

Nutrition, Phytochemicals, Antioxidants, and Cytotoxicity of Sweet Potato Leaf Tea

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Abstract

Due to their nutritional loadings and the value-added aspect, fresh leaves of 5 sweet potato varieties were screened for phytochemicals and used to produce herbal teas. The teas were observed for their proximate components, antioxidants, antioxidative activity, cytotoxicity and colour parameters under brewing at 60, 80 and 100 °C for 3, 5, 7 and 10 min. Tannins, phenolic compounds, saponins, flavonoids, phytosterols and alkaloids were contained in almost all varieties, whereas cardiac glycosides were dominant only in WP (white skin and purple flesh root) fresh leaves. Results for proximate components found that WP tea was prominent with the enrichment of crude fat (16.71 g/100 g of tea), chlorophyll b (29.41 mg/100 g of tea), cyanidin-3-glucoside (83.59 mg/100 g of tea), pelargonidin-3-glucoside (72.30 mg/100 g of tea) and tannins (1,994.81 mg TAE/100 g of tea). This was followed by RO (red skin and orange flesh root) with more protein content (0.13 g/100 g of tea). In the antioxidant test, OO (orange skin and orange flesh root) had the highest total phenolic content (1,260.92 mg GAE/100 g of tea) and offered the greatest potential of antioxidative activity (12,218.44 mg GAE/100 g of tea). No teas showed any signs of cytotoxicity ($LC_{50} > 1,000 \mu\text{g/mL}$). Almost all the tea was green in colour, but a yellow colour indicating lutein or its influence was also detected. In conclusion, WP, RO and OO tea were predominantly served as a low-price healthy beverage with high nutrient levels and antioxidative properties.

Keywords: Sweet potato leaves, Phytochemicals, Antioxidant activity, Cytotoxicity, Colour parameter

Introduction

Globally, sweet potato is grown in many developing countries, covering an area of 8.62 million hectares and producing a yield of 105.19 million tons [1]. In Asia, the major producers such as Israel, Japan, Korea, China and Thailand could produce flesh sweet potato with averages of 33.3, 24.7, 20.9, 20 and 12 tons/hectare, respectively [2]. In Thailand, sweet potatoes can be cultivated in all parts of the country, generally cultivated after the rice season (from October to April). In 2018, Thailand was the thirtieth ranked exporter of sweet potato (valued at 1.07 million USD) and had a market share of 45.37 % of global exports [3]. In addition to the edible storage roots, leaves can also be edible, although they are not favoured, with the average yields of 800 to 910 kg/hectare [4].

Fresh leaves of sweet potato have been documented as a source of nutritive enrichment and offer the potential for bioactivities. In China, sweet potato leaves were reported to have high proximate components, especially crude protein, crude fat and dietary fibre (24.04, 4.39 and 11.33 g/100 g DW, respectively) [5]. In Malaysia, methanolic leaf extracts of 6 varieties possessed high contents of phytochemicals and showed a relative capacity of antioxidative activity (total phenolic content at 5.35 g GAE/100 g DW, flavonoid content at 96 - 263.5 $\mu\text{g/g}$ and IC_{50} at 372.4 - 597.61 $\mu\text{g/mL}$) [6,7]. In 5 from 6 Malaysian varieties, the IC_{50} values of the leaf extracts were more effective than vitamin C (IC_{50} at 569.6 $\mu\text{g/mL}$) [7]. Sweet potato leaves, especially at the apical shoot, contained caffeoyl quinic acid that acted as an angiotensin-converting enzyme (ACE) inhibitor [8]. The functions of the ACE inhibitor were to control vascular contraction and treat hypertension and congestive heart failure [8]. Due to high polyphenols in leaves of Suioh varieties, this part was proved to delay the lag phase of LDL oxidation and to prevent relative thiobarbituric acid production, which resulted in a decrease in LDL oxidation [9]. Also, the extract of sweet potato leaves could relieve hyperglycemia of type II diabetes by stimulating

glucagon-like peptide-1 secretion [10]. Although sweet potato leaves had significant medicinal properties, they were not edible favourites; therefore, this study applied the fresh leaves for use as herbal teas to offer another choice for consumption.

Currently, the trend for health and wellness care is increasing and has induced the growth of herbal tea consumption. Many kinds of herbal teas such as mulberry leaves (*Morus alba* L.), mugwort (*Artemisia vulgaris* L. var. *indica* Maxim), Jamaica sorrel fruit (*Hibiscus sabdariffa* L.), bael fruit (*Aegle marmelos* L.), rose flower (*Rosa hybrida*) and chrysanthemum flower (*Chrysanthemum indicum* L.) are believed to be primary sources of antioxidants and to offer anti-disease properties [11]. In addition to traditional tea drinking, the new generation has also turned to select healthy beverages instead of true tea from *Camellia sinensis* L. Kuntze. Herbal teas have various tastes, colours and medicinal properties according to plant species, plant parts and chemical constituents that can serve a wider market. Sweet potato leaves might be another choice for herbal teas due to the reviews of proximate components and bioactivity; however, the associated information and food safety standards after tea processing needs further research.

Therefore, this study aimed to screen the phytochemicals in the fresh leaves of 5 varieties of sweet potato and to investigate the properties of sweet potato leaf teas by focusing on the proximate components, antioxidants, antioxidative activity and cytotoxicity. Also, the tea quality was investigated through colour parameters.

Materials and methods

Plant materials

Five varieties of sweet potato (*Ipomoea batatas* L.) were used in this study. These varieties were popular cultivation in the northeast of Thailand that the propagation was performed according to traditional techniques. The plant names in this study were named followed by the local farmer callings, which were considered from the colours of skin and flesh storage roots. These were (i) red skin and orange flesh root (RO), (ii) white skin and purple flesh root (WP), (iii) red skin and white flesh root (RW), (iv) dark purple skin and dark purple flesh root (DD) and (v) orange skin and orange flesh root (OO) (Figures 1 and 2), which the description of root storage and vegetative characteristics is shown in Table 1. The specimens with the code numbers MSUT 7648 for RO, MSUT 7649 for WP, MSUT 7650 for RW, MSUT 7651 for DD and MSUT 7652 for OO were collected in Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University, Thailand (MSUT). All plants were cultivated in December 2019 in the same location at Phosai subdistrict, Si Somdet district, Roi Et province (16°7'28.4232 and 103°28'20.4456). Soil texture was silt (1.25 % sand, 97.5 % silt and 1.25 % clay) with a pH value of 7.53 and EC_e value at 59.62 μS/cm. Soil minerals were composed of 1.84 ppm Ca, 0.007 ppm K, 0.31 ppm Mg and 0.0007 ppm Na. Soil analysis was followed by Land Development Department [12]. All plants were subject to identical management practices and harvested in March 2020.

