout" (Stephens, 2009 *).* Broccoli is classified in the Italica cultivar group of the species *Brassica oleracea*. Broccoli has large flower heads, usually dark green in color, arranged in a tree-like structure branching out from a thick, edible stalk which is usually light green. The mass of flower heads is surrounded by leaves. Broccoli resembles cauliflower, which is a different cultivar group of the same *Brassica* species. Broccoli is a particularly rich source of vitamin C and vitamin K. Contents of its characteristics are a particularly rich source of vitamin C and vitamin K. Contents of its characteristic sulfur-containing glucosinolate compound, isothiocyanates and sulforaphane, are diminished by boiling, but are better preserved by steaming, microwaving or stir-frying (Nugrahedi, *et al.*, 2015*).*

Description of the Plant

There are three commonly grown types of broccoli. The most familiar is Calabrese broccoli, often referred to simply as "broccoli", named after Calabria in Italy. It has large (10 to 20 cm) green heads and thick stalks. It is a cool season annual crop. Sprouting broccoli has a larger number of heads with many thin stalks. Purple cauliflower is a type of broccoli grown in Europe and North America. It has a head shaped like cauliflower, but consists of tiny flower buds. It sometimes, but not always, has a purple cast to the tips of the flower buds. Broccoli is a fast-growing

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annual plant that grows 60–90 cm (24–35 inches) tall. Upright and branching with leathery leaves, broccoli bears dense green clusters of flower buds at the ends of the central axis and the branches. If left unharvested, those buds bear yellow flowers with four petals and produce silique fruits (a dry capsule). Broccoli thrives in moderate to cool climates and is propagated by seeds, either sown directly in the field or in plant beds to produce transplants. The heads, or florets, reach harvest in 60 to 150 days, depending upon the variety and the weather. *(*Dixon, 2007)

The majority of broccoli cultivars are cool-weather crops that do poorly in hot summer weather. Broccoli grows best when exposed to an average daily temperature between 18 and 2 °C (64 and 73 °F). When the cluster of flowers, also referred to as a "head" of broccoli, appear in the center of the plant, the cluster is generally green. Garden pruners or shears are used to cut the head about an inch from the tip. Broccoli should be harvested before the flowers on the head bloom bright yellow *(*Smith, 1999).

Scientific Classification

Kingom : Plantae
Order : Brassicas
Family : Brassicaceae
Genus : Brassica
Species : *B. oleracea*
Botanical name: *Brassica oleracea* L.
Common name: Broccoli

(A) (B)

Figure 1. (A) Broccoli plant, (B) broccoli side shoot

Nutrition of Broccoli

A 100 gram reference serving of raw broccoli provides 34 calories and is a rich source (20% or higher of the Daily Value, DV) of vitamin C (107% DV) and vitamin K (97% DV) (table). Raw broccoli also contains moderate amounts (10–19% DV) of several B vitamins and the dietary mineral manganese, whereas other micronutrients are low in content (less than 10% DV). Raw broccoli is 89% water, 7% carbohydrates, 3% protein, and contains negligible fat. (USDA, 2018)

Material and Methods

The fresh broccoli was collected from Htan-ta-bin Township, Yangon Region, Myanmar. Then, the broccolis were cut into small piece and air-dried. The dried sample was powdered by a grinder. The powdered samples of broccoli were stored in air-tight containers. Preliminary phytochemical investigation of broccoli sample was carried out by test tube method (M-Tin Wa, 1972). The screening of antioxidant activity of the water and ethanol extracts from broccoli were carried out by DPPH method using UV spectrophotometer.

Results and Discussion

Preliminary Phytochemical Investigation of Broccoli

Preliminary phytochemical investigation of broccoli samples was carried out by test tube methods. It is observed that these tests show the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, reducing sugars and saponins in the sample. Tannins and starch were absent in the sample.

According to the phytochemical investigation, it can be seen the presence of vital nutrient phytochemical compounds.

Screening of Antioxidant Activity of Broccoli Extracts by DPPH Method

For this purpose, DPPH free radical scavenging activity method using UV spectrophotometer was employed. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature which accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine. A color change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of the sample. In the determination of radical scavenging by DPPH method base on the change in absorbance of water and ethanolic extracts solutions in various concentrations. The concentrations; 100 µg/mL, 50 µg/ mL, 25 µg/ mL, 12.5 µg/ mL and 6.25 µg/ mL were prepared by dilution with ethanol as solvent. Ascorbic acid was used as standard sample and ethanol was employed as control. Blank solution was also prepared by mixing sample and ethanol. The absorbance values were measured at wavelength at and the control. These values are used to calculate the percentage inhibition of DPPH radical against the samples.

