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Research Article

Contents of Important Phenolic Compounds in Indigowoad (*Isatis indigotica* Fort.) and Plains Wild Indigo (*Baptisia bracteata*) Roots

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Abstract

The levels of some phenolic compounds were determined for Indigowoad Root (IR) and the Plains Wild Indigo Root (PWIR) to provide insights on their health benefits. The IR is a well-known medicinal plant as well as an edible plant root similar to daikon, sweet potatoes, yam, ginseng and carrots. The PWIR roots, leaves and seeds have traditionally been used by Native Americans for medicinal purposes and its extracts are consumed to enhance health. Total phenolic, total flavonoid, p-coumaric acid, gallic acid, syringic acid and vanillic acid contents were determined. Overall, the phenolic compounds of PWIR and IR either exceeded or compared favorably with other commonly consumed root vegetables. The phenolic compounds of the PWIR generally exceed those of IR. Based on the findings of this study, further research on IR and PWIR may be warranted to determine their possible use as additional sources of phenolic compounds and other desirable constituents to benefit human health.

Key words: Indigowoad root, phenolic compound, plains wild indigo root, p-coumaric acid, vanillic acid

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There is worldwide interest in constituents of plant materials that may benefit human health. Phytonutrients such as phenolic compounds are known to be present in most unprocessed whole plants and this has led to more demand for knowledge regarding natural sources of these constituents from various plants (Balasundram *et al*., 2006). Phenolic compounds can act as antioxidants because of their capability to provide electrons or hydrogen and because of their intermediates that prevent oxidation of many food ingredients such as fatty acids and oils (Prakash *et al*., 2007; Cuvelier *et al*., 1992; Maillard *et al*., 1996). It is well known that phenolic compounds are major contributors to the total antioxidant capacity of vegetables, fruits and grains (Heo *et al*., 2007; Jacobo Velazquez and Cisneros Zevallos, 2009). Enrichment of human diets with food products that have high levels of phenolic compounds will provide antioxidative, antimutagenic, antifungal and antiglycemic properties (Friedman, 1997; Canbek *et al*., 2014). There are many types of phenolic compounds but they can be roughly categorized into two major types: flavonoids and phenolic acids. Several studies present the benefits of flavonoids which includes reduced risk of coronary disease (Knekt *et al*., 1996). Research on some specific phenolic acids, such as p-coumaric acid, gallic acid, syringic acid and vanillic acid have shown many benefits for human health. A study of the phenolic fractions of ginseng foundthat it had high levels of p-coumaric acid and vanillic acid, which exhibited peroxynitrite and hydroxide scavenging activity (Kang *et al*., 2006). Also, p-coumaric acid is a potent inhibitor of 5-S-cysteinyl-dopamine induced neurotoxicity, which implies possibilities for inhibiting Parkinson's disease (Vauzour *et al*., 2010). Vanillic acid together with syringic acid, significantly decreased transaminase activity, suppressed the disorganization of the hepatic sinusoids and suppressed cytokine levels, which is associated with inflammation (Itoh *et al*., 2009). Syringic acid was shown to have strong antiendotoxin activity (Liu *et al*., 2003). Gallic acid has been shown to induce death of tumor cells (Inoue *et al*., 1994) and to act as an agent in prevention of renal damage (Canbek *et al*., 2014).

Different plants and different parts of each plant have different levels of phenolic compounds, so each plant needs to be analyzed in order to assess its potential benefit as a health product. Many researchers have focused on plants that have a history of health benefits with hopes that they may have higher levels of phenolic compounds than most plants. This study focuses on the roots of two plants with a history of health benefits which have not yet been well analyzed for phenolic compounds-indigowoad (*Isatis indigotica* Fort.) and plains wild indigo (*Baptisia bracteata*). There is no known literature that reports the levels of some of the more important phenolic compounds for these plants.

Indigowoad root is considered to be a safe medicinal herb, which has been planted and used all over Asia for centuries and has been recorded in ancient Chinese books (Li, 2003). It is believed to have strong activity in removing toxins and eliminating inflammation (Wei *et al*., 2011). It is also consumed by millions of people throughout the world, especially in Asian diets where, it is an edible plant root similar to daikon, sweet potatoes, carrots, yam, ginseng, etc. In China, indigowoad is even used as a beverage base (like orange juice or blended juice).

