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Characterization of Simplicia, Extract, and Mineral Content of Broccoli (*Brassica oleracea var. italica*)

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ABSTRACT

Bioactive compounds and minerals in medicinal plants make them both therapeutic agents and raw materials for producing contemporary medications. Broccoli (*Brassica Oleracea* var Italica) is one of the possible therapeutic plants that features several nutrients that are high in minerals. This study produced Broccoli ethanol extract and characterized the water, ash, and phytochemical test of simplicia and extract. The mineral content of simplicia, such as calcium, potassium, sodium, and iron, used the flame test Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method. This method is specifically for analyzing atomic elements or ions, which causes them to be excited and emit light at specific wavelengths. The result of water and ash content of Broccoli simplicia and ethanol extract within a range of values 2-9 – 5.8 %. Positive results for alkaloids, flavonoids, saponins, and tannins in phytochemical tests of simplicia and ethanol extract. The mineral content showed that calcium, potassium, sodium, and iron were 109.52 ± 0.25 mg/100 g, 375.58 ± 0.81 mg/100 g, 9.54 ± 0.092 mg/100 g, and 1.93 ± 0.00 mg/100g, respectively. It was concluded that as a natural ingredient, broccoli contains higher potassium than other minerals, and secondary metabolites in simplex and ethanol extracts have health benefits.

Keywords: Broccoli, Ethanol extract, ICP-OES, Mineral, Phytochemical test

1. INTRODUCTION

Minerals are required in the physiological process of living organisms to keep the enzymes and the organs working. It is divided into two groups: macrominerals and microminerals. Macro minerals are necessary or present in relatively large quantities, including Ca, P, K, Na, Cl, S, and Mg [1]. Microminerals are smaller amounts of minerals generally found in tissues in minimal concentrations, including Fe, Mo, Cu, Zn, Mn, Co, I, and Se. Mineral content assessment is one of the initial parameters used to assess food quality. Mineral deficiency can cause health problems such as anemia, goiter, osteoporosis, and osteomalacia [2]. The fulfillment of mineral requirements in humans requires the intake of food from both vegetable (plant-derived minerals) and meat (animal-derived minerals) sources [3].

Broccoli (*Brassica Oleracea var Italica*) is a preferred crop compared with several other fibre-containing crops as it contains nutrients and is high in vitamins and minerals [4]. It provides a variety of essential minerals such as calcium, potassium, sodium, iron, and selenium [5]. It is also higher in calcium than a glass of milk and is known to pack more fiber than a slice of whole-meal bread. Flavonoids, fiber, and vitamins also enrich the nutritional content of broccoli. The vitamins in broccoli include vitamins A, C, E, K, B1, and B6, which are sufficiently high and work with functional fiber that may cause the unavailability of nutrients such as fat-soluble vitamins [4].

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The main ICP-OES hardware is a gas-assisted plasma that atomizes elements from ground state energy to excitation state while emitting light energy. It can identify and measure all the elements simultaneously in a short time using only ± 5 mL of sample. It is suitable for measuring all element concentrations from minor to significant levels. However, detection limits are generally low for most typical elements, ranging from 1-100 mg/L [6].

2. METHODS

The research occurs in the Pharmacy Laboratories of Universitas Pakuan, Bogor, West Java, Indonesia.

2.1. Equipment/ Materials

Inductively coupled plasma optical emission spectroscopy or ICP-OES (Agilent 700 series), maceration flask, Whatman No. 42 filter paper, spirits lamp, porcelain chair, rotary evaporator (R-300 Buchi), Pyrex glassware, water bath.

Broccoli flowers were taken from traditional markets in Bogor City. Concentrated of HCl, H_2SO_4, ethanol, HNO_3, $(NH_4)_2C_2O_4$, NH_3, $FeCl_3$, Mg, standards of calcium, potassium, sodium, iron, and deionized water were purchased from Sigma-Aldrich, Indonesia.

2.2. Experiment

2.2.1. Simplicia Preparation

Ten kilograms of broccoli flowers simplicia are cleaned and washed thoroughly in running water. After sun-drying, the simplicia is cut into smaller pieces, and the dry simplicia is calculated for the final weight.

2.2.2. Broccoli Flower Extract Preparation

Two hundred grams of simplicia was macerated with two liters of 96% ethanol solvent for 72 hours. The filtrate results are concentrated using a rotary evaporator to collect the thick extract. The extract solution is loaded into a round bottom flask with a rotary vacuum evaporator. The maceration method is accompanied by heating at 50ºC and stirring at 200 rpm. The concentration process ceases when its volume shrinks and its color looks darker [7].

