Food Research 5 (1) : 216 - 224 (February 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Total phenolic, flavonoid, flavonol contents and antioxidant activity of Inca peanut (*Plukenetia volubilis L.*) leaves extracts

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Article history:
Received: 11 July 2020
Received in revised form: 16 August 2020
Accepted: 21 September 2020
Available Online: 20 December 2020

Keywords:
Antioxidant,
Flavonoids,
Inca peanut,
Phenolic,
Plukenetia volubilis L.

DOI:
https://doi.org/10.26656/fr.2017.5(1).346

Abstract

Inca peanut (*Plukenetia volubilis* L.) leaves were used to make tea and sold as a local product in Thailand but there is no research on the bioactivity of Inca peanut leaves. The present study was carried out to evaluate the phytochemical constituents and antioxidant activity of Inca peanut leaves. Fresh leaves were extracted with water (FW) and hot water (FH). Dried leaves (DH), roasted leaves (RH) and commercial tea leaves (CH) were extracted with hot water. Their phytochemical constituents, the amount of phenolic compounds (TPC), total flavonoid content (TFC) and total flavonol content (TFoC) were analyzed. The in vitro antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) radical cation scavenging activity. The phytochemical screening revealed the presence of phenols, flavonoids, tannin, cardiac glycosides, steroids, and terpenoids. RH contained the highest TPC (21.36±1.90 µg GAE/mg), TFC (8.65±0.16 µg QE/mg), TFoC (0.249±0.004 µg QE/mg) and exhibited the most potent antioxidant activity by DPPH assay (IC_{50} = 135.97±6.71 µg/mL) and ABTS assay (IC_{50} = 37.53±3.87 µg/mL). Flavonoid has a positive correlation with DPPH radical scavenging activity. These results suggested that the antioxidant activity of Inca peanut leaves might be attributed to the presence of flavonoid compounds. Roasted leaves extract exhibited the highest antioxidant activities. Therefore, Inca peanut leaves extracts could be considered as a good source of antioxidant and developed as a functional food.

1. Introduction

Aging is regarded as one of the most common concerns in modern society, and it is a complex certain process in human life. Among the theories purposed for explaining the mechanism of aging, free radical or oxidative stress theory is one of the most accepted (Liguori *et al.,* 2018). The free radical theory of aging purposes that the accumulation of free radical and the declining antioxidant defense leading to oxidative stress. This phenomenon is implicated in the pathogenesis of a variety of human and animal diseases and potentially important contributors to the aging process (Wickens, 2001). For this purpose, the use of antioxidants to prevent aging is important.

Inca peanut (*Plukenetia volubilis* L.) is a perennial, oleaginous plant of the Euphorbiaceae family. It grows in Amazon region of South America that includes parts of Peru and northwestern Brazil in an environment with water and well-drained acidic soil (Gonzalez-Aspajo *et al.*, 2015). It has a star-shaped fruit capsule which the colour turns from green to blackish brown when the fruit matures. The fruit capsules contain edible dark brown oval seeds. The seeds have been utilized for oil production because they are guaranteed to be beneficial from several types of research. In recent years, there has been growing interest in developing Inca peanut plant as a novel source of oil rich in unsaturated fatty acid. Inca peanut is being developed in Southeast Asia because of its great potential as an economic crop (Chandrasekaran and Liu, 2015). It was introduced in Thailand 6 years ago and widely cultivated in Northern Thailand such as Phayao, Lampang, Chiang Mai, and Chiang Rai due to the appropriate geographical location and climate. The main composition of fatty acids in Inca peanut oil cultivated in Thailand was studied and found the presence of linoleic acid or ω6 (45.72%), linolenic acid or ω3 (42.27%), palmitic acid (6.42%) and stearic acid (4.53%) (Wuttisin, 2017). Some amount of oleic acid or ω9 (8.7-9.6%) was also detected (Guillén *et al*., 2003;

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eISSN: 2550-2166

Follegatti-Romero *et al*., 2009; Chirinos *et al*., 2013). It also contains essential amino acids such as cysteine, tyrosine, threonine, and tryptophan as well as vitamin E, polyphenols, and minerals (Wang *et al*., 2018). Inca peanut oil is now available as edible oil. Roasted seeds were served with salt as a snack, while fresh and cooked leaves were part of traditional dishes in many countries. Inca peanut leaves were used to make tea and sold as local products in Thailand. However, there is no research which studies about the bioactivity of Inca peanut leaves and Inca peanut tea. Hence, the aims of this study were to screen phytochemical components and determine the antioxidant activity of Inca peanut leaves. The data might be useful for supporting the benefit of Inca peanut leaves in the future.

