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Determination of Mineral Content and Estimation of Total Flavonoid Content of *Brassica oleracea* L.var. italica plenck.

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Abstract: *In this research work, the Brassica oleracea L.var. italica plenck., Myanmar name Pan Gaw Bi Sein, Broccoli, which is one of the most commonly consumed vegetables by the Myanmar population was selected to qualify and quantify the flavonoids present in it. The fresh broccoli was purchased from the local market, Chan Mya Thar Si Township, Mandalay Region, Myanmar. Firstly, the mineral contents of selected sample were measured by EDXRF method. Moreover, the fresh broccolis were crushed to obtain the expressed juice with 95% EtOH which is the liquid product. This extract was checked for qualitative test of flavonoids. In addition, total flavonoid content of B. oleracea L.var. italica plenck. was evaluated by the aluminum chloride ($AlCl_3$) method using UV spectrophotometer (UV-1800, SHIMADZU, UV spectrophotometer) at 415 nm. The total flavonoid content of this selected sample was determined as 10.17± 0.16 mg quercetin equivalent (QE) per 100 g fresh weight.*

Keywords : *Brassica oleracea* L.var. italica plenck., EDXRF method, UV spectrophotometer, flavonoids, quercetin.

1. Introduction

Diets rich in fruit and vegetables have long been associated with reduced risk of chronic disease, particularly cardiovascular disease, cancers and type 2 diabetes [1]. Oxidative stress from increased amounts of reactive oxygen species (ROS) can cause extensive damage to cell structures, and is considered a major factor in the pathogenesis of these chronic diseases [2]. Evidence suggests that regular consumption of fruit and vegetables minimizes some of these harmful effects, which has been somewhat accredited to the presence of compounds possessing antioxidant properties [3]. The major antioxidants present in fruit and vegetables are: vitamin C, vitamin E, carotenoids and polyphenols, especially flavonoids, which all provide protection against free radicals [4]. The quality and quantity of these antioxidant components are major attributes to the health benefits of fruit and vegetables that are associated with reduced risk of chronic disease [2]. For example, regular consumption of dark green leafy vegetables has shown a protective effect against two common eye diseases, cataract and macular degeneration, caused by free radicals generated by sunlight, metabolism and infection. These vegetables contain the pigments lutein and zeaxanthin, which accumulate in the eye and eradicate free radicals, thus preventing harm to the eye's sensitive tissues [5]. The high fibre content of fruit and vegetables also provide a protective effect. The bulking and softening action of indigestible fibre can reduce pressure inside the intestinal tract and calm the irritable bowel [6].

Broccoli (*Brassica oleracea* L. var. italica Plenck), which originated from the eastern Mediterranean region of Europe, is one of the most commonly consumed green vegetable worldwide. Broccoli possesses a wide range of bioactive compounds that have several health benefits, and are rich in both nutritional as well as non-nutritional antioxidants such as vitamins C and E, phenolic compounds, and glucosinolates (GSLs) {7-9]. Several epidemiological studies have shown that consumption of broccoli is positively associated with reduced risk of several types of cancers, type 2 diabetes, and cardiovascular diseases [10-11]. Furthermore, broccoli is known to possess antioxidant and anti-proliferative activities [3, 8]. These beneficial properties can be attributed to the presence of health-promoting phytochemicals such as GSLs, vitamins, carotenoids, phenols, flavonoids, and minerals [7, 9].

Phenolic compounds present in broccoli help neutralize or quench free radicals [12] and are often considered to be the most abundant antioxidants in the human diet [13]. Flavonoids and their derivatives possess antioxidant properties due to their ability to scavenge reactive oxygen species and inhibit oxidative stress [14]. All the antioxidants present in

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broccoli show stronger interactive antioxidative properties when they work in groups as they function synergistically to reduce reactive oxygen species. The concentration of these phytochemicals and their properties in broccoli are dependent on both the genotype and environment. Several studies have confirmed significant changes in these phytochemicals based on plant genotype [9], growing season [15, 16], plant parts [17, 8], developmental stages [15], fertilization levels [18], temperature and irrigation [19], postharvest storage conditions [20], and even the cultivation year [21].