Plains wild indigo grows naturally in the Southern plains of the USA and has historically been used to enhance human health. It was mentioned by USDA, Casey and Wynia (2010) as a "Culturally significant plant" and its roots, leaves and seeds have traditionally been used for medicinal purposes. Native Americans made tea from the dried leaves to treat colds, concentrate bile and aid the liver. It was also used to treat cuts, bruises, sore arms and legs, stomach cramps, open cuts, eye disease and rheumatism (Casey and Wynia, 2010). In addition, tea made from the roots was consumed to aid digestion or used as mouthwash to treat periodontal disease and sore throats. Plains wild indigo seeds have been reported to scavenge free radicals and to be a good antioxidant (Borchardt *et al*., 2008). The most common uses for wild indigo are as an antibacterial, a fungicide and to treat lymphatic disorders (Foster and Duke, 1999).

Thus, indigowoad and plains wild indigo roots are believed to have many beneficial chemicals. In this research, the contents of total phenolic compound, total flavonoid, p-coumaric acid, gallic acid, syringic acid and vanillic acid were determined for indigowoad root and plains wild indigo root. These values will help determine if these plants have the potential to be included as a valuable biomaterial for humans.

MATERIALS AND METHODS

Plant test materials: The plant materials studied were Indigowoad Roots (IR) and Plains Wild Indigo Roots (PWIR). All samples were prepared and analyzed in triplicate. Four different samples of the indigowoad (*Isatis indigotica* Fort.) roots were analyzed separately. They were commercial products that were purchased from four different stores located in Luodong city of Yilan County in Taiwan, because, they were commercial products, there was no control over how they were grown or handled. Consequently, four different samples were purchased to determine the level of variation in the chemical contents within these commercially available products. They were already dried and clean when purchased from the stores. Those samples were then freeze-dried, milled to a powder and stored at 4°C until the chemical tests were conducted. Plains wild indigo roots (*Baptisia bracteata* var. leucophaea) were harvested from 10 plants that were grown in a pasture in central Texas in the summer of 2013. These plants grew in the wild and there were no fertilizers, pesticides or other chemicals applied to the plants. These root samples were cleaned and dried in the sun for four days and taken to the laboratory at night time. The samples were freeze-dried, blended together, milled to a powder and stored at 4°C, until the chemical tests were conducted.

Chemicals: All the standards had a purity that was over 95%. The p-coumaric acid, ferulic acid, syringic acid, vanillic acid, quercetin, folin-ciocalteu's phenol reagent, sodium carbonate, hydrochloric acid (37%) and aluminum chloride hexahydrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid, methanol and sodium hydroxide were purchased from Merck Chemical Co. (Darmstadt, Germany). Diethyl ether and sodium sulfate were purchased from Nihon Shiyaku Reagent (Tokyo, Japan). The n-hexane was purchased from ALPS Chemical Co., Ltd. (Hsinchu, Taiwan). Ascorbic acid was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethylene dinitrilo tetra-acetic acid (EDTA) and acetic acid were purchased from J.T. Baker Chemical Co. Phillipsburg, USA. Ethyl acetate was purchased from Lab-Scan Chemical Co. (Patumwan, Bangkok, Thailand).

Extraction of total phenolic compounds: In this study, total phenolic compound olextraction values = water soluble (non-bounded type) phenolic compound extraction values+bound phenolic compounds extracted via alkaline hydrolysis values+bound phenolic compounds extracted via acid hydrolysis values. Extraction of the total phenolic compounds was carried out according to the method presented by Krygier *et al.* (1982) with a few minor modifications.

Removal of fatty acids: Prior to any of the extractions of phenolic compounds, the fatty acids were removed from the plant samples by the following procedure. Samples of the five plant powders were weighed (0.5±0.0001 g each) and placed into separate 50 mL centrifuge tubes. Next, 15 mL of n-hexane and 5 drops of 10 M sodium hydroxide were added to each tube and the mixtures were subjected to ultrasound vibrations (Leo Ultrasonic, Model LEO-150, Taiwan) for 10 min. Then, the top clear liquid part (which was n-hexane with the fatty acids) was removed. The remaining precipitate (by now, all possible fatty acids were removed by the n-hexane) was the material used for all the following extractions. Each individual extraction method is described in the following sections.