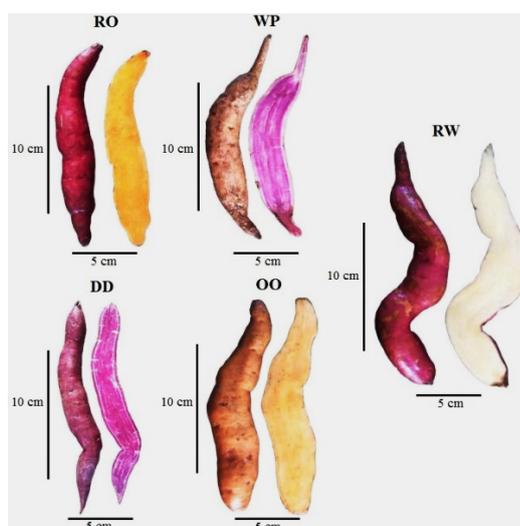


Figure 1 Storage roots of red skin and orange flesh root (RO), white skin and purple flesh root (WP), red skin and white flesh root (RW), dark purple skin and dark purple flesh root (DD) and orange skin and orange flesh root (OO).

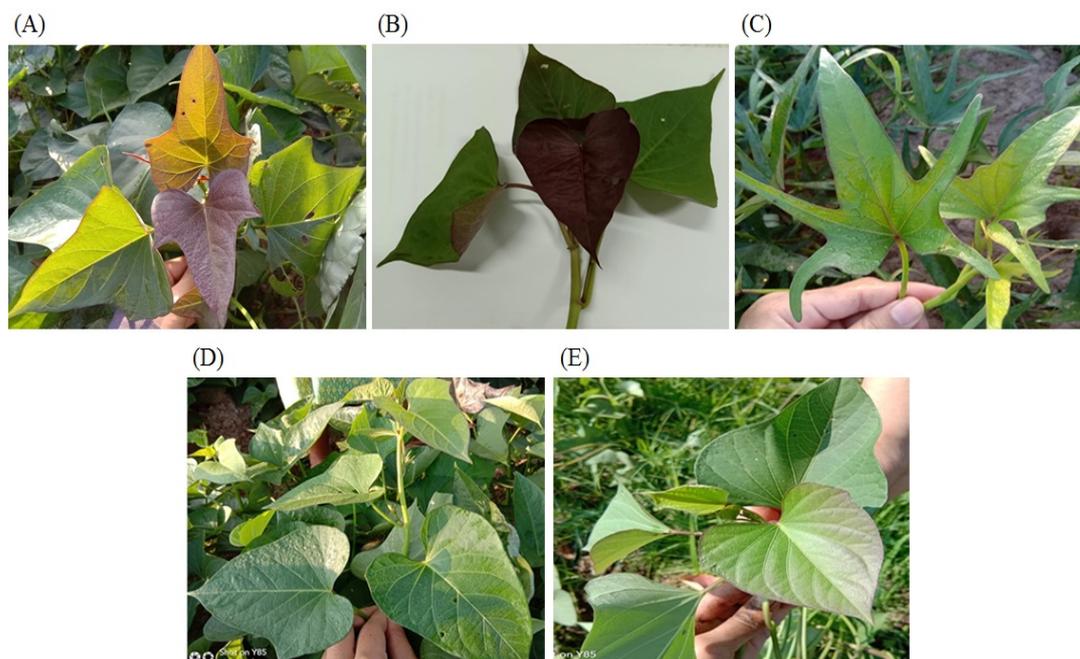


Figure 2 Leaf characteristics of sweet potato varieties in this study. (A) red skin and orange flesh root (RO); (B) white skin and purple flesh root (WP); (C) red skin and white flesh root (RW); (D) dark purple skin and dark purple flesh root (DD) and (E) orange skin and orange flesh root (OO).

Table 1 Root storage and vegetative characteristics of sweet potato in this study.

Sweet potato variety	Morphological characteristics		
	Storage root	Stem	Leaves
RO	Storage roots formed disperse with long oblong shape. Skin colour was red, and flesh colour was orange.	Average length of the erect plant was 93.2 cm long with green to purple colour. The shoots had pubescent.	Young blades at the apical shoots were light purple, and at the next positions were green colour with purple margin. Mature blades were green. Shapes of the young leaf outline were triangular with no lateral lobes, or lobed slightly. Shapes of the mature leaf outline were cordate with no lateral lobes, or lobed slightly. Number of leaf lobes was 1 - 3. Average width and length of the mature leaves was 10.5×9.5 cm. Mean petiole was 12 cm long.
WP	Storage root formed disperse with long oblong shape. Skin colour was white to cream, and flesh colour was purple. Anthocyanin pigmentation distributed covering most of the flesh.	Average length of the erect plant was 85.1 cm long with green colour. The shoots had pubescent.	Young blades at the apical shoots were dark purple, and at the next positions were green colour at the dorsal side and purple colour at ventral side. Mature blades were green. Shape of the young and mature leaf outline was cordate with no lateral lobes. Number of leaf lobes was 1. The petioles was pubescent. Average width and length of the mature leaves was 11.5×12.5 cm. Mean petiole was 11.8 cm. long.
RW	Storage root formed disperse with long irregular shape. Skin colour was red, and flesh colour was white.	Average length of the erect plant was 114.2 cm long with green colour. The hairiness in stem was glabrous.	Both young and mature blades were green with dark purple margin and dark purple vein. Mature blades were thick. Shape of the leaf outline was hastate with deep lobes. Number of leaf lobes was 5. Average width and length of the mature leaves was 8.5×10 cm. Mean petiole was 10.4 cm long.

