Determination of Antioxidant Activity of *Spinaceaoleraceae* L. (Spinach)

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Abstract

In this research work, Spinach was selected for chemical analysis. Firstly, moisture and ash contents in Spinach weredetermined by using oven drying method and Muffel Furnace method. Protein content in Spinach was determined by Kjeldahl's method. Mineral composition of Spinach was determined by EDXRF spectroscopy. In antioxidant activity study, ethanolic extract of Spinach was used to investigate radical scavenging activity by (1, 1-diphenyl-2-picrylhydrozyl) DPPH assay.

Keyword: Spinach, EDXRF, Kjeldahl's method, DPPH, Antioxidant

Introduction

A plant has been co-opted by humans to fulfill a particular need. Earth is a green planet due to the presence of plants. The importance of plants lies in that they contribute greatly to human life and the environment. There are a list of useful plants which are medicine or drugs, or edible, vegetable and fruit and other economical purpose.

The World Health Organizaiton (WHO) in 2004 launched the global strategy on Healthy Eating, which establishes certain guidelines with the main objective of reducing risk factors related to such diseases based on a healthy diet, physical activity and health. Among the recommendations is the increase in the intake of vegetables and fruits as well as vitamins and minerals, since these foods contain bioactive compounds such as carotenoids, phytoestrogens, glycosimolates, etc.. Bioactive compounds are normally accumulated in all parts of plants, but their concentration varies according to the part of plant(Felipe de Lima Franzen, 2019.

Food science has a growing literature about fruits and vegetables imparting greater benefits to humans. Considerable epidemiological evidence suggests an association between consumption of diet high fruits and vegetables and decreased risk of cardiovascular diseases, hypertension, diabetes, stroke and various forms of cancer. Although fruits and vegetables account for only 10% of total calories consumed, they make a significant contribution to overall health. Fruits and vegetables have a regular place in traditional India cuisine(Farnsworth, 1992).

The important of leafy vegetables in the developing countries has been recognized only now due to their nutritional and medicinal value. Green leafy vegetables occupy an important place among food crops as theyprovide adequate amount of crude fibre, carotene, a precusor of vitamin A, vitamin C, riboflavin, folic acid and mineral salts like calcium, iron, phosphorus etc. Green leafy vegetables are highly seasonal and are available in plenty at a particular season and can be easily cooked. Green leafy vegetables are rich source of a number of micronutrients and

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phytochemical that appeared to provide much of the disease fighting power(Farnsworth, 1992).

Vegetables, apart from providing nutrition, contribute to the appetizing colour, texture and flavours to the food. Vegetables are classified into group's based on their growing season, or based on the parts of plant consumed and shape or appearance, but none of the classifications is either complete or satisfactory(Felipe de Lima Franzen, 2019).

Most vegetables are consumed fresh. However, vegetables can be stored for prolonged periods depending on their type. Root and tuber vegetables such as potatoes, carrots, celery, onions, cabbage and turnips can be stored for months while leafy vegetables such as lettuce and spinach and others such as beans, peas, cauliflower, cucumbers, tomatoes and asparagus can be stored for a few days only. Storage of vegetables brings about changes in their composition mainly due to storage temperature(Felipe de Lima Franzen, 2019).

The compounds of antioxidant action used by the food industry can be synthetic or natural, and those of greater use are those of a synthetic nature. Antioxidants are substances that can prevent, stop or reduce oxidative damage which causes by free radicals. Antioxidants donate one of their own electrons to free radicals. Because they are stable in either form, they do not themselves become free radicals. Generally, antioxidants function in two ways. First, they can actually prevent free radicals forming second, they can break chains by scavenging oxygen radicals(Kaur, C., Kapoor, H.C., 2008).

Spinach is green leafy vegetable. The biochemical components present in green leafy vegetables are great pharmacological or medicinal important. Thus, in this results work, antioxidants activity of spinach was determined.

Botanical Description

Figure 1.The Leaves of*Spinaceaoleraceae* L. (Hin-nu-nwe)

Family	Chenopodiaceae
Botanical name	*Spinaceaoleraceae* L.
English name	Spinach
Myanmar name	Hin-nu-nwe
Part used	The leaves

Experimental

Sample Collection

The Leaves of*Spinaceaoleraceae*L**. (**Spinach) were collected from HinYwet Su Village, PatheinGyi Township, Mandalay Region. They were cut into small pieces and air-dried in the shade for few days. These pieces were used as the sample.