Water soluble (non-bounded type) phenolic compounds extraction: Fifteen milliliter of 80% methanol was added into the precipitated test material and it was subjected to ultrasound vibrations for 30 min. The mixture was centrifuged at 2500 rpm for 15 min and the upper part of clear solution and the precipitate portions were separately collected. The precipitate portion was processed further as described in the next section. The separated clear solution was vacuum-concentrated at 35°C until dry and 5 mL of pure methanol was poured into this dried material. Ultrasound vibration was applied to the mixture to dissolve the dried precipitate and this material was filtered with 0.45 µm filter membrane (Minisart NML syringe filter). The resulting clear liquid (Test material "I") was put into a sealed glass bottle (protected from light) and stored at 4°C, until it could be analyzed for levels of total phenolic compound, total flavonoid and specific phenolic acids.

Bound phenolic compounds extraction-via alkaline hydrolysis: The precipitate portion obtained from the previous section was the test material used in this extraction. A solution of 10 M sodium hydroxide, 1% ascorbic acid and 10 mM EDTA was premixed and 5 mL of this solution was added to the precipitated material within a centrifuge tube. The air in the head space of this centrifuge tube was displaced with nitrogen gas by injecting the gas into the head space for 1 min. The centrifuge tube was sealed immediately with a lid coated with para film and this tube was stored for 16 h to allow for the alkaline hydrolysis to take place. After 16 h, the parafilm and lid were removed and a 37% concentration of sulfuric acid was added to adjust this solution to a pH of 1-2. Fifteen milliliter of a solution of ether/ethyl acetate (1:1 v/v) was added to extract the phenolic acids. This mixture was centrifuged at 2500 rpm for 15 min and the upper part of clear solution (which is the desired alkaline hydrolysis bounded phenolic compounds extraction) was collected and placed into a beaker (Beaker A). This process was repeated twice for the bottom precipitate part and the upper part of clear solution from these two repeated procedures were added into the same beaker (Beaker A). All of the precipitated material that was separated from the clear solution in these three replications was used as the test material in the next section. The solution in Beaker A was vacuum-concentrated at 35°C, until dry and 5 mL of methanol was poured into this fully dried material. This material was subjected to ultrasound vibration to dissolve the dried precipitate and this solution was filtered with a 0.45 μm membrane. The clear liquid (Test material "II") was sealed in a bottle and stored at 4°C, until it was needed for further analysis of total phenolic compound, total flavonoid and specific phenolic acids.

Bound phenolic compounds extraction-via acid hydrolysis: The precipitate portion obtained from the previous section was the test material used in this extraction. The precipitated material and 2.5 mL of hydrochloric acid (37% concentration) were added to a centrifuge tube and it was maintained at 85°C in a thermostatic water bath for 30 min for acid hydrolysis to take place. Next, 10 M sodium hydroxide was added to titrate the solution inside of this centrifuge tube to obtain a pH value of 1-2. Then, 15 mL of a solution of ether/ethyl acetate (1:1 v/v) was added it to this material to extract the phenolic acids. This material was centrifuged at 2500 rpm for 15 min and the upper part of clear solution (which is the desired acid hydrolysis bounded phenolic compounds extraction) was collected and placed it into a beaker (Beaker B). This process was repeated twice for the bottom precipitate part and the upper part of clear solution of this two times repeated procedure was added into the same beaker (Beaker B). The solution in Beaker B was vacuum-concentrated at 35°C until dry, 5 mL of methanol was added, it was subjected to ultrasound vibration to dissolve the dried precipitate and the solution was filtered with a 0.45 μm membrane. The clear liquid (Test material "III") was sealed in a bottle and stored at 4°C, until it could be further analyzed for total phenolic compound, total flavonoid and specific phenolic acids.

Determination of total phenolic content: Determination of total phenolic content was carried out according to the methods presented by Taga *et al*. (1984) and Quettier-Deleu *et al*. (2000) with a few minor modifications:

- **Part A:** First, 0.5 mL of test material "I" was mixed with 0.5 mL of folin-ciocalteu's phenol reagent and 5 mL of 20% sodium carbonate and incubated for 30 min at room temperature. After incubation, the absorbance of the mixture was measured with a spectrophotometer (Metertech, Model SP-830, Taiwan) set at 750 nm and the total phenolic content values were obtained from the regression curve developed in part C of this section. The total phenolic content results were presented using the standard expression of mg Gallic Acid Equivalent (GAE)/g dry weight plant material
- **Part B:** The same procedures described in part A were followed for test material "II" and for Test material "III"
- **Part C:** To obtain the gallic acid standard curve, readings were determined for five standard solutions of gallic acid (50, 100, 200, 400 and 500 ppm) by following the procedure in part A. The linear regression standard curve was developed from these values

