EXTRACTION, CHARACTERIZATION AND TOTAL PHENOLIC CONTENT OF LOCAL (MALAYSIAN) GREEN SWEET POTATO \( (IPOMOEA BATATAS) \) LEAVES

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ABSTRACT:
Sweet potato leaves (SPL) have been used by malay rural folks as traditional topical medication for eczematous skin conditions especially psoriasis. Local Chinese consume green SPL as vegetable. SPL contains high amount of polyphenols, a proven antioxidant and free radical scavengers. Local green SPL was extracted for hydrophilic and lipophilic compounds using water, ethanol and hexane as solvents. Water extraction method gave the best yield per kg fresh weight. Total phenolic content was 4.48 mg GAE/g dw SPL, slightly lower of Japan’s and USA’s averages. HPLC analysis revealed significant amount of caffeic acid as one of its major phenolic components beside the dominant chlorogenic acid.

Keywords: sweet potato, polyphenols, HPLC, total phenolic content (TPC), caffeic acid, antioxidant

Introduction
Sweet potato or the scientific name \( Ipomoea batatas \) belongs to Convolvulaceae, a food plant of morning glory family. This plant is a native to tropical America and widely cultivated in tropical and the warmer temperature climates. This family is dominated by twining or climbing woody or herbaceous plants that often have heart-shaped leaves and funnel-shaped flowers [1]. The genus \( Ipomoea \) occurs in the tropics of the world although some species also reach temperate zones [2]. The species of this genus are mainly distributed throughout the South and Central America countries, and Tropical Africa territories.

One of the most noticeable anatomical characteristics of the Convolvulaceae is the existence of cells, which secrete resin glycosides in the foliar tissues and in the roots of the plants. These glycoresins constitute one important chemotaxonomic marker of this family and are responsible for the purgative properties of some species of the Convolvulaceae [3]. Sweet potato stems are usually long and trailing and bear lobed or unlobed leaves that vary in shape. The flowers, borne in clusters in the axils of the leaves, are funnel shaped and tinged with pink or rose violet. The edible part is the much-enlarged tuberous root, varying in shape from fusiform to oblong or pointed oval. Root colours range from white to orange and occasionally purple inside, and from light buff to brown or rose and purplish red outside. The pulp consists largely of starch, and orange-fleshed varieties are high in carotene [4].

Sweet potato leaves are consumed as vegetables around the world, especially in Southeast Asia [5]. In Malaysia, the tubers are more widely consumed and only the green SPL is being consumed as vegetable, mostly among the Chinese. The Malays use the leaves mainly as traditional topical medication for eczematous skin diseases especially psoriasis. Chen et al.[6] confirmed that sweet potato leaves was rich in bioactive compounds, especially carotenoids and polyphenols (flavonoids). They are also rich in vitamin B, iron, calcium, zinc and protein, and the crop is more tolerant of diseases, pests and high moisture than many other leafy vegetables grown in the tropics. Due to sweet potato tops can be harvested several times a year, their annual yield is much higher than many other green vegetables [7].

A review by Meira et al.[8] recorded several traditional uses of \( I. batatas \) which works as treatment of tumors of the mouth and throat, while the leaves decoctions are used as alterative, aphrodisiac, astringent, bactericide, demulcent, fungicide, laxative and tonic. The sweet potato is also used to treating asthma, bugbites, burns, catarrh, ciguatera, convalescence, diarrhea, dyslactea, fever, nausea, renosis, splenosis,
stomach distress, tumors and whitlows [9]. In region of Kagawa, Japan, a variety of white sweet potato has been eaten raw to treating anemia, hypertension and diabetes [10].

The observation that the Samoans, who eat plenty of sweet potato and its leaves, and are reportedly totally free from psoriasis [11] adds some more weightage on possible potential of SPL to have medicinal value especially on auto immune skin disease such as psoriasis. Though little information is available on the use of SPL bioactive compounds on psoriasis, it was noted that SPL, in particular the purple type, has been researched on its ability to modulate immune response, in particular, its anti inflammatory and anti oxidant properties [12]. As psoriasis is an auto immune disease of T-cell mediated inflammation, our ability to control immune hyper-reactivity especially of T-cells could potentially offer safe, cheap and viable treatment of psoriasis.