2. Materials and methods

2.1 Chemical and reagents

Gallic acid, Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, and quercetin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo., U.S.A.). Other chemicals and reagents used in this study were analytical grades.

2.2 Plant Materials

Inca peanut plant was grown in Chiang San, Chiang Rai, Thailand. The leaves (5 months old) were collected during June 2018 (Figure 1). Commercial tea leave was purchased from local product in Baan Tha Khan Thong, Chiang San, Chiang Rai, Thailand. Inca peanut tea was prepared by sun-dried and roasted on pan-fired.

Figure 1. Appearance of Inca peanut leaves (left), dried leaves (middle) and commercial tea leaves (right)

2.3 Preparation of Inca peanut leaves extracts

The leaves (fresh or dried) were cut into small pieces by blending in the blender (Sharp/EM-11) and then extracted with water (30 mins) in a ratio of 1:10 (w/v) resulting in five extracts: fresh leaves extracted with water (25°C) (FW), fresh leaves extracted with hot water (90°C) (FH), dried leaves (air-dried under shade for 7 days) extracted with hot water (90°C) (DH), roasted leaves (hot air oven 60°C, 48 hrs) extracted with hot water (90°C) (RH) and commercial tea leaves extracted with hot water (90°C) (CH). The extracts were then filtered through Whatman® paper No.1 and dried using freeze dryer (Labconco). The percentages of yield were calculated by the following equation:

% Yield = [Inca peanut extract (g)/Inca peanut leaves (g)] × 100

2.4 Phytochemical screening of Inca peanut leaves extracts

Inca peanut leaves extracts were dissolved in water (5 mg/mL) and determined for their phytochemical constituents as following.

2.4.1 Test for phenols

5% ferric chloride solution (0.2 mL) was added into test tube containing 1 mL of the extract. The purple colour indicates the presence of phenolic compounds (Harborne, 1973).

2.4.2 Test for saponins

The extract (2 mL) was added into the test tube containing 2 mL of distilled water. The mixture was shaken vigorously for 2 mins and warmed (37°C). The formation of stable foam indicates the presence of saponins (Banso and Adeyemo, 2006).

2.4.3 Test for flavonoids

2.4.3.1 Alkaline reagent test

10% sodium hydroxide solution (0.2 mL) was added into 1 mL of the extract. The intense yellow colour indicates the presence of flavonoids (Tiwari *et al*., 2011).

2.4.3.2 Lead acetate test

10% lead acetate solution (0.2 mL) was added into 1 mL of the extract. The white or yellow precipitate indicates the presence of flavonoids (Bargah, 2015).

2.4.4 Test for steroids

According to Salkowski's test, 1 mL of the extract was mixed with 1 mL of chloroform and 1 mL of concentrated sulfuric acid. The red colour in the lower chloroform layer indicates the presence of steroids (Harborne, 1973).

2.4.5 Test for terpenoids

The extract (2 mL) was dissolved in 2 mL of chloroform and evaporated to dryness. Concentrated sulfuric acid (5 mL) was then added and heated for 2 mins. Development of a grayish colour indicates the presence of terpenoids (Bargah, 2015).

2.4.6 Test for alkaloid

Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. An aliquot (0.5 mL) of this solution was added to 1 mL of the extract (50 mg/mL). A brown colored precipitate indicates the presence of alkaloids (Abdullahi *et al*., 2013; Joshi *et al*., 2013).

2.4.7 Test for tannins

Leave extract (50 mg) was separately dissolved in distilled water (10 mL). The mixture is boiled for five mins and then filtered. The filtrate was added with 0.5 mL of 5% ferric chloride. Black or blue-green colouration or precipitate was taken as a positive result for the presence of tannins (Akinjogunla *et al.*, 2010).

2.4.8 Test for glycosides

Leave extract (0.5 g) was shaken with distilled water (5 mL). Glacial acetic acid (2 mL) containing one drop of 5% ferric chloride was added, followed by concentrated sulfuric acid (1 mL) along the side of the test tube. The formation of a brown ring at the interface gives a positive indication for cardiac glycoside and a violet ring may appear below the brown ring (Ayoola *et al*., 2008).