2.2.3. Ash Content

As much as 1 g of extract was weighed carefully and put into a porcelain cup that had been heated and weighed previously. It was then heated slowly in the furnace (with the temperature gradually increased to 600 ± 250C). The higher the temperature, the more ash is present, but dirt and dust might be brought in during dehydration. The ash content of the extract should not be more than 16.0 % [8].

2.2.4. Water Content

The main factor determining the quality of Simplicia is the water content. Oven drying for a standardized period is often the legal method of determination. Add approximately 10 grams of extract and weigh it carefully in a tared porcelain cup. Dry at 105ºC for 5 hours, then weigh it in a desiccator until cool. The water content should be less than 10%. If the content exceeds 10%, it will cause enzymatic and microbial damage [8].

2.2.5. Phytochemical Screening

Harfiani et al. (2020) conducted phytochemical tests on flavonoids, alkaloids, tannins, and saponins [9].

2.2.6. Alkaloid Test

Add half a gram of broccoli extract to a tube, then 2% HCl. Separate the solute into two tubes. The first tube adds 2-3 drops of Dragendorff reagent, and the second adds Mayer reagent. The alkaloid's positive result gives brick red (Dragendorff reagent) and white precipitate or yellowish (Mayer reagent).

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2.2.7. Flavonoid Test

Half a gram of broccoli extract is added to a bit of magnesium powder (Mg), mixed thoroughly, then added to concentrated hydrochloric acid (Shinoda test). Orange, red, or yellow colors appear, showing positive results.

2.2.8. Tannin and Saponin Test

Half a gram of broccoli extract boiled with 20 mL of water. Filter it, then add a few drops of 1% $FeCl_3$. The greenish brown or dark blue color appeared from the sample, earning positive tannin results.

Add half a milliliter of hot water to half a gram of broccoli extract. Shake vigorously for ten seconds until foam appears. Add 1% HCl and wait for ten minutes. When the foam does not disappear, the extract is positive for saponins.

2.2.9. Mineral Calibration Curve Preparation

Determination of mineral content according to the Association of Official Analytical Chemists (AOAC, 1999) [10, 11].

The calibration curve and analysis of calcium, potassium, sodium, and iron of Broccoli flower simplicial were carried out by Savić et al. 2019 [9]. Calcium standard series 1, 2, 4, 8, 10, 20 are prepared from 998 µg/mL solution. A total of 0.05, 0.1, 0.2, 0.4, 0.5, and 1.0 mL of standard solution pipetted into a 50 mL volumetric flask. The absorbance was then measured at a wavelength of 317.933 nm.

Potassium standard series 15, 20, 40, and 60 are prepared from a 997 µg/mL solution. A total of 0.75, 1.00, 2.00, and 3.00 mL of standard solution was pipetted into a 50 mL volumetric flask. The absorbance was then measured at a wavelength of 766.491 nm.

Sodium standard series 2, 4, 6, 8, 10, and 20 µg/mL are prepared from 1000 µg/mL solution. A total of 0.1, 0.2, 0.3, 0.4, and 0.5 mL of standard solution was pipetted into a 50 mL volumetric flask. The absorbance was measured at a wavelength of 568.821 nm.

Iron standard series 0.2, 0.4, 0.8, 1.0, 2.0, and 3.0 mL made from 999 µg/ml solution. A total of 0.01, 0.02, 0.04, 0.05, 0.10, and 0.15 mL of standard solution pipetted into a 50 mL volumetric flask. The absorbance was measured at a wavelength of 238.204 nm.

2.2.10. Analysis of Minerals in Samples

After 0.5-1 g of sample is put into the vessel, 10 mL of concentrated HNO3 is added, and the vessel is closed. It is then put into a digested microwave at 150ºC for 15 minutes. The solution is then filtered through a 45µm cellulose filter into a 50 mL volumetric flask. It is then diluted with deionized water until it reaches the limit mark, homogenized, and measured in the ICP-OES system.

2.2.11. Flame Test

Heat on a platinum wire in the reducing flame of the bunsen, moisten with hydrochloric acid, and reheat. The specific presence of calcium, potassium, sodium, and iron [12].

3. RESULTS AND DISCUSSION

3.1. Simplicia and Extract

They produced 255 grams of dry Simplicia with a yield of 2.55%. The preparation of broccoli flower extract follows the maceration method using two liters of 96% ethanol solvent combined with 200 g of Simplicia powder. The concentrated extract yields 17.4%. The higher the yield value, the greater the compounds drawn by the solvent. Ethanol, as a polar solvent, plays a vital role in the extraction yield of broccoli and is more effective in the quantitative recovery of extracted polar substances [13, 14].

