Pharmacogn J. 2021; 13(1): 52-58
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Phytochemical Screening and Antioxidant Activities of the Seedling Extracts from Inca Peanut *Plukenetia volubilis*

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ABSTRACT

Background: *Plukenetia volubilis* L., Inca peanut is an oleaginous plant, widely cultivated as commercially in South East Asia, especially in Thailand. The oil from the seed plant is a greatest interesting a natural source. **Objectives:** The aims of this study were investigated phytochemical screening, to evaluated the total flavonoids and phenolic compound contents as well as antioxidant activities of seedling extract from *P. volubilis*. **Methods:** The dried *P. volubilis* seedlings of 21 days growing period were extracted by using different solvent including aqueous (ASS), 50% ethanolic (HESS), and 95% ethanolic (ESS) extracts. The phytochemical screenings were determined on total phenolic compound (TPC) and flavonoid (TFC) contents. The antioxidation were tested by using 2,2-diphenyl-1-picrylhydrazy radical scavenging (DPPH), 2,2 -azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^+$) assay, and ferric reducing antioxidant power (FRAP). **Results:** The results found that the ESS were significantly highest amount on total phenolic compound (23.0809±0.8632 mgGE/gExt) and flavonoid (466.3839±1.5580 mgQE/gExt) contents. In this study, ascorbic acid (IC_{50} =0.016±0.0003 mg/mL) and Trolox® (IC_{50} =0.044±0.0008 mg/mL) as standard substances were showed more potent than all of the extracts from *P. volubilis* seedlings. Surprisingly, the ESS has more potent on free radical scavenging higher than different solvents; DPPH=0.007± 0.001 (IC_{50}=mg/mL), ABTS= 1.4065± 0.0505 (IC_{50}=mg/mL),and FRAP= 74.4960± 2.6067 (mg=TE/gExt). **Conclusion:** the plant seedling extracts composed with high amount of flavonoids and phenolic compound contents possess valuable to antioxidant activities. The seedling extracts from the plant could apply to supplementary food, cosmetic, pharmaceutical, and horticultural industries. Next study, chemical compositions, the major active compound(s), and biological activities will be clarified.

***Key Words*:** Phytochemical screening, Flavonoids, Phenolic compounds, *Plukenetia volubilis*, Inca peanut seedling, Antioxidant activity.

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History

- Submission Date: 21-09-2020;
- Review completed: 28-10-2020;
- Accepted Date: 21-11-2020.

DOI : 10.5530/pj.2021.13.8

Article Available online

http://www.phcogj.com/v13/i1

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INTRODUCTION

The secondary metabolites, flavonoids, phenolic compounds present in different parts of the plants provide protection against also the pathogens and protect the plants from UV radiations[1]. Flavonoid(s) is a phenolic compound derivative, component in nature and distributed in many parts of plant[2]. The molecular structure is process to stop oxidation of cells within human body[3]. The phenolic compounds in plant against chronic conditions including cardiovascular diseases, neurodegeneration, and certain kinds of cancer[4]. The oxidative stress results from an improper balance between Reactive Oxygen Species (ROS) and their metabolites and antioxidant defense cause some factor in the pathogenesis of various diseases[5]. The reaction of free radical such as hydrogen peroxide and superoxide radical yields the hydroxyl radical (•OH) which is highly reactive and damaging to most biomolecules[6]. Antioxidant mechanisms, electron donated, hydrogen atom transfer (HAT), single electron transfer (SET), and the ability to chelate transition metals. The importance of antioxidant mechanisms is to understand the biological meaning of antioxidants, their possible uses, their production by organic synthesis or biotechnological methods, or for the standardization of the determination of antioxidant activity[7].