Sweet potato variety	Morphological characteristics		
	Storage root	Stem	Leaves
DD	Storage root formed disperse with long irregular shape. Skin and flesh colour were dark purple. Anthocyanin pigmentation distributed covering all flesh.	Average length of the erect plant was 65.4 cm long with green to purple colour. Only apical shoots had pubescent.	Both young and mature blades were green with light purple margin. Shapes of the leaf outline were cordate with no lateral lobes, or lobed slightly. Number of leaf lobes was 1 - 3. The leaf dorsal side and petioles were pubescent. Average width and length of the mature leaves was 8×9.2 cm. Mean petiole was 6.5 cm long.
OO	Storage root formed disperse with long irregular shape. Skin and flesh colour were orange.	Average length of the erect plant was 62.3 cm long with green to purple colour. The hairiness in stem was glabrous.	Young blades were purple green. Mature blades were green. Shape of the leaf outline was cordate with no lateral lobes. Number of leaf lobes was 1. Average width and length of the mature leaves was 11.3×10 cm. Mean petiole was 12.5 cm long.

Phytochemical screening

The phytochemical qualitative tests were used to screen plant secondary metabolites by considering the intensity of colour in a chemical reaction or the formation of a precipitate. The preliminary screening was performed on sweet potato fresh leaves for tannins, phenolic compounds, saponins, coumarin, flavonoids, phytosterols, cardiac glycosides, terpenoids, alkaloids and anthraquinone. The assays followed Banu and Cathrine [13] and Harborne [14].

Tannin, phenolic compound and saponin test

One g of fresh leaf samples was extracted by deionized water and heated at 60 - 80 °C for 15 min. Then, the aqueous solution was filtered and tested for the presence of tannins, phenolic compounds and saponins. For tannins and phenolic compounds, the positive result was indicated by a dark green presence after adding a neutral 5 % ferric chloride solution. The identification of saponins was by foaming ability after shaking for 15 min.

Coumarin, flavonoid, phytosterol, cardiac glycoside, terpenoid, alkaloid and anthraquinone test

One g of fresh leaf samples was extracted by 80 % ethanol and concurrently shaken for 30 min. The extract was filtered and the filtrate was used to indicate coumarin, flavonoids, phytosterols, cardiac glycosides, terpenoids, alkaloids and anthraquinone. The coumarin test was observed a dark yellow in an aqueous solution after adding 10 % sodium hydroxide. For the flavonoid test, the positive presence was indicated from a yellow fluorescence after adding 10 % ammonium hydroxide solution. For phytosterol screening, acetic anhydride solution and a few drops of concentrated sulfuric acid were added to an aqueous sample solution. Change of the aqueous solution colour was used for identifying phytosterols. For cardiac glycoside detection, 1 % ferric chloride solution and glacial acetic acid were added to an aqueous solution, followed by a gentle addition of concentrated sulfuric acid. The positive result for cardiac glycosides was indicated from a brown ring appearance. Terpenoids were detected from a red colour present in an aqueous solution after adding chloroform and concentrated sulfuric acid. For alkaloids, the positive result was concluded if the aqueous solution formed precipitates after adding hydrogen chloride solution. The positive anthraquinone test was indicated from a pink colour presentation in an aqueous solution after adding ammonium acetate solution.

Tea procedure

In tea procedure, 5 young leaves from the apical shoot (both folded and unfolded leaves) were plucked in the early morning of March 10, 2020. After collecting, fresh leaves were washed 3 times with tap water, 1 time with deionized water and indoor withered for 30 min. Tea processing started by slicing leaves approximately 0.5 cm thick, pan-firing at 150 - 160 °C for 15 min [11], and finally rolling for 10 min. Before packaging, sweet potato leaf teas were dried in an oven at 60 °C until the stable weight and sorted for quality. The fresh leaves of RO, WP, RW, DD and OO variety could produce tea approximately 21.75, 22.05, 21.96, 22.09 and 19.00 % (w/w), respectively.

Each tea sample was divided into 2 parts. First, the sample provided for analysis of ash, crude fat, protein, total soluble sugar, chlorophyll a and b, anthocyanins (cyanidin-3-glucoside and pelargonidin-3-glucoside), and colour parameter test. Second, the sample was prepared to produce hydrophilic crude extracts for analysis of total phenolic content, tannins, antioxidant activity and cytotoxicity.

Extraction of hydrophilic fractions

The hydrophilic fractions of 20 g powder sweet potato leaf tea were extracted by 70 % ethanol at 80 °C using Soxhlet apparatus and repeated 8 times. All extracts were pooled, evaporated at 60 °C, and lyophilized to powders. The teas of RO, WP, RW, DD and OO varieties could produce crude extracts of approximately 22.38, 37.93, 21.26, 20.43 and 22.78 % (w/w), respectively.

Determination of ash

The assay of ash followed Department of Medical Sciences [15]. Before starting, the crucibles were heated at 525 ± 25 °C for 1.5 h, cooled down in a desiccator, and weighed. Five g tea sample was weighed in the same crucible and combusted at 525 ± 25 °C until white ash remained. Ash was determined from the differences in dried weights before and after combustion.

Determination of crude fat

The assay followed the solvent extraction method [16]. Three g of dried sample was extracted by 40 mL petroleum ether for 25 min and further flooded for 30 min. The sample was then dried at 125 °C until the stable weight. Crude fat was determined from the differences in dried weights before and after extraction.

Determination of protein

The protein assay followed Bradford [17]. The process started from adding 1.5 mL of Bradford reagent to 30 µL of desired sample concentration, thoroughly mixed, and left in the dark condition at room temperature for 15 min. The samples were measured at the absorbance of 595 nm. The amount of protein was calculated from a standard curve using bovine serum albumin solution.

Determination of total soluble sugar

The determination of total soluble sugar followed the phenol-sulfuric acid method [18]. One g of sample was extracted by 95 % ethanol and then 1 mL 5 % (w/w) phenol and 5 mL concentrated sulfuric acid were added sequentially. The extract was mixed thoroughly and left at the room temperature for 30 min. The sample was measured at the absorbance of 600 nm. The amount of total soluble sugar was calculated from a standard curve of glucose solution.