Despite the studies on phytochemical and nutritional values of sweet potato leaves, no extensive research on developing this valuable natural source as potential functional food ingredient or pharmaceutical product, unlike its flesh (tuber). Sweet potato flesh has been transformed to purees and powders for functional food ingredients with high levels of anthocyanins (purple-fleshed varieties) and β-carotene (orange-fleshed varieties) [13].

Several studies on polyphenols in sweet potatoes leaves have been carried out, mostly in Japan [13]. The polyphenolic extracts from sweet potato leaves and roots were shown to exhibit high radical-scavenging activity, antimutagenicity, potential chemopreventive properties, and antidiabetic effects [14-17].

Various methods of extraction for leave samples have been reported as sample preparation prior to sample analyses. Essentially, various methods were employed in order to extract different compounds, some are hydrophilic (using water/ethanol mixture) and some are lipophilic (using hexane). Table 1 below lists several references for methods used in sweet potato leaves extractions.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Journal</th>
<th>Solvent(s)</th>
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<tbody>
<tr>
<td>Truong, V.-D. Mcfeeters, R.F. Thompson, R.T., Dean, L.L.andShofran, B. 2007</td>
<td>Phenolic acid content and composition in leaves and roots of common commercial sweetpotato (Ipomea batatas L.) cultivars in the United States. Journal of Food Science 72(6): 343-349</td>
<td>Boiling ethanol (80%)</td>
</tr>
<tr>
<td>Huang, M-H et al., 2010</td>
<td>Inhibitory effects of sweet potato leaves on nitric oxide production and protein nitration. Food Chemistry 121: 480-486</td>
<td>Boiling water</td>
</tr>
<tr>
<td>Hue, S-W., Boyce, A.N and Somasundram, C. 2011</td>
<td>Comparative study on the antioxidant activity of leaf extract and carotenoids extract from Ipomoea batatas var. oren (sweetpotato leaves). World Academy of Science, Engineering and Technology. 58: 584-587</td>
<td>Methanol, Acetone</td>
</tr>
<tr>
<td>Everette, J.D and Islam, S. 2012</td>
<td>Effect of extraction procedures, genotypes and screening methods to measure the antioxidant potential and phenolic content of orange-fleshed sweet potatoes (Ipomoea batatas L.). American Journal of Food Technology 7(2): 50-61</td>
<td>Hexane, Methanol</td>
</tr>
</tbody>
</table>

Table 1: References on sweet potato leaves extractions. Solvents used vary depending on constituents of interest.
As for HPLC analysis, Islam et al. [17] analyzed the phenolic composition in the leaves of various sweet potato genotypes using gradient high-performance liquid chromatography (HPLC) with 20% to 70% methanol and a run time of more than 60 min per sample. Six phenolic acids in sweet potato leaves were isolated and identified by nuclear magnetic resonance (NMR) spectroscopy. Other investigators used a similar HPLC method to analyze the phenolic composition in the root extracts of sweet potato cultivars commonly used in the processing of several Japanese traditional food products [18]. A new phenolic acid derivative, 4,5-di-O-caeffeoyldaucic acid, has been recently isolated and identified by HPLC in the root extracts of a sweet potato cultivar collected in Peru [19].

Materials and Methods

Ethanol (99.4%) and n-hexane (95%, Fisher Chemical) were purchased from Fisher Scientific (M) Sdn Bhd. Sample of sweet potato leaves was purchased from local market and identified by Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia, Serdang. For total phenolic content, Folin-Ciocalteu’s (FC) phenol reagent (Sigma Aldrich), Sodium carbonate (Merck), methanol (FriendemannSchimidt) and gallic acid (FlukaChemigmnbh) were used. For characterization, high performance liquid chromatography (HPLC) machine was of Agilent 1100 Series System and caffeic acid (Sigma) as standard was used.

Water Extraction

The extraction procedure was conducted based on Huda-Faujan et al. [20] with some modifications. Briefly, the sweet potato leaves with stalks were removed from the stem and cleaned under tap water, air-dried using fan, kept in zipped polyethylene bag and frozen at -18°C for overnight. The sample was then blended with distilled water with the ratio of 1:3 (leaves: water), centrifuged at 10,000 rpm for 15 min, filtered and concentrated by using rotary evaporator (Eyela Tokyo Rikakikai Co. Ltd). The crude extract obtained was kept in amber vial and stored in refrigerator at 4°C.