2.5 Determination of total phenolic contents (TPC)

The amount of TPC was determined according to the method described by Waterman and Mole (1994) with some modifications. Inca peanut leaves extracts were dissolved in water (2 mg/mL) and determined for their TPC. Each extract (20 µL) was added with Folin-Ciocalteu reagent (100 µL). Three mins later, 7.5% w/v sodium carbonate (80 µL) was added into the mixture which was then shaken and allowed to stand for 1 hr at ambient temperature. After incubation time, the absorbance was measured at 760 nm (SPECTROstar Nano Microplate Reader, BMG Labtech). Gallic acid was used as a reference compound. A calibration curve of gallic acid was prepared in the range of 1 to 10 µg/mL. The result was expressed as µg gallic acid equivalent per mg of extract (µg GAE/mg).

2.6 Determination of total flavonoid content (TFC)

Inca peanut leaves extracts were dissolved in water (2 mg/mL) and determined for their TFC. TFC was determined using the aluminum colorimetric method with some modifications using quercetin as the standard (Iqbal *et al*., 2015). A calibration curve of quercetin was prepared in the range of 0.5 to 12 µg/mL. Briefly, extract (100 µL) or standard (100 µL) were placed in different test tubes and 10% aluminum chloride (50 µL), 1M potassium acetate (50 µL), 80% methanol (750 µL) and distilled water (1.4 mL) were added and mixed. A blank was prepared in the same manner where 100 µL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 mins. The absorbance was taken at 415 nm (UV-VIS Spectrophotometer, Thermo Scientific). The concentration of flavonoid was expressed as µg quercetin equivalent per mg of extract (µg QE/mg).

2.7 Determination of total flavonol content (TFoC)

Inca peanut leaves extracts were dissolved in water (2 mg/mL) and determined for their TFoC. TFoC content was analyzed using aluminum chloride colorimetric method with some modifications (Pattanayak *et al*., 2011; Pallab *et al*., 2013). In this method, quercetin was used to make a standard calibration curve in the range of 0.5 to 4 µg/mL. In different test tubes, each extract (100 µL) and standard solutions (100 µL) were placed and then 2% aluminum chloride (300 µL), 5% sodium acetate (0.9 mL) were added and mixed well. All tubes were incubated at room temperature for 20 mins. The absorbance of standard and sample was taken at 440 nm. Results were expressed as µg quercetin equivalent per mg of extract (µg QE/mg).

2.8 Determination of antioxidant activities

Inca peanut leaves extracts were dissolved in water at various concentrations (2.0, 1.0, 0.5, 0.25 and 0.125 mg/mL) and determined for their antioxidant activities as following.

2.8.1 DPPH radical (DPPH•) scavenging activity

DPPH scavenging activity was determined according to colorimetric method with some modifications (Gülçin *et al*., 2003). Each sample was prepared by mixing 20 µL of each extract with 180 µL of DPPH solution (0.1 mmol/L). A mixture containing 180 µL of DPPH solution and 20 µL of 95% ethanol was used as control. After incubation in the dark place for 30 min, the absorbance of each mixture was measured spectrometrically at 517 nm (SPECTROstar Nano Microplate Reader, BMG Labtech). Trolox was used as a reference compound. Similar concentration extract without DPPH solution was used as the blank to eliminate interference. The ability to scavenge the DPPH• was calculated by the following equation:

$$\% \text{ DPPH}^{\bullet} \text{ scavenging} = [A0-(A1-A2)/A0] \times 100$$

Where A0 = Absorbance of the control without standard

or sample, A1 = Absorbance of the mixture containing standard or samples, and A2 = Absorbance of the blank (extract without DPPH solution)

The calibrations curve between the percent inhibition and the trolox concentration was established. The DPPH$^{\bullet}$ scavenging activity was expressed as trolox equivalent antioxidant capacity (µg TEAC/mg) and IC_{50} values (µg/mL), indicating the concentrations of extracts scavenge 50% of DPPH$^{\bullet}$.

