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Research Article

Total Active Compounds and Mineral Contents in *Wolffia globosa*

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Wolffia globosa, or watermeal, is an aquatic plant belonging to the Lemnaceae family that is consumed as food and sold in local markets of Thailand. The aim of this study was to quantify selected active compounds and minerals in *W. globosa* ethanolic extract and evaluate its antioxidant activity. Total phenolic, flavonoid, and anthocyanin contents were analyzed. High-performance liquid chromatography was used for the determination of beta-carotene, ferulic acid, luteolin-7-O-β-D-glucoside, and kaempferol. Mineral contents (iron, potassium, calcium, magnesium, zinc, and sodium) were determined by atomic absorption spectroscopy. Antioxidative activity was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azobis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays. The beta-carotene, ferulic acid, luteolin-7-O-β-D-glucoside, and kaempferol contents of the extract were 2.52 ± 0.10, 1.40 ± 0.10, 2.42 ± 0.50, and 1.57 ± 0.14 mg/g extract, respectively. The highest mineral content in the *W. globosa* extract was magnesium. The wet extract of *W. globosa* showed higher amounts of all minerals than the dry extract. Freshly prepared and boiled *W. globosa* extracts showed radical scavenging activity at 1000 μg/milliliter with $75.77 \pm 0.93\%$ and $67.10 \pm 0.20\%$ inhibition of DPPH and $70.40 \pm 7.20\%$ and $59.78 \pm 3.16\%$ inhibition of ABTS, respectively. This plant is a promising novel source of natural phytochemical constituents and antioxidants and has potential for development as a plant-based nutraceutical product for the treatment of diseases caused by free radicals.

1. Introduction

Several classes of natural compounds generally found in plants have been reported to have antioxidant properties and reduce oxidative stress. Various kinds of plants such as *Moringa oleifera* and *Glycine max* (L.) Merr are sources of phytonutrients and minerals which have many biological activities [1–4]. These include flavonoids, phenolics, carotenoids, alkaloids, and some minerals [5, 6]. The accumulation of damage caused by free radicals arising from oxidative stress plays a crucial role in aging and diseases such as cancer, cardiovascular disease, and neurodegenerative diseases [7, 8]. The scavenging and deactivation of free radicals is one important mechanism of antioxidant activity. Free radicals are unstable atoms or molecules with unpaired electrons that react with proteins and DNA in human cells. Antioxidant defense mechanisms in plants and animals deactivate and remove these reactive molecules to prevent damage to tissues, but under conditions of oxidative stress, free radicals can overwhelm this defense system. Dietary intake of antioxidant compounds can prevent cell damage from oxidative stress and stabilize damaged cells by supplying electrons to free radicals and then eliminating them from the body. Many kinds of plants are used as sources of active compounds and elements in herbal products, nutraceuticals, cosmeceuticals, and food products.

Identifying active compounds and minerals in plants have received much attention, not only because of the perceived safety of natural products but also due to the synergistic mechanisms among the various kinds of compounds contained in plants.

Wolffia globosa, known as watermeal, khai nam, or khai phum, is a small, aquatic plant belonging to the Lemnaceae family of duckweeds as shown in Figure 1. The leaves and stem of this plant are fused together in a highly reduced rootless structure called a frond, which is less than 1 mm in diameter with a globular or oval shape, flattened upper surface [9, 10]. It has been reported that *W. globosa* fronds absorb nutrients through the underside, which is in contact with the water. This plant grows readily and is commonly found in nature. *W. globosa* is consumed as a vegetable in Myanmar, Laos, and the northern parts of Thailand. This plant has high protein content (approximately 40% dry weight) and high amounts of chlorophyll, carotenoids, flavonoids, and vitamins such as cyanocobalamin. All nine essential amino acids are also found in this plant [11]. Previous studies have revealed the presence of 20–35% protein, 4–7% fat, and 4–10% starch in plants from Lemnaceae, and *W. globosa* has been used as a protein source in animal feed and as a raw material for many industrial products including bioalcohol and biodegradable plastics [12, 13].

The nutritional value of this plant has long been recognized in the culinary traditions of Southeast Asia including Thailand. One study showed that consumption of *W. globosa* might have beneficial postprandial glycemic effects [14]. Another showed that the presence of cyanocobalamin, iron, and folic acid in this plant improved the health of prediabetic patients when included in a standard Mediterranean style diet [15]. *W. globosa* was also found to help maintain iron and folic acid status in humans and completely reverse iron deficiency anemia in an experimental rat model [16].