The IC_{50} values of various extracts were calculated from the percentage inhibitions at various concentrations. The results of the free radical scavenging activity of broccoli were assessed by DPPH assay that was summarized by IC_{50} using method of linear regression. The lower the value of $IC_{50,}$ the higher is the antioxidant property.

From the screening of antioxidant activity, IC_{50} values of standard ascorbic acid, watery and ethanolic extracts were observed with 5.15, 20.6 and 15.25 µg/mL, respectively. Therefore, it can be seen that the broccoli possesses the antioxidant activity. The antioxidant activity of ethanolic extract is more potent than that of watery extract. The antioxidant activities of standard ascorbic acid and extract samples of broccoli are shown in Table 1 and 2 and Figure 2, 3, 4, 5 and 6.

Table 1. Percent Inhibition of Standard Ascorbic Acid and Broccoli Extracts (DPPH Scavenging Assay Method)

No.	Concentration (µg/mL)	Ascorbic acid (Standard)	Water extract	Ethanol extract
1	6.25	32.51	36.61	31.42
2	12.5	52.60	50.00	48.92
3	25	69.40	53.83	62.30
4	50	81.42	67.21	72.40
5	100	90.44	81.15	90.98

* Absorbance of DPPH (Control) = 0.366

$$\% \text{ RSA} = \frac{\text{Abs}_{\text{DPPH}} - [\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Blank}}]}{\text{Abs}_{\text{DPPH}}} \times 100$$

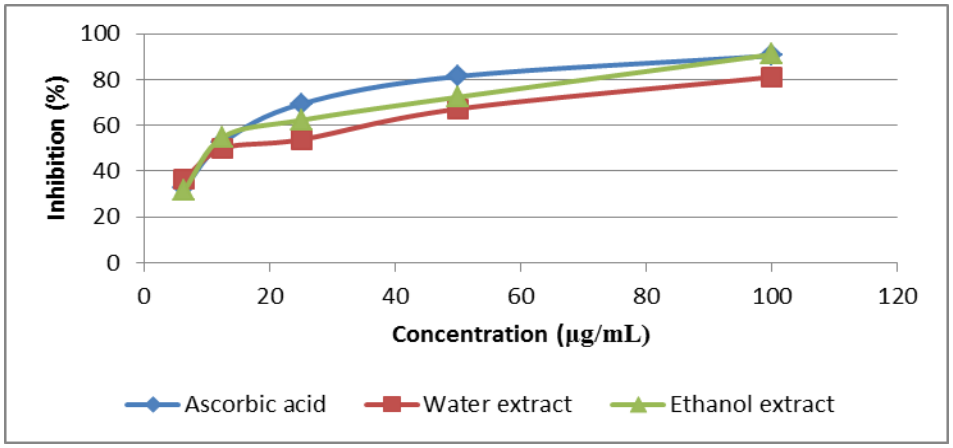

Figure 2. % Inhibition of standard ascorbic acid, watery and ethanolic extract with concentrations