Determination of Ash Content of *Spinaceaoleraceae*L.

The ash of a sample is the inorganic residue remaining after the organic matter has been burnt away. Sample (ca. 5g) was introduced into a predried and cooled procelain crucible in air-tight desiccator and accurately weighed. Then, it was heated gently over a burner until the sample was thoroughly charred. The crucible and sample content were then transferred to the electric furnace at 600°C for two hours until the residue was free from carbon. Then the crucible containing residue was cooled in adesiccator and weighed. Heating, cooling and weighing were repeated until constant weight was attained.

The ash content of the sample was calculated using the following equations.

$$\text{Ash} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$

Determination of Moisture Content of *Spinaceaoleraceae*L.

The moisture content of sample was determined by oven drying method. The moisture content of sample is the weight lost due to the evaporation of water at the drying temperature.

Sample (ca. 5g) was placed in the crucible. The crucible with the sample placed in an oven and dried for 30 minutes at 100°C. Then, they were removed from the oven and cooled in the air-tight desiccator at room temperature and weighed. The procedure was repeated until the loss in weight had not been changed. The moisture content can be calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{loss in weight (g)} \times 100}{\text{weight of sample (g)}}$$

Determination of Protein Content of *Spinaceaoleraceae*L. by Using Kjedahl's Method

The protein contents of samples were determined by (Official Methods of Analysis, AOAC, 2000) method with nitrogen estimation system using the conversion factor of N x 6.25.

(a) Digestion :1g of sample was weighted and placed in the Kjeldahl's digesting flask. 5g of K_2SO_4+(0.5g)$CuSO_4.5H_2O$ and 10 mL of 98% sulphuric acid 10 mL of distilled water and were added into it in such a way as to wash solid adhering to the neck. The flask was shaken until the contents were thoroughly mixed and it was heated till the mixture became colorless. The digestion was continued for half an hour to make sure that all nitrogen in the sample was converted to ammonium sulphate. Then it was allowed to cool.

(b)Distillation: The Kjeldahl's distillation apparatus was setup, taking care that the tip of the condenser extended below the surface of the 4% boric acid solution 50 mL in the receiver. The digested solution was poured into the flask together with 50 mL

of 40% sodium hydroxide to make mixture strongly alkaline. The sample is distilled until 100 mL of distillate are collected in 50 mL of 4% boric acid. The evolved ammonia was distilled off.

(c) Titration: Add 2-3 drops of methyl red indicator to the conical flask containing boric acid and titrate it with 0.1MHCL until a faint pink color is obtained. A blank determination was carried out without sample using the reagents as in the case of sample.

Determination of Mineral Contents of*Spinaceaoleraceae*L.

The mineral contents of sample were determined by Energy Dispersive X-rays Fluorescence Spectroscopy measured at Department of Physics, University of Mandalay.

Determination of Antioxidant Activity *of Spinaceaoleracea*L.by DPPHRadical Scavenging Assay

The DPPH radical scavenging method was used to evaluate the antioxidant, ascorbic acid activity was compared with that of the natural antioxidant. DPPH solution (0.002%) was prepared in the brown colored bottle by dissolving (2mg) of DPPH powder in the 100 mL of ethanol. It must be stored in the refrigerator for no longer than 24 hours. The stock solution (20µg/mL) of the sample was prepared by dissolving (2mg) of sample in 100mL of ethanol. This stock solution was diluted with ethanol to get the sample solution with the concentrations of 400, 200, 100, 50, 25 and 12.5 µg/mL. Blank solution was prepared by mixing the sample solution (1.5 mL) with ethanol (1.5 mL). The control solution was prepared by mixing 1.5mL of 0.002% DPPH solution and 1.5 mL of ethanol in brown bottle. DPPH radical scavenging activity was determined by UV-visible spectrophotometer. The sample solution was also prepared by mixing 1.5mL of 0.002% DPPH solution and 1.5mL of test sample solution. These bottles were incubated at room temperature and were shaken by shaker for 30 min. After 30 min, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a UV-visible spectrophotometer. It was determined at University Research Center(URC), Yangon.

Results and Discussion

Ash Content, Moisture Content and Protein Content of

***Spinaceaoleraceae*L.**

Ash content, moisture content and protein content of***Spinaceaoleraceae*L.** were determined and the results were shown in the following Table (1).