2.8.2 ABTS radical cation (ABTS$^{\bullet+}$) scavenging activity

The antioxidant activity was determined by ABTS scavenging activity with some modifications (Re *et al*., 1999). The stock solution of ABTS cation chromophore was prepared by the reaction between 7 mM ABTS solution (100 mL) and 2.45 mM potassium persulfate (final concentration) (100 mL) in the dark place at ambient temperature for 16 h. The ABTS$^{\bullet+}$ solution was diluted with phosphate buffer (50 mM, pH 7.4) to an absorbance of 0.70±0.02 at 734 nm. A mixture containing 180 µL of ABTS$^{\bullet+}$ solution and 20 µL of phosphate buffer was used as control. The extract (20 µL) was added to 180 µL. ABTS$^{\bullet+}$ solution and incubated for 30 min at ambient temperature. The absorbance was measured at 734 nm (SPECTROstar Nano Microplate Reader, BMG Labtech). Ascorbic acid was used as a reference compound. Similar concentration extract without ABTS$^{\bullet+}$ solution was used as the blank to eliminate interference. The percent inhibition of ABTS$^{\bullet+}$ was calculated by the following equation:

$$\% \text{ ABTS}^{\bullet+} \text{ scavenging} = [A0-(A1-A2)/A0] \times 100$$

Where A0 = Absorbance of the control without standard or sample, A1 = Absorbance of the mixture containing standard or samples, and A2 = Absorbance of the blank (extract without ABTS$^{\bullet+}$ solution)

The calibrations curve between the percent inhibition and the ascorbic acid concentration was established. The ABTS$^{\bullet+}$ scavenging activity was expressed as mg ascorbic acid equivalent antioxidant capacity per gram extract (mg AEAC/g) and IC_{50} values (µg/mL), indicating the concentrations of extracts scavenge 50% of ABTS$^{\bullet+}$.

2.9 Statistical analysis

All assays were performed triplicate in three independent and separate experiments. The data were presented as mean±standard deviations (SD). SPSS 23.0 was employed for all data analyses. One-way analysis of variance (ANOVA) Post Hoc multiple comparisons by Duncan's multiple range test was used to evaluate the difference between sample groups. The level of significance was at $P<0.05$. A linear correlation analysis was performed in order to determine the relationship between TPC, TFC, TFoC and antioxidant activities.

3. Results and discussion

3.1 Phytochemical screening of Inca peanut leaves extracts

The phytochemical screening of the Inca peanut leaves extracts revealed the presence of some active compounds such as phenols, flavonoids, tannins, cardiac glycosides, steroids, and terpenoids as shown in Table 1. Phenol is considered the simplest class of phenolic compounds. Flavonoids are the largest group of plant phenols. Tannins are compounds of high molecular weight phenolic polymers which are found commonly in grapes, tea and legume (Saxena *et al*., 2013). Cardiac glycosides are steroids having the ability to exert specific powerful action on the cardiac muscle valuable in the treatment of congestive heart failure. It could be also found in some plants belonging to Euphorbiaceae (Hollman, 1985). Steroids and terpenoids were reported to be active against antibacterial activity (Bargah, 2015). The same finding was reported by Nascimento *et al*. (2013) that they described the presence of phenolic compounds, flavonoids, steroid and terpenoids in Inca peanut leaves. These phytochemicals are known to possess therapeutic activities including antimicrobial, cytotoxicity, anti-inflammatory, antitumor activity, anticarcinogenic and antioxidant with beneficial effects in the human diet. Only fresh leaves extracts contain saponins and alkaloids which were in accordance with the previously studied that roasting enables the reduction of saponins and alkaloids which are considered as phytotoxins then it was recommended to avoid high and chronic consumption of fresh leaves (Srichamnong *et al*., 2018). The preliminary phytochemical screening tests

Table 1. Phytochemical screening of Inca peanut leaves extracts

	FW	FH	DH	RH	CH
Phenols	+	+	+	+	+
Flavonoids					
Alkaline reagent test	+	+	+	+	+
Lead acetate test	+	+	+	+	+
Tannins	+	+	+	+	+
Glycosides	+	+	+	+	+
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Saponins	-	+	-	-	-
Alkaloids	+	-	-	-	-

Where, + indicates the presence of phytochemicals, and - indicates the absence of phytochemicals.

help to detect the bioactive compounds. Further steps such as purification and characterization are necessary.

3.2 TPC of Inca peanut leaves

Extraction yields of Inca peanut leaves are given in Table 2. After extraction, DH and RH provided a higher yield than FW and FH. There are no studies in the previous works of literature concerning yields of Inca peanut leaves extracts. TPC of Inca peanut leaves was estimated and the result revealed in Table 2 that DH, RH and FW exhibited a high significance level ($P<0.05$) of TPC followed by FH and CH. Phenolic compounds are the largest category of phytochemicals and the most widely distributed in the plant kingdom (Saxena *et al*., 2013). They are mostly composed of flavonoids, phenolic acids, stilbenes, coumarins and tannins (Islam *et al*., 2015). Phenolic compounds have redox properties, which allow them to act as antioxidants (Soobrattee *et al*., 2005). Their antioxidant ability is facilitated by their hydroxyl groups via scavenging or stabilizing free radical through hydrogenation or complexion with oxidizing species (Uddin *et al*., 2020). TPC could be used as a basis for rapid screening of antioxidant activity. Inca peanut seeds have been assessed for TPC ranging from 64.6 to 80 mg GAE/100g which is lower than in leaves extract 20 folds (Wuttisin, 2017).