Plukenetia volubilis L., is a climbing shrub plant commonly known as sacha peanut, mountain peanut or inca peanut, a perennial plant with somewhat hairy leaves, belonging to the Euphorbiaceae family. It is widely also being cultivated commercially in South East Asia, especially in Thailand[8-9]. The seed obtained with mainly oil, which is characterized by having a high unsaturated fatty acid content including oleic, linoleic, and linolenic acid. The oil extraction process generates a sub-product with high protein contents[10]. Inca peanut seeds contained the largest amount of protein (62.07%) and carbohydrates (82.68%). Moreover, it also contains with essential amino acid such as Lysine, leucine, histidine, and phenylalanine[11]. The seeds were contained 25–27% protein and 41–54% of oil which comprises approximately 90% unsaturated fatty acids (oleic, linoleic, and linolenic acid) and is rich in vitamins E (alpha-tocopherols) and A (carotenoids)[12-14]. Phytochemical constituents from seed of the plant composed of fatty acids, phytosterols, tocopherols, phenolic compounds, total carotenoids, and hydrophilic and lipophilic capacities[15]. Several secondary metabolites such as phenolics, flavonoids, alkaloids derived from plants show superfluous antioxidant potential. The

Cite this article: Puangpronpitag D, Tankitjanon P, Sumalee A, Konsue A. Phytochemical Screening and Antioxidant Activities of the Seedling Extracts from Inca Peanut *Plukenetia volubilis*. Pharmacog J. 2021;13(1): 52-8.

unsaturated fatty acid such as omega-3 are very important to the nutraceutical industrial because associated with a number of health benefits, such as prevent fatal cardiovascular disease, coronary heart disease prevention, diabetes prevention, hypertension prevention, and others[16]. Inca peanut grain is an excellent source of bioactive molecules such as phytosterols which decrease the risk of certain types of cancer, reduce cholesterol in the blood, prevent cardiovascular diseases, decrease the risk of heart and other chronic diseases, increase HDL (high density lipoprotein) blood quantities, prevent cancer and relief the effects of auto-immune diseases, rheumatoid arthritis, and depression[17]. The oil from the plant is a greatest interesting a natural source with potential to applications in the food, cosmetic, and pharmaceutical industries[18-19].

Although, *P. volubilis* were widely used for supplementary food, cosmetic, and pharmaceutical industries. There are so many literatures that have been reported to the effect of the seed from *P. volubilis* but still not yet any article of plant seedling. The purposes of this study were investigated phytochemical screening and antioxidant activities of seedling extract from the plant.

MATERIALS AND METHODS

Sample collection

P. volubilis seeds were acquired from Loei Province, Northeastern of Thailand. The specimens were identified and deposited at the Faculty of Medicine, Mahasarakham University, Thailand (code; *P. volubilis*: MSU. MED- PV0001/ AK). The white *P. volubilis* seedlings were cultivated at the experimental farm of Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand. The seeds eliminated when they were deformed. The seed soaking at room temperature for 12 hr at before sowing in seed sponge with 2.54 x 2.54 x 2.54 cm. given to distilled water daily during 21 days. After day 21st, the fresh seedlings were cleaned and dried at 50°C for 48 hr in a hot air oven, then powdered.

Preparation of extracts

The aqueous extract (ASS) was prepared by boiling with distilled water for 15 min (1:10 w/v). The boiling process was repeated twice. The hydro-ethanolic extracts (HESS) and ethanolic extracts (ESS) were macerated with 50% ethanol and 95% ethanol for 7 days (1:5 w/v). The residue powder was excluded by using filter papers (Whatman, Germany). The filtrate was evaporated using by a rotary evaporator (Heidolph Laborota 4000, Germany) and then freeze-dried to obtain dark brown extract. The extracts were kept in the refrigerator at temperature below 4 °C until be used.

DETERMINATION OF PHYTOCHEMICAL SCREENING

Total flavonoid content assay

Flavonoid content was estimated using the aluminum chloride colorimetric method[20]. The extracts from recipe will be mixed with 100 µL of 5% aluminum chloride (w/v), 400 µL of 2.5% Na_2NO_3 After 5 min, 500 µL of 5% $AlCl_3$. The mixture will be allowed to stand at room temperature for 10 min. The solution was mixed 2,000 µL distilled water. The results were measured at 415 nm. The TFC was calculated from a standard Quercetin equivalent (mgQE/gExt).

Total phenolic compound content assay

Total phenolic compound content was determined according to a modified procedure[21]. Sample (100 µL) will be oxidized with 500 µL of 0.2 N Folin-Ciocalteu's reagent and neutralized by adding 400 µL of 7.5% Na_2CO_3. The absorbance measured at 765 nm after mixed will be allowed to stand at room temperature for 30 min. The results were expressed as gallic acid equivalents (mgGE/gExt).