In addition to this, domestic cooking can dramatically reduce activities of antioxidant components, as many of these compounds are very sensitive to heat and are soluble in water [22]. Absorption of water during boiling can dilute and cause leaching of antioxidant compounds and thus decrease their antioxidant content [3]. There is a great amount of literature available concerning levels of antioxidant properties in raw fruit and vegetables [23–24, 2, 25]. However, there is less literature regarding the content of antioxidants in vegetables as usually eaten, i.e. after cooking. Variation in both cooking treatment and cooking duration may affect the nutritional value of vegetables [26]. Broccoli is normally cooked by boiling in water or microwaving before consumption [22]; thus, it is essential to determine which domestic cooking method and cooking duration are best for retaining antioxidants in this vegetable [27-28]. The aim of this study was to investigate the mineral content and to estimate the total flavonoid content of *B. oleracea* L.var. italica plenck.

1.1 Botanical Description

Family : Brassicaceae (Cruciferae)
Botanical name : *Brassica oleracea* L.var. italica plenck.
English name : broccoli
Myanmar name : Pan Gaw Bi Sein
Part used : Flower

Figure 1. Flower of *Brassica oleracea* L.var. italica plenck.

2. Materials and Methods

2.1 Collection and Preparation of Sample

The flowers of *B. oleracea* L.var. italica plenck. were collected from local Market, Chan Mya Thar Si Township, Mandalay Region, Myanmar. It was cut into small pieces and dried in the air for about four weeks. It was stored in a well-stoppered bottle and used for the determination of mineral content.

2.2 Determination of Mineral Content from the *Brassica oleracea* L.var. italica Plenck.

Mineral content of the *B. oleracea* L.var. italica plenck. was measured at the Department of Physic, University of Mandalay, Myanmar by applying EDXRF (Energy Dispersive X-ray Fluorescence Spectroscopy).

2.3 Extraction Procedure

100 g of fresh broccoli was crushed with 100 mL of 95 % EtOH by blender. These juices were squeezed, filtered and then centrifuged with 5000 rpm for 30 minutes. 105 mL of expressed juice which is the liquid product was obtained.

2.4 Qualitative Test for Flavonoids

Ferric Chloride Test:

A few drops of neutral ferric chloride solution were added to 1 mL of extract solution. Formation of blackish red color indicates the presence of flavonoids.

Shinoda's Test:

To 1 mL of extract solution, a small piece of magnesium ribbon or magnesium foil was added and a few drops of concentrated HCl were added. Change in pink red colour shows the presence of flavonoids.

Lead- acetate Test:

To 1 mL of extract solution, a few drops of aqueous basic lead acetate solution were added. Formation of precipitate indicates the presence of flavonoids.

2.5 Quantitative Determination of Total Flavonoid Content

2.5.1 Principle

The basic principle of Aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard quercetin solutions of various concentrations were used to build up the calibration curve [29-32].

2.5.2 Preparation and Determination of Standard Quercetin

10 mg of the standard quercetin was taken in a test tube. 100 mL of MeOH was added to the standard compound. The stock solution was obtained. It was diluted with MeOH in various ratios to obtained four ranges of concentration, such as 25 μg/mL, 50μg/mL, 75 μg/mL, and 100 μg/mL respectively. Then, 4.0 mL of solution was prepared

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for each concentration. 0.5 mL of each standard quercetin solution was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added separately to each tubes.

These tubes were left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The calibration curve was plotted by using the resulted absorbance data of standard quercetin solutions at concentrations 25 µg/ mL to 100 µg/ mL in methanol. The calibration curve of standard quercetin is shown in Figure 2 [29-32].

2.5.3 Determination of Total Flavonoid Content of *Brassica oleracea* L.var. italica Plenck.

The total flavonoid content of *B. oleracea* L.var. italica plenck. was measured by aluminium chloride ($AlCl_3$) according to the spectrophotometric method using quercetin as a standard. Firstly, 0.5 mL of extract solution was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL distilled water were added into tube.

This tube was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The assay was performed in triplicate. The total flavonoid content of *B. oleracea* L.var. italica plenck. was expressed as mg quercetin equivalent (QE) /100 g fresh weight [29-32].

3. Results and Discussion

3.1 Determination of Mineral Content from the *Brassica oleracea* L.var. italica Plenck.

The mineral contents of selected sample were described in Table 1.

Table 1. Mineral Contents in the *Brassicea oleracea* L.var.italica Plenck.