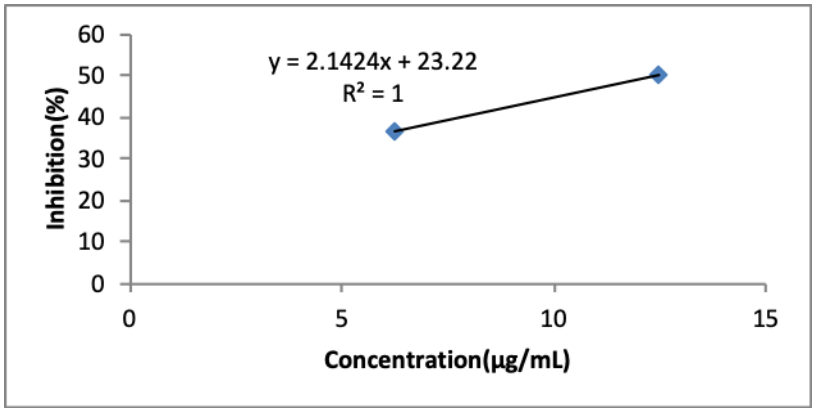

Figure 3. Linear regression equation for IC_{50} value of standard ascorbic acid

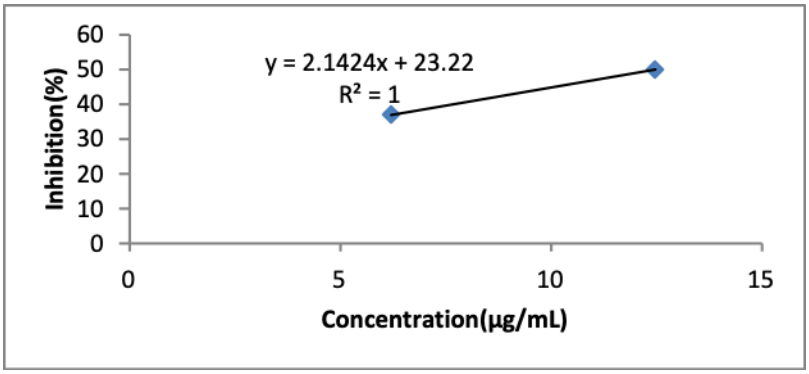

Figure 4. Linear regression equation for IC_{50} value of water extract

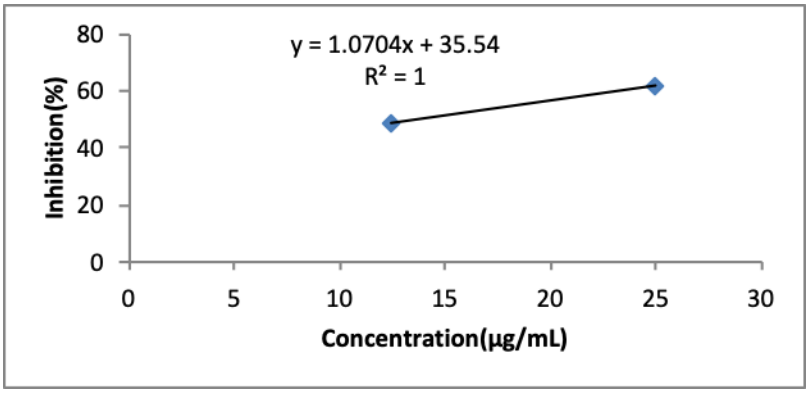

Figure 5. Linear regression equation for IC_{50} value of ethanol extract

Table 2. The Linear Regression Equations and IC_{50} Values

No.	Test Solution	Regression Equations	IC_{50} (ppm)
1	Ascorbic acid	y = 0.5271x + 44.848	9.77
2	Water extract	y = 0.4274x + 41.2	20.60
3	Ethanol extract	y = 0.5278x + 41.953	15.25

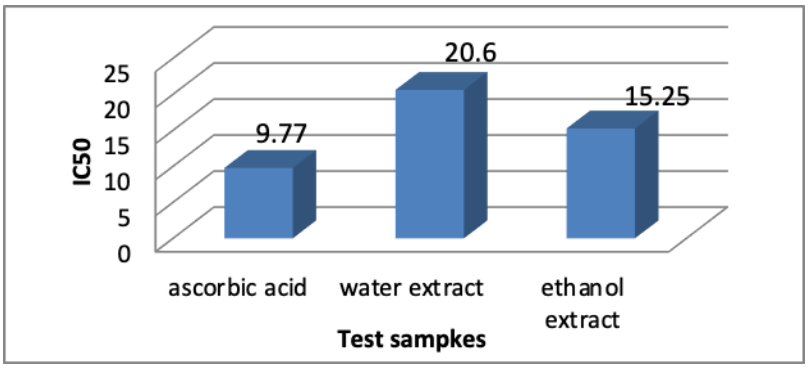

Figure 6. IC_{50} values of standard ascorbic acid and broccoli extracts

Conclusion

This research project deals with the study of phytochemical constituents and antioxidant activity of broccoli. The preliminary phytochemical investigation of broccoli was carried out by test tube method. From the investigation, alkaloids, carbohydrates, glycosides, phenolic compounds, reducing sugars and saponins were present in the sample. Starch and tannins were absent in the sample.

The antioxidant activities of watery and ethanolic extracts of broccoli were screened by DPPH method. In the screening, ascorbic acid was used as the standard. The absorbances of the test samples with the various concentrations were determined by UV spectrophotometer. Then, the various percent inhibitions of the test samples were calculated by the reported equation. The IC_{50} values of standard ascorbic acid, watery and ethanolic extracts were obtained by the linear regression equations from each graph of percent inhibitions vs. concentrations. From the screening, the IC_{50} values of standard ascorbic acid, watery and ethanolic extract of broccoli were observed as 9.77, 20.6 and 15.25 μg/mL, respectively. Therefore, it can be seen that the broccoli possesses the antioxidant activity. The antioxidant activity of ethanolic extract is more potent than that of watery extract.

According to the present research, the broccoli possesses effective phytoconstituents and antioxidant activity. Therefore, the broccoli may be used as the nutritional food as well as the antioxidant for human health.

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