Thus, this plant is a rich, potential source of biologically active compounds. However, although the health-promoting effects of this plant have been reviewed, the plant components responsible for such biological effects have not yet been completely identified. Therefore, the aim of this research was to determine the biologically active components and minerals present in *W. globosa* and investigate its antioxidant activity in support of developing this plant as a nutraceutical product.

2. Materials and Methods

2.1. Materials and Equipment. *W. globosa* was collected from Nonmuang area, Muang District, Khon Kaen, Thailand. A specimen of the plant material (voucher number: SD10) was identified and authenticated by Assistant Professor Dr. Prathan Luecha, Division of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.

Folin-Ciocalteu phenol reagent, β-carotene, sodium acetate, ferulic acid, luteolin-7-O-β-D-glucoside, Trolox, kaempferol, and quercetin were purchased from Sigma-Aldrich (SM Chemical supplies Co., LTD., Bangkok, Thailand), ethanol, methanol, and ethyl acetate were purchased from VWR Chemicals BDH (SM Chemical supplies Co., LTD., Bangkok, Thailand), and gallic acid, hexane, and hydrochloric acid were purchased from Merck (Merck, Bangkok, Thailand). Sodium carbonate (Loba chemie, India), aluminum chloride (Ajax Finechem, Australia), potassium chloride (QRëC, New Zealand), acetone (RCI Labscan, Thailand), and ultrapure water were also used in this study. A rotary vacuum evaporator (Buchi: R-114, Switzerland), a freeze dryer (Labconco: 710611130, USA), an atomic absorption spectrophotometer (Perkin Elmer Inc: PinAAcle 900F, USA), a high-performance liquid chromatograph (Agilent Technologies:1260, USA), and an incubator microplate reader (Perkin Elmer Inc: HH3400, USA) were used.

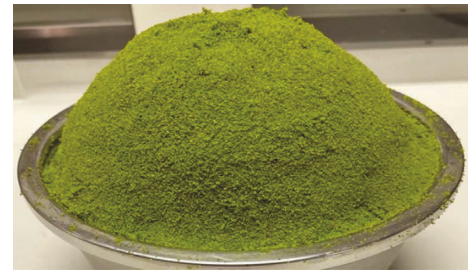

Figure 1: Fresh *Wolffia globosa* (watermeal, khai nam, or khai phum).

2.2. Preparation of Crude Extract. *W. globosa* was washed under running water until the water was clean and then boiled for 15 min. Then, the raw materials were dried at 50 degree Celsius and macerated with 95% ethanol (1000 ml) twice within 7 days. The extracts were filtered and evaporated using a vacuum rotary evaporator. The crude extracts were stored at −20 degree Celsius until needed for analysis.

2.3. Determination of Total Phenolic Content. The total phenolic content in *W. globosa* extract was determined using the Folin-Ciocalteu method [17, 18]. In brief, 20 microliters of the extract solution (1 mg of extract dissolved in 1,000 μl of ethanol) were added into 100 μl of the Folin-Ciocalteu reagent (10%) and 80 μl of sodium carbonate (7%). The reaction is shown as follows. After 30 minutes of incubation in the dark, the absorbance was measured at 760 nm using a microplate reader. The calibration curve of standard gallic acid solution was prepared in the range of 10–100 μg/ml and used to calculate the gallic acid equivalent content from the measured absorbance. The otal phenolic content is expressed as milligram gallic acid equivalents per gram of the extract (mg GAE/g extract).

$$\mathrm{Mo}(VI) + e^{-}\ (\text{antioxidant}) \mathrm{Mo}(V) \longrightarrow \mathrm{Mo}(V)\,(\lambda\mathrm{max} = 760\,\mathrm{nm}). \quad (1)$$

2.4. Determination of Total Flavonoid Content. The total flavonoid content in the extract was determined using the aluminum chloride colorimetric method [19]. The principle of this method involves the addition of $AlCl_3$ which could

form a stable acid complex with the ketone groups, hydroxyl groups, or orthodihydroxyl groups in flavonoid compounds which have a maximum absorption at a wavelength of 432 nm. In brief, twenty microliters of the extract solution were added to 15 μl of aluminum chloride, 20 μl of sodium acetate (10%), and 145 μl of distilled water. After 15 minutes of incubation in the dark, the absorbance was measured at 430 nm with a microplate reader. The calibration curve of standard quercetin solution (5–100 μg/ml) was prepared and used for the calculation of the quercetin equivalent from the measured absorbance. The otal flavonoid content is expressed as milligram quercetin equivalents per gram of the extract (mg QE/g extract).

2.5. Determination of Total Anthocyanin Content. The total anthocyanin content was determined using the pH differential method by measuring the absorbance at pH 1.0 and pH 4.5 with a UV-visible spectrophotometer [20]. Twenty microliters of the extract were mixed with 100 μl of 0.025 m potassium chloride solution (pH 1) or 100 μl of 0.4 m sodium acetate solution (pH 4.5). The absorbance was measured at 535 and 700 nm with a microplate reader. The total anthocyanin content is expressed as milligram cyanidin-3-glucoside equivalents per gram of the extract (mg c3g/g extract) and was calculated according to the following equation:

$$\text{monomeric anthocyanin} = (A \times M \times D \times 1000) \div (\text{molar absorptivity} \times 1), \quad (2)$$

where A is the absorbance, M is the molecular weight of a reference pigment (cyanidin-3-glucoside: 449.2 g/mol), D is the dilution factor = 10, and the molar absorptivity is the reference anthocyanin (extinction factor 26,900 $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$).

2.6. HPLC Analysis of Beta-Carotene. The analytical method for beta-carotene was adopted from the study by Khamphukdee et al. [21]. The analysis was carried out using HPLC with a diode array detector (Agilent Technologies: 1260, USA). The extract solution (10 mg in 1 ml of 80% acetone) was injected into a Hypersil ODS column (Thermo Scientific: 30105–254030, 250 × 4.0 mm i.d.; 5 μm particle size). The mobile phase consisted of acetonitrile, dichloromethane, and methanol in the ratio of 70 : 20 : 10. The flow rate for all the analyses was 1.0 ml/min. The absorbance was measured at a wavelength of 450 nm. The beta-carotene content was determined using the standard curve plotted between the peak area and concentration of standard solutions (0.25–30 μg/ml) and calculated.

2.7. HPLC Analysis of Ferulic Acid, Luteolin 7-O-β-D-Glucoside, and Kaempferol and Validation Method. The modified HPLC method with a diode array detector (Agilent Technologies:1260, USA) was carried out for the analysis of these three compounds [22]. A reversed phase HPLC column (ACE Generix 5, C18, 150 × 4.6 mm) was used. The mobile phase consisted of solvent A (ultrapure water) and solvent B (0.25% acetic acid in 80% methanol) which was run in gradient elution (0–3 min, solvent A was changed from 98 to 80%; 3–15 min, solvent A was changed from 80 to 0%; 15–20 min, solvent A was changed from 0 to 10%; and 20–25 min, solvent A was changed from 10 to 98%). The flow rate was 1.0 ml/min, and the total run time was 25 min. The detection wavelength was set at 340 nm. The analytical method was validated using some parameters to ensure the reliability. Accuracy, precision, linearity, limit of detection, and limit of quantitation were tested.

2.8. Elemental Analysis by Atomic Absorption Spectroscopy (AAS). An atomic absorption spectrophotometer was used for the analysis of potassium, sodium, magnesium, calcium, iron, and zinc. The type of hollow cathode lamp and the detection wavelengths were selected depending on each mineral (λ589.0 for sodium, λ248.33 for iron, λ213.86 for zinc, λ766.49 for potassium, λ422.67 for calcium, and λ285.21 for magnesium). An air-acetylene flame was used for the analysis. Samples were prepared by the dry ashing method. Two kinds of raw materials, wet and dry samples, were analyzed in this study. Dry samples were dried at 50 degree Celsius. For the wet sample, three grams of *W. globosa* were weighed in a porcelain dish and burned in a muffle furnace at 250 degree Celsius for 20 min and then 480 degree Celsius for 8 hr. The sample ash was cooled, 50 milliliters of 25% nitric acid were added, and the mixture was filtered. The filtrate was analyzed by AAS. For dry samples, one gram of the dried sample was burned using the same process as for the wet sample, and the filtrate was then analyzed by AAS. Stock standard solutions of all elements (40 ppm) were diluted to 0.8–4.8 ppm for the determination of iron, potassium, and calcium, 0.032–0.8 ppm for the determination of magnesium, 0.4–2 ppm for the determination of zinc, and 0.48–3.84 ppm for the determination of sodium. Standard curves of each set of standard solutions were prepared for the determination of each element by AAS.

2.9. Determination of Radical Scavenging Activity Using the DPPH Reagent. The free-radical scavenging method was adopted according to the method of some researchers [23]. In brief, the DPPH reagent was prepared by dissolving DPPH (7.9 mg) in ethanol (100 ml), which was then stored at −20 degree Celsius prior to use. The extract (10 mg/milliliter, 100 microliters) and DPPH reagent (100 microliters) were transferred into microplate wells and kept for 30 minutes at room temperature. Then, the absorbance was measured at 517 nm using a microplate reader (Perkin Elmer Inc: HH3400, USA). A calibration curve was plotted for

10–50 μM concentrations of Trolox, the reference standard. The inhibitory percentage of DPPH was calculated by the following formula:

$$\%\text{inhibition} = \left[\left(A_{\text{DPPH}} - A_{\text{sample}}\right) \div \left(A_{\text{DPPH}} - A_{\text{blank}}\right)\right] \times 100, \quad (3)$$

where A_{DPPH} is the absorbance of DPPH radical solution (without sample or standard) and A_{sample} is absorbance of a DPPH solution (with sample or control). The inhibitory concentration at 50% (IC50) was then calculated.

2.10. Determination of Radical Scavenging Activity Using ABTS Radical Cation Decolorization. This method was adapted from a method formerly published [21, 24]. The $ABTS^{\bullet+}$ was prepared by incubating ABTS with potassium persulfate and keeping in the dark at room temperature for 12 hr. One milliliter of $ABTS^{\bullet+}$ solution was diluted by adding ethanol (50 milliliter) to obtain an absorbance of 0.70 ± 0.02 at wavelength 734 nm. The extract (50 μl) in various concentrations and $ABTS^{\bullet+}$reagent (100 μl) were transferred into microplate wells and kept at room temperature for 2 hr. Absorbance was measured at 734 nm. The calibration curve fromTtrolox standard solution (10–50 μM) was plotted.

The percentage inhibition of ABTS was calculated using the following formula:

$$\%\text{inhibition} = \left[\left(A_{\text{ABTS}} - A_{\text{sample}}\right) \div \left(A_{\text{ABTS}} - A_{\text{blank}}\right)\right] \times 100, \quad (4)$$

where A is the absorbance. Inhibitory concentration at 50% (IC50) was then calculated.

2.11. Statistical Analysis. All results are expressed as the mean ± standard deviation (SD) of three replicates for the antioxidant activity assays and the determination of the active content of the samples.

3. Results and Discussion

3.1. The Content of Total Active Compounds and Beta-Carotene in W. globosa Extract. Total active contents were analyzed using standard curves of quercetin and gallic acid for the total flavonoid and total phenolic content. The total anthocyanin content was calculated as described above. The beta-carotene content in *W. globosa* extract was determined by HPLC and using a standard curve of beta-carotene. The total flavonoid, total phenolic, total anthocyanin, and beta-carotene contents of *W. globosa* extract are shown in Table 1. Phenolic, flavonoid, and beta-carotene contents found in the *W. globosa* extract in the present study were similar to those of other studies [25, 26]. Anthocyanin content has not been previously reported for *W. globosa*. These compounds have previously been shown to have biological activities, especially in protection against cell damage by oxidative stress, which plays an important role in aging, cancer, cardiovascular disease, and Alzheimer's disease.

An HPLC method was developed and used for the analysis of ferulic acid, luteolin 7-O-β-D-glucoside, and kaempferol in the *W. globosa* extract. All three peaks were separated in the HPLC chromatogram, and the resolution between peaks was more than 2, which shows complete separation. The validation of the HPLC method for determination of these compounds was satisfactory for some important parameters including accuracy (% recovery is in the range of 80–110), within-day precision, between-day precision (% RSD less than 7.3), linearity (r^2 more than 0.995), limit of detection, and limit of quantitation (Table 2). Thus, this developed HPLC method was appropriate to analyze these compounds in the *W. globosa* extract. Fingerprint HPLC chromatograms of the *W. globosa* extract are shown in Figure 2. The ferulic acid, luteolin 7-O-β-D-glucoside, and kaempferol contents in *W. globosa* extract were 1.40 ± 0.10, 2.42 ± 0.50, and 1.57 ± 0.14 mg/g extract, respectively, from three replicates. These three active compounds might be used as markers for the quality control of *W. globosa* raw materials, and their levels support the development of a nutraceutical product from this plant [21, 23]. However, this study had the limitation that the confirmation to improve the identification of each compound should be performed by some specific techniques such as liquid chromatography-mass spectrometry.

Table 1: Total active contents of the *W. globosa* extract.

Active compounds	Content (mg/g extract ± SD, three replications)
Total flavonoid	38.99 ± 0.44
Total phenolic	40.83 ± 4.99
Total anthocyanin	0.47 ± 0.08
Beta-carotene	2.52 ± 0.10

3.2. Mineral Content in the W. globosa Extract by AAS. Potassium, sodium, magnesium, calcium, iron, and zinc were analyzed by atomic absorption spectrophotometry. The analytical method was tested to confirm the reliability of the method, and the contents of all minerals are shown in Table 3. A comparison of the mineral content of wet and dry samples is shown in Figure 3.

The highest mineral content in both wet and dry *W. globosa* samples was magnesium.The mineral content in the wet sample was higher than in the dry sample except calcium. However, no definite conclusion could be drawn as which method is the best. This study was the only case study which showed the effect of different characteristics of samples on the mineral content. Probably, this might be due to the heating effect of the drying minerals which do escape or vaporize, and as such, lower values of minerals such as Mg was seen in this study. A previous study showed that the iron content in *W. globosa* extract was bioavailable and efficient at treating iron deficiency anemia in a rat model [16]. The important value-added elements of this plant are iron, potassium, calcium, magnesium, zinc, and sodium. Magnesium is an essential mineral for humans. One of magnesium's main roles is acting as an enzyme cofactor in biochemical reactions involved in energy creation, protein formation, gene maintenance, muscle movement, and nervous system regulation. Magnesium has also been shown to reduce symptoms of depression and have beneficial effects against type 2 diabetes [27]. Magnesium can also exhibit

Table 2: Validation results of the analysis of ferulic acid, luteolin 7-O-β-D-glucoside, and kaempferol in the *W. globosa* extract by the HPLC method.

Parameters	Ferulic acid	Luteolin 7-O-β-D-glucoside	Kaempferol
Range (μg/milliliter)	4–15	20–100	4–15
Linearity			
Regression equation	$y = 73.341x - 65.298$	$y = 59.273x - 68.148$	$y = 57.638x - 40.183$
Coefficient of determination	0.9988	0.9951	0,9989
Percentage recovery	102.59 ± 1.57	98.77 ± 4.67	101.39 ± 4.48
Precision (%RSD)			
Within-day	0.45–1.39	1.02–1.72	1.81–2.54
Between-day	1.81–5.38	2.99–3.64	3.82–6.20
Limit of detection (μg/milliliter)	2.0	2.5	2.0
Limit of quantitation (μg/milliliter)	4.0	10.0	4.0
Retention time (minute)	11.2	11.8	15.3
Wavelength detection (λ, nm)	340	340	340

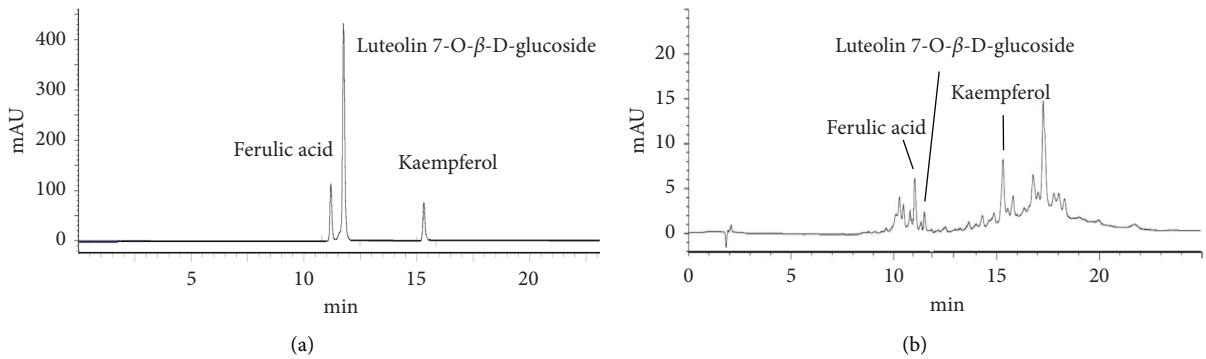

Figure 2: HPLC chromatograms of standard ferulic acid, luteolin 7-O-β-D-glucoside, and kaempferol solutions (a) and the fingerprint HPLC chromatograms of *W. globosa* extract (b) at wavelength 340 nm.

Table 3: The contents of all minerals in *W. globosa* and their validation results.

Parameters	Fe	K	Ca	Mg	Zn	Na
Range (μg/milliliter)	1.60–4.80	0.80–4.00	0.80–4.00	0.032–0.800	0.40–2.00	0.48–3.84
Linearity						
Regression equation	$y = 0.039x - 0.424$	$y = 0.231x + 0.281$	$y = 0.052x + 0.094$	$y = 0.981x + 0.073$	$y = 0.267x + 0.109$	$y = 0.210x + 0.090$
Coefficient of determination	0.9971	0.9923	0.9988	0.9938	0.9927	0.9943
Percentage recovery	99.33 ± 2.85	100.49 ± 10.71	100.99 ± 2.83	101.84 ± 4.87	101.50 ± 5.18	103.39 ± 6.54
Precision (% RSD)						
Within-day	0.00–1.89	1.18–1.70	2.32–3.76	0.49–4.57	0.00–0.91	1.01–2.98
Between-day	0.00–2.79	1.43–2.81	0.33–0.76	1.09–3.78	0.49–2.21	0.56–5.56
Limit of detection (μg/milliliter)	0.037	0.003	0.016	0.001	0.009	0.009
Limit of quantitation (μg/milliliter)	0.120	0.012	0.058	0.005	0.030	0.030
Amount						
Wet sample (mg/g)	0.419 ± 0.020	1.007 ± 0.044	0.059 ± 0.006	1.447 ± 0.321	0.425 ± 0.020	1.087 ± 0.543
Dry sample (mg/g)	0.137 ± 0.011	0.279 ± 0.036	0.282 ± 0.054	0.937 ± 0.076	0.001 ± 0.000	0.647 ± 0.259

anti-inflammatory benefits, and low magnesium intake has been linked to chronic inflammation, one of the drivers of aging, obesity, and chronic disease [28]. *W. globosa* might be counted among pumpkin seeds, boiled spinach, boiled swiss chard, dark chocolate, black bean, cooked quinoa, almonds, cashews, avocado, and salmon as excellent food sources of magnesium [28]. Sodium and potassium were the minerals with the succeeding highest values found in *W. globosa* in this study. These minerals can stimulate cell proliferation and mitochondrial activity, which decreased the expression of some aging markers and showed beneficial effect on keratinocytes damaged by UV exposure [29].

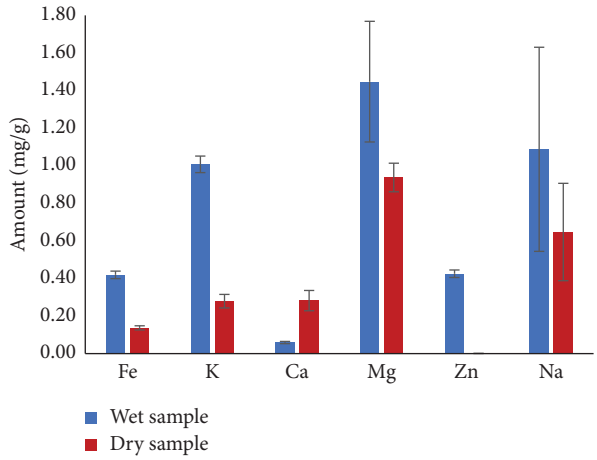

Figure 3: The content of all minerals in wet and dry extracts of *W. globosa*.

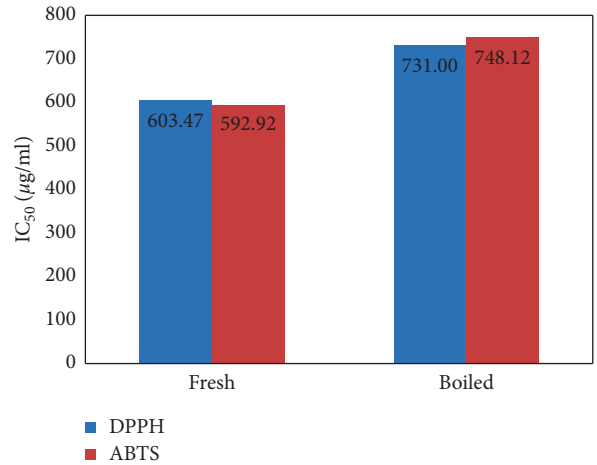

Figure 4: IC_{50} of the free-radical scavenging assay of extracts from fresh and boiled *W. globosa*.

However, the origin of a plant and the season of harvesting can affect the levels of minerals [30]. Generally, *W. globosa* is harvested in the rainy season. Thus, changes in the growth conditions, the harvest season, and the source or origin of the plants should all be studied to improve the plant materials and increase the potential of this plant to be a super food in the future.

3.3. Radical Scavenging Activity of the W. globosa Extract by DPPH and ABTS. The free-radical scavenging ability of freshly prepared and boiled samples of the *W. globosa* extract was tested using DPPH and ABTS assays. At a concentration of 1000 μg/milliliter, the fresh and boiled samples showed percentage inhibition of scavenging activity by DPPH at $75.77 \pm 0.93\%$ and $67.10 \pm 0.20\%$, respectively. By ABTS, the fresh and boiled samples showed percentage inhibition of scavenging activity of $70.40 \pm 7.20\%$ and $59.78 \pm 3.16\%$, respectively, at the same concentration. IC_{50} of the standard Trolox solution was $26.07 \pm 0.02\,\mu$M for DPPH and $21.05 \pm 0.07\,\mu$M for ABTS. Their IC_{50} is shown in Figure 4.

This result corresponded with a previous report that showed freshly prepared *W. globosa* extract had higher radical scavenging activity than boiled *W. globosa* extract [25]. The scavenging activity of *W. globosa* might be related to the amounts of the specific phytochemical constituents found in *W. globosa*. A previous study revealed that the total phenolic and beta-carotene contents of the boiled *W. globosa* extract were less than of the freshly prepared sample. This might be due to the effects of high temperature during boiling, which can cause degradation and loss of some phytochemical constituents. The antioxidant activity of *W. globosa* has previously been attributed to the phenolic compounds and flavonoids present in extracts [26]. Phenolic and flavonoid compounds have been reported to be associated with antioxidation activity in various plants [31–33]. The result of this study conformed to the study by Tipnee in 2017, which reported the anti-inflammatory and antioxidant activities were as a result from the phytosterols, carotenoids, and tocopherols. Moreover, the *W. globosa* extract was not toxic to the human fibroblast (HDFn) cells [26]. Antioxidant activities from these natural compounds might perform many mechanisms of action such as radical scavenging, chain breaking, and metal chelation. Several types of compounds found in the plant raw material might show synergistic effect. The phytochemical compounds found in this study were flavonoids, phenolics, anthocyanins, and carotenoids. Because different phytochemical constituents in this plant could show different mechanisms of action, further study about the other antioxidant tests should be carried out to get enough information about its activities. A few studies examined the phytochemical constituents of this plant. The phytochemical content could be used in the quality control aspect. This could be used to confirm the quality of raw material in different batches or different sources of the raw material. Moreover, a previous study revealed a moderate correlation between the potassium content of vegetables and their antioxidative activity, as determined by DPPH and ABTS radical scavenging assays [34]. Thus, the total active compounds and minerals found in *W. globosa* in the present study could be a key performance indicator for antioxidant activity of *W. globosa*. This study revealed some interesting aspects regarding to the value-added and sustainable food sources. The biodiversity of plants is related to the chemical constituent in the plant. Thus, knowledge about the quality control of the raw material will be the appropriate channel to improve the potential of this plant.

4. Conclusions

This study was an overall view of the phytochemical composition of *W. globosa* that included the determination of the total phenolic, flavonoid, and anthocyanin contents, the identification of potential biomarkers (beta-carotene, ferulic acid, luteolin 7-O-β-D-glucoside, and kaempferol), and the levels of selected minerals (iron, potassium, calcium, magnesium, zinc, and sodium), coupled with the evaluation of its radical scavenging activity. This plant exhibited free-radical scavenging activity that correlated with the beta-carotene, ferulic acid, luteolin- 7-O-β-D-glucoside, and kaempferol

phytochemical contents, all of which have been shown to have antioxidant activity. However, as this study covered only some antioxidant mechanisms, further investigation of other mechanisms is needed prove the efficiency and potential of this plant as a new promising source of nutraceutical compounds. The comparison among sample characteristics should be further investigated to confirm the content of minerals in the raw material.

Data Availability

The main part of the data is included within the content. Other datasets can be made available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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