Ethanol Extraction

The cleaned and oven-dried (50°C, 18 hrs) weight sweet potato leaves with stalk (dried weight 50 gram) were ground to powder form. Then, it was stirred in ethanol for 5 hrs in a ratio 1:5 (leaves: ethanol) and was filtered with Büchner funnel. The ethanol extraction was repeated twice with the extraction time 3 hrs and the combined extracts were evaporated at 40°C. The crude extract was kept in amber vial and stored at 4°C.

Hexane Extraction

Powder of oven-dried leaves with stalks was stirred for 5 min in hexane in a ratio of 1:5 (leaves: hexane) and the mixture was filtered using Büchner funnel. The hexane extraction was repeated twice and the combined extracts were evaporated at 40°C using a rotary evaporator. Extracts obtained were later concentrated with nitrogen gas flow and weighed. The crude extract obtained was kept in amber vial and stored in refrigerator at 4°C. This method of extraction was adapted and modified from Teow et al.[21].

Total Phenolic content

Total phenolic content was determined by a colorimetric FC method as described by Amin et al.[22]. All samples and readings were prepared and measured in triplicate. 750 gram of fresh SPL was freeze dried to yield 20 gram of dry SPL, 0.5 g of which was mixed with 10 mL of methanol and ground and filtered. 1 mL of sample was transferred into test tube and diluted with 1.25 mL of methanol. 0.5 mL of FC reagent and 3.0 mL of sodium carbonate (200g/L) were added and vortexed. The mixture was left standing at room temperature for 15 minutes and further diluted with 10 mL of distilled water. The resulting blue complex was read after 15 minutes at 640nm against blank using a spectrophotometer. The concentration of the extracted phenolic compounds was calculated based on comparison with standard curve prepared by plotting absorbance against varying concentrations of gallicacid in methanol (25-500 µg/mL). The total phenolic
Characterization & quantification of selected phenolic compound using HPLC

The ethanolic extract was subjected to characterization and quantification of selected phenolic compound based on available standard (caffeic acid was chosen). The extract was produced from 50g dry weight of SPL and analyzed using an Agilent HPLC system equipped with diode array detector. 4 mL of the extract was diluted with 12 mL of distilled water (dilution by factor of 4) and filtered twice. 1.5 ml of the diluted extract was transferred to sample vial for HPLC. The HPLC separation employed isocratic method with operating conditions as follows: auto sampler at room temperature, injection volume 20 microLiter, eluent flow rate 1.2 mL/minute, mobile phase 0.1% formic acid in acetonitrile and methanol in 80:20 ratio. Column used was chromolith RP-18e and the chromatogram was monitored at 280nM for 60 minutes.

Result & Discussion

Extraction

As per table 2, The highest percentage on yield was obtained from water extraction. However, it was based on fresh weight of sample. It was also noted that standard deviation was very high probably due to water and moisture content which may influence the weight of extract. The slimy characteristic of water extract was believed from the presence of mucilage as suggested by a study conducted by Huang et al.[23] on sweet potato root storage mucilage.

The efficiency ethanol in producing extracts was very steeply lower compared to water despite long hour extraction with repeating procedure. The dark green colour of the extract was due to chlorophyll content present in the leaves and being extracted by ethanol. Wasmund et al.[24] reported the ethanol as recommended solvent in extracting chlorophyll. A hydroethanolic extract of sweet potato leaves demonstrated the presence of triterpenes and/or steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids as secondary metabolites with potential biological activities [25].

In this study, the least yield obtained was by hexane extraction. Everette and Islam [26] extracted orange-fleshed sweet potato leaves by using hexane to examine its lipophilic fractions and found out antioxidant activity correlated poorly when compared in methods studied. Another study by Menelaou et al.[27] on lipid soluble constituents of sweet potato leaves by using acetone and hexane showed the leaves are an excellent source of dietary lutein, one of the major carotenoids present in the human retina.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>% Yield of extract ± SD</th>
<th>Description of extract</th>
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<tbody>
<tr>
<td>Water^A</td>
<td>34.93 ± 9.96</td>
<td>Dark brown. Viscous and slimy.</td>
</tr>
<tr>
<td>Ethanol^B</td>
<td>8.87 ± 1.15</td>
<td>Very dark green to almost black. Viscous.</td>
</tr>
<tr>
<td>Hexane^B</td>
<td>2.61 ± 0.46</td>
<td>Dark green with orange in colour. Viscous but lighter than ethanol extract.</td>
</tr>
</tbody>
</table>