Determination of chlorophyll

The chlorophyll assay followed Arnon [19] and Hiscox and Israelstam [20]. Sweet potato leaf tea was extracted by 90 % acetone. After that, the extract was measured at the absorbance of 645 and 663 nm for chlorophyll a and b contents as follows:

$$\begin{aligned} \text{Chlorophyll a (g/L)} &= 0.0127 (A_{663}) - 0.00269 (A_{645}) \\ \text{Chlorophyll b (g/L)} &= 0.0029 (A_{663}) - 0.00468 (A_{645}) \end{aligned} \quad (1)$$

Determination of anthocyanins

The anthocyanin assay followed the pH-differential method [21]. Each leaf tea sample was divided into 2 parts. The 1st part was extracted with potassium chloride buffer at pH 1.0, and the 2nd part was extracted with sodium acetate buffer at pH 4.5. The tested sample was left at the room temperature for 30 min and measured at the absorbance of 510 and 700 nm. The amount of anthocyanins was calculated as follows:

$$\text{Anthocyanins} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{A \times MW \times df \times 10^3}{\epsilon \times l} \quad (2)$$

where A is $(A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH } 1.0} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH } 4.5}$; MW is a molecular weight of cyanidin-3-glucoside (449.2 g/mol) or pelargonidin-3-glucoside (306.7 g/mol); df is a dilution factor; ϵ is a molar absorptivity (26,900 L/mol/cm for cyanidin-3-glucoside and 31,100 L/mol/cm for pelargonidin-3-glucoside); l is a path length in cm.

Determination of total phenolic content

The total phenolic assay followed the Folin-Ciocalteu method [22]. Fourteen microliters of 100 µg/mL crude tea extract were reacted with 65.3 µL DI water and 14 µL Folin-Ciocalteu reagent for 6 min. After that, 187 µL of 2 % sodium carbonate solution was added to the aqueous solution and then left under the dark condition for 60 min. The samples were measured at the absorbance of 600 nm. The amount of total phenolics was calculated from a standard curve of gallic acid solution.

Determination of tannins

The assay of tannin determination followed Makkar *et al.* [23]. The crude tea extract was attenuated to 8 - 12 mg/mL concentrations. The sample solution was reacted with 5 mL Folin-Ciocalteu reagent and 10 mL of 7 % sodium carbonate solution at the room temperature for 30 min. The samples were measured at the absorbance of 762 nm. The amount of tannins was calculated from a standard curve of tannin acid solution.

Determination of antioxidative activity

The DPPH inhibition assay followed Moreno *et al.* [24]. The crude tea extract was attenuated to 1 - 15,000 µg/mL concentrations. Seven microliters sample solution and 273 µL DPPH solution were reacted under the dark condition for 45 min. The samples were measured at the absorbance of 517 nm and the percentages of DPPH inhibition were calculated. Median inhibitory concentration (IC₅₀) was calculated from the correlation of absorbance and sample concentration. Gallic acid was a standard antioxidant that was treated in the same manner.

Determination of cytotoxicity

The determination of median lethal concentration (LC₅₀) followed Meyer *et al.* [25]. Ten healthy brine shrimps (*Artemia salina*) at the nauplii stage were added to 24-well plates for the cytotoxicity test. Tea sample was attenuated to 5 - 25 mg/mL concentrations. At 24 h after treatment, the dead animals have counted and calculated the percentages and LC₅₀ values. Animal research ethics for scientific work was approved by the ethics committee on animal experimentation, Maharakham University (research project code no: IACUC-MSU-017/2020).

Determination of colour of tea water

To determine the colour of tea water, 1 g of tea sample was brewed with 120 mL DI water under various temperatures (60, 80 and 100 °C) and times (3, 5, 7 and 10 min). The colour of tea water was measured via Colorimeter (Konica Minolta, version Chroma Meter CR-400) and expressed as L^* , a^* , and b^* value. Lightness (L^*) ranged from 0 - 100 (black to white). a^* presented between $-a^*$ to $+a^*$ (green and redness). b^* expressed $-b^*$ to $+b^*$ (blue and yellow). Chroma (C^*) and hue angle (H°) from calculation determined colour intensity and sample colour, respectively. C^* calculated from $C^* = ((a^*)^2 + (b^*)^2)^{1/2}$. H° calculated from $H^\circ = \tan^{-1}(b^*/a^*)$, if a^* and $b^* > 0$, and $H^\circ = \tan^{-1}(b^*/a^*) + 180^\circ$, if $a^* < 0$ and $b^* > 0$. Hue angles at 0° , 90° , 180° , 270° and 360° represented red, yellow, green, blue and purple, respectively [26].

Statistical analysis

Mean \pm standard deviation (SD) was calculated from 3 replications for each analysis and analyzed for a significantly different variance by using one-way ANOVA and Duncan test at $p < 0.05$. Correlation was used to examine the relationship between antioxidants and antioxidative activity, and also brewing conditions and colour parameters at $p < 0.05$ and $p < 0.01$.

Results and discussion

Qualitative tests of phytochemicals on sweet potato fresh leaves

Table 2 presents the results for the preliminary phytochemicals of sweet potato fresh leaves. Ten compounds (tannins, phenolic compounds, saponins, coumarin, flavonoids, phytosterols, cardiac glycosides, terpenoids, alkaloids and anthraquinone) were screened. Tannins, phenolic compounds and flavonoids were the most common in sweet potato leaves, whereas terpenoids and anthraquinone could not be observed. Saponins, phytosterols and alkaloids could be found in RO, WP, RW and DD leaves, but not in the OO variety. In particular, coumarin occurred in WP and OO leaves and cardiac glycosides appeared only in WP leaves.

Table 2 Phytochemical screening test on sweet potato fresh leaves.

Compounds	Sweet potato varieties				
	RO	WP	RW	DD	OO
Tannins	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Saponins	+	+	+	+	–
Coumarin	–	+	–	–	+
Flavonoids	+	+	+	+	+
Phytosterols	+	+	+	+	–
Cardiac glycosides	–	+	–	–	–
Terpenoids	–	–	–	–	–
Alkaloids	+	+	+	+	–
Anthraquinone	–	–	–	–	–

(+) refers to chemical presence and (–) refers to chemical absence.

Sweet potato leaves had diverse phytochemicals. Preliminary screening of purple sweet potato 70 % ethanolic leaf extract revealed the presence of tannins, saponins, flavonoids, triterpenoids and alkaloids [27], with the result proving similar to this study. Through GC-MS analysis, several bioactive chemicals, especially 1,4-benzenediol hydroquinone, benzenesulfonic acid 4-hydroxy and hexadecanoic acid (palmitic acid) presented in purple sweet potato ethanolic leaf extract. These compounds were a class of phenol that had the identity of antimicrobials [27]. Quercetin, myricetin and luteolin were also exposed in purple sweet potato leaves, whereas apigenin in the flavone class could be found in green sweet potato leaves [28]. All these compounds were a class of flavonoids, which were classified as nutraceuticals with antioxidant properties. Moreover, apigenin was also active in terms of anti-hyperglycemic, anti-inflammatory and anti-apoptotic effects (in myocardial ischemia) [29]. Flavonoids in the present were a candidate as a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) inhibitor with potential equal to or more effective than chloroquine (CLQ) [30]. The research showed that flavonoids including hesperetin, myricetin, linebacker and caflanone (FBL-03G) could bind with the angiotensin-converting enzyme 2 (ACE2) receptor, the cellular entrance of SARS-CoV-2 and inhibit the virus entry [30]. Anthocyanins, another class of flavonoids, also acted as antioxidants and presented in various colours (from red to purple or blue) depending on the pH value of the solution [31]. In this study, apical shoots of all 5 varieties showed dominantly purple anthocyanin presentation which was one of the reasons for the research, especially concerning WP. In WP apical shoots, young leaves at the 1st and 2nd position exhibited a very dark purple colour in both dorsal and ventral sides, whereas at the 3rd to 5th position they expressed a purple colour at the ventral side (**Figure 2**). Cardiac glycosides are another compound found in the WP leaves which can potently increase the capacity of the pumping of the heart muscle through action on the Na⁺-K⁺ exchange [32]. Alkaloids, a significant toxic chemical in the liver, could be found in RO, WP, RW and DD fresh leaves, but could not be observed in OO fresh leaves.

Proximate composition of sweet potato leaf teas

The proximate composition of sweet potato leaf teas is presented in **Table 3**. The results showed that ash and total soluble sugar contents were not significantly different among teas. Ash values ranged from 2.03 to 2.79 g/100 g of tea, and soluble sugar contents were between 0.23 and 0.31 g/100 g of tea. As for crude fat, the values ranged from 8.58 to 16.71 g/100 g of tea. WP and OO tea contained the highest and the lowest fat values, respectively, but there were no significant differences among WP, RW and DD teas. Protein quantity was prominent in RO tea (0.13 g/100 g of tea), whereas the others showed no significant differences. With the exception of WP tea, chlorophyll a contents were greater than chlorophyll b contents, with the highest chlorophyll a and b values found in DD and WP tea (69.55 and 29.41 mg/100 g of tea, respectively). Cyanidin-3-glucoside and pelargonidin-3-glucoside, the 2 groups of anthocyanins, were highest in WP leaf tea (83.59 and 72.30 mg/100 g of tea, respectively). Cyanidin-3-glucoside was remarkable for being greater than pelargonidin-3-glucoside for all teas.

The information of proximate variables of sweet potato leaf teas was rare, but it could be found for the fresh leaves. Among 40 varieties, the proximate components of sweet potato fresh leaves varied; ash ranging from 7.4 to 14.7 g/100 g DW, crude fat ranging from 2.1 to 5.3 g/100 g DW, crude protein ranging from 16.7 to 31.1 g/100 g DW, and dietary fibre ranging from 9.2 to 14.3 g/100 g DW [5]. Compared with this study, only crude fats of sweet potato leaf teas (8.58 - 16.71 g/100 g of tea) were

higher than that of the review, even after tea processing. Omega (ω)-3 essential fatty acid α -linolenic acid (18:3n-3) was mainly found in sweet potato leaves; however, the other healthful groups such as ω -6 essential fatty acid linolenic acid (18:2n-6) and saturated fatty acid palmitic acid (16:0) could also be observed [33]. In true tea (*Camelia sinensis* L. Kuntze), crude fat was not high, with the range between 1.59 - 4.15 g/100 g of tea [34]. Sweet potato fresh leaves from Taiwan were recommended as a good and rich source of all essential amino acids, except methionine plus cysteine, in doses that exceeded the score of the WHO reference [33]. Sucrose and galactose were the main soluble sugars found in sweet potato fresh leaves [35]. Sucrose was the main component of starch granule formation, and galactose was a component of galactolipids which form polyunsaturated fatty acids. Chlorophyll is a pigment to use for separating green tea from oolong and black tea [11]. The process applied to green tea of immediately heating (250 - 300 °C for 3 - 5 min) after harvesting could prevent polyphenol oxidase activity and reduce the chance of chlorophyll degradation to pheophorbides and pheophytins [36]. Chlorophyll concentrations of commercial green teas from China, India, Japan, Russia and Vietnam varied from 118 to 198 mg/100 g of tea [37], which were greater than the values reported for the sweet potato leaf teas in this study. This may be because the technique used in the tea process in this study was not good enough to protect the chlorophyll pigment. Anthocyanins possessed many bioactive properties such as antioxidation, anti-obesity, anticancer, anti-aging, anti-proliferation, anti-angiogenic activity, anti-inflammation activity, and anti-hydrolytic enzymes (such as α -amylase, lipase and protease) [38-41]. The antioxidative capacity of anthocyanins was diverse according to the types and numbers of free hydroxyl groups around pyrone rings; for example, pelargonidin was more effective on hydroxyl radicals, whereas delphinidin was stronger in scavenging superoxide radicals than cyanidin and pelargonidin [40]. However, cyanidin was remarked to be a major anthocyanin in sweet potato fresh leaves [41]. Consistently, from the HPLC results, cyanidins (cyanidin 3-(6,6'-caffeoyl-p-hydroxybenzoylsophoroside)-5-glucoside and cyanidin 3-(6,6'-dicafeoylsophoroside)-5-glucoside) were the major components in the leaves of 3 Japanese varieties [42]. The cyanidin type was superior to the peonidin type in antioxidative activity and antimutagenicity [42], and that had significant meaning for the sweet potato leaf teas in this study.

Table 3 Proximate composition of sweet potato leaf teas.

Proximate composition	Sweet potato varieties				
	RO	WP	RW	DD	OO
Parameter (g/100 g of tea)					
Ash	2.25 ± 0.12 ^a	2.40 ± 0.56 ^a	2.63 ± 0.25 ^a	2.79 ± 0.31 ^a	2.03 ± 0.37 ^a
Crude fat	12.52 ± 0.31 ^b	16.71 ± 0.80 ^a	15.35 ± 1.57 ^{ab}	16.14 ± 1.87 ^a	8.58 ± 1.59 ^c
Protein	0.13 ± 0.00 ^a	0.10 ± 0.00 ^b	0.09 ± 0.00 ^b	0.09 ± 0.00 ^b	0.10 ± 0.00 ^b
Total soluble sugar	0.25 ± 0.05 ^a	0.29 ± 0.03 ^a	0.24 ± 0.05 ^a	0.31 ± 0.04 ^a	0.23 ± 0.05 ^a
Parameter (mg/100 g of tea)					
Chlorophyll a	43.83 ± 0.45 ^c	26.82 ± 0.81 ^c	35.03 ± 0.39 ^d	69.55 ± 0.31 ^a	50.58 ± 0.38 ^b
Chlorophyll b	19.09 ± 0.10 ^d	29.41 ± 1.19 ^a	23.13 ± 0.11 ^b	19.43 ± 0.06 ^d	21.78 ± 0.24 ^c
Cyanidin-3-glucoside	63.09 ± 7.78 ^b	83.59 ± 7.23 ^a	54.40 ± 2.17 ^c	52.92 ± 3.60 ^c	61.56 ± 8.10 ^b
Pelargonidin-3-glucoside	52.05 ± 7.15 ^b	72.30 ± 6.26 ^a	47.06 ± 1.87 ^c	45.77 ± 3.11 ^c	55.69 ± 11.11 ^b

Note: Values are means ± SD. ^{a,b,c} different letters within the same row are significantly different at $p < 0.05$.

Antioxidants and antioxidative activity of sweet potato leaf teas

The results of antioxidant and antioxidative activity are presented in **Table 4**, and the results of the correlation coefficients are shown in **Table 5**. The highest total phenolic content was found in OO tea (1,260.92 mg GAE/100 g of tea) and the levels diminished in the sequence RO, WP, RW and DD (829.17, 675.95, 447.51 and 343.2 mg GAE/100 g of tea, respectively). As for tannins, WP tea contained the highest value (1,994.81 mg TAE/100 g of tea), subsequently followed by RO, OO, DD and RW (1,945.32, 1,124.24, 544.93 and 290.22 mg TAE/100 g of tea, respectively). Compared with gallic acid, OO tea showed the most powerful antioxidative activity (12,218.44 mg GAE/100 g of tea), followed by RO, WP, DD and RW tea (943.84, 178.52, 79.17 and 64.96 mg GAE/100 g of tea, respectively).

Antioxidative activity of sweet potato teas showed a strongly positive relationship with total phenolic compounds ($r = 0.994$, $p < 0.01$) and tannins ($r = 0.719$, $p < 0.05$). Moreover, the total phenolic compounds of all teas were positively associated with tannins ($r = 0.773$, $p < 0.05$).

Table 4 Antioxidants and antioxidative activity of sweet potato leaf teas.

Sweet potato varieties	Properties		
	Total phenolic contents (mg GAE/100 g of tea)	Tannins (mg TAE/100 g of tea)	Antioxidative activity (mg GAE/100 g of tea)
RO	829.17 ± 1.20 ^b	1,945.32 ± 15.84 ^a	943.84 ± 72.24 ^b
WP	675.95 ± 2.03 ^c	1,994.81 ± 14.76 ^a	178.52 ± 22.30 ^c
RW	447.51 ± 1.34 ^d	290.22 ± 2.29 ^d	64.96 ± 1.93 ^c
DD	343.27 ± 1.09 ^d	544.93 ± 3.82 ^c	79.17 ± 1.96 ^c
OO	1,260.92 ± 2.11 ^a	1,124.24 ± 6.50 ^b	12,218.44 ± 192.27 ^a

Note: Values are means ± SD. ^{a,b,c,d} different letters within the same column are significantly different at $p < 0.05$.

Table 5 Partial correlation between antioxidants and antioxidative activity of sweet potato leaf teas.

	Total phenolic compounds	Tannins	Antioxidative activity
Total phenolic compounds	1.000		
Tannins	0.773*	1.000	
Antioxidative activity	0.994**	0.719*	1.000

Correlations were significant at the 0.01 (**) and 0.05 (*) level (2-tailed), respectively.

Sweet potato leaves have been reported to have a high level of antioxidant properties. Chinese Vardaman sweet potato leaves had a total phenolic concentration of 8.11 mg/g DW and resulted in higher antioxidant activity than the comparable vitamin C (IC₅₀ 184.3 and 238.6 µg/mL, respectively) [43]. From HPLC analysis, the main polyphenols were observed in Chinese Pushu 53 sweet potato leaves. These were 5-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, caffeic acid, 4,5-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid and 3,4,5-tri-O-caffeoylquinic acid, while the highest quantity was 3,5-di-O-caffeoylquinic acid followed by 4-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid, respectively. Boiling sweet potato leaves decreased all the previously mentioned amounts; however, steaming could increase 4,5-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid and antioxidative activity [44]. Antioxidative activity in sweet potato leaves was in accordance with the quantity of caffeoylquinic acid derivatives [45]. Also, tannins could be assumed to be antioxidants in sweet potato leaves. Condensed tannins, the structure composed of flavonoid units, could generate anthocyanidins under variant mediums, and hydrolysable tannins which the structure derives from gallic acid and its derivatives [46].

Cytotoxicity

The results for cytotoxicity via brine shrimp assay are shown in **Figure 3**. After 24 h of treatments, it was found that the most significant effect came from DD tea crude extract (LC₅₀ 6,110.11 µg/mL) followed by RO, OO, WP and RW tea (LC₅₀ 6,121.56, 6,825.70, 8,465.74 and 9,914.95 µg/mL, respectively).

For the polyphenol-rich sweet potato leaves on inducing cancer cell apoptosis [47], we were aware of the safety of sweet potato leaf teas. Therefore, the cytotoxicity analysis was performed to observe the bioactive cellular metabolism of the brine shrimp model. The results indicated that all teas had no or few effects on cellular metabolism with all LC₅₀ values over 1,000 µg/mL. That is based on the LC₅₀ acceptance [25,48]. Although sweet potato leaf extract could disturb the cell cycle progression of prostate cancer PC-3 cells in many steps, and finally induced antiproliferation and apoptosis both *in vitro* and *in vivo*, the extract had no significant effects on normal prostate epithelial cells with very low cytotoxicity impact [47]. In traditional remedies, sweet potato leaves were used to heal irritation of the mouth and

throat, relieve rashes and reduce appetite and symptoms of metabolic issues [49]. And currently, people in the islands of the Pacific Ocean, Africa and Asian countries still consume sweet potato leaves that offers no economic value but high bioactive potential [49].

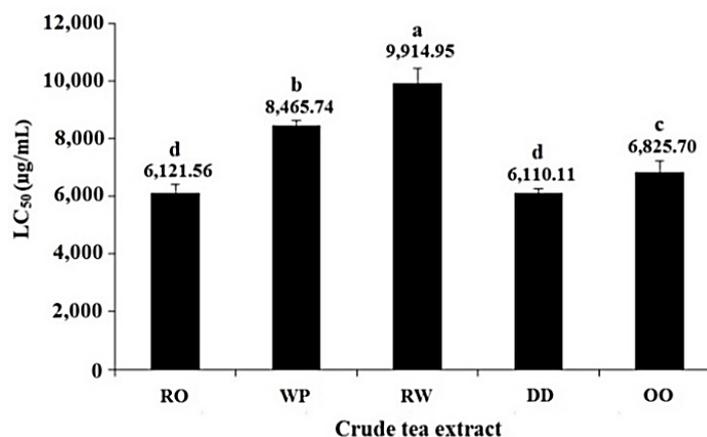


Figure 3 LC₅₀ of crude extracts of sweet potato leaf teas via brine shrimp test.

Correlation of tea water colour under different brewing temperatures and brewing times

Under various brewing temperatures (60, 80 and 100 °C) and brewing times (3, 5, 7 and 10 min), the colour parameters (L^* , a^* , b^* , C^* and H^*) of tea water were distributed and shown in **Figure 4**. Lightness (L^*) of tea water ranged from 19.96 to 24.74, with the greatest frequency at 23 to 24. Results of the a^* values were mostly at the green position (-2.31 to -1.06), except for RO tea water brewed at 100 °C for 10 min which were at the red position (1.22). All b^* values were at the yellow position (2.98 to 5.49), with the highest value for OO tea water brewed at 100 °C for 7 min. Colour intensity (C^*) ranged from 3.77 to 5.62, with the highest value for OO tea water brewed at 100 °C for 10 min. Almost all the final colours (H^*) of the tea samples were of a green colour (167.86 to 187.02), except for RO tea brewed at 60 °C for 7 min which gave a yellowish-green colour (140.13), and WP tea brewed at 80 °C for 7 min which gave a purple colour (371).

The correlations between brewing conditions (temperatures and times) and colour parameters of tea water are presented in **Table 6**. The results showed that lightness (L^*) of tea water had an opposite relationship with brewing times, and more special was that RW tea water was also influenced by temperatures. In particular, redness ($+a^*$), indicating anthocyanins, was positively associated with brewing times in WP tea ($r = 0.584$, $p < 0.05$), and with brewing temperatures in OO tea ($r = 0.755$, $p < 0.01$). As for the yellow colour ($+b^*$), indicating carotenoids, it was positively significant with brewing times in RO, WP and DD tea ($r = 0.694$, $p < 0.05$; $r = 0.852$, $p < 0.01$; $r = 0.672$, $p < 0.05$, respectively), and with brewing temperatures in OO tea ($r = 0.743$, $p < 0.01$). As for RW tea, the yellow colour ($+b^*$) of tea water was positively related to both brewing temperatures and brewing times ($r = 0.655$, $p < 0.05$ and $r = 0.738$, $p < 0.01$). Colour intensity (C^*) of RO, WP and DD tea water was positively associated with brewing times ($r = 0.640$, $p < 0.05$; $r = 0.693$, $p < 0.05$; $r = 0.887$, $p < 0.01$, respectively), while the colour intensity of OO tea water was positively related to brewing temperatures ($r = 0.735$, $p < 0.01$). However, there was only RW tea water for which C^* was positively influenced by both brewing temperatures and brewing times ($r = 0.647$, $p < 0.05$ and $r = 0.739$, $p < 0.01$). The results showed that dissolving more yellow colour from carotenoids in RO, WP, RW and DD tea involved a brewing time-dependent manner, whereas the increased redness of anthocyanins in OO tea was related to high brewing temperatures.

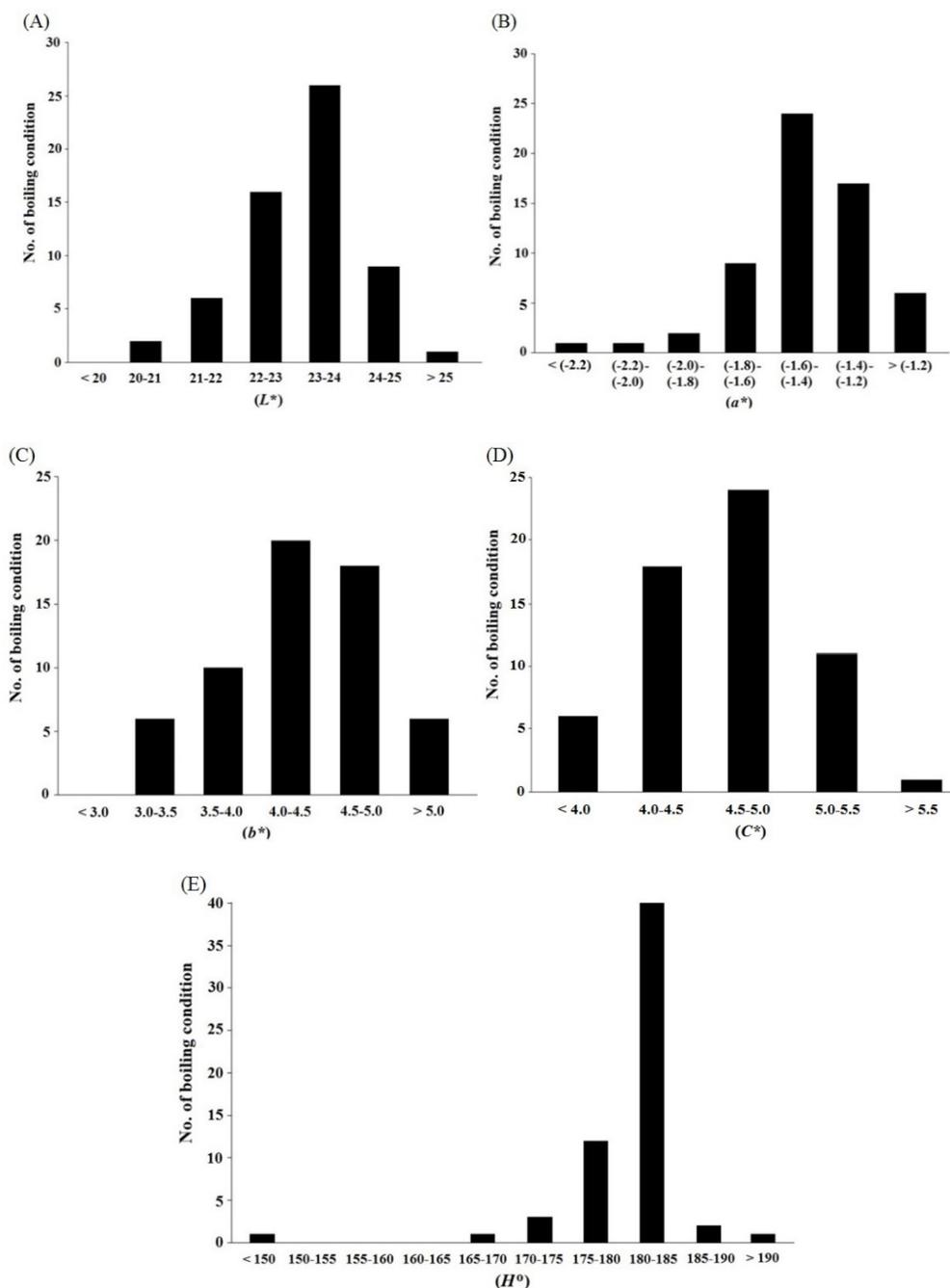


Figure 4 Colour parameters (L^* , a^* , b^* , C^* and H°) of tea water distribution under various brewing temperatures (60, 80 and 100 °C) and brewing times (3, 5, 7 and 10 min). A, L^* values: B, a^* values: C, b^* values: D, C^* values and E, H° values.

Table 6 Pearson’s correlation coefficients of brewing temperatures, brewing times and colour parameters of tea water.

Groups	Colour parameters				
	L^*	a^*	b^*	C^*	H°
RO tea water					
Brewing temperature	-0.572	0.272	0.372	0.423	0.334
Brewing time	-0.594*	0.512	0.694*	0.640*	-0.252
WP tea water					

Groups	Colour parameters				
	<i>L</i> *	<i>a</i> *	<i>b</i> *	<i>C</i> *	<i>H</i> ^o
Brewing temperature	-0.610*	-0.465	-0.142	0.040	0.001
Brewing time	-0.378	0.584*	0.852**	0.693*	0.112
RW tea water					
Brewing temperature	-0.621*	0.212	0.655*	0.647*	-0.303
Brewing time	-0.626*	0.094	0.738**	0.739**	-0.112
DD tea water					
Brewing temperature	-0.308	-0.121	-0.170	0.417	-0.233
Brewing time	-0.871**	0.502	0.672*	0.887**	-0.370
OO tea water					
Brewing temperature	-0.453	0.755**	0.743**	0.735**	-0.533
Brewing time	-0.580*	0.382	0.559	0.562	0.293

Correlations were significant at the 0.01 (**) and 0.05 (*) level (2-tailed), respectively.

The yellow colour of sweet potato leaf teas results from lutein, lycopene, or β -carotene. Lutein is an important carotenoid contained in the human retina, known as retinal pigment, which filters light and protects the eyes from sunlight or high-energy waves such as ultraviolet rays. Also, lutein provides important nutrition in protecting against age-related macular degeneration (AMD), and especially blindness. Therefore, the consumption of dietary lutein-rich plants leads to an improvement in vision and eye health. Lutein not only offers retina protection but has also been reported to act against dioxin toxicity via inhibited aryl hydrocarbon receptor (AhR) transformation [50]. Dioxins, the unintentional products from incomplete combustion, are known as tumour promoters in liver, skin, lung and ovarian cancers [51]. Sweet potato leaves have been indicated to be excellent lutein sources with the range from 0.38 to 0.58 mg/g FW in Beauregard, Bienville, L99-35, L00-8, L01-145 and L01-29 genotypes [4]. The yellow colour could also be the result of lycopene and β -carotene. Lycopene and β -carotene are antioxidants in many vegetables and fruits, and prefer to scavenge peroxy radicals (RO[•]) from lipid peroxidation to a greater extent than other chemical species; therefore, lipoprotein in the cellular membrane could be protected through carotenoid activity. After scavenging, free radicals would be delocalized in a high number of conjugated double bonds of long-chain carotenoids and are responsible for stability [52].

Another interesting colour presented in sweet potato leaf teas was red and purple that is associated with anthocyanins or theaflavins. During the tea withering or fermentation step, some colours, such as carotenoids, might continually be oxidized and produced specific fragrances, which could be used to identify tea types [11]. Anthocyanins, the dominant pigment found in flesh sweet potato leaves in this study, presented colours from red to purple. Theaflavins were orange to red substances that contribute significantly to the astringency, briskness, brightness and some extended colour of the tea beverage. Theaflavins, the main pigments in true tea (*C. sinensis* L.), were documented to potentiate in inhibiting the activation of nuclear factor-kappaB (a transcription factor relative to proinflammatory cytokines) and could also suppress interleukin-2 (IL-2) secretion and IL-2 gene expression [53].

Conclusions

This work found that edible sweet potato leaves from 3 varieties could represent value-added by producing healthy tea beverages. Teas from WP, RO and OO leaves contained high levels of nutrients, especially crude fat, chlorophyll, cyanidin-3-glucoside, pelargonidin-3-glucoside, total phenolic compounds, tannins and also antioxidative activity. The yellow colour in tea water also showed signs of lutein expression which was the extra loading nutrient in sweet potato leaves.

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