The Journal of Agricultural Sciences - Sri Lanka
Vol. 14, No. 3, September 2019. Pp 181-200
http://doi.org/10.4038/jas.v14i3.8602

Open Access Article

X-ray Fluorescence Analysis and Consumer Preference Assessment Unveils that Sweet Potato Shoot-Tops are a Safer Alternative to Water Spinach, a Leafy Vegetable Prone to Bioaccumulation of Heavy Metals

A.P.D.T. Ranathunga, R.D.G.A. Ranasinghe, H.S.M. Jayarathne, S.M.N.K. Thilakarathne, S.I. Karunarathne, L.T. Ranaweera, G.K.S. Ananda, S.K. Kannangara, S.W. Meepegamage, and S.D.S.S. Sooriyapathirana*

Received: 20th November 2018 / Accepted: 8th January 2019

ABSTRACT

Purpose: *Water spinach (WS) (Ipomoea aquatica) is a popular leafy vegetable. However, WS is highly prone to bioaccumulation of toxic heavy metals. Sweet potato (SP) (Ipomoea batatas) is primarily a root tuber crop with fast growing shoots that can be used to replace WS to safe guard the human and livestock health. However, SP is an under-utilized leafy vegetable and no studies have been conducted to assess the consumer preference and food safety except photochemical assessments to profile its high nutritious value. Therefore, in the present study, we assessed the heavy metal content, consumer preference and phytochemical contents to test the applicability of using shoot-tops of SP to replace bioaccumulation prone WS if grown in polluted sites. We also conducted a DNA barcoding analysis to discriminate SP from WS.*

Research Method: *We obtained three greenhouse grwon shoot-top samples from WS (WS1, WS2 and WS3) and two greenhouse grown SP samples (SP1 and SP2) for all the analyses and two additional WS collected from the market (WSC) and a polluted site (WSP) a for XRF analysis. To assess the consumer preference we carried out a taste panel with 30 human subjects. For qualitative detection of the phytochemical contents, we performed routine laboratory tests. Finally, we carried out molecular based analyses using agarose gel electrophoresis and DNA barcoding using the markers rbcL and ITS.*

Findings: *The sensory analysis revealed that human subjects equally preferred both WS and SP dishes. According to phytochemical assessment, SP contained higher amounts of anthocyanin, flavonoids, phlobatannins, reducing sugars, tannins and terpenoids. The XRF analysis revealed that SP shoot-tops did not accumulate toxic heavy metals while WS shoot-tops grown in the same garden soil accumulated toxic heavy metals in trace amounts. Commercially available WS in Sri Lanka contained Hg (4500 mg/Kg), Cd (1056 mg/Kg), As (598 mg/Kg) and Cr (74 mg/Kg). WSP contained Hg (1820 mg/Kg), Cd (228 mg/Kg), As (126 mg/Kg), Cr (138 mg/Kg), Sb (114 mg/Kg), Sn (464 mg/Kg) and Pb (2100 mg/Kg) and the highest amount of Fe (6894 mg/Kg).*

Research Limitation: *It is imperative to study the heavy metal profiles of WS samples grown in diverse locations of Sri Lanka in collaboration with state consumer protection agencies to profile the food safety levels.*

Originality/ Value: *It is apparent from the present study that the consumption of WS is unsafe to human and animal health. DNA barcoding assays can be successfully employed by the consumer protection agencies to confirm the identity of SP. Out of the two tested markers, ITS is more straight forward in exhibiting the length polymorphism. The sequence data confirms band sizes detected using agarose gels and ITS is more informative in studying the genus Ipomoea than rbcL.*

Keywords: *Healthy and safe green leafy vegetables, Ipomoea aquatica, Ipomoea batatas, Toxic heavy metals, XRF for heavy metal assessment in vegetables*

[1*]Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, 20400, Sri Lanka
sunethuop@gmail.com
ORCID http://orcid.org/0000-0002-5592-1742

INTRODUCTION

Ipomoea aquatica Forssk. [(water spinach) (WS)] Vern. *Kangkong*, of family Convolvulaceae, is a globally consumed green leafy vegetable (GLV). WS mainly grows in aquatic and semi-aquatic habitats (Van Ooststroom and Hoogland, 1953; Fang and Staples, 1995; Austin 1998). The origin of WS is South East Asia (Filatenko *et al*., 2003; Li, 1970) and commercially cultivated as an edible GLV in many Asian countries such as China, India, Japan, Myanmar, Bangladesh, Indonesia, Malaysia, Philippines, Thailand and Sri Lanka (Wiser, 1955; Wills *et al*., 1984; Clarke, 1885; French, 1986; Westphal, 1993; Manandhar, 2002). The optimum growth of WS occurs in the temperature range of 24-30 °C (Rabatzky and Yamaguchi, 1997). WS grows free floated in water bodies and rooted when touches the moist soil (Duc *et al*., 1999).

Human beings consume the tender upper parts of the foliage (shoot-tops) of WS. The farmers use the remainder of the shoots as livestock fodder or mulching material. WS has high nutritional value as a GLV (Umar *et al*., 2007). It also possesses significant medicinal properties, such as laxative in indigenous herbal medicinal preparations (Read, 1936; Burkill, 1966; Van Valkenburgh and Bunyapraphatsara, 2001), treatment for ringworm lesions (Burkill, 1966; Van Valkenburgh and Bunyapraphatsara, 2001) and anti-poisonous therapy for the toxicity due to the intake of Opium, Arsenic (As) and polluted water. Also, WS has significantly higher glycemic activity and is a component of herbal drugs to treat people with diabetes (Uphof, 1968; Kapoor and Kapoor, 1980; Iwu, 1993; Villansenor *et al*., 1998; Van Malalavidhane *et al*., 2000; Valkenburgh and Bunyapraphatsara, 2001).

However, the consumption of WS is unsafe due to the bio-accumulation of toxic elements such as Pb, Cd, Hg and As when WS is grown in polluted habitats (Gothberg *et al*., 2002; Kananke *et al*., 2014). Most of the shoot-tops of WS harvested in Sri Lanka are coming from the unhygienic habitats polluted with the wastewater released from the urban drainage systems and industrial effluents (Pers. Comm.). The presence of toxic elements would be variable and under the safe limits (Kananke *et al*., 2014) imposed by WHO/FAO or can be even higher making it treacherous for the human consumption. However, WS is popular among human beings as a GLV with a lot of demand and consumption. In Sri Lanka, WS is a very common herb in the hotel based and household cuisines. There is no problem of consuming of WS if it is growing in farms utilizing safe growing media. Such isolated WS farms are very rare in Sri Lanka, and most of the harvest is coming from unhygienic habitats (Pers. Comm.). Thus, there must be an alternative GLV in place of WS for the human consumption. There are no detailed studies on the exploration of an alternative GLV for bioaccumulation-prone WS. In this context, the tender shoot-tops of sweet potato (SP) [*Ipomoea batatas* (L.) Lam.] can be used as an alternative. SP is a globally consumed tuber crop (Watson and Dallwitz, 1992), native to tropical America and naturalized in many parts of the world. The SP vines grow very rapidly and cover the ground within a shorter period making the minimal use of herbicides for weed control. Because of the rapid growth, the insects find less time to infest shoots making the use of insecticide to a minimum level. Although the tubers of SP are consumed worldwide, consumption of SP as a leaf vegetable is restricted to the ethnic range and some rural areas of the countries such as China, Africa, Taiwan, and Japan (Li *et al*., 2017). The SP leaves are rich sources of polyphenols and flavonoids and possess very high free radical scavenging activity compared to other vegetables (Chu *et al*., 2000; Tang *et al*., 2000; Islam *et al*., 2002; Lin *et al*., 2004). Although SP leaves possess significant food, nutrient, and medicinal properties, they are still recognized as an under-exploited GLV and mainly ended up as an animal fodder or mulching material.

Therefore, we conducted the present study to assess the toxic heavy metal contents, consumer preference, relative presence of essential phytochemicals and evolutionary relationships between the commonly grown SP varieties as a safe alternative GLV to common WS varieties grown and consumed in Sri Lanka.

MATERIALS AND METHODS

Plant material

We collected WS and SP plant material from the home gardens except for commercially grown WS variety (WS1) from an authenticated seed store in Kandy, Sri Lanka. We gathered the other two WS selections; WS selection-*Kandy* (WS2) and WS selection-*Kurunegala* (WS3); from *Manikhinna* (7.293878 °N, 80.697103 °E) of *Kandy* District and *Mawathagama* (7.185197 ° N, 80.280404 °E) of *Kurunegala* District, Sri Lanka. We also gathered the SP selection with purple leaves (SP1) and the SP selection with green leaves having purple veins (SP2) from *Peradeniya* (7.259952 ° N, 80.596570 °E) of *Kandy* District. We planted all five WS and SP selections in a greenhouse at Peradeniya. We maintained WS plants according to the instructions given in Temperate Climate Permaculture, (2018) and SP plants according to the crop recommendations given by the Department of Agriculture, Sri Lanka (Horticultural Crop Research and Development, 2018).

Assessment of the leaf morphological variation

We randomly collected ten fully grown leaves from greenhouse-grown WS and SP plants to measure the morphological parameters; petiole length, leaf parameters; length, width, area, shape, base, tip, margin, the pattern of venation and the leaf arrangement on the stem. We followed the key available at Norton Brown Herbarium, (2016) to record the morphological measurements.