DETERMINATION OF ANTIOXIDANT ACTIVITIES

DPPH free radical scavenging assay

2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacities of wheat extracts were estimated by the reduction of the reaction color between DPPH solution and sample extracts as previously described by prior method[22]. DPPH was dissolved in ethanol to a 0.039 mg/mL. The plant extract at various concentrations was diluted with distilled water to get sample solution. Then, 100 µL of the sample solution following which 900 µL DPPH (0.1 mM) working solution. After a 30 min reaction kept in the dark at ambient temperature then absorbance of the solution was measured at 515 nm. In this study, will be used Trolox® and ascorbic acid as standard substances. Blanks were run in each assay. DPPH radical ability was expressed as IC_{50} (mg/mL) and the inhibition percentage calculated using the following formula: DPPH scavenging activity (%) = $(A_0-A_1)/ A_0$ x 100 where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

$ABTS^+$ radical scavenging assay

In ABTS assay, the plants extract will be allowed to react with $ABTS^+$, a model stable free radical derived from 2,2-azinobis (3-ethylvenzothiazolin-6-sulphonic acid) ($ABTS^+$) assay was performed[23]. The $ABTS^+$ (900 µL) was added to the extracts (100 µL) and thoroughly mixed. The mixture was held at room temperature for 6 min, and absorbance was immediately measured at 734 nm. Trolox® and ascorbic acid solution in 80% ethanol was prepared and assayed under the same conditions. ABTS scavenging ability was expressed as IC_{50} (mg/mL) and the inhibition percentage calculated using the following formula: ABTS scavenging activity (%) = $(A_0-A_1)/ A_0$ x 100 where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing ability of the extract will be measured at low pH[24-25]. Sample (100 µL) will be oxidized with FRAP reagent 900 µl (300 mM Acetate buffer (pH 3.6): 10 mM tripyridyl triazine (tptz) solution: 20 mM Ferric chloride solution of 10: 1: 1). This will be incubated for 5 min at 37 °C. An intense blue coloured complex will be formed when Fe^{3+}-TPTZ complex could be reduced to the ferrous (Fe^{3+}) form. The absorbance at 593 nm will be recorded. The reducing power of the samples increased with the absorbance values. The results were expressed as Trolox® equivalent (mgTE/gExt).

Statistical analysis

All data were expressed as mean± standard error of mean (SEM) from four separate experiments (n = 4). Total variation of data carried out using one-way analysis of variance (ANOVA). Differences between means were determined using Duncan's multiple range tests. Differences at $p < 0.05$ considered significantly.

RESULTS

Total phenolic compound content

Total phenolic compound content showed that 95% ethanolic extract had TPC higher than different extracts. The TPC found that ESS (23.0809±0.8632 mgGE/gExt) was higher than hydro-ethanolic and aqueous extracts (1.7144±0.0346, 1.5319±0.0286 mgGE/gExt) respectively (Table 1).

Total flavonoid content

Total flavonoid content showed that 95% ethanolic extract had TFC higher than different extracts. The TFC found that ESS (466.3839±1.5580 mgQE/gExt) was higher than hydro-ethanolic and aqueous extracts (310.1624±16.6718 and 284.8477±7.8958 mgQE/gExt) respectively (Table 1).

Antioxidant activities

DPPH free radical scavenging activity

In this study, standard substances, ascorbic acid (IC_{50} =0.016±0.0003 mg/mL) and Trolox® (IC_{50} =0.044±0.0008 mg/mL) were showed more potent than all of the extract from Inca peanut seedlings. The ESS (IC_{50} =0.007±0.001 mg/mL) was exerted on anti-oxidation higher than HESS and ASS (IC_{50} =4.4675±0.2450 and 4.9150±0.2669 mg/mL) respectively (Table 2).

$ABTS^+$ radical scavenging activity

The $ABTS^+$ assay, the standard substances, ascorbic acid (IC_{50} =0.0250 ±0.0010 mg/mL) and Trolox (IC_{50} =0.0320±0.0010 mg/mL) were still showed more potent than all of the various solvent extracts from Inca peanut seedlings. The ESS (IC_{50}= 1.4065±0.0505 mg/mL) had been more potent than HESS and ASS (IC_{50} = 1.5398±0.0305 and 1.7215±0.0756 mg/mL) respectively (Table 2).