3.3 TFC of Inca peanut leaves

DH and RH exhibited the highest ($P<0.05$) TFC followed by CH while FH and FW exhibited the lowest TFC (Table 2). Flavonoids are probably the most important natural phenolics. TFC in the extract was determined by the spectrophotometric method with aluminum chloride. The flavonoids combine with aluminum to form a complex flavonoid-aluminum that could be measured at 415 nm (Quettier, 2000). The most abundant flavonoid which has a good antioxidant property is quercetin.

3.4 TFoC of Inca peanut leaves

Table 2 shows that RH and FW exhibited the highest TFoC ($P<0.05$) followed by DH, CH and FH. Flavonols are one type of flavonoids. TFoC was less in all extracts. In this study, RH exhibited the highest TPC, TFC and TFoC when compared to other extracts. However, further research is needed to identify the flavonoids and flavonols components in Inca peanut leaves.

3.5 Antioxidant activities

3.5.1 DPPH scavenging activity

The free radical scavenging activities of the extracts were determined using DPPH scavenging assay and the results were displayed in Table 3. The results were expressed with TEAC range from 58.49±3.47 to 70.77±2.35 mg TEAC/g. The significant highest antioxidant activity ($P<0.05$) is from DH (IC_{50} = 134.35±5.07 µg/mL) and RH (IC_{50}= 135.97±6.71 µg/mL) as compared with other extracts. DPPH scavenging method offers the first approach for evaluating the antioxidant potential of plant extract. This assay measures the ability of the plant extract to donate an electron or H^+ ion. The $DPPH^{\bullet}$ is a stable free radical and

Table 2. Total phenolic, total flavonoid and total flavonol contents of Inca peanut leaves extracts

Extracts	% Yield	Total phenolic content (µg GAE/mg)	Total flavonoid content (µg QE/mg)	Total flavonol content (µg QE/mg)
FW	10.26	21.29±0.89^{a}	4.34±0.61^{c}	0.235±0.005^{a}
FH	8.68	16.94±1.80^{b}	4.75±0.12	0.111±0.016^{d}
DH	19.34	21.48±0.72^{a}	8.78±0.15^{a}	0.194±0.017^{b}
RH	19.29	21.36±1.90^{a}	8.65±0.16^{a}	0.249±0.004^{a}
CH	17.83	13.79±0.82^{c}	6.74±0.04^{b}	0.151±0.041^{c}

Values were presented as mean±SD of three independent measurements. Different letters in the same column indicates significant differences ($P<0.05$)

Table 3. DPPH scavenging activity and ABTS scavenging activity of Inca peanut leaves extracts

	DPPH (mg TEAC/g)	IC_{50} (DPPH) (µg/mL)	ABTS (mg AEAC/g)	IC_{50} (ABTS) (µg/mL)
FW	58.49±3.47^{b}	148.30±7.17^{b}	135.92±2.79^{a}	39.59±1.57^{a}
FH	60.11±0.62^{b}	147.49±3.42^{b}	121.61±3.02^{b}	43.58±1.89^{bc}
DH	68.15±2.57^{a}	134.35±5.07^{a}	118.12±3.04^{b}	44.72±2.60^{c}
RH	70.77±2.35^{a}	135.97±6.71^{a}	138.36±5.24^{a}	37.53±3.87^{a}
CH	59.84±1.70^{b}	149.42±3.40^{b}	122.31±5.96^{b}	42.19±2.78^{b}
Trolox	-	6.96±0.60	-	-
Ascorbic acid	-	-	-	3.88±0.50

Values were presented as mean±SD of three independent measurements. Different letters in the same column indicates significant differences ($P<0.05$)

shows maximum absorbance at 517 nm. DPPH$^{\bullet}$ accepts electron or hydrogen from antioxidant molecules to become a stable molecule resulting in a decrease in absorbance (Ahmed *et al*., 2013). With reference to the positive control Trolox, the scavenging ability of the Inca Peanut leaves extracts on DPPH$^{\bullet}$ was shown. The lower the IC_{50} values are, the higher the antioxidant capacity of the leaves extracts become. Inca Peanut leaves contain flavonoids, flavonols and related polyphenols are able of donating a hydrogen atom to a free radical to neutralize it.