Determination of total flavonoid content: Extraction of total flavonoid content was carried out according to the method presented by Quettier-Deleu *et al*. (2000) with a few minor modifications:

- **Part A:** First, 0.5 mL of test material "I" was mixed with 0.5 mL of 2% aluminum chloride hexahydrate and incubated for 10 min at room temperature. When the incubation was completed, a spectrophotometer (Metertech, Model SP-830, Taiwan) set at 430 nm was used to measure the absorbance of the mixture and the total flavonoid values were obtained from the regression curve developed in part C of this section. A blank test (with methanol) was done with the same device prior to the sample measurement to zero the device. The flavonoid content results were presented using the standard expression of mg Quercetin Equivalent (QE)/g dry weight plant material
- **Part B:** The same procedures described in Part A of this section were followed for test material "II" and for test material "III"
- **Part C:** To obtain the quercetin standard curve, spectrophotometer readings were determined for six standard solutions of quercetin (1, 5, 10, 25, 50, 100 ppm) by following the process described in part A of this section. The linear regression standard curve was developed from these values

Determination of p-coumaric acid, gallic acid, syringic acid and vanillic acid content: Determination of these phenolic acids was carried out according to the method presented by Rao and Muralikrishna (2002) with a few minor modifications. The phenolic acids (p-coumaric acid, gallic acid, syringic acid and vanillic acid) were determined by HPLC analysis. The HPLC had a C-18 column (4.6×250 mm, 5 mm; Phenomenex, Inc., USA) with column temperature set at 50±1°C. It had a UV-VIS detector (UV-2070 Plus, JASCO, Japan) and a chromatography data handling system (Peak-ABC Software). There were two mobile phases and the flow rate of each was set at 1 mL min^{-1}. The injection volume of each test sample or standard solution was 20 μL and the ultraviolet detector wavelength was

280 nm. For mobile phase 1, the elution solvent was 4% acetic acid dissolved in water. For mobile phase 2, the elution solvent was a lab analytical methanol solution. Gradient elution was used and the initial gradient was 92% of mobile phase 1 solution and 8% of mobile phase 2 solution. These values gradually changed to 75% of mobile phase 1 solution and 25% of mobile phase 2 solution, over the period from 0-15 min. Then, the gradients stayed at 75% of mobile phase 1 and 25% of mobile phase 2 over the next period from 15-40 min. Next, the gradients gradually changed back to 92% for mobile phase 1 and 8% for mobile phase 2 over the last stage from 40-70 min.

Statistical analysis: All samples were prepared and analyzed in triplicate. Significant differences between means of the different plant samples were analyzed by one-way analysis of variance (ANOVA) and further analyzed with Duncan's multiple range test to determine significant differences between means ($p<0.05$) using SAS (SAS 9.3, SAS Institute Inc., Cary, NC).

RESULTS

Results of total phenolic content in the four samples of Indigowoad Root (IR 1, 2, 3 and 4) and in the Plains Wild Indigo Root (PWIR) sample have been presented in Table 1. The results were presented for each of the three extraction methods (water soluble, water insoluble (bound)by alkaline hydrolysis and water insoluble (bound) by acid hydrolysis), plus the total of all the methods. Results of total flavonoid content in the four samples of Indigowoad Root (IR 1, 2, 3 and 4) and in the Plains Wild Indigo Root (PWIR) sample were presented in Table 2. The results were presented for each of the three extraction methods (water soluble, water insoluble (bound) by alkaline hydrolysis and water insoluble (bound) by acid hydrolysis), plus the total of all the methods. Results of specific individual phenolic acids in the two samples of indigowoad root (IR 2 and 4) and in the sample of Plains Wild Indigo Root (PWIR) were presented in Table 3. The results were presented for each of the three extraction methods (water soluble, water insoluble (bound) by alkaline hydrolysis and water insoluble (bound) by acid hydrolysis), plus the total of all the methods.