No	Elements	Symbols	Result (%)
1.	Potassium	K	2.73700
2.	Phosphorus	P	0.70070
3.	Calcium	Ca	0.56290
4.	Sulphur	S	0.37730
5.	Chlorine	Cl	0.33400
6.	Aluminium	Al	0.18420
7.	Iron	Fe	0.02119
8.	Silicon	Si	0.02100
9.	Zinc	Zn	0.00743
10.	Titanium	Ti	0.00691
11.	Manganese	Mn	0.00555
12.	Rubidium	Rb	0.00534
13.	Barium	Ba	0.00340
14.	Vanadium	V	0.00282
15.	Copper	Cu	0.00108

According to the resulted data, broccoli consists of K, P, Ca, S, Cl, Al, Fe, Si, Zn, Ti, Mn, Rb, Ba, V, and Cu, respectively. Among them, it was found that potassium (K), Phosphorus (P), calcium (Ca), sulphur (S) and chlorine (Cl) contain the higher amount than other in this sample and moderate contents of aluminium (Al), iron (Fe) and silicon (Si) could be observed.

3.2 Estimation of Total Flavonoid Content in *Brassica oleracea* L.var. italica Plenck.

Special Test for Flavonoid

The fresh juice obtained by crushing the *B. oleracea* L.var. italica plenck. was examined by using the special qualitative tests of flavonoid. The resulted data were tabulated in Table 2.

Table 2. The results of qualitative test for flavonoid

No	Experiment	Observation	Inference
1.	Ferric Chloride Test:	Blackish red colour was appeared	Flavonoid may be present
2.	Shinoda's Test:	Colour turns to pink red colour	Flavonoid is present
3.	Lead Acetate Test:	Precipitate was produced	Flavonoid is present

From these results, it was observed that the selected sample consists of flavonoid compounds.

The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Figure 2.

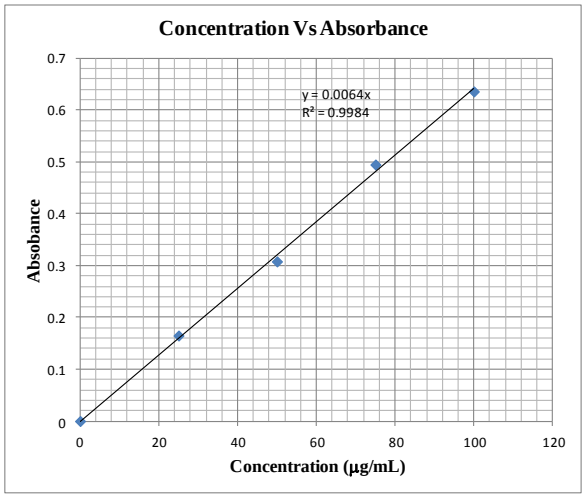

Figure 2: Concentration absorbance calibration curve for standard quercetin

In addition, the total flavonoid content of the *B. oleracea* L.var. italica plenck. was carried out by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbance values of prepared sample solutions were measured by UV spectrophotometer at 415 nm with respect to the blank solution. From these results, the amount of total flavonoid content of analyzed sample was obtained by using the standard graph. The results are described in Table 3.

Table 3. The results of total flavonoid content of extract solutions of *B. oleracea* L.var. italica Plenck.

No	Name of Sample	Flavonoid (mg/100 g)	Flavonoid (mg/100 g) Mean ± Standard Deviation
1.	*B. oleracea* L.var. italica Plenck.	10.17	10.17 ± 0.16
		10.34	
		10.01	

The total flavonoid content present in the selected vegetable (Broccoli) was found as 10.17± 0.16 mg quercetin equivalent (QE) per 100 g fresh weight.

4. Conclusion

In this research work, one of Myanmar well known vegetables, *B. oleracea* L. var. italica plenck., Myanmar name Pan Gaw Bi Sein, Broccoli, which is flavonoid rich vegetable, was selected to determine the mineral content and to evaluate the total flavonoid present in it qualitatively and quantitatively. The mineral content of selected sample was determined by EDXRF method. It was found that potassium contains the highest amount in this sample. In accordance with the qualitative test of flavonoid, it was confirmed that this fresh juice contains the flavonoid compounds. Moreover, the total flavonoid content of the extract obtained from the selected sample could be evaluated by UV spectrophotometer using the aluminium chloride method at 415 nm. It was found that the total flavonoid content of *B. oleracea* L. var. italica plenck. is 10.17± 0.16 mg quercetin equivalent (QE) per 100 g fresh weight. The resulted data of the current study showed that the selected sample, the broccoli, had the considerable amount of total flavonoid compounds. Flavonoid compounds that are secondary metabolites are antioxidants. Quercetin, the most abundant dietary flavanol, is a potent antioxidant because it has all the right structural feactures for free radical scavening activity.

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