Ferric reducing antioxidant power (FRAP) activity

In this study, standard substances, ascorbic acid and Trolox were showed more potent than all the extract of Inca peanut seedlings The ESS (74.4960±2.6067 mgTE/gExt) was exerted on antioxidation higher than HESS and ASS (34.6960±1.3396, 28.0588±0.7758 mgTE/gExt) respectively (Table 2).

DISCUSSION

Currently, *P.volubilis*, Sacha inchi, or Inca peanut has been cultivated into sustainable agricultural systems provides new developmental opportunities and can increase the resilience of food production systems. It has a high potential for replacing illicit crops and the establishment of commercial plantations generate positive environmental impacts because it could be install on degraded soils[26]. Its grows on the tropical humid monsoon regions where annual rainfall is high and the wetting–drying cycles resulted in temporal changes in soil structure[27]. The parameter of seedling germination was approximately stimulated by vermiculite, continuous light, and 30 °C temperature[28].

The oil has a higher omega-3 (40%-50%) and omega-6 (30%-40%) contents and a lower oleic acid (10%) content than olive oil. The omega-6 fatty acid linoleic acid plays a role in the regeneration of the lipid barrier structure in the seed plant. Natural antioxidants, including tocopherols and polyphenol have been found in the oil. The present study, the seedling extracts showed stronger on free radical scavenging which the extract composition with the tocopherols, known as vitamin E is an important minor component in oil that may be antioxidative and slow down the process of oxidation[29]. Moreover, phenolic compounds, flavonoids were detected in seedling extracts that are good for the oil's oxidative stability of polyunsaturated fatty acids imparting the characteristic flavor to the oil. So many polyphenols have beneficial effects on various diseases including hypertension and atherosclerosis, play roles in the prevention of certain cancers and modification of immune responses. The high content of tocopherols could play a major antioxidant role in the oil, or in synergy with phenolic compounds, which might be also adding more nutritional value as antioxidants and are probably the main components responsible for oxidative stability[30].

The reviewed literature, some cereals such as Inca peanut were recommended compositions with phenolic substance and α-Tocopherol presence high antioxidant activity, most investigators are using a combination of antioxidant tests. Many investigators showed the importance of the use of antioxidant assays in assessment of bioactivity of natural products, which related mainly to the bioactivity of their phenolic compounds. It was shown that these natural products are active in prevention and treatment of various diseases including heart diseases and even of cancer[31]. While several species of *Plukenetia* have ornamental value, the genus is best known for the health promoting properties of *P. volubilis*, it is the consumption of Ω-3 and -6 fatty acids have been shown to prevent cardiovascular diseases, heart diseases, and cancer, and can have a hypocholesterolemic effect[31-32].

Recently issues, Non-communicated diseases (NCD) such as cancer, diabetes, arteriosclerosis, inflammatory disease, autoimmunity, cardiovascular disease, and Alzheimer's have been associated with the increase of reactive oxygen species (ROS) or the inability of the organism to reduce these ROS that was normally produced by the organism cells, a process known as oxidative stress. Antioxidants are important substances, which have the ability to protect the organism from the damage caused by oxidative stress. Due to this ability,

Table 1: Phytochemical screening methods including total phenolic (TPC) and flavonoid (TFC) contents of various solvent extracts from Inca peanut seedlings.

	TPC (mgGE/gExt)	TFC (mgQE/gExt)
Aqueos extract	1.5319 ± 0.0286a	284.8477 ± 7.8958a
Hydro-ethanolic extract	1.7144 ± 0.0346b	310.1624 ± 16.671b
Ethanolic extract	23.0809 ± 0.8632c	466.3839 ± 1.5580c

TPC was measured with gallic acid equivalents (mgGE/gExt). TFC was measured with quercetin equivalent (mgQE/gExt). Different letters indicated significantly difference at *p*-values less than 0.05.

Table 2: Antioxidant activities showed IC_{50} of various solvent extracts from Inca peanut seedlings.