DISCUSSION

Comparison of total phenolic content: Table 1 shows that the total phenolic content of PWIR was significantly greater ($p<0.05$) than for all of the IR samples. The PWIR also had significantly higher contents than the IR samples for each of the extraction methods, except for insoluble-bound with alkaline hydrolysis where PWIR and IR 2 were statistically the same. Among the four indigowoad root samples, IR 2 showed significantly higher amounts of total phenolic content for all the extraction methods than did the other IR samples. There was wide variation in total phenolic content for all the extraction methods than did the other IR samples. There was wide variation in total phenolic content among the four samples of indigowoad root (5.14-13.63 mg GAE g^{-1}). This variation may be due to different growing or handling conditions and more research is needed to better determine how growing and handling conditions affect phenolic content of these plants.

Table 1: Profile of total phenolic contents in four samples of indigowoad root and in plains wild indigo root as determined by different extraction methods

	mg GAE/g dry weight				
Parameters	PWIR	IR 1	IR 2	IR 3	IR 4
Water soluble	4.72±0.20^{a}	0.44±0.075^{d}	2.65±0.093^{b}	0.63±0.11^{d}	1.43±0.04^{c}
Insoluble-bound (alkaline hydrolysis)	5.85±0.13^{a}	1.01±0.13^{c}	5.87±0.041^{a}	1.67±0.23^{b}	1.37±0.08^{bc}
Insoluble-bound (acid hydrolysis)	5.85±0.42^{a}	3.69±0.42^{cd}	5.11±0.19^{b}	2.99±0.13^{d}	4.13±0.12^{c}
Total	16.43±0.51^{a}	5.14±0.41^{d}	13.63±0.14^{b}	5.28±0.35^{d}	6.94±0.16^{c}

Values are expressed as Mean±SD (n = 3), ^{a,b,c,d}When comparing values in the same row, different letter superscripts indicate significant differences between the means at $p<0.05$ statistical level, IR: Indigowoad root, PWIR: Plains wild indigo root, GAE: Gallic acid equivalent

Table 2: Flavonoid content in four samples of indigowoad root and in plains wild indigo root as determined by different extraction methods

	mg QE/g dry weight				
Parameters	PWIR	IR 1	IR 2	IR 3	IR 4
Water soluble	1.15±0.00^{a}	0.14±0.00^{c}	0.17±0.01^{b}	0.080±0.00^{e}	0.120±0.01^{d}
Insoluble-bound (alkaline hydrolysis)	0.13±0.01^{a}	ND^{b}	0.01±0.00^{b}	0.002±0.00^{b}	0.002±0.00^{b}
Insoluble-bound (acid hydrolysis)	0.07±0.02^{ab}	0.06±0.00^{bc}	0.08±0.00^{a}	0.030±0.00^{d}	0.050±0.00^{c}
Total	1.35±0.02^{a}	0.19±0.01^{c}	0.26±0.01^{b}	0.120±0.00^{d}	0.170±0.00^{c}

Values are expressed as Mean±SD (n = 3), ^{a,b,c,d}When comparing values in the same row, different letter superscripts indicate significant differences between the means at $p<0.05$ statistical level, ND: Not detectable, IR: Indigowoad root, PWIR: Plains wild indigo root, QE: Quercetin equivalent

Table 3: Phenolic acid (p-coumaric, gallic, syringic and vanillic) contents in two samples of IR and PWIR as determined by different extraction methods