3.5.2 ABTS scavenging activity

Table 3 shows the antioxidant activities of Inca peanut leaves extract determined by ABTS scavenging activity. The results revealed that RH and FW exhibited the highest antioxidant activities ($P<0.05$) with the IC_{50} values of 37.53±3.87 µg/mL and 39.59±1.57 µg/mL, respectively. ABTS scavenging assay depends on the antioxidant compound ability to scavenge ABTS$^{\bullet+}$. This assay can measure the antioxidant capacity of hydrophilic compounds (Awika *et al*., 2003). In the same way, ABTS inhibition mechanism was similar to DPPH scavenging assay. We observed that RH exhibited more potent antioxidant activity than other extracts when determined by DPPH and ABTS scavenging assay, then it is the most interesting for further roles in health-promoting as an antioxidant.

3.6 Correlation between TPC, TFC, TFoC and antioxidant activity

The correlation between the TPC, TFC, TFoC and their antioxidant activities were done and displayed in correlation coefficients (R^2) values from linear regression analysis (Table 4). A positive correlation was observed between antioxidant activity and phenolic compounds. The results showed that flavonoid has a positive correlation with DPPH antioxidant activity (R^2 = 0.8153) while flavonols have a positive correlation with ABTS antioxidant activity (R^2 = 0.6051). The correlation indicated that the richness in phenolic compounds especially higher flavonoids contents lead to better DPPH scavenging activity (Felhi *et al*., 2016). The results suggested that the antioxidant activity of Inca peanut leaves might be attributed to the presence of flavonoids which are more react with DPPH$^{\bullet}$ better than ABTS$^{\bullet+}$ (Wuttisin and Boonsook, 2019). Thus, flavonoids can be used to predict the antioxidant activity of Inca peanut leaves. The positive correlation between flavonol content and ABTS antioxidant activity strengthens the results observed in the DPPH. This investigation confirms that an increase in total phenolic compounds will increase the antioxidant activity of extracts which in accordance with previously reported (Bakari *et al*., 2015). Polyphenols have a metal chelating potential and their redox properties can be justified by their chemical structure (Li *et al*., 2008; Schvab *et al*., 2015). For this reason, the high polyphenolic content in the extracts may explain the high antioxidant activity of the extracts. In addition, the antioxidant activity might be attributed to the presence of non-phenolic compounds and Inca peanut leaves might be contained other synergistic or antagonistic compounds (Tomsone *et al*., 2012). Further studies are required to identify other chemical components in Inca peanut leaves. The presence of flavonoids such as quercetin and other phytochemicals are recommended to be characterized by HPLC analysis. Furthermore, quercetin is suggested to use as a reference compound in DPPH and ABTS radical scavenging activity for prediction the antioxidant activity of Inca peanut leaves due to the positive correlation between flavonoid and antioxidant activity.

Table 4. Correlation matrix of phenolic, flavonoids, flavonols and antioxidant activities against DPPH and ABTS radical

Parameters	ABTS	DPPH	Flavonols	Flavonoids
Phenolic	0.226	0.3111	0.5953	0.0787
Flavonoids	0.0082	0.8153	0.1234	
Flavonols	0.6051	0.242		
DPPH	0.0245			

4. Conclusion

The result of this study shows the presence of some phytochemicals such as phenols, flavonoids, tannin, cardiac glycosides, steroids, and triterpene in Inca peanut leaves extracts. Roasting enables the reduction of saponins and alkaloids in Inca peanut leaves extracts. Roasted leave extract with hot water exhibited the highest phenolic, flavonoids, and flavonols contents. The result also revealed that roasted leaves extract showed the highest antioxidant activity by DPPH assay and ABTS assay. The antioxidant properties of the extracts might be due to the presence of flavonoid and other phytochemicals present in Inca peanut leaves. Therefore, Inca peanut leaves and Inca peanut tea could be consumed as a source of antioxidant for preventing free radicals mediated oxidative stress. This is an ongoing study and further work is being carried to investigate its biological activities.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by Mae Fah Luang University's grant, Chiang Rai, Thailand.

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eISSN: 2550-2166