	DPPH (IC_{50}=mg/mL)	ABTS (IC_{50}=mg/mL)	FRAP (mg=TE/gExt)
Aqueos extract	4.9150 ± 0.2669d	1.7215 ± 0.0756d	28.0588 ± 0.7758a
Hydro-ethanolic extract	4.4675 ± 0.2450c	1.5398 ± 0.0305c	34.6960 ± 1.3396b
Ethanolic extract	0.007 ± 0.001b	1.4065 ± 0.0505b	74.4960 ± 2.6067c
Ascorbic acid	0.016 ± 0.0003a	0.0250 ± 0.0010 a	-
Trolox®	0.044 ± 0.0008a	0.0320 ± 0.0010a	-

DPPH radical scavenging, $ABTS^+$ and FRAP assay were used Trolox® and ascorbic acid as standard substances. Different letters indicated significantly difference at *p*-values less than 0.05.

there is a special interest in the presence of natural antioxidants in medicinal plant that may help an organism to keep the normal balance of ROS[33]. Antioxidants could be use for the management of some pathophysiological conditions, which involve free radicals. Flavonoid and phenolic compounds may be useful as antioxidants from natural sources. The distribution of phenolic compounds varies between different parts of the plant[34]. Inca peanut seedlings enhanced the capacity of antioxidants, which increased the capacity of antioxidant enzymes and decreased the level of ROS[35]. Our data found that the Inca peanut seedling composed of flavonoids and phenolic compounds and antioxidant activities, it is might be prevented and improve some systemic diseases. The seedling extracts presence of flavonoids and phenolic compounds have shown that the antioxidant activities may be relate to compound ingredients that cloud be prevented the biological and chemical substances from radical induced oxidation damage. Because radical oxidation of substrates occurs through a chain reaction involving three stages (i.e., initiation, propagation, and termination), antioxidants show their effects through various mechanisms[36].

In any way, the seedling consumption, Heat processing should be apply before consumption Inca peanut in order to reduce phytotoxins and potential health risks. Roasting could be most effectively reducing these phytotoxins, and hence, thermal processing should be applied before the consumption of Inca peanut[31,37]. The approximately heating inducing on Inca peanut seeds (at 160ºC for 15 mins) might be increased total phenolic compound contents and improved antioxidant inhibitory activity has been reported for the plant seeds[38]. The results obtained in this study indicated that the seedling is an important source of antioxidant from phenolic compounds. It contained with condensed tannins (93.1%), the main family of phenolic compounds present in plants. The other phenolic families in seeds (hydrolyzed tannin, free and bound phenolic acids, flavanoids, flavonoids, and lignans) were present in amounts. Thus, our results indicated that the seedling has a high potential as an alternative and novel source of antioxidant phenolic compounds from an agro-industry by product that could be derive to the functional food industry[39]. It is of growing economic importance in the supplementary food, pharmaceutical, cosmetic, and horticultural industries[40].

In our finding, the phytochemical screening were detected flavonoids and phenolic compound contents of plant seedling extracts which their composition has more potent on antioxidant activity (DPPH, ABTS, and FRAP) properties. Thus, should be additional study on germination, survival, cultivation, and harvesting practice to obtained major or/and active components in the seedling.

CONCLUSION

In conclusion, the *P. volubilis* seedling extracts were carried out to evaluated phytochemical screening also the flavonoids and phenolic compound contents and the antioxidant activities (DPPH, ABTS, and FRAP) ability. The results demonstrated that the seedling extracts composed with high amount of flavonoid and phenolic compound contents possess valuable to antioxidant activities. The seedling extracts from the plant could be apply to supplementary food, cosmetic, pharmaceutical, and horticultural industries. Future study, chemical compositions, the major active compound(s), and biological activities will be clarified.

ACKNOWLEDGEMENT

The authors sincerely thank Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand for financial supported.

FINANCIAL SUPPORT AND SPONSORSHIP

The study was partially financially supported by Faculty of Medicine, Mahasarakham University, Maha Sarakham, Thailand.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare and are responsible for the content and writing of the manuscript.

ABBREVIATIONS

ASS: Aqueous extract; HESS: 50% Ethanolic extract; ESS: 95% Ethanolic extract; ABTS: 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; mgGE/gExt: Gallic acid equivalent; mgQE/gExt: Quercetin equivalent; TE/gExt: Trolox® equivalent; Trolox®: 2-carboxy-2,5,7,8-tetramethyl-6-chromanol; TFC: Total flavonoid content; TPC: Total phenolic compound content.

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