	Phenolic acids contents (mg/100 g dry weight)				
Samples	Extraction method	p-coumaric acid	Gallic acid	Syringic acid	Vanillic acid
PWIR					
Water soluble	Methanol extraction	13.65 ± 0.42^{b}	23.13 ± 6.56^{a}	10.10 ± 0.20^{b}	ND^{c}
Insoluble-bound	Alkaline hydrolysis	14.51 ± 0.25^{b}	ND^{a}	12.91 ± 0.64^{b}	2.21 ± 0.27^{a}
Insoluble-bound	Acid hydrolysis	25.50 ± 0.22^{b}	ND^{a}	10.25 ± 0.28^{c}	5.08 ± 0.04^{a}
Total		53.67 ± 0.27^{b}	23.13 ± 6.56^{a}	33.26 ± 0.97^{c}	7.29 ± 0.31^{a}
IR 2					
Water soluble	Methanol extraction	15.50 ± 0.05^{a}	ND^{b}	13.11 ± 0.34^{a}	5.70 ± 0.12^{a}
Insoluble-bound	Alkaline hydrolysis	16.30 ± 0.10^{a}	ND^{a}	17.52 ± 0.35^{a}	ND^{b}
Insoluble-bound	Acid hydrolysis	29.22 ± 0.61^{a}	ND^{a}	15.51 ± 0.76^{a}	ND^{c}
Total		61.02 ± 0.61^{a}	ND^{b}	46.15 ± 1.10^{a}	5.70 ± 0.12^{b}
IR 4					
Water soluble	Methanol extraction	14.68 ± 0.55^{ab}	ND^{b}	13.03 ± 0.65^{a}	3.10 ± 0.32^{b}
Insoluble-bound	Alkaline hydrolysis	14.32 ± 0.05^{b}	ND^{a}	12.88 ± 0.22^{b}	ND^{b}
Insoluble-bound	Acid hydrolysis	ND^{c}	ND^{a}	12.16 ± 0.30^{b}	0.35 ± 0.07^{b}
Total		29.00 ± 1.05^{c}	ND^{b}	38.06 ± 2.00^{b}	3.45 ± 0.39^{c}

Values are expressed as Mean±SD, ^{a,b,c}When comparing values of the same phenolic acid and same extraction method among the different plant samples, different letter superscripts indicate significant differences between the means at p<0.05 statistical level. ND: Not detectable, IR: Indigowoad root, PWIR: Plains wild indigo root

There have been a number of studies reporting total phenolic content of commonly consumed plants (Vinson *et al*., 1998; Ismail *et al*., 2004; Kaur and Kapoor, 2004; Zheng and Wang, 2001). They expressed total phenolic content in a variety of forms that makes direct comparisons of values difficult. For example, many reported the values in terms of fresh weight without reporting moisture contents or they used various methods for extracting the phenolic compounds. Vinson *et al*. (1998) reported total phenol content of some plants in terms of μmol g^{-1} dry weight. They stated that if one assumes an average molecular weight of 290 for all the phenolic compounds, then the total phenolic content could be expressed in mg g^{-1} dry weight. Based on this assumption, the results from Vinson *et al*. (1998) yield the following total phenolic contents (mg g^{-1} dry weight) of some plant roots: Beet = 15.5, carrot = 4.44, garlic = 9.95, yellow onion = 6.64, potato = 1.71 and sweet potato = 3.97. The PWIR had a total phenolic content of 16.4 mg g^{-1} dry weight and IR had values that ranged from 5.14-13.6.

Comparison of total flavonoid content: Table 2 shows that the total of the flavonoid content of PWIR was significantly greater (p<0.05) than for all of the IR samples. For the unbounded water soluble type of total flavonoid content, PWIR was much higher than all the IR samples. The results for content values of bounded type of total flavonoid from alkaline hydrolysis showed that PWIR is greater than all the IR samples and there was no statistical difference between IR 1, 2, 3 and 4. The content values of bounded type of total flavonoid from acid hydrolysis were all low and the differences among all samples were small. Miean and Mohamed (2001) reported the total flavonoid levels for a variety of plants and the following are values in units of mg g^{-1} dry weight for some plant roots: garlic = 0.96, carrot = 0.23, white radish = 0.065 and turmeric = 0.093. The total flavonoid content of PWIR was 1.35 and IR ranged from 0.12-0.26.

Comparison of specific individual phenolic acid contents: For p-coumaric acid, the total content of IR2 was significantly higher (p<0.05) than for PWIR and the total content of PWIR was significantly higher than for IR 4 (Table 3). For the insoluble type of total phenolic compound from alkaline hydrolysis extract, the p-coumaric acid content of IR 2 was significantly higher than PWIR and IR 4. For the insoluble type of p-coumaric acid from acid hydrolysis, IR 2 was greater than PWIR and IR 4 did not contain acid-bound p-coumaric acid. Mattila and Hellstrom (2007) reported p-coumaric acid contents for some plants that were reported in units of mg/100 g fresh weight. If one assumes moisture contents, then p-coumaric acid contents can be estimated on a dry weight basis for comparison to the values in Table 3. Estimated p-coumaric acid contents in mg/100 g dry weight (and assumed moisture content) of some plant roots based on Mattila and Hellstrom (2007) are as follows: garlic = 0.26 (65%), onion = 1.62 (87%), carrot = 8.63 (87%), radish = 112 (95%) and red beet = 5.0 (87%).

