Research Article

Nutritional Properties of Nine Microgreens Consumed in Thailand

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Abstract
Microgreens are considered to be novel functional foods based on their nutritional properties. Compositional data of bioactive compounds and mineral nutrients with different conditions are crucial for customers. This study aimed to determine the nutritional values of some microgreens that were commonly consumed in Thailand. Nine microgreens from five families were analyzed for total anthocyanins, β-carotene and antioxidant activity by microplate reader, while vitamin C was assayed by a reflectometer. Mineral compositions of calcium, potassium, iron, and zinc were analyzed using flame atomic absorption spectroscopy with air acetylene. Microgreens were found to be a good source of antioxidant, vitamin C, K and Ca. Chinese radish accumulated the highest amount of vitamin C and Fe, at 404 µg/g fresh weight (FW) and 3.79 mg/g dry weight (DW), respectively, while red cabbage was the good source of β-carotene, accounting for 2.01 mg/g FW and Ca, at 283 mg/g DW. Water spinach and red sorrel were rich in potassium, at approximately 376 and 301 mg/g DW, respectively. However, people with impaired kidney conditions could choose sunflower and Chinese radish microgreens to avoid the adverse effects on heart function, while still receiving other nutrients from them. In conclusion, this study provided crucial information for microgreens selection that are consumed in Thailand.

Keywords: Vegetable seedlings, Microgreens, Bioactive compound, Mineral composition, Correlation

1 Introduction

Microgreens are a new group of specialty crops, defined as immature vegetables produced from the seeds of vegetables, herbs, or grains usually harvested aboveground within 7–21 days [1]. These products gain popularity due to their vivid colors, fresh textures, and also high contents of phytonutrients [1], [2]. Microgreens are a great source of food nutrients and are considered as superfoods or functional foods due to the benefits of nutrients that lessen the risk of diseases. The study from the U.S. Department of Agriculture (USDA) and University of Maryland reported the amount of vitamins and minerals from microgreens were much higher, compared with the regular vegetables [1]. For example, microgreens contained higher amount of vitamin C for 6 times, 400 times in vitamin E and 60 times in vitamin K comparing to the vegetables in a regular ripening stage, which are considered to fulfill daily intakes of these vitamins based on WHO recommendations [1]. These three vitamins are known as the antioxidant vitamins. Vitamin A is an important bioactive compound needed in small amount in human to function in the optical system, in growth and development; and maintain of immune system. Provitamin A, such as β-carotene can be found in green leafy vegetables (e.g. amaranth, kale and young leaves of vegetables), yellow vegetables (e.g. yellow squash and carrots) and yellow-to-orange non-citrus fruits (e.g. papayas and mangoes) [3]. Vitamin C (or ascorbic acid) is an essential nutrient involving in many physiological functions in human body, such as collagen synthesis, neuromodulator and maintenance of immune system. Vitamin C deficiency commonly results in scurvy. Fruits are well-known as the main dietary sources of vitamin C, especially in citrus fruits. However, vegetables, such as cabbage, broccoli, bean sprouts and bell pepper may be more important sources of vitamin C due to the longer harvesting periods [4]. Antioxidants are substances that can prevent the damage of free radicals and reactive oxygen species (ROS). Dietary antioxidant is defined as a substance in food that essentially reduces the adverse effects of ROS and/or reactive nitrogen species (RNS) in human. The damage from these radicals results in inflammatory diseases, heart diseases, cancers or neurological disorders e.g. Alzheimer's disease, Amyotrophic lateral sclerosis (ALS) disease and muscular dystrophy [5]. Anthocyanins are also considered as other sources of antioxidants. They can be found in many microgreen species with red or purple pigment in the seedlings such as rosette, red cabbage and red amaranth. There have been many articles reporting the health benefits of anthocyanins in many disease, such as cardiovascular diseases, anticancer effect, diabetes and antimicrobial function [6]–[10].