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3.2. Phytochemical Test

The phytochemical tests of alkaloids with Dragendroff reagent resulted in an orange and red-colored precipitate. Meanwhile, the Mayer test produced white and yellowish precipitate. The flavonoid test shows the formation of an orange color. The tannin test changed the precipitate to a greenish-brown color, and the saponin test showed foam formation after shaking the sample solution. Broccoli is a source of flavonol derivatives, mainly sophorosides of quercetin, kaempferol, and hydroxycinnamic acid derivatives [14]. The content of alkaloids, flavonoids, and tannins in natural sources has anti-microbial properties [15]. Phytochemical analysis is crucial for evaluating a plant's possible medicinal utilities and determining the active principles responsible for its known biological activities.

3.3. Ash and Water content

The gravimetric method determines the ash and water content in the broccoli flower powders and extracts. The water content of the simplicia reached 5.7%, while the water content of the broccoli flower extract reached 5.8%. The determination of water content relates to the purity of the extract. Higher water content, exceeding 10%, may cause microbial growth, which will reduce the stability of broccoli extracts and powders [7].

The ash content in the simplicia was 4.9%, while in the Broccoli ethanol extract was 2.1%. The ash content may refer to the inorganic or mineral residue after removing organic matter through high heating. Minerals content in Broccoli such as Ca, Cu, Fe, K, Mg, Mn, P, and Zn are minor nutritional elements [12]. The ash content in this study was higher than researchers before (1.3±0.2%) [13]. This may cause ash, which is one of the results of coal combustion, a toxic by-product found in the environment or as a contaminant and has unhealthy impacts [14]. The location and the fat, water, and ash content of each species of macroalgae are also unknown. This is important to know because environmental factors can affect the survival of macroalgae, including the fat, water, and ash content in macroalgae.

3.4. Mineral Calibration Curve

ICP-OES and calibration curve methods are applied to determine the metal content in broccoli flowers. The wavelength values for each element are close to previous research from Savić et al., 2019 [11]. The different wavelength results are due to the many other energy levels. Excited atoms and ions can then emit their characteristic radiation simultaneously. This result will produce different emissions and allow the detection of several elements simultaneously.

There should be at least five concentration points on the sample. Based on the results from Table 1, there is a linear relationship between concentration and the corrected intensity with a coefficient of determination (R^2) 0.9970 - 0.9995. R^2 more than 0.8 means it indicates a perfect regression [18]. These values indicated that the greater the value, the more sensitive the method. Meanwhile, the intercept value in the linear regression equation showed that the smaller or closer to zero the value, the smaller the interference from other factors.

3.5. Mineral Content Results

Referring to the research results on the presentation of mineral content using ICP-OES, the high calcium content in broccoli flowers can cause hypertension. To reduce its calcium content, this research suggests boiling broccoli before consumption. Besides high calcium levels, the extract is also rich in vitamin C, which is suitable for consumption. They also contain glucoraphanin, natural anticancer sulforaphane compounds, and active thiocyanate compounds. Consuming them is helpful for anticancer [19]. The result shown in Table 1 is that potassium has a higher concentration than the other mineral content.

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Potassium plays a vital role in balancing physiological activities. This mineral also influences elements' growth and yield and broccoli's dry weight [20].

3.6. Flame Test

This qualitative analysis with platinum wire is a preliminary study to determine the mineral content. The result showed a positive flame color for calcium (brick red), potassium (lilac), sodium (bright yellow), and iron (brownish orange) [12].

The disadvantage of this flame test is the difficulty of detecting some elements in small quantities. Too many amounts also fade the flame's color and make it completely invisible.

Table 1. Calibration curve result

Parameter	Calcium	Potassium	Sodium	Iron
Linear regression	Y = 56424x + 28613	y = 61377x + 139877	y = 866.11x + 266.09	y = 41436x + 563.65
Coefficient of determination (R^2)	0,9996	0,9973	0,9995	0,9989
Mineral Content	109.52 ± 0.25 mg/100 g	375.58 ± 0.81 mg/100 g	9.54 ± 0.09 mg/100 g	1.93 ± 0.00 mg/100 g

4. CONCLUSION

According to the results, this research concludes that the simplistic characteristics based on the flame test produce positive values for potassium and calcium. The phytochemical test of the four compounds also produced positive values in the extract characteristics. Simplism and extracts' ash content is 4.9% and 2.1%, while water content and extracts are 5.7% and 5.8%.

The content of calcium, potassium, sodium, and iron in 100g of extract or simplicial is 109.52 ± 0.247 mg/g, 375.58 ± 0.805 mg/g, 9.54 ± 0.092 mg/g, and 1.93 ± 0.00 mg/g. Broccoli's mineral and secondary metabolite content can be developed into functional foods to prevent and treat certain chronic disorders.

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