Only the PWIR contained gallic acid which was found only in the water soluble form. For the water soluble syringic acid content, IR 2 and 4 were statistically the same and both were statistically greater than PWIR. The alkaline-bound syringic acid content of IR 2 was significantly greater than PWIR and IR 4. For the insoluble acid-bound syringic acid content, IR 2 was significantly greater than IR 4 and IR 4 was significantly greater than PWIR. Mattila and Hellstrom (2007) reported syringic acid contents for some vegetables that were reported in units of mg/100 g fresh weight. If one assumes moisture contents, then syringic acid contents can be estimated on a dry weight basis for comparison to the values in Table 3. Estimated syringic acid contents in mg/100 g dry weight (and assumed moisture content) of some plant roots based on Mattila and Hellstrom (2007) are as follows: garlic = not detectable (65%), onion = not detectable (87%), carrot = not detectable (87%), radish = not detectable (95%) and red beet = 3.9 (87%).

The water soluble vanillic acid content of IR 2 was significantly greater than IR 4 and PWIR did not contain water soluble vanillic acid. Only PWIR contained insoluble alkaline-bound vanillic acid. For the insoluble acid-bound vanillic acid, the content of PWIR was significantly greater than IR 4 and IR 2 did not contain acid-bound vanillic acid. Mattila and Hellstrom (2007) reported vanillic acid contents for some plants that were reported in units of mg/100 g fresh weight. If one assumes moisture contents, then vanillic acid contents can be estimated on a dry weight basis for comparison to the values in Table 3. Estimated vanillic acid contents in mg/100 g dry weight (and assumed moisture content) of some plant roots based on Mattila and Hellstrom (2007) are as follows: garlic = not detectable (65%), onion = 0.46 (87%), carrot = 7.54 (87%), radish = 1.4 (95%) and red beet = 2.6 (87%).

Overall, the results of this study showed that many beneficial constituents of the plains wild indigo roots generally exceed those of the well-known medicinal plant-indigowoad root. Also, the phenolic compounds of PWIR and IR either exceeded or compared favorably with other commonly consumed plant roots (Vinson *et al*., 1998; Miean and Mohamed, 2001). Phenolic compounds and flavonoids have been reported to have a number of benefits in the human diet including antioxidant and antimicrobial benefits (Puupponen-Pimia *et al*., 2001). In a review, on nutritional values of vegetables and antioxidant potential, Nahak *et al*. (2014) stated that the phenolic content of a plant is a generally good indicator of its antioxidant potential. Also, PWIR contained gallic acid which is believed to induce cell death of tumor cells (Inoue *et al*., 1994). Other researchers have shown that the seeds of plains wild indigo have some antimicrobial activity against *Staphylococcus aureus* which could potentially have beneficial health effects related to infected epithelial tissue (Borchardt *et al*., 2008; Mukku *et al*., 2013). Based on the findings of this study, further research on PWIR and IR may be warranted to determine their possible use as additional sources of phenolic compounds and other desirable constituents. More research is needed on other potential bioactive constituents in these plants-for example, a group of experienced organoleptic panelists noticed a fragrance of saponins from PWIR which is a major bioactive component of ginseng.

The values presented here for phenolic compounds in PWIR and IR are only for the set of conditions for which the sample plants were grown and handled prior to analysis. The variation of phenolic compounds in various samples of IR found in this research demonstrate that growth and handling conditions may have a significant effect on the levels of chemicals in plant tissues. Nahak *et al*. (2014) stated that environmental factors (light intensity, season, climate and temperature) during plant growth and the extraction methods used may contribute to the wide variation in total phenols and antioxidant activities found in plants by various researchers. Chang *et al*. (2013a, b) found that the time of day that plant tissue is collected may significantly affect levels of some chemicals. Rezazadeh *et al*. (2012) indicated that soil conditions may affect phenolic levels and antioxidant activity of plants.

CONCLUSION

This study can be concluded as the phenolic compounds of PWIR and IR either exceeded or compared favorably with other commonly consumed root vegetable. The phenolic compounds of the PWIR generally exceed those of IR. More research is needed to identify factors that will affect chemical levels in plant tissue so plant can be grown and harvested under optimum conditions to enhance desirable chemical levels. More research is also needed on possible negative aspects of PWIR and IR, such as potential toxicity at certain levels.

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