Table 2: Percentage yield of extract obtained with different type of solvents with the descriptions of the extracts. SD:Standard deviation (n=4); ^A Yield of extraction based on fresh weight; ^B Yield of extraction based on dried weight.
Total phenolic content (TPC)

A linear calibration curve of gallic acid with $R^2$ value of 0.8232 was obtained with resultant equation of $y = 1.4052x + 1.6867$, whereby $y =$ absorbance at 640nm and $x =$ concentration of total phenolic compounds in mg GAE per mL of the methanolic extract. Putting in the spectrophotometric reading of the sample and allowing for dilution, the TPC of the sample was 4.48 mg GAE/g dw of SPL. This was slightly low compared to reported range of 6.3-76.1 mg GAE/g dry weight by Islam et al. [17] done on purple SPL. Purple SPL was also researched by Chen et al. [6] and regarded the purple SPL had the highest TPC of all types of SPL. Some researchers prefer to express the TPC of SPL in the form of chlorogenic acid equivalent per 100 gram of fresh weight (mg CAE/100g fw). Apparently, freezed drying the sample (used in this research) did not confer any advantage in term of maintaining TPC as opposed to conventional oven drying used by other researchers.

Characterization & quantification of selected phenolic compound using HPLC

As per figure 1, 22 peaks were identified with the first peak appeared at 1.859 minute and followed by three most prominent peaks at around the 3rd minute (table 3, only first 13 peaks are shown here). The peaks were very close together that they were almost overlapping. The other 18 peaks were also easily identified albeit with much smaller area under the curves. All peaks appeared within 60 minutes of elution time. Available literatures suggested the most prominent peak could either be of caffeic acid or chlorogenic acid and usually by isocratic method, chlorogenic acid would appear first followed by caffeic acid [13, 17]. The chromatogram was compared to caffeic acid standard (not shown here) and the third peak which appeared at 3.338 minute corresponded to it.

Equation derived from various standard concentrations of caffeic acid chromatogram yielded $y=724x + 19589$ (with $R^2=0.957$) with $y =$ area under the curve and $x =$ concentration of the caffeic acid in mg/mL. Area under the curve for the extract was 34726 unit giving the concentration of caffeic acid of the sample as 2.09 mg/mL. As the sample was diluted 4 times, the actual concentration of caffeic acid from 50 gram dried SPL was actually 8.36 mg/mL (or 16.71 mg/mL per 100 gram dry weight of SPL)

Figure 1. HPLC of phenolic compounds from ethanolic extract of SPL via isocratic method
Table 3: Peaks’ detail of isocratic elution HPLC of the ethanolic extracts (the first thirteen peaks).

The third peak (underlined) corresponds to caffeic acid.

Islam et al. [28] reported in their study of 20 sweet potato genotypes in Japan that concentration of caffeic acids ranged from 29.2 to 30.7 mg/mL per 100 gram dry weight of SPL. This study showed our local SPL’s content of caffeic acid was almost half less compared to those found in Japan. It could be a true figure but very likely the figure could also be attributed to drying method and solvent used for extraction. Furthermore, different parts of sweet potato contain different level of phenolic compounds, caffeic acid included. Nevertheless, most literatures are in agreement that the stalks and the leaves contain the most phenolic compounds than the rest of the plant [15, 21, 28]. While antioxidant activity of SPL was largely attributed to its dominant chlorogenic acid content [13, 17, 19], the role of caffeic acid should also be considered as it did have significant scavenging property [29, 30].

Conclusion

Sweet potato leaves contain various compounds from polyphenols to carotenoids that could be beneficial for health. While polyphenols could be extracted via aqueous or ethanolic solvents due to its hydrophilic nature, the carotenoids compound which is fat-soluble need to be extracted by lipophilic solvent such as hexane but the yield is rather low. Conventional oven drying is as good as freeze-drying in term of maintaining total phenolic content. Local green (Malaysian) SPL contain significantly less TPC as compared to overseas’ SPL such as in Japan and USA. For separation using HPLC, isocratic elution with acidified methanol can separate the components within short period of time (less than 60 minutes). Caffeic acid is a major component beside the dominant chlorogenic acid that make up the polyphenols in SPL.

Acknowledgement

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References

http://cals.arizona.edu/herbarium/sites/cals.arizona.edu.herbarium/files/old_site/assoc/people/daustin/convolv.html.