Table 1. Ash Content, Moisture Content and Protein Content in Spinach

No.	Content	Value
1.	Ash	7.35%
2.	Moisture	23.28 %
3.	Protein	2.78 %

The ash content is a measure of the total amount of minerals present within a vegetable. The results showed that Spinach contains appreciable amount and type of minerals.

Moisture content can affect the physical and chemical properties of vegetable, which directly correlate to the freshness and stability of vegetable for consumers. The moisture content in spinach was high ranging. According to moisture content spinach can be stored for a few days only.

Protein content was estimated (2.78 %) in Spinach. Protein is required for the growth and maintenance of tissues. Body's protein needs are dependent upon health and activity level.

Elemental Composition in *Spinaceaoleraceae*L. by EDXRF

Table 2. Results of Elemental Composition by EDXRF

No	Element	Symbols	Relative Abundance (%)
1	Potassium	K	5.546
2	Calcium	Ca	2.188
3	Phosphorus	P	0.7480
4	Chlorine	CL	0.2886
5	Sulphur	S	0.2046
6	Silicon	Si	0.2010
7	Aluminium	Al	0.1999
8	Iron	Fe	0.00356
9	Titanium	Ti	0.00268
10	Vanadium	V	0.00244

Mineral content is a measure of the amount of specific inorganic components present with a food, such as Ca, Na, K and Cl. Some minerals are essential to a healthy diet. According to this table, potassium content was high as compare to other minerals. Potassium helps the body regulate fluid, send nerve signals and regulate muscle contraction. Calcium was second abundant mineral found in Spinach. This mineral is essential for bone health and a signaling molecule for nervous system, heart and muscles. Iron was also found in appreciable amount in Spinach. It is key element in the metabolism of almost all living organisms. In human, iron is an essential component of hundreds of proteins and enzyme and then probable concentrations of trace minerals are found in spinach.

Antioxidant Activity of *Spinaceaoleraceae*L. by DPPH Assay Method

Spinach samples were available for antioxidant activity by DPPH assay method. The presence of a stable free radical (DPPH) antioxidant donates a hydrogen atom to quench the stable free radical. This method is associated with the change in the absorbance. Ascorbic acid was used as standard sample. Six kinds of concentrations of each Spinach samples 12.5 μg/m1, 25 μg/ml, 50 μg/ml, 100 μg/ml

and 200 μg/ml and 400 μg/ml were prepared by dilution method. The IC_{50}values of standard ascorbic acid and Spinach samples were described in Table (3).

Determination absorbance of control solution, blank solution, sample solution and standard ascorbic acid solution were carried out at wavelength 517nm.

Table 3. % Inhibition of Various Concentrations of CrudeExtract and Standard Ascorbic Acid

Extracts	Concentration (μg/ml)	Mean % inhibition	IC_{50} (μg/ml)
Ethanol extract (sample)	12.5	1.20	195.60
	25	4.20	
	50	15.32	
	100	32.72	
	200	52.37	
	400	72.05	
Ascorbic acid	1.25	28.71	2.23
	2.5	50.97	
	5.0	70.65	
	100	9161	

Free radicals are by products of metabolism. They can cause oxidative stress, which triggers accelerated aging and increases risk of cancer and diabetes. However, Spinach contains antioxidant, which fights oxidative stress and helps reduce the damage it causes.

Conclusion

In this research work, Spinach was selected for chemical analysis. Ash content, moisture content and protein content of Spinach were determined and it was found that ash content (7.35 %), moisture content (23.28 %) and protein content (2.78 %). Protein provides various parts of body structure, strength and elasticity.

From EDXRFstudy, spinach was very rich source of essential nutrients such as K, Ca, Fe and palpable concentrations of trace minerals which are beneficial for human health.

In addition, the determination of antioxidant activity of ethanol crude extract was performed by DPPH assay. Ascorbic acid was used as standard antioxidant. IC_{50}values of Spinach had acceptable antioxidant activity.The results showed that the spinach had a certain antioxidant activity. It can be concluded that spinach contains maximum amount of moisture and protein, appreciable amount of essential nutrients acceptable antioxidant activity. Therefore, Spinach should be taken daily to getextremely healthy and linked to numerous health benefits.

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