Assessment of toxic heavy metals

We obtained shoot-top samples from greenhouse grown SP1 and SP2 and pooled them for XRF analysis. We also obtained greenhouse grown WS1, WS2 and WS3 samples and pooled them for XRF analysis. In addition to the greenhouse grown SP and WS samples, we purchased commercially available WS (WSC) and collected WS shoot-tops from a polluted aquatic site (WSP) in Kandy Municipal Council area (7.278971 ° N, 80.618917 ° E). Next, we oven dried the samples at 80 °C for three days. We then crushed the dried shoot-tops to obtain finely powdered samples. Then we subjected the five powdered samples in multiple replicates of each WS and SP types to X-ray fluorescence (XRF) analysis using Fischerscope X-Ray Xan analyzer and obtained the elemental contents in mg/ kg (dry weight basis). During the analysis, we calibrated the XRF analyzer for 16 elements and a set of readings were also obtained for the blank samples. Finally, we corrected the elemental readings for SP and WS samples with reference to the readings obtained for the blank samples.

Preparation of dishes for sensory assessment

We harvested the tender shoot-tops from greenhouse-grown WS and SP and cooked according to the most common basic recipe used to prepare WS in Sri Lanka. For each WS and SP selections separately, we weighed 1 kg of shoot-tops and cut into 3-5 cm pieces. Then we mixed shoot-top-pieces with 100 g of onion, 50 g of chopped green chili and table salt for the required taste. We made sure to mix gently to avoid the squeezing the content. We then tempered the mixture for three mins in 5 ml of coconut oil under low-medium heat in a closed pan. We allowed the mixture to settle for 15 mins before subjecting to the taste panel analysis. We employed 30 human subjects to rank the preferred levels of color, aroma, texture, bitterness and overall taste separately for all five dishes using a hierarchical scale of one, two and three. Except for bitterness, one represented the least preferred category and three for the highest

preferred. For the bitterness, three served the most upper bitterness felt by the panelists.

Qualitative measurement of the phytochemicals

We harvested the shoot-tops of WS and SP selections from the greenhouse and cleaned thoroughly using distilled water to remove debris. Then we left the shoot-tops to dry for 24 hours in the room temperature on a laboratory bench. We weighed 30 g from each sample, ground and dissolved in approximately 200 ml of distilled water. Next, we sonicated the mixtures for 30 mins and filtered the mixture through an 8 µm Whatman® qualitative filter paper, Grade 2 (Cat No.: 1002-125) three times until solution became clear. Through the filtration, we were able to recover about 50 ml of filtrate. We stored all the filtrates at 4 °C until used them for phytochemical analyses.

Anthocyanin test

We mixed 2 ml of leaf extract with 1 ml of 2M NaOH. The presence of anthocyanin was indicated by the appearance of a bluish green color (Harborne, 2014).

Flavonoid test

We added 5 ml of diluted NH_3 to 1 ml of leaf extract and observed yellow coloration. Then we added conc. H_2SO_4 gradually. The disappearance of intense yellow color with the addition of conc. H_2SO_4 indicated the presence of flavonoids. The number of flavonoids present in the sample is proportional to the volume of H_2SO_4 added (Hossain *et al*., 2013).

Phlobatannin test

We boiled a mixture containing few drops of 2 % HCl solution and 1 ml of leaf extract in a water bath. A red precipitate was visible indicating the existence of phlobatannins (Auwal *et al*., 2014).

Reducing sugar test

We added few drops of Benedict's solution to 1 ml of leaf extract and boiled the mixture for few minutes. The development of brick-red precipitation confirmed the presence of reducing sugars (Benedict, 1909).

Saponin test

We shook a sample of leaf extract vigorously to detect the formation of a persistent froth as an indicator of the presence of saponin (Singh *et al*., 2012).

Tannin test

We added few drops of freshly prepared 10 % $FeCl_3$ to 1 ml of leaf extract and the presence of tannins was given by the development of greenish or bluish black color in the mixture (Auwal *et al*., 2014).

Salkowski test for terpenoids

We added 2 ml of chloroform to 1 ml of leaf extract and mixed well. Then we incorporated 3 ml of conc. H_2SO_4 to the mixture along with the wall of the tube. The presence of terpenoids was confirmed by the formation of reddish-brown color at the interface of the solvents (Singh *et al*., 2012).

pH and ascorbic acid

We measured the pH in triplicate for each sample using a pH meter. Simultaneously, we measured the ascorbic acid content by adding three drops of the indicator-starch solution to 5 ml of leaf extract. Then we titrated the resulted mixture using a 0.005M iodine solution. We observed the formation of the starch-iodine complex at the endpoint as a dark bluish black coloration. We repeated the titration three times for each replicate. Finally, we employed the following equation to calculate the ascorbic acid concentration.

$$\text{Ascorbic acid}_{(aq)} + I_{2(l)} \rightarrow 2I^-_{(aq)} + \text{Dehydroascorbic acid}_{(aq)}$$

DNA extraction, PCR, and Sequencing

We picked tender leaves from greenhouse-grown WS and SP plants for DNA extraction using the CTAB method (Doyle and Doyle, 1990). We further purified the extracted DNA using chloroform and isoamyl alcohol to remove proteins and other contaminants. We

precipitated the extracted DNA using ice cold isopropanol. We washed the pellet with 70 % alcohol and ammonium acetate to remove the salts and alcohol remnants to neutralize the charge on the DNA backbone to lessen the hydrophilic attribute of DNA. Finally, we dissolved the DNA pellet in TE buffer which protected DNA from degradation. We PCR amplified the extracted DNA samples using the standard DNA barcoding primer pairs; *rbcL* (5'-3' ATG TCA CCA CAA ACA GAG ACT AAA GC/ GTA AAA TCA AGT CCA CCR CG) (Wang *et al*., 2001) and *ITS* (5'-3' TCC GTA GGT GAA CCT GCG G/ TCC TCC GCT TAT TGA TAT GC) (White *et al*., 1990). We amplified the *rbcL* locus using the following cycle. The initial denaturation at 94 °C for 3 mins; 35 cycles of denaturation at 94 °C for 30 secs, primer annealing at 55 °C for 1 min, synthesis at 72 °C for 90 secs and final synthesis at 72 °C for 4 mins (Bolson *et al*., 2015). We amplified the *ITS* locus using the following cycle. The initial denaturation at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min, synthesis at 64 °C for 1 min and final synthesis at 72 °C for 10 mins. We prepared 15 µl PCR solution mixtures with 1× Go Taq® Green Master Mix (Promega Corporation, Madison, Wisconsin, USA), 1.0 µM of forward and reverse primers and 1.0 µl of DNA template. We visualized the PCR products in agarose gel electrophoresis and purified using the Wizard SV gel® and PCR clean-up system (Promega Corporation, Madison, Wisconsin, USA). Finally we subjected the purified PCR products to DNA sequencing using the ABI 3500 Genetic Analyzer (Cat. No. 4405186).

Analysis of morphological, heavy metal, sensory and phytochemical data

We analyzed the quantitative XRF, leaf morphological measurements using ANOVA procedure in the in Statistical Package SAS 9.4 (SAS Institute, NC, Cary, USA). We subjected the ranked data generated from the taste panel analysis to the FREQ procedure in SAS and calculated the row and column percentages of the significant association to interpret the relative preference on SP dishes in relation to WS dishes. We analyzed the phytochemical data descriptively as well as statistically using principal component analysis (PCA) and cluster procedures in SAS.

Analysis of DNA barcorde sequences

We created two alignments separately for *ITS* and *rbcL.* We aligned the sequences of *ITS* region using the CLUSTAL W algorithm (Thompson *et al*., 1997) in MEGA V7 (Kumar *et al*., 2016). In contrast, we aligned the *rbcL* sequences manually in MEGA V7 (Kumar *et al*., 2016) without affecting the reading frame. We converted the final alignment into amino acid sequences to check the presence of stop codons. We used both alignments separately to draw distance trees. We prepared two distance trees independently for both markers using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. We calculated the pairwise distances between sequences using uncorrected *p* distances. We visualized the distance trees and further modified the trees using FigTree v 1.4.3 (Rambaut, 2014). Finally we created the barcodes for *rbcL* and *ITS* markers using BIO-RAD barcode generator (BIO-RAD, 2018).

RESULTS

Morphological variation of the shoot-tops and leaves

We observed the significantly highest mean leaf petiole length in SP1 and SP2 (18.40 cm and 18.50 cm respectively) followed by in WS1 (10.42 cm) and the least in WS1 and WS2 (5.71 cm and 5.73 cm). We found the significantly most extended leaves in WS1 (mean leaf length=18.43 cm) the broadest leaves in SP2 (mean blade width=18.95 cm). As an essential attribute of GLV, we noticed the significantly largest leaves in SP2 (mean leaf area=132.60 cm^2) followed by SP1 (94.90 cm^2) (Table 1, $P<0.05$). Also, Table 1 summarizes all the

qualitative leaf morphological parameters. As appealing GLVs, both WS and SP did not produce hairs on the leaves. WS exhibited 'slippery' textured leaves whereas SP exhibited 'mucilaginous' textured leaves that are ideal for the preparation of various delicacy. Figure 1 shows the representative shoot-top and leaf images of the three WS and two SP selections studied.