Essential minerals are other necessary nutrients for human health. They can be distinguished into 2 groups: macroelements (e.g. Ca, Mg, P, K and Na) and microelements (e.g. Fe, Zn and Mn). Malnutrition in these elements can cause metabolic disorders, organ damage or severe symptoms needing hospitalization. For example, calcium (Ca) plays an important role in bone mass and metabolism. Hypocalcemia results in several symptoms including memory loss, muscle cramps and easy fracturing of bones, which are critical for aging adults [11]. A recent study showed that microgreens provided a higher content of several essential elements including Ca, Mg, Fe and Zn [12]. Potassium (K) is another macroelement that controls nerve function, muscle metabolism and regulates fluid balance in the human body. K deficiency causes chronic vomiting, diarrhea and breathing difficulties. Based on the World Health Organization recommendation for K consumption (at least 3,500 mg/day), the risk of insufficient K intake is considered to be low for a generally healthy population. However, when kidneys fail, they are not able to remove excess K, resulting in chronic kidney disease (CKD). These patients are suggested not to take K-rich food, such as raw leafy vegetables. Although the concentrations of K in different species of microgreens range from 82 to about 400 mg/100 g fresh weight (FW), the amount of K accumulation in mature leafy vegetables is higher than in microgreens [12], [13]. Iron (Fe) is another necessary mineral that the body needs for growth and development. Fe has many essential functions in the body, especially for serving as an oxygen carrier in the red blood cell. Additionally, Fe functions as a co-factor for several enzymes including enzymes for steroid hormone synthesis; enzymes for liver detoxification and enzymes for controlling neurotransmitters [14], [15]. Zinc, one of the essential microelements, is important for the maintenance of the immune system and response to antioxidant stress, especially for aging adults. Due to aging, the intake of Zn declines from inadequate diet or intestinal malabsorption, causing an increased incidence of age-related degenerative diseases [16].

There have been several studies that reported the nutrient compositions in microgreens (Table 1). In Thailand, microgreen consumption has been become a healthy trend. However, many studies focused on commercial microgreens that were used internationally. There rarely were studies on the nutritional values of microgreens in Thailand, and thus, the aim of this study was to investigate the nutritional composition of nine microgreens being consumed in Thailand.

Table 1: Studies of nutrient compositions in some microgreens

Microgreen	Nutrient	Reference
Amaranth	Beta-carotene, vitamin C	[17]
Basil	Vitamin A, vitamin C, vitamin E, Ca, Mg, K, Na, Fe, Zn	[18]
Broccoli	P, K, S, Mg, Mn, Zn, Fe, Ca, Na, Cu, Al, B	[19]
Carrot	Vitamin A, vitamin C, vitamin E, Ca, Mg, K, Na, Fe, Zn	[18]
Kale	K, Ca, Mg, P, Na, Fe, Mn, Zn, Cu	[20]

Table 1: Studies of nutrient compositions in some microgreens (Continued)

Microgreen	Nutrient	Reference
Lettuce	Ca, Mg, P, Fe, Mn, Cu, Zn, Mo	[12]
Mungbean and soybean	Vitamin C, Ca, Fe, Zn	[21]
Spinach	Carotenoids, vitamin B9, vitamin C, vitamin E, vitamin K, Ca, Mg, K, P, Na, Fe, Zn	[1], [18]
30 varieties of Brassicaceae microgreeens	Ca, K, Mg, P, Na, Fe, Cu, Mn, Zn	[13]

2 Materials and Methods

2.1 *Plant materials*

Greenhouse-grown microgreens of nine vegetable seed varieties (Figure 1, Table 2) were obtained at Kasetsart University, Bangkok, Thailand. The experiment was designed as completely randomized design (CRD) with three replications. The vegetable seeds were planted by soaking in warm water for 12 h, planting in a plastic box (8.3 × 11.6 cm) and peat moss was used as a growing substrate, then placed in the greenhouse conditions (at the average temperature and relative humidity as 30.4 °C and 73.1%, respectively) with natural light condition (12 h light exposure and 12 h dark time). Microgreens were harvested aboveground parts in 10–12 days after sowing (DAS) to reach the 2-true leaf stage, depending on plant variety, and measured for the content of bioactive compounds and mineral compositions afterward.

2.2 *Bioactive compound assays*

2.2.1 Anthocyanin assay

One gram of each microgreen was extracted in 1% HCl in methanol as described by Mizukami *et al.* [22], then incubated at room temperature in dark for 24 hours. After that, samples were centrifuged for supernatants, which were measured at 530 nm and calculated for the amount of anthocyanin content as shown in Equation (1). The results were calculated in mg/g FW.

$$\text{Total Anthocyanin (mg/g FW)} = ((27.208 \times A_{530} + 0.059) \times \text{solvent (mL)}) \times ((10/1000) \times \text{FW (g)}) \quad (1)$$

Figure 1: Aboveground parts of the microgreens used in the experiment at the harvesting stages: (a) Sunflower, (b) Chinese radish, (c) Indian mustard, (d) water spinach, (e) Thai rat-tailed radish, (f) sugar pea, (g) red sorrel, (h) red cabbage, and (i) cabbage.

Table 2: Common names, plant family names, scientific names and harvesting times of 9 vegetable varieties used in the experiment

Common Name	Family	Scientific Name	Harvest Time (DAS)
Sunflower	Asteraceae	*Helianthus annuus*	10
Chinese radish	Brassicaceae	*Raphanus sativus* var. *longipinnatus*	10
Indian mustard	Brassicaceae	*Brassica juncea*	12
Water spinach	Convolvulaceae	*Ipomoea aquatica*	10
Thai rat-tailed radish	Brassicaceae	*Raphanus sativus* var. *caudatus*	10
Sugar pea	Fabaceae	*Pisum sativum*	12
Red sorrel	Malvaceae	*Hibiscus sabdariffa*	12
Red cabbage	Brassicaceae	*Brassica oleracea* var. *rubra*	12
Green cabbage	Brassicaceae	*Brassica oleracea* var. *capitata*	12

2.2.2 β-carotene assay

β-carotene content was determined using the modified method from Nagata and Yamashita [23] and results were expressed as mg/g FW. Each fresh microgreen sample (1 g) was grounded in 4:6 acetone-hexane solvent and incubated at room temperature for 1 h. Samples were centrifuged and collected supernatants, which were measured in microplate reader (Thermo Fisher Scientific, Massachusetts, USA) at 435, 505, 645 and 663 nm then calculated the amount of β-carotene from Equation (2) below.

$$\beta\text{-carotene (mg/g FW)} = 0.216A663 - 1.22A645 - 0.304\,A_{505} + 0.452A_{453} \quad (2)$$

2.2.3 Vitamin C measurement

Ascorbic acid in microgreen samples was measured by the modified method from Pantelidis *et al.* [24]. Fresh microgreen (5 g) was ground and dissolved in reverse osmosis water (RO) then filtered. Filtered samples were spotted on strip paper and measured ascorbic content in a reflectometer (Merck, Darmstadt, Germany). The results were shown in μg/g FW.

2.2.4 Antioxidant activity assay (DPPH radical scavenging activity test)

Two grams of each fresh microgreen were collected and extracted in 4 mL ethanol as described by Sensoy *et al.* [25]. Samples were measured with a stable radical, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) as described by Ingkasupat *et al.* [26] with some modifications, by carrying out in a 96-well microplate using Microplate Reader. 22 mL of each plant extract was added to 200 μL of 0.1 mm DPPH radical solution in ethanol and incubated for 30 min at room temperature under dark condition. Each mixed solution was measured at 517 nm, by using 95% aqueous ethanol as a control. The DPPH radical scavenging activity (%) was calculated using the Equation (3) below.

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

2.3 *Element composition assay*

Microgreen samples were dried in the oven for 72 h at 60 °C to constant dry weights. 0.5 g of dried samples were digested in 10 mL concentrated nitric acid (AnalaR grade, BDH 69%) at 40 °C for 1 h then 140 °C for at least 3 h. After cooling down, samples were topped up with double de-ionized water to 40 mL then filtered through Whatman no. 1 filter paper (Buckinghamshire, UK). Samples were stored in acid-washed pill boxes and analyzed using an air-acetylene flame atomic absorption spectroscopy (Perkin-Elmer model Analyst 800, Massachusetts, USA). Standard solutions for Ca, K, Fe and Zn were prepared from 1,000 ppm stock solution provided by Merck Titrisol [27]. The concentrations were calculated in mg/g DW.

2.4 *Statistical analysis*

Results are presented as means of three replications along with standard deviations. Differences in nutrient compositions across varieties were determined using analysis of variance (ANOVA) followed by multiple comparisons as (DMRT) at $P = 0.05$ to determine significant differences. Pearson correlation coefficients among traits were determined by pairwise correlation. All statistical analyses were performed using R software [28].

3 Results and Discussion

3.1 *Bioactive compounds and antioxidant activity*

Anthocyanin, β-carotene, vitamin C contents and antioxidant activity are listed in Table 3. Anthocyanins are considered an efficient antioxidant in scavenging free radicals [29]. Anthocyanin content was highest in red cabbage (79.76 mg/g FW), followed by sugar pea (36.21 mg/g FW), red sorrel (14.56 mg/g FW), and cabbage (12.43 mg/g FW), respectively. Even though anthocyanins are the red-to-blue pigments in fruit and vegetables, we found that it was not necessary related to the purple color in microgreens. These results were similar to the study by Jones-Baumgardt *et al.* [30], which found that red mustard cv. 'Ruby Streaks' had the highest concentration of total anthocyanins followed by green cabbage, red kale cv. 'Red Russian' and arugula, respectively, in every light intensity.

Table 3: Content1 of anthocyanins, β-carotene, vitamin C content and antioxidant activity measurement in nine varieties of Thai microgreens per 1 g edible portion of fresh weight

Micro Greens	Antho Cyanins (mg)	β-carotene (mg)	Vitamin C (μg)	Antioxidant Activity (%)
Sunflower	7.32 ± 0.77[b]	0.623 ± 0.045[b]	110.67 ± 58.73[c]	20.18 ± 0.90[de]
Chinese radish	5.71 ± 0.46[b]	0.017 ± 0.006[b]	404.67 ± 123.00[ab]	69.79 ± 2.87[a]
Indian mustard	11.77 ± 0.96[b]	0.647 ± 0.015[b]	162.00 ± 33.05[bc]	62.24 ± 2.18[abc]
Water spinach	9.67 ± 1.46[b]	0.493 ± 0.012[b]	318.67 ± 66.04[abc]	13.77 ± 1.52[e]
Thai rat-tailed radish	11.49 ± 0.59[b]	0.243 ± 0.042[b]	212.00 ± 14.00[abc]	32.26 ± 3.66[abcde]
Sugar pea	36.21 ± 2.94[b]	1.870 ± 0.197[a]	483.33 ± 85.66[a]	27.79 ± 3.32[cde]
Red sorrel	14.56 ± 0.47[b]	0.313 ± 0.006[b]	NA	29.31 ± 4.06[bcde]
Red cabbage	79.76 ± 4.45[a]	2.010 ± 0.079[a]	137.33 ± 30.02[bc]	69.01 ± 2.67[ab]
Cabbage	12.43 ± 0.61[b]	0.263 ± 0.012[b]	80.00 ± 18.33[c]	59.42 ± 4.50[abcd]

[1] Values are means ± standard deviation. Means within a column with different upper case letters are significantly different at $P < 0.05$. NA = not available.

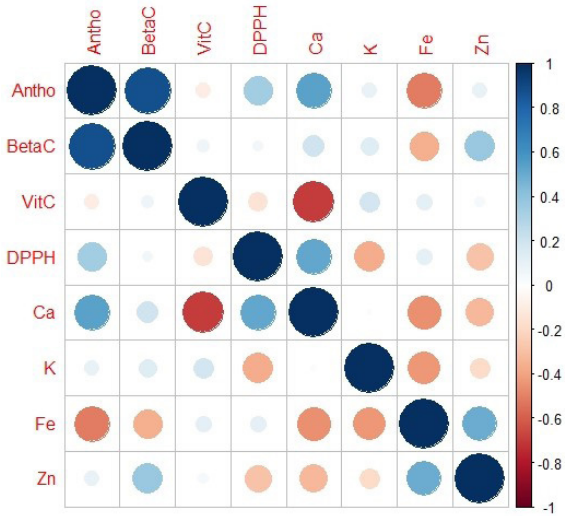

Figure 2: Correlation matrix calculating from Pearson correlation among bioactive compounds and mineral compositions in nine microgreens. Circles show the correlations between two traits including Antho (anthocyanin content), BetaC (β-carotene content), VitC (vitamin C content), DPPH (antioxidant activity), Ca (calcium), K (potassium), Fe (iron), and Zn (zinc). Blue circles show positive correlations between traits whereas red circles represent negative correlations between traits.

In this study, the highest amount of β-carotene content was found in red cabbage (2.01 mg/g FW), followed by sugar pea (1.87 mg/g FW). In adults and elderly, World Health Organization (WHO) recommends people to uptake vitamin A at about 600 μg RE/day (which equals to 2,400 μg in β-carotene form) [4]. Consuming red cabbage microgreen about 12 g/day or 13 g/day for sugar pea seedling provides enough β-carotene for vitamin A's daily requirement. Considering to the Pearson correlation between anthocyanin and β-carotene accumulation, there was a strong positive correlation between these two bioactive compounds (Figure 2), corresponding to the study by El-Nakhel *et al.* [31] who found that anthocyanin and β-carotene accumulation tended to positively correlate in three Brassica microgreens. This result was different from the accumulations of anthocyanin and β-carotene in mature plants, which would have significantly negative correlations between these bioactive compounds [32], [33].

Due to our research, the amount of ascorbic acid was highest in sugar pea, which accounted for 483 μg/g FW, followed by Chinese radish and water spinach at 405 and 319 μg/g FW, respectively. Based on WHO recommendation, vitamin C intake should be around 45 mg/day for adults and elderly. Thus, intake of these microgreens between 9–15 g/day should be sufficient for daily need of vitamin C. Many studies including our results found that many microgreens, such as pea sprouts, broccoli, and cauliflower and water spinach contained a higher concentration of vitamin C over 100 μg/g FW, which would be sufficient for daily requirements [34].

Antioxidant properties have the potential beneficial effects in protecting against diseases. β-carotene and other carotenoids, vitamin C and phenolic compounds are considered to have antioxidant activity [35]. In our study, antioxidant activity from DPPH inhibiting reaction in nine microgreens ranged from 20.18 to 69.79% with high inhibitions in Chinese radish

(69.79%), red cabbage (69.01%), Indian mustard (62.24%) and cabbage (59.42%). All high antioxidant microgreens belonged to family Brassicaceae due to their high phytochemical contents including vitamin C, β-carotene and total anthocyanin contents, which was similar to the results from Xiao *et al.* [34]. Moreover, the correlation matrix also shows the positive correlation between antioxidant compounds, including anthocyanin and β-carotene, and the antioxidant activity measuring from DPPH scavenging procedure (Figure 2).

3.2 *Mineral composition*

In order to determine the mineral element composition of microgreens, macro- and microelement minerals including calcium (Ca), potassium (K), iron (Fe) and zinc (Zn) were measured in nine microgreens (Table 4). The concentrations of each element varied with the species of microgreens. The most abundant macroelement in most microgreens was K, followed by Ca, Fe and Zn, respectively. These results were similar to Xiao *et al.* [13] who found that the concentration of K was the highest in every varieties, followed by P, Ca, Mg and Na, while the microelements had much lower concentrations than macroelements. Potassium was found in the highest concentration in water spinach and red sorrel at 377 and 302 mg/g DW, respectively. Since potassium is an essential mineral for electrolyte balance and DNA synthesis, WHO recommendation is a consumption of K for 3,500 mg/day in normal adults to reduce the possibility of cardiovascular sickness [36]. Therefore, microgreens, especially for water spinach and red sorrel, about 9–12 g DW should be sufficient for daily K intake. However, certain groups of patients, such as people with kidney diseases should be considered the amount of K intake due to their impaired kidney excretion of potassium, which could cause the adverse effects of increasing K intake on heart function associated with the concentration K in plasma [37]. They were able to consume K at approximately 1,500 mg/day. Microgreens from sunflower and Chinese radish had the least K accumulation, approximately at 139 and 147 mg/g DW, respectively, which were considered food with relatively low K contents. Thus, these microgreens may be an alternative choice for impaired kidney people because they are not only low K contents, they also have the highest concentration of iron (Fe) at 3.3 and 3.8 mg/g DW, respectively, along with high vitamin C content in Chinese radish.

Table 4: Content1 of mineral compositions (Ca, K, Fe and Zn) measurement in nine varieties of Thai microgreens per 1 g edible portion of dry weight

Microgreen	Ca (mg)	K (mg)	Fe (mg)	Zn (mg)
Sunflower	153.67 ± 1.46[b]	138.78 ± 5.95[c]	3.29 ± 0.05[ab]	0.83 ± 0.03[ab]
Chinese radish	122.15 ± 14.48[b]	147.22 ± 8.54[c]	3.79 ± 1.23[a]	0.58 ± 0.16[ab]
Indian mustard	173.92 ± 2.17[ab]	195.92 ± 4.39[bc]	2.87 ± 0.50[abc]	0.63 ± 0.06[ab]
Water spinach	124.04 ± 4.04[b]	376.81 ± 22.66[a]	1.59 ± 0.39[bcde]	0.55 ± 0.08[ab]
Thai rat-tailed radish	180.18 ± 4.94[ab]	201.03 ± 6.50[bc]	1.16 ± 0.13[cde]	0.58 ± 0.03[ab]
Sugar pea	76.46 ± 9.49[b]	244.47 ± 30.27[bc]	2.54 ± 1.18[abcd]	0.89 ± 0.31[a]
Red sorrel	121.00 ± 12.96[b]	301.81 ± 33.48[ab]	0.36 ± 0.11[e]	0.65 ± 0.10[ab]
Red cab-bage	283.03 ± 39.37[a]	226.83 ± 9.54[bc]	0.79 ± 0.12[de]	0.62 ± 0.01[ab]
Cabbage	284.65 ± NA[a]	294.61 ± NA[ab]	2.46 ± NA[abcd]	0.52 ± NA[b]

[1] Values are means ± standard deviation. Means within a column with different upper case letters are significantly different at $P < 0.05$. NA = not available.

The Ca contents in this study ranged from 76 to 285 mg/g DW. Comparing to Xiao *et al.* [13], our study found the 3–5 times higher Ca contents in all microgreens. This may be affected by differences in mineral assays or vegetable varieties. To maintain Ca balance in the body, WHO suggests that adults need 1,000 mg/day of Ca, whereas adolescents (10–18 years old) and elderly requires additional 300 mg of Ca to continue the peak of the growth phase in adolescents and reduce the bone loss in aging adults [4]. From our study, cabbage and red cabbage had the highest Ca contents at around 280 mg/g DW. Consumption of cabbage microgreens around 3.5–4.6 g DW per day will receive enough Ca for every generation. However, microgreens containing high Ca content were relatively low accumulation of vitamin C (Figure 2), because vitamin C is the substrate of calcium oxalate formation. Increase of calcium (in calcium oxalate) accumulation results in the reduction of vitamin C content [38].

Iron (Fe) and zinc (Zn) are microelements that are required low amounts but necessary to keep the body function normally. Fe contents in nine microgreens ranged from 0.36 to 3.79 mg/g DW, which were 1–7 times higher than the previous study in Brassicaceae varieties [13]. Fe intakes required are different in sex, age and menstruation period [39]. Unfortunately, foods that have high availability in Fe are from non-vegetable sources, such as meats and fish. Nevertheless, germinations of grains are able to improve the fractional iron absorption from low to moderate bioavailability due to the reduction of phytates that inhibits the absorption of Fe as well as Zn [40].

On the other hand, Zn contents in our study varied from 0.52 to 0.89 mg/g DW, while the previous study ranged from 0.22 to 0.51 mg/100 g FW [11]. In adults, men need a Zn intake of 1.4 mg/day whereas women require 1.0 mg/day. However, several studies show the effect of zinc contents in different types of food on fractional zinc absorption. Similar to Fe, Zn bioavailability can be improved by seed germination. Thus, vegetarians can reduce the amount of food with low Zn bioavailability to consume microgreens with moderate Zn bioavailability by half [41].

In addition, we found a negative correlation of Fe accumulation with both anthocyanin and β-carotene contents (Figure 2). This might result from the microgreens that accumulate other pigments besides chlorophyll requiring less Fe in the mechanism of chlorophyll formation than microgreens with low anthocyanin and β-carotene [42].

4 Conclusions

This study supplied information on nutrients in nine microgreens consumed in Thailand including bioactive compounds, antioxidant activity and selected element compositions. Some microgreens were found to be a potential source of vitamin C, calcium, potassium and iron. The study also revealed some microgreens that were suitable for specific groups of people, such as kidney-defected patients or vegetarian people. Thus, this should be a guideline for microgreens consumption by different types of people in Thailand.

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