Analysis on Phytochemicals, Minerals and Total Flavonoid Content of Spinach (*Spinacia oleracea* Linn.)

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Abstract

The present study was conducted for quantitative determination of flavonoids in spinach using spectrophotometric method. Quercetin was used as the standard for calibration. Phytochemical screening of the selected sample revealed the presence of phenolic, glycoside, reducing sugar, tannin, steroid, polyphenol, terpene, saponin, flavonoid and lipophenol. Qualitative tests for flavonoids such as ferric chloride test, Shinoda's test, lead-acetate test were also performed. In addition, physicochemical parameters such as total ash and water soluble ash content of the selected sample were also determined. Furthermore, the elemental analysis of the selected sample was carried out by EDXRF (Energy Dispersive X-ray Fluorescence) method.

Keywords: EDXRF, flavonoids, phytochemical, quercetin

1. Introduction

Phytochemicals are naturally occurring, bioactive chemical compounds found in plants. Phytochemicals provide health benefits for humans. Recently, plant-derived substances have become of great importance due to their versatile applications. Among phytochemicals, flavonoids are known to display a wide range of pharmacological and biochemical properties. Flavonoids and other phenolic compounds serve as antioxidants by scavenging the free radicals with their hydroxyl groups and are also effective metal chelators.[1]

Fruits and vegetables contain abundant antioxidant compounds such as flavonoids. Spinach (*Spinacia oleracea*) is an important nutritive vegetable rich in antioxidants. Spinach contains several active antioxidant components including flavonoids, *p*-coumaric acid derivatives and uridine.[2]

The purpose of this research is to investigate the total flavonoid content in fresh spinach. To achieve this aim, preliminary phytochemical screening and qualitative tests for flavonoid were carried out. Moreover, total ash, water soluble ash and mineral content in fresh spinach were also examined.

2. Materials and Methods

2.1. Apparatus and Chemicals

Common laboratory apparatus were used throughout the course of this research work. Analytical grade chemicals were used.

2.2. Instruments

Readings were recorded using UV-visible spectrophotometer (UV-1800, Shimadzu).

2.3. Sample Collection and Preparation

Fresh spinach was purchased from local Market, Mandalay. The edible part of spinach was used for the experiments.

The edible portion of fresh spinach sample (100 g) was cut into small pieces and mixed with 100 mL ethanol and then, they were homogenized by blender. The extract was then separated from the residue by filtration. The remaining residue was re-extracted twice and the extracts were combined. The obtained ethanolic extract (250 mL) was centrifuged and the clear supernatant solution was used for qualitative and quantitative analysis of flavonoids.

2.4. Preliminary Phytochemical Screening of Spinach

Phytochemical tests were done according to standard procedures.[3]

2.5. Determination of Mineral Content

Mineral contents were measured by applying Energy Dispersive X-ray Fluorescence (EDXRF).

2.6. Determination of Total Ash and Water Soluble Ash Content

Total ash content was determined by ashing the samples in the muffle furnace at 600 °C until the samples were thoroughly carbonized. Then, they were cooled at room temperature and weighed. The process of heating, cooling and weighing was repeated until the constant weight was obtained (AOAC, 2000). The total ash (1 g) was boiled with 25

mL of distilled water for about 5 min. The insoluble matter was collected on filter paper and rinsed with hot water and ignited to constant weight at low temperature. The weight difference between the total ash and the residue represents the water soluble ash.

2.7. Qualitative Tests for Flavonoids[4]

2.7.1. Ferric chloride test: A few drops of ferric chloride solution were added to 1 mL of ethanolic extract. Formation of blackish red color indicated the presence of flavonoids.

2.7.2. Shinoda's test: A small piece of magnesium ribbon or magnesium foil was added to 1 mL of sample solution and a few drops of concentrated HCl were added. The appearance of red colour solution showed the presence of flavonoids.

2.7.3. Lead-acetate test: A few drops of aqueous basic lead acetate solution were added to 1 mL of sample solution. The formation of reddish brown bulky precipitate indicated the presence of flavonoids.

2.8. Estimation of Total Flavonoid Content (Aluminium Chloride Colorimetric Method)

The total flavonoid content of spinach was measured by applying aluminum chloride ($AlCl_3$) colorimetric method and quercetin was used as a standard.

2.8.1. Preparation of Standard Quercetin Solution for Calibration Curve: Quercetin was used to construct the standard calibration curve. The stock quercetin solution was prepared by dissolving 10 mg of standard quercetin in 100 mL of distilled water. The obtained stock solution was diluted with distilled water to attain five different concentrations such as 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL. Then, the reaction mixture was prepared for the measurement of absorbance. 5.0 µL of standard quercetin solution at five different concentrations was put separately in five test tubes. Then, 150 µL of 5% $NaNO_2$ was added to each test tube. After 5 min, 150 µL of 10% $AlCl_3$ was added. After further 5 min, 1 mL of 1 M NaOH was added. The resulting solution was diluted to a final volume of 5 mL with distilled water and mixed. After each standard solution was prepared, the absorbance of the solutions was measured with a UV spectrophotometer at 510 nm with respect to the blank solution.

2.8.2. Preparation of Test Sample Solution: Firstly, 5 uL of ethanolic extract was taken in a test tube. 150 uL of 5% $NaNO_2$ was added to the test tube. After 5 min, 150 uL of 10% $AlCl_3$ was added. After further 5 min, 1 mL of 1 M NaOH was added to the mixture. The solution was diluted to a final volume of 5 mL with distilled water and mixed. After mixing with distilled water, the absorbance of this prepared sample solution was measured by UV-Vis spectrophotometer at 510 nm using distilled water as a blank. The total flavonoid content of the ethanolic extract of spinach was expressed as quercetin equivalent (QE) mg/g of fresh weight.

3. Results and Discussion

3.1. Preliminary Phytochemical Screening of Spinach

According to the phytochemical test, fresh spinach contained glycoside, flavonoid, polyphenol, phenolic, reducing sugar, saponin, tannin, terpene and terpenoid, alkaloid, steroid and lipophenol.

3.2. Determination of Mineral Content

According to the results of EDXRF method, fresh spinach contained potassium, calcium, chlorine, phosphorus, aluminium, sulfur, silicon, iron, manganese, zinc and copper. Potassium is the highest content in the sample.

Table 1. Elemental analysis of fresh spinach

No.	Elements	Symbols	Concentration (%)
1	Potassium	K	4.300
2	Calcium	Ca	1.987
3	Chlorine	Cl	1.328
4	Phosphorus	P	0.4851
5	Aluminium	Al	0.1589
6	Sulfur	S	0.1175
7	Silicon	Si	0.1020
8	Iron	Fe	0.03929
9	Manganese	Mn	0.00931
10	Zinc	Zn	0.00483
11	Copper	Cu	0.00304

Potassium is one of the essential mineral in the body and it is required for the heart, kidneys and other organs to work properly. High potassium in the diet helps to reduce blood pressure and it helps to regulate fluid balance, muscle contractions and nerve impulse. Calcium plays a vital role in muscle contraction, regulating heartbeat, formation of healthy bones and teeth, blood clotting, nerve impulse and fluid balance within cells.[5] Phosphorus together with calcium plays an important role in the building of healthy bone and tooth structure. It is also essential in the structure of cell membrane. Chlorine is involved in the regulation and maintenance of body water and salt balance. It is essential for functioning of nerves and muscle healthily. The sample also contained other trace elements such as sulphur, iron, copper, zinc and manganese. These trace elements have specific biochemical function in the human body. For example, iron is necessary for the formation of haemoglobin, and it is essential component in many enzyme reactions. It has an important role in the immune system. Copper is also a component of

many enzymes and zinc serves as cofactor in many enzymatic reactions.[6]

3.3. Determination of Total Ash and Water Soluble Ash Content

The percentage of total ash content was found to be 14.91±0.02%. The results were shown in Table 2.

Table 2. Percentage of total ash

No.	Weight of the sample (g)	Weight of ash obtained (g)	Percentage of total ash
1.	1.0000	0.1493	14.93
2.	1.0000	0.1490	14.90
3.	1.0000	0.1489	14.89
			14.91±0.02

The percentage of water soluble ash content was found to be 18.65±0.03%. The results were shown in Table 3.

Table 3. Percentage of water soluble ash

No.	Weight of the total ash (g)	Weight of water soluble ash (g)	Percentage of water soluble ash (in 1 g of total ash)
1.	1.0000	0.1865	18.65
2.	1.0000	0.1862	18.62
3.	1.0000	0.1868	18.68
			18.65±0.03

3.4. Qualitative Tests for Flavonoids

From qualitative analysis, it was observed that the ethanloic extract of selected sample contained flavonoid compounds.

Table 4. Results of qualitative tests for flavonoids

No.	Experiment	Observation	Inference
1.	Ferric chloride test	Blackish red color solution	Flavonoid may be present
2.	Shinoda's test	Red colour solution	Flavonoid is present
3.	Lead acetate Test	Reddish brown colour ppt.	Flavonoid is present.

3.5. Estimation of Total Flavonoid Content (Aluminium Chloride Colorimetric Method)

The principle of the aluminium chloride colorimetric method is that aluminium chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids. Moreover, aluminium chloride can also form acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.[7] 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.

Table 5. Concentration and corresponding absorbance of standard quercetin solution

	Sample	Concentration (µg/mL)	Absorbance at 510 nm
1	Std Q 1	10.0000	0.013
2	Std Q 2	20.0000	0.029
3	Std Q 3	30.0000	0.046
4	Std Q 4	40.0000	0.057
5	Std Q 5	50.0000	0.080

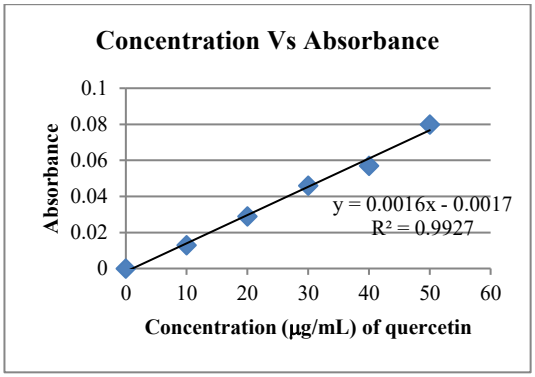

Figure 1. Standard calibration curve of quercetin for the determination of total flavonoid content

Table 6. Absorbance and concentration of fresh spinach

No of Test	Absorbance at 510 nm	Concentration (µg/mL)
1	0.061	39.19
2	0.063	40.44
3	0.058	37.31

The total flavonoid content in ethanolic extract of fresh spinach was calculated from the standard calibration curve of quercetin.

From the resulted data (Table 6), the total flavonoid content of ethanolic extract of fresh spinach was found to be 19.49±0.64 mg quercetin equivalent (QE) per g fresh weight.

Table 7. Flavonoid content in ethanolic extract of fresh spinach

No	Name of Sample	Flavonoid Content (mg QE/g of fresh weight)	Mean±Standard Deviation (mg QE/g of fresh weight)
1	Fresh Spinach	19.59	19.49±0.64
		20.22	
		18.66	

4. Conclusion

This study was carried out to investigate the phytochemicals, minerals, total ash, water soluble ash and total flavonoid content of fresh spinach. The phytochemical screening of the sample indicated the presence of glycoside, flavonoid, polyphenol, phenolic, reducing sugar, saponin, tannin, terpene and terpenoid, alkaloid, steroid and lipophenol. According to EDXRF analysis, the sample contained three of five major minerals in the body such as potassium, calcium and phosphorus. The other trace elements such as sulphur, iron, copper, zinc and manganese were found in the sample. The total ash and water soluble ash contents of the sample were found to be 14.91±0.02% and 18.65±0.03% respectively. In addition, the total flavonoid content in ethanolic extract of fresh spinach was 19.49±0.64 mg QE/ g of fresh weight. The results determined in this research showed that spinach is a rich source of phytochemicals, minerals and flavonoids.

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