Table 01: Variation of the leaf morphological parameters

Parameter	Water spinach			Sweet potato	
	WS1	WS2	WS3	SP1	SP2
Petiole length (cm)	5.71^{c}	10.42^{b}	5.73^{c}	18.40^{a}	18.50^{a}
Leaf length (cm)	18.43^{a}	11.86^{c}	7.76^{b}	12.23^{c}	14.50^{b}
Leaf width (cm)	3.11^{e}	6.42^{c}	4.51^{d}	14.59^{b}	18.95^{a}
Leaf area (cm^2)	44.30^{d}	65.2^{c}	24.8^{e}	94.90^{b}	132.60^{a}
Leaf shape	Sagittate	Sagittate	Sagittate	Palmatifid	Palmatifid
Leaf base shape	Auriculate	Auriculate	Auriculate	Auriculate	Auriculate
Leaf tip shape	Acuminate	Acuminate	Acuminate	Cuspidate	Cuspidate
Leaf margin	Entire	Entire	Entire	Entire	Entire
Presence of hairs	No	No	No	No	No
Leaf texture	Slippery	Slippery	Slippery	Mucilaginous	Mucilaginous
Venation pattern	Pinnate	Pinnate	Pinnate	Palmate	Palmate
Leaf arrangement	Spiral	Spiral	Spiral	Alternate	Alternate

Means denoted by the same letters within the rows are not significantly different at P< 0.05

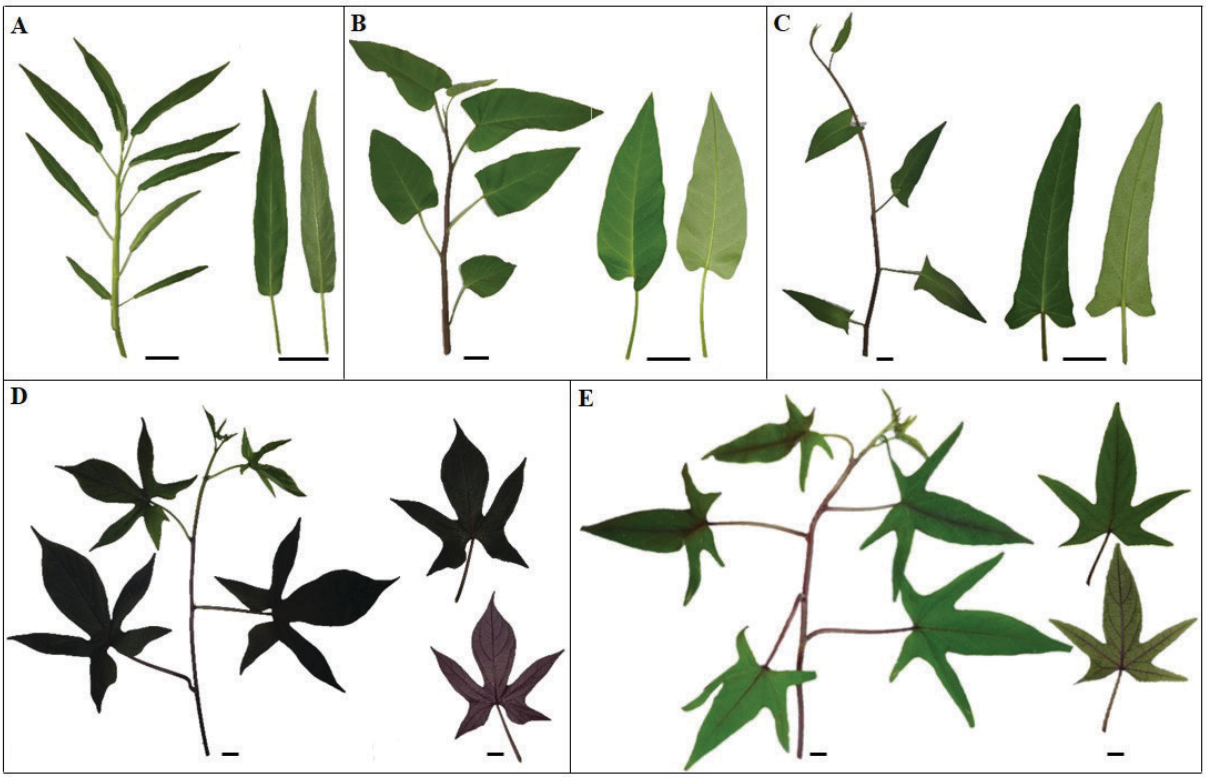

Figure 01: Edible shoot-tops of the three water spinach (WS) and two sweet potato (SP) collections. A representative shoot-top and, adaxial and abaxial surfaces of the leaf are shown. A: WS1; B: WS2; C: WS3; D: SP1; E: SP2. Scale bars represent 1 cm for each specimen separately.

Toxic heavy metal content

We observed the significantly highest mean K content in SP (27506 mg/Kg), WSC (26590 mg/Kg) and greenhouse grown WS (25178 mg/Kg) and the least amount in WSP (19130 mg/Kg). We detected the significantly highest mean amount of Ca in WSC (14992 mg/Kg) and the least amount in WSP (3736 mg/Kg). We observed the significantly highest mean S content in WSP (22100 mg/Kg). We detected the significantly highest mean contents of Zn (814 mg/Kg), Cu (1942 mg/Kg), Ni (744 mg/Kg), Co (846 mg/Kg) and Mn (928 mg/Kg) in WSC. The Fe contents were significantly higher in WSP (6894 mg/Kg) and WSC (3664 mg/Kg). We did not detect the heavy metals Hg, Cd, As, Cr, Sb, Sn and Pb in SP. However, we detected trace amounts of above heavy metals in pooled samples of greenhouse grown WS although we grew both SP and WS on the same potting medium (Table 2, P<0.05). WSC had the significantly highest mean amounts of Hg (4500 mg/Kg), Cd (1056 mg/Kg) and As (598 mg/Kg). We detected significantly highest mean amounts of all the heavy metals Hg (1820 mg/Kg), Cd (228 mg/Kg), As (126 mg/Kg), Cr (138 mg/Kg), Sb (114 mg/Kg), Sn (464 mg/Kg) and Pb (2100 mg/Kg) in the WSP samples. The study revealed that the toxic heavy metal Pb was significantly highest in the WSP (2100 mg/Kg) (Table 2, $P<0.05$).

Consumer preference

The WS1 exhibited an attractive green color (Figure 2A) compared to the WS2 (Figure 2B) and WS3 (Figure 2C). When comparing two SP dishes, SP1 exhibited a gorgeous purple color (Figure 2D) whereas SP2 had equally appealing dark green color (Figure 2E). The overall appearance of WS dishes was unique because the stem pieces were more prominent in them compared to SP dishes leading to differences in crunchiness when eating.

We observed in the association analysis between the sensory attributes and the individual WS/SP selections that the tasters had significantly different preferences on the colors of the dishes prepared (Pearson $\chi^2=28.88$ at P=0.00). We observed the highest preferred color perception on SP2 and WS3 and the least color preference in WS3 (Figure 3A). All the other sensory attributes; aroma, texture, bitterness and the overall taste did not significantly associate with WS/SP selection (Figures 3B, 3C, 3D and 3E respectively) indicating that there were no perceived differences for the parameters assessed except color ($P>0.05$).

Table 02: The elemental contents detected for WS and SP samples

Element	Elemental content (mg/kg)			
	Greenhouse grown WS	WSC	WSP	SP
K	25178^{a}	26590^{a}	19130^{b}	27506^{a}
Ca	8462^{b}	14992^{a}	3736^{c}	8974^{b}
S	17100^{b}	13152^{b}	22100^{a}	16050^{b}
Zn	63^{c}	814^{a}	250^{b}	42^{b}
Cu	218^{b}	1942^{a}	104^{b}	54^{b}
Ni	281^{b}	744^{a}	346^{a}	0^{a}
Co	131^{b}	846^{a}	328^{a}	270^{a}
Mn	27^{c}	928^{a}	178^{b}	0^{b}
Fe	1709^{c}	3664^{b}	6894^{a}	348^{c}
Hg	135^{c}	4500^{a}	1820^{b}	0^{c}
Cd	154^{b}	1056^{a}	228^{b}	0^{c}
As	31^{c}	598^{a}	126^{b}	0^{c}
Cr	86^{b}	74^{b}	138^{a}	0^{c}
Sb	67^{b}	0^{a}	114^{a}	0^{a}
Sn	219^{b}	0^{b}	464^{a}	0^{b}
Pb	72^{b}	0^{b}	2100^{a}	0^{b}

Means denoted by the same letters within the rows are not significantly different at P< 0.05

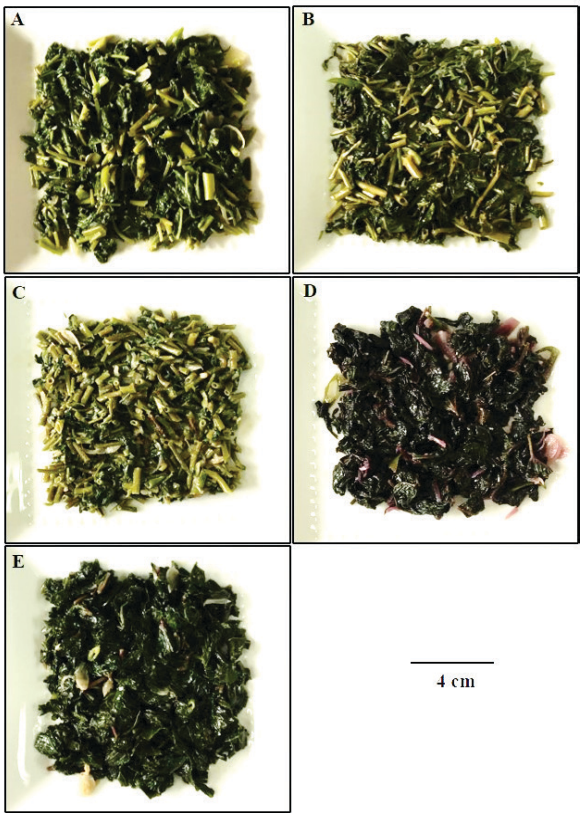

Figure 02: Culinary preparations of three WS and two SP selections subjected to the taste panel analysis. A: WS1; B: WS2; C: WS3; D; SP1; E: SP2.

We combined the sensory data of WS selections and SP selections separately as two different species and reassessed the association between the sensory attributes and the two species. Similar to the associations detected with individual selections, here also we detected a significant association between the species and the perceived color preference of the dish prepared (Pearson χ^2=7.73 at P=0.02). We observed from the percentage respondents in each category that tasters preferred the color of the SP dishes more compared to the WS dishes making SP is more appealing GLV than WS (Figure 4A). For all the other tested parameters; aroma, texture, bitterness and overall texture, we did not observe any significant association with the species (Figures 4B, 4C, 4D and 4E respectively) (P>0.05).

Phytochemical assessment

We found that the leaf extracts got significantly similar pH values in the range of 6.4-6.7. However, we measured a significantly higher ascorbic acid content in WS3 (9.0×10^{-5} mol/dm^3) compared to the others (Table 3) (P<0.05). Table 4 shows the relative amounts of seven phytochemicals WS and SP collections. We did not positively detect saponins. We noted that the collection SP1 got ranked highest for other phytochemicals. We calculated PCs for the qualitative measurements and drew a scatter plot between the two major PCs to display overall content of phytochemicals in WS and SP accessions. Also using all PCs, we constructed a dendrogram to verify the separation in the scatter plot. The scatter plot between two major PCs displays the overall difference of the phytochemical compounds WS and SP collections (Figure 5A). According to the grouping in the scatterplot, it is evident that the three WS selections got the similar type of phytochemical profiles whereas two SP selections got the similar type of phytochemical profiles. We observed the same outcome in the dendrogram where it has two major clusters of WS and SP depending for the differences of phytochemical profiles (Figure 5B).

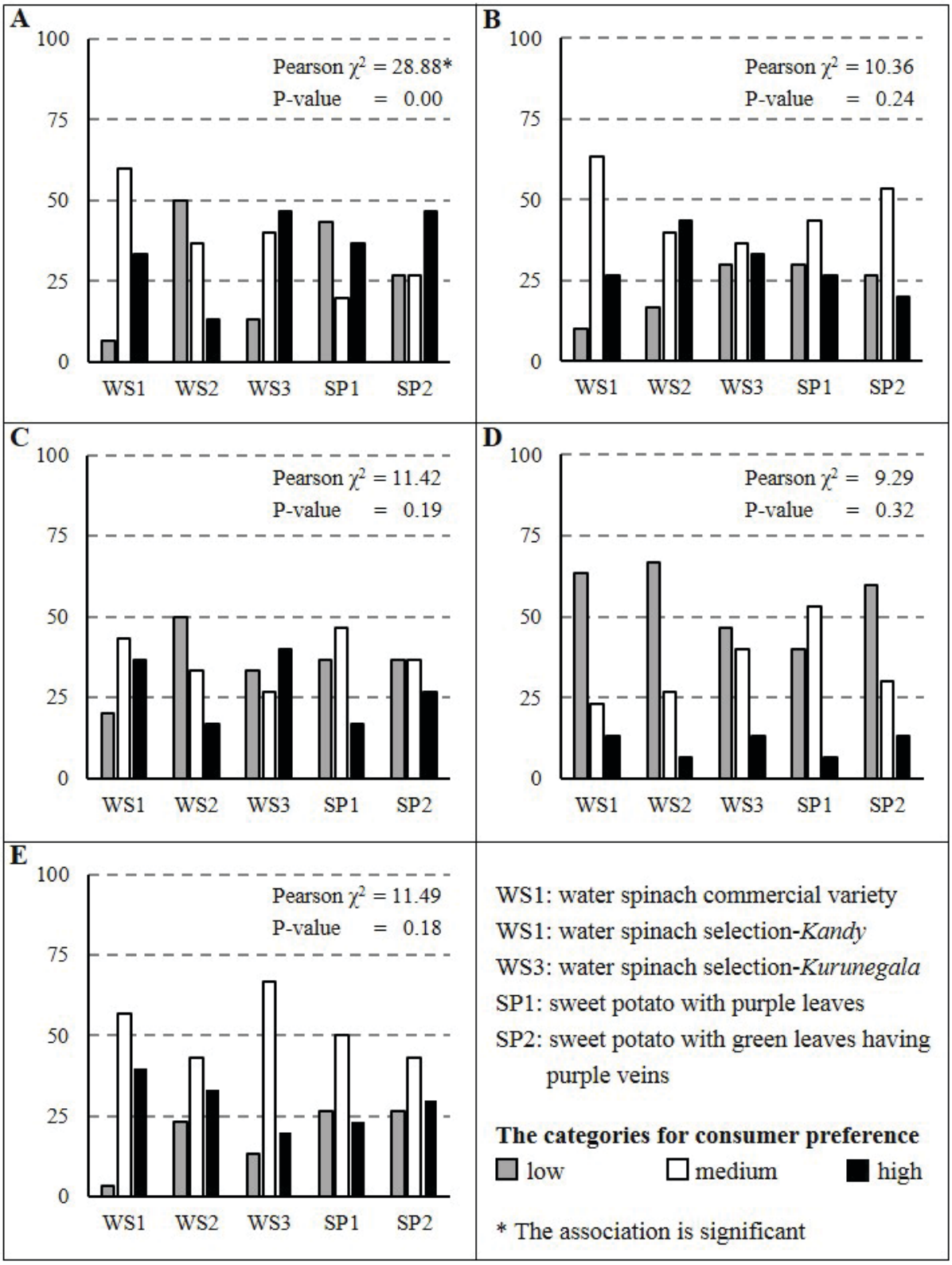

Figure 03: **The Comparison of the strength of the association analysis (i.e., magnitude of the chi-square (χ^2)) between sensory parameters (color, aroma, texture, bitterness and overall taste) and individual WS/SP selections. A: Color; B: Aroma; C: Texture; D: Bitterness; E: Overall taste. Y-axis represent the percentage respondents.**

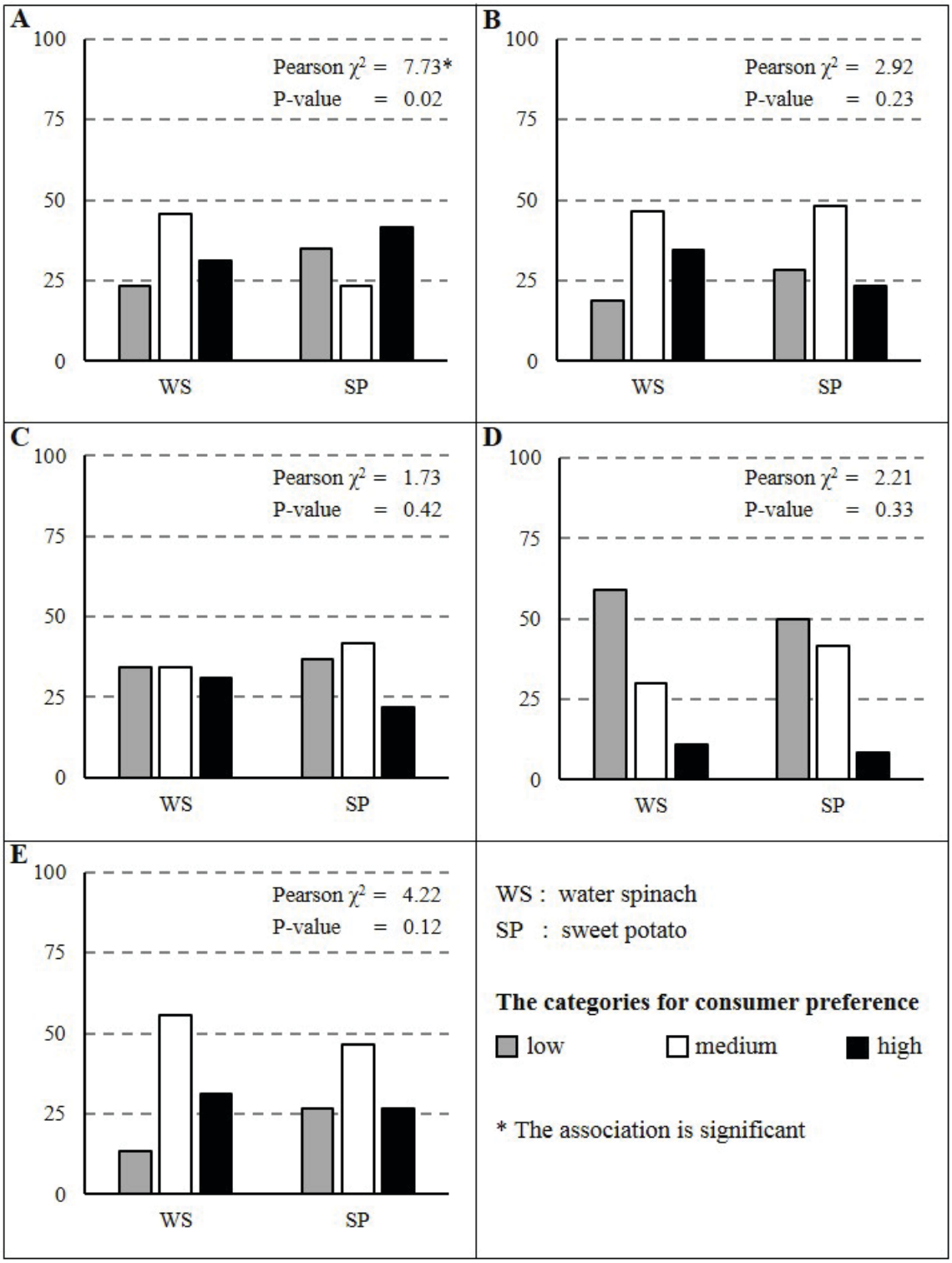

Figure 04: **Comparison of the strengths of the association analysis (i.e., magnitude of the chi-square (χ^2)) between sensory parameters (color, aroma, texture, bitterness and overall taste) and WS and SP selections combined species wise. A: Color; B: Aroma; C: Texture; D: Bitterness; E: Overall taste. Y-axis represent the percentage respondents.**

Table 03: Variation of the three biochemical parameters among the WS and SP selections

Parameter	WS1	WS2	WS3	SP1	SP2
pH	6.58[a]	6.52[a]	6.70[a]	6.45[a]	6.54[a]
Ascorbic acid content (mol/dm^3)	3.67×10^{-5b}	3.67×10^{-5b}	9.0×10^{-5a}	3.67×10^{-5b}	3.67×10^{-5b}

Means denoted by the same letters within the rows are not significantly different at P< 0.05

Table 04: The relative abundance of phytochemicals in WS and SP

Parameter	WS1	WS2	WS3	SP1	SP2
Anthocyanin	3	4	1	5	2
Flavonoids	2	2	1	3	1
Phlobatannins	2	3	1	4	1
Reducing sugars	2	3	1	4	3
Saponnins	0	0	0	0	0
Tannins	3	2	1	4	3
Terpenoids	3	2	1	4	3

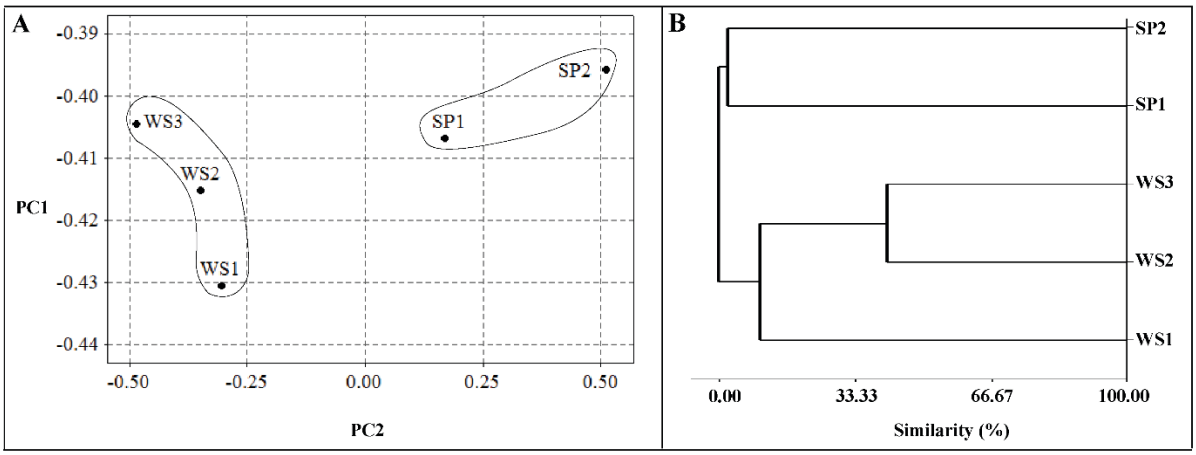

Figure 05: The overall phytochemical profiles in WS and SP selections. A: Scatter plot between two PCs calculated for the data in Table 3. B: Dendrogram drawn based on all PCs calculated for the data in Table 3. The WS and SP selections got clearly clustered onto species distinctions.

DNA barcoding

The DNA barcoding locus *rbcL* yielded a monomorphic band having the length of ~1350 bp for WS, SP and rice (positive control). Therefore, the length polymorphism of *rbcL* cannot be used to discriminate WS and SP. For the *ITS* locus, a band with ~675 bp was obtained for WS and ~650 bp fragment was observed for SP indicating clearly distinguishing bands in 2.5 % agarose gel electrophoresis (Figure 6). When *rbcL* PCR products were subjected to sequencing, 1250 bp long sequences were observed for both WS and SP. In contrast when *ITS* PCR products were subjected to sequencing, 633 bp and 606 bp long sequences were observed for WS and SP respectively. The sequence lengths for both loci are compatible with the band sizes observed in Figure 6. The generated *rbcL* and *ITS* sequences for three WS and two SP selections were submitted to GenBank. The accession numbers of for five *rbcL* sequences ranges from MH796544 - MH796548 and accession numbers for five *ITS* sequences ranges from MH792114 - MH792118.

In *rbcL*, only ten SNPs were obtained for the entire length of 1250 bp (Table 5). In the *ITS* locus of 637 bp, 44 SNPs and 35 INDELs (single-base INDELs) were detected and the exact length difference between WS and SP was 27 bp (Table 6). Figure 7A and 7B showed the DNA barcodes generated for WS1, WS2 and WS3 and SP1 and SP2 for *rbcL* and *ITS* sequences. The *rbcL* barcodes did not represent any length polymorphisms where the *ITS* barcode showed a length difference between WS and SP. The observed sequence polymorphism produced distance trees as shown Figure 7C for *rbcL* and Figure 7D for *ITS*. For both *rbcL* and *ITS* loci, there was no intra-species diversity detected for SP. However, for WS, *rbcL* shows intra-species diversity between WS1 and other two varieties with a 4.0625×10^{-4} uncorrected pairwise genetic distance. Similarly for *ITS*, WS1 was different from WS2 and WS3 with an uncorrected pairwise genetic distance of 0.0008.

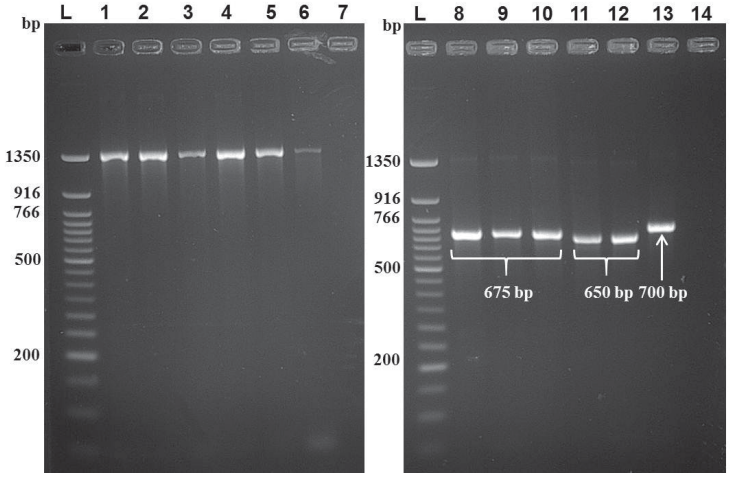

Figure 06: The length polymorphism detected for the loci *rbcL* and *ITS*.1-7: *rbcL* PCR products; 8-14: *ITS* PCR products; L: 50 bp ladder; 1 and 8: WS1; 2 and 9: WS2; 3 and 10: WS3; 4 and 11: SP1; 5 and 12: SP2; 6 and 13: Positive control (rice DNA as the template); 7 and 14: Negative control (no template in PCR)

Table 05: Allelic difference between SP and WS for *rbcL* marker

No.	SNPs	
	Position (bp)	Allelic difference
1	4	G (WS), C (SP)
2	63	G (WS), A (SP)
3	81	C (WS), T (SP)
4	375	T (WS), C (SP)
5	378	T (WS), A (SP)
6	444	A (WS), T (SP)
7	589	A (WS), C (SP)
8	688	C (WS), A (SP)
9	1047	G (WS), T (SP)
10	1230	A (WS), G (SP)

Table 06: Allelic difference between SP and WS for *ITS* marker

No.	SNPs		No.	INDELs	
	Position (bp)	Allelic difference		Position (bp)	Allelic difference
1	15	G (WS), T (SP)	1	38	-(WS), G (SP)
2	31	A (WS), G (SP)	2	45	-(WS), C (SP)
3	33	C (WS), G (SP)	3	72	G (WS), - (SP)
4	44	G (WS), T (SP)	4	73	G (WS), - (SP)
5	46	A (WS), G (SP)	5	90	A (WS), - (SP)
6	52	A (WS), G (SP)	6	167	-(WS), T (SP)
7	61	C (WS), A (SP)	7	168	-(WS), T (SP)
8	81	G (WS), T (SP)	8	219	A (WS), - (SP)
9	96	A (WS), G (SP)	9	220	G (WS), - (SP)
10	99	G (WS), A (SP)	10	450	T (WS), - (SP)
11	148	G (WS), A (SP)	11	519	G (WS), - (SP)
12	153	A (WS), G (SP)	12	520	A (WS), - (SP)
13	162	C (WS), A (SP)	13	521	A (WS), - (SP)
14	164	T (WS), G (SP)	14	522	C (WS), - (SP)
15	165	T (WS), A (SP)	15	523	A (WS), - (SP)
16	173	A (WS), C (SP)	16	524	A (WS), - (SP)
17	185	T (WS), A (SP)	17	525	G (WS), - (SP)
18	191	G (WS), A (SP)	18	526	C (WS), - (SP)
19	193	T (WS), A (SP)	19	527	C (WS), - (SP)
20	199	A (WS), C (SP)	20	528	C (WS), - (SP)
21	212	G (WS), A (SP)	21	529	C (WS), - (SP)
22	214	C (WS), G (SP)	22	530	A (WS), - (SP)
23	297	A (WS), G (SP)	23	531	C (WS), - (SP)
24	402	G (WS), A (SP)	24	532	C (WS), - (SP)
25	420	A (WS), G (SP)	25	533	C (WS), - (SP)
26	423	C (WS), T (SP)	26	534	C (WS), - (SP)
27	440	T (WS), A (SP)	27	535	G (WS), - (SP)
28	441	A (WS), G (SP)	28	536	A (WS), - (SP)
29	455	G (WS), C (SP)	29	537	G (WS), - (SP)
30	458	T (WS), C (SP)	30	538	G (WS), - (SP)
31	467	C (WS), T (SP)	31	539	G (WS), - (SP)
32	469	G (WS), A (SP)	32	540	A (WS), - (SP)
33	470	A (WS), G (SP)	33	541	T (WS), - (SP)
34	473	A (WS), C (SP)	34	542	G (WS), - (SP)
35	475	G (WS), A (SP)	35	555	C (WS), - (SP)
36	489	G (WS), A (SP)			
37	510	G (WS), A (SP)			
38	511	A (WS), G (SP)			
39	546	G (WS), C (SP)			
40	548	C (WS), T (SP)			
41	563	A (WS), G (SP)			
42	567	G (WS), T (SP)			
43	581	T (WS), C (SP)			
44	609	A (WS), T (SP)			

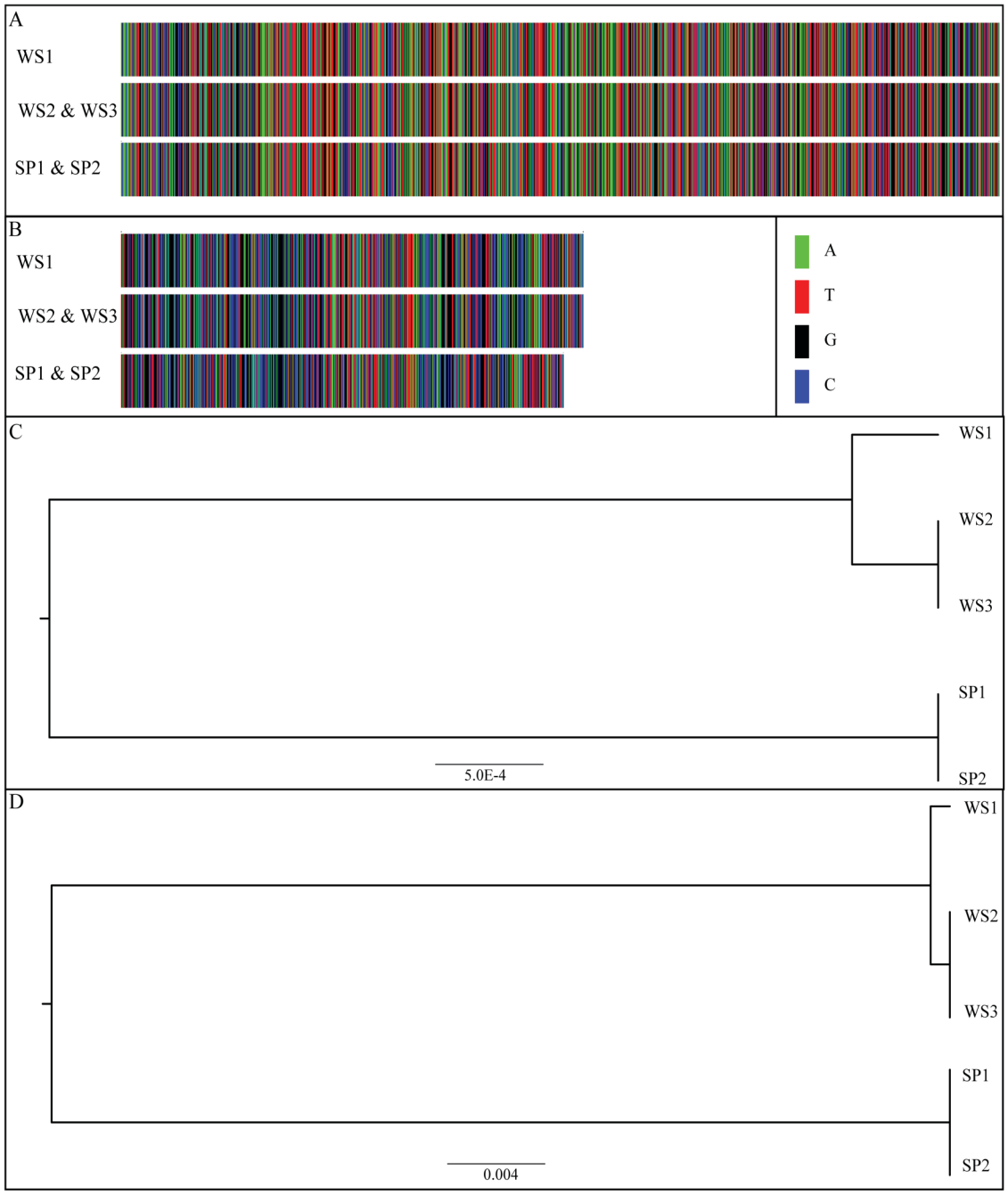

Figure 07: DNA barcode polymorphism (*rbcL* and *ITS*) for WS and SP. A: The DNA barcodes of *rbcL*; B: The DNA barcodes for *ITS* marker; C: Distance tree drawn for *rbcL* marker; D: Distance tree drawn for *ITS* marker. The scale of the distance trees represent the uncorrected pairwise genetic distance. In the DNA barcodes, the bases are depicted according to the colors given in the legend showed at bottom-right of the Panel B.

DISCUSSION

WS and SP are two plant species belonging to the genus *Ipomoea* (World Crops for Northern United States, 2018). WS is a popular leafy vegetable consumed by wide range of ethnic groups all over the world (Marcussen *et al*., 2008). The evolutionary closest relative for WS is SP (Islam, 2014; World Crops for Northern United States, 2018). The tubers of SP are the staple food in some parts of Brazil (Dhaliwal, 2017; World crops for Northern United States,

2018), Africa, Taiwan, Japan and China. So far, the edibility of shoot-tops of SP remains under-evaluated (Woolfe, 1992; Johnson and Pace, 2010). According to the morphological analyses, we detected that these two species show similarities in parameters leaf base shape, leaf margins and absence of hairs despite their distinct species differences. Both WS and SP show intra-species diversities for the leaf parameters length, width and area. However, the petiole lengths of SP do not show any intra-species variations while WS shows variation. Surprisingly, WS3 and SP2 show similarities in leaf lengths (Table 1; Figure 1).

Since SP is not an aquatic plant there is no way of collecting SP samples from polluted aquatic habitats. We pooled the greenhouse grown SP samples and WS samples separately to obtain more representative measurements for heavy metal contents (Table 2). To display the elemental profiles and to provide the inferences about the food safety of SP and WS, we collected WSC samples from the market and WSP samples from an aquatic habitat with polluted water coming from urban and industrial activities. We noted that SP shoot-tops are safe to consume because of the absence of toxic heavy metals Hg, Cd, As, Sb, Sn and Pb. Also, SP lacked Ni and Mn that could be toxic under higher concentrations. The WSC samples usually purchased by the customers contain significantly higher mean amounts of Hg, Cd and As indicating that they are coming from habitats polluted with heavy metals or grown in agricultural soils prone to heavy metal contaminations. The WSP samples contained all the heavy metals in significantly higher amounts. Although it has not been documented, it is well known in Sri Lanka that the shoot-tops are harvested from these unsafe sites and sold in the markets. Even though, greenhouse grown SP and WS were established using the same potting medium, WS contains trace amounts of toxic heavy metals, thereby indicating that WS bioaccumulates heavy metals whereas SP does not undergo that process (Table 2, $P< 0.05$). The present study confirmed that WSC and WSP contain toxic heavy metals and not safe for the consumption. The bioaccumulation of heavy metals by WS has been reported in Auwal *et al*., (2014), Gothberg *et al*., (2002), and Marcussen *et al*., (2008) and the results reported are comparable with the observations made in the present study.

For the first time in Sri Lanka, we reported the consumer preference on SP shoot-tops in comparison to WS when prepared as culinary dishes (Figures 2, 3 and 4). We noted that consumers equally prefer SP for its color, aroma, texture, bitterness and overall taste indicating that SP shoot-tops are a good substitute for WS. The color preference is a major concern when novel food item is released to the market and we observed that despite color variations between SP and WS dishes consumers equally or highly preferred SP dishes compared to WS. However, we observed that there is a slippery texture for the WS leaves while SP leaves are mucilaginous in texture which is ideal for the preparation of various delicacies. However, at present, the prominent stem pieces of the WS make the WS dishes more attractive than the SP dishes (Figure 2).

The qualitative phytochemical assessment revealed that SP1 is highly nutritious compared to all WS samples and SP2. However, after assigning qualitative scores for the phytochemicals (anthocyanin, flavonoids, phlobatannins, reducing sugars, tannins, terpenoids) once subjected to nutritional analysis and clustering; all WS and SP samples got clustered differently showing two clear groups (Figures 5A and 5B). The phytochemicals present in the shoot-tops specially anthocyanin and polyphenols are known to result in many physiological / medicinal properties of SP (Islam, 2014; Sun *et al*., 2014; Dhaliwal, 2017; Li *et al*., 2017). Accordingly, some studies have found that SP shoot-tops contain vital nutrients required to promote human health (Johnson and Pace, 2010). The SP shoot-tops possess medicinal properties such as anti-oxidative activity, anti-mutagenicity, anti-carcinogenesis, anti-hypertension, antimicrobial activity, anti-inflammation, anti-diabetic effect, anti-

HIV, reduction of liver injury, relief from constipation and ultra-violet protection effects. Thereby, previous studies have reported that consumption of SP can combat malnutrition especially in developing countries (Sun *et al*., 2014). Other than using for consumption, WS is popular for its medicinal effects. It is a known laxative and recommended for piles (Austin, 1998). Also, WS is a great source of nutrition (Austin, 1998; SkipThePie.org - The Nutrition Search Engine, 2018).

Therefore, the consumption of SP provides higher nutritional value compared to WS other than the safety incurred by SP over WS. The public awareness programs to popularize consumption of SP shoot-tops and to discourage the consumption of suspected WS coming from potentially polluted habitats are important. Simply if the origin is not known for WS, it should not be consumed. However, as we previously mentioned most of WS shoot-tops available in the market are coming from polluted aquatic habitats. If the general public and consumer protection agencies accept based on the scientific information to popularize the SP over WS, DNA based detection methods are required to differentiate SP from WS. This is because the assessment of heavy metals content to differentiate WS from SP would be subjective and less robust compared to DNA barcoding which is the standard method available for detecting species identities in the world. The present study represents two barcodes for *rbcL* and *ITS* and their internal SNP and INDEL polymorphisms (Figures 6 and 7; Tables 5 and 6). It is clear from the DNA barcoding analysis that *ITS* can be used to discriminate two species without DNA sequencing which provides easy, quick and low-cost platform to discriminate SP from WS. The marker *rbcL* displayed fewer mutations with only ten SNPs compared to *ITS* indicating that *ITS* would be a promising candidate DNA barcoding locus to study the intra-generic variations of *Ipomoea*. (Tables 5 and 6) (Gielly and Taberlet, 1994). Surprisingly, there was a unique SNP (in addition to above 9) at the 543rd position for WS1 for *rbcL* in which WS1 has the base C and all other four varieties have the base A. Thus, *rbcL* could discriminate WS1 from all other WS species and SP species studied in the present study indicating an intra-species variation. WS1 that was purchased from commercial seed store could be an exotic introduction although the exact origin is unknown. Also, the morphological analysis conducted in the present study demonstrated that morphology of WS1 is quite different compared to WS2 and WS3 (Figure 1). If this difference is considered along with the differences of nucleotide divergence depicted in Figures 9C and 9D, it could be logical to think that WS1 could be a different species or sub species within *I. aquatica*. However, further studies on more barcoding loci are required to test this hypothesis.

CONCLUSIONS

In the present study, we tested the applicability of using shoot-tops of SP to replace bioaccumulation prone WS if grown in polluted sites. The consumer preference analysis showed that human subjects equally prefer SP as WS. The phytochemical assessment revealed that SP contains higher amounts of anthocyanin, flavonoids, phlobatannins, reducing sugars, tannins and terpenoids. The XRF analysis revealed that SP shoot-tops do not accumulate toxic heavy metals. However, WS shoot-tops grown in the same garden soil accumulate toxic heavy metals in trace amounts [Hg (135 mg/Kg), Cd (154 mg/Kg), As (31 mg/Kg), Cr (86 mg/Kg), Sb (67 mg/Kg), Sn (219 mg/Kg) and Pb (72 mg/Kg)]. WSC in Sri Lanka contains Hg (4500 mg/Kg), Cd (1056 mg/Kg), As (598 mg/Kg) and Cr (74 mg/Kg). WSP contains Hg (1820 mg/Kg), Cd (228 mg/Kg), As (126 mg/Kg), Cr (138 mg/Kg), Sb (114 mg/Kg), Sn (464 mg/Kg) and Pb (2100 mg/Kg) and highest amount of Fe (6894 mg/Kg). It is apparent from the present study that the consumption of WS is unsafe unless WS is grown in authenticated growth media or hydroponic cultures certified for the absence of heavy metals. If consumer protection agencies want to replace WS with SP, DNA barcoding

assays can be used to confirm species identity. Out of the two assessed DNA barcodes (*rbcL* and *ITS*), *ITS* is more straight forward because it exhibits a length polymorphism in agarose gels where *ITS* generates ~675 bp band for WS and ~650 bp band for SP that are differentially migrating in agarose gels. The DNA band sizes detected in agarose gels were confirmed using sequence analysis and it is apparent that *ITS* is more informative in studying genus *Ipomoea* than *rbcL*

Data Availability Statement

The nucleotide dataset generated during and/or analyzed during the current study are available in the GenBank, and https://www.ncbi.nlm.nih.gov/nuccore.

The other datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

ACKNOWLEDGEMENT

We greatly acknowledge the University of Peradeniya, Sri Lanka for proving funds for this research. *Research Grant (URG/2016/59/S)*

REFERENCES

Austin, D.F. (1998) The genus *Aniseia* (Convolvulaceae), *Systematic Botany*. 411-20. DOI: https://doi.org/10.2307/2419373

Auwal, M.S., Saka, S., Mairiga, I.A., Sanda, K.A., Shuaibu, A., and Ibrahim, A. (2014) Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of (*Acacia nilotica* Thorn mimosa), *Veterinary Research Forum: an International Quarterly Journal*. 5(2): 95-100.

Benedict, S.R. (1909) A reagent for the detection of reducing sugars. *The Journal of Biological Chemistry*. 15(5): 485-487.

BIO-RAD (2018). Available at: http://biorad-ads.com/DNABarcodeWeb. Accessed on 25th July 2018.

Bolson, M., Smidt, E.C., Brotto, M.L., and Silva-Pereira, V. (2015) ITS and trnH-psbA as efficient DNA barcodes to identify threatened commercial woody Angiosperms from Southern Brazilian Atlantic rainforests, *PLOS ONE*.10(12): e0143049. DOI: https://doi.org/10.1371/journal.pone.0143049

Burkill, I.H. (1966) A dictionary of the economic products of the Malay Peninsula (2nd edition). Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia.

Chu, Y.H., Chang, C.L., and Hsu, H.F. (2000) Flavonoid content of several vegetables and their antioxidant activity, *Journal of the Science of Food and Agriculture*. 80: 561–566. DOI: https://doi.org/10.1002/(SICI)1097-0010(200004)80:5<561::AIDJSFA574>3.0.CO;2-%23

Clarke, C.B. (1885) Convolvulaceae Flora of British India Volume 4, London: Reeve & Co., 179-228 pp.

Dhaliwal, M.S. (2017). Tuber vegetable crops in Handbook of Vegetable Crops.3rd ed. Kalyani Publishers.

Doyle, J.J. (1990) Isolation of plant DNA from fresh tissue, *Focus*. 12: 13-15.

Duc, B.M., Humphries, D., Mai, L.T.B., Dao, H.A., Co, T.M., Nga, H., and Kim, P.T. (1999) Iron and vitamin content of commonly consumed foods in Vietnam, Asia Pacific *Journal of Clinical Nutrition*. 8(1): 36-38. DOI: https://doi.org/10.1046/j.1440-6047.1999.00078.x

Fang, R.C., and Staples, G.W. (1995) Convolvulaceae. Flora of China. Gentianaceae through Boraginaceae, St. Louis: Missouri Botanical Garden. 271-325 pp.

Filatenko, A.A., Pistrick, K., Knüpffer, H., and Hammer, K. (2003) "EN SINSKAYA'S inventory of plant taxa in the basic and dependent areas of the historical development of the flora of cultivated plants in Rudolf Mansfeld and Plant Genetic Resources. Proceedings of a Symposium dedicated to the 100th Birthday of Rudolf Mansfeld, Gatersleben, Germany. 222-256.

French, B.R. (1986) Food Plants of Papua New Guinea. A Compendium. Sheffield, Tasmania.

Gielly, L., and Taberlet, P. (1994) The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences, *Molecular Biology and Evolution*. 11(5): 769-77. DOI: https://doi.org/10.1093/oxfordjournals.molbev.a040157

Gothberg, A., Greger, M., and Bengtsson, B. (2002) Accumulation of heavy metals in Water Spinach (Ipomoea aquatica) cultivated in the Bangkok region, Thailand, *Environmental Toxicology and Chemistry*. 9: 1934-1939. DOI: https://doi.org/10.1002/etc.5620210922

Harborne, J.B. (2014) Introduction to ecological biochemistry. Academic press.

Horticultural Crop Research and Development. (2018) Sweet Potato. Available at: www.doa.gov.lk/HORDI/index.php/en/crop-2/49-sweet-potato-e. Accessed on 25th May 2018.

Hossain, M.A., Khulood, A.S.R., Zawan, H.M., Afaf, M.W., and Qasim, A.R. (2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*, *Asian Pacific Journal of Tropical Biomedicine*. 3(9): 705-710. DOI: https://doi.org/10.1016/S2221-1691(13)60142-2

Islam, S. (2014) Nutritional and medicinal qualities of sweet potato tops and leaves. Cooperative Extension Service, University of Arkansas.

Islam, S.M., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., and Yamakawa, O. (2002) Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) genotypes, *Journal of Agricultural and Food Chemistry*. 50: 3718-3722. DOI: https://doi.org/10.1021/jf020120l

Iwu, M.M. (1993) Handbook of African Medicinal Plants, London: CRC Press. 183-184 pp.

Johnson, M., and Pace, R.D. (2010) Sweet potato leaves: properties and synergistic interactions that promote health and prevent disease, *Nutrition Reviews*. 68(10): 604-615. DOI: https://doi.org/10.1111/j.1753-4887.2010.00320.x

Kananke, T., Wansapala, J., and Gunaratne, A. (2014) Heavy metal contamination in green leafy vegetables collected from selected market sites of Piliyandala area, Colombo district, Sri Lanka, *American Journal of Food Science and Technology*. 5: 139-144. DOI: https://doi.org/10.12691/ajfst-2-5-1

Kapoor, S.L., and Kapoor, L.D. (1980) Medicinal plant wealth of the Karimnagar district of Andhra Pradesh, Bull Med Ethnobot Res, v. 1, pp.120-144.

Kumar, S., Stecher, G., and Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, *Molecular Biology and Evolution*. 33(7): 1870-1874. DOI: https://doi.org/10.1093/molbev/msw054

Li, H.L. (1970) The origin of cultivated plants in Southeast Asia, *Economic Botany*. 24:3-19. DOI: https://doi.org/10.1007/BF02860628

Li, M., Jang, G.Y., Lee, S.H., Kim, M.Y., Hwang, S.G., Sin, H.M., Kim, H.S., Lee, J., and Jeong, H.S. (2017) Comparison of functional components in various sweet potato leaves and stalks, *Food Science and Biotechnology*. 26(1): 97-103. DOI: https://doi.org/10.1007/s10068-017-0013-6

Lin, Y.L., Hu, S.P., and Liu, J.F. (2004) The Bioavailability and Effect of Dietary Polyphenol from Leafy Ipomoea batatas on Human Antioxidant Status (Master's thesis), Taipei, Taiwan: Taipei Medical University.

Malalavidhane, T.S., Wickramasinghe, S.H.D.N., and Jansz, E.R. (2000) Oral hypoglycemic activity of I aquatica, *Journal of Ethno pharmacology*. 72: 293-298. DOI: https://doi.org/10.1016/S0378-8741(00)00217-8

Manandhar, N.P. (2002) Plants and People in Nepal, Portland, Oregon: Timber Press Inc.

Marcussen, H., Joergensen, K., Holm, P.E., Brocca, D., Simmons, R.W., and Dalsgaard, A. (2008) Element contents and food safety of water spinach (Ipomoea aquaticaForssk.) cultivated with wastewater in Hanoi Vietnam, *Environmental Monitoring and Assessmentv*. 139(1): 77-91. DOI: https://doi.org/10.1007/s10661-007-9817-9

Norton Brown Herbarium, College of Agriculture and Natural Resources. (2013). Available at: http://www.nbh.psla.umd.edu/guides/navikeyguide.html. Accessed on 25th May 2018.

Rambaut, A., Drummond, A.J., and Suchard, M. (2014) Tracer v1. 6. Available at: http://beast. bio. ed. ac. uk. Accessed on 26th May 2018.

Read, H.E. (1936) Surrealism, Toledo: Scholarly Pr.

Rubatzky, V.E., and Yamaguchi, M. (1997) World vegetables: Principles, production, and nutritive values, New York: Chapman & Hall. 711–713 pp.

Singh, D., Singh, B., and Goel, R.K. (2012) Role of saponins for the anticonvulsant effect of adventitious roots of Ficusreligiosa, *Pharmaceutical biology*. 50: 816-822. DOI: https://doi.org/10.3109/13880209.2011.636057

SkipThePie.org: The Nutrition Search Engine. (2018) Nutritional info of: Spinach, raw vs Sweet potato leaves, raw. Available at: https://skipthepie.org/vegetables-and-vegetable-products/spinach-raw/compared-to/sweet-potato-leaves-raw/. Accessed on 26th May 2018.

Sun, H., Mu, T., Xi, L., Zhang, M., and Chen, J. (2014) Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods, *Food chemistry*. 156: 380-389. DOI: https://doi.org/10.1016/j.foodchem.2014.01.079

Tang, S.C., and Chao, P.Y. (2000) Studies on the Antioxidative Capacity of Taiwan Indigenous Red Vegetables Extracts (Master's thesis). Taipei, Taiwan: Chinese Culture University.

Temperate Climate Permaculture. (2018) Permaculture Plants: Water Spinach, Kangkong, Ong Choy. Available at: http://tcpermaculture.com/site/2014/01/29/permaculture-plants-water-spinach-kangkong/. Accessed on 25th May 2018.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Research*. 25(24): 4876-4882. DOI: https://doi.org/10.1093/nar/25.24.4876

Umar, K.J., Hassan, L.G., Dangoggo, S.M., and Ladan, M.J. (2007) Nutritional composition of water spinach (*Ipomoea aquatica* Forsk.) leaves, *Journal of Applied Sciences*. 7(6): 803-809. DOI: https://10.3923/jas.2007.803.809

Uphof, J.C.T., and Cramer, J. (1968) Dictionary of economic plants.

Van Ooststroom, S.J., and Hoogland, R.D. (1953) Convolvulaceae, *Flora Malesiana*. 4: 388-512.

Van Valkenburg, J.L.C.H., and Bunyapraphatsara, N. (2001) *Stachytarpheta jamaicensis* (L.) Vahl in Medicinal and Poisonous Plants 2, Backhuys Publishers. 510-513 pp.

Villansenor, I.M., Cabrera, W.A., Meneses, K.B., and Rivera, V.R. (1998) Comparative antidiabetic activities of some medicinal plants, *Philippine Journal of Science*. 127: 261–266.

Wang, H.W., Kawaguchi, S., Horiguchi, T. and Masuda, M. (2001) A morphological and molecular assessment of the genus *Prionitis* J Agardh (Halymeniaceae, Rhodophyta), *Phycologicalresearch*. 49: 251-261. DOI: https://doi.org/10.1046/j.1440-1835.2001.00245.x

Watson, I., and Dallwitz, M.J. (1992) The families of flowering plants: Descriptions, illustrations, identification and information retrieval, Australia.

Westphal, E. (1993) Ipomoea aquatica Forsskal in Plant Resources in South-East Asia in Vegetables, Siemonsma, J.S., and Piluek, K. (eds.), Wageningen: Pudoc Scientific Publishers. 181-184 pp.

White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetic, PCR Protocols: A Guide to Methods and Applications. 18: 15-322.

Wills, R.B.H., Wong, A.W.K., Scriven, F.M., and Greenfield, H. (1984) Nutrient composition of Chinese vegetables, *Journal of Agricultural and Food Chemistry*. 32: 413-416. DOI: https://doi.org/10.1021/jf00122a059

Wiser, C.V. (1955) The food of a Hindu village in North India, *Annals of the Missouri Botanical Garden*. 42: 303-412. DOI: https://10.2307/2394594

Woolfe J.A. (1992) Sweet potato: an untapped food resource, Cambridge: Cambridge University Press.

World Crops for Northern United States (2018) Water Spinach: *Ipomoea aquatica*. Available at: https://World Crops.org/crops/water-spinach. Accessed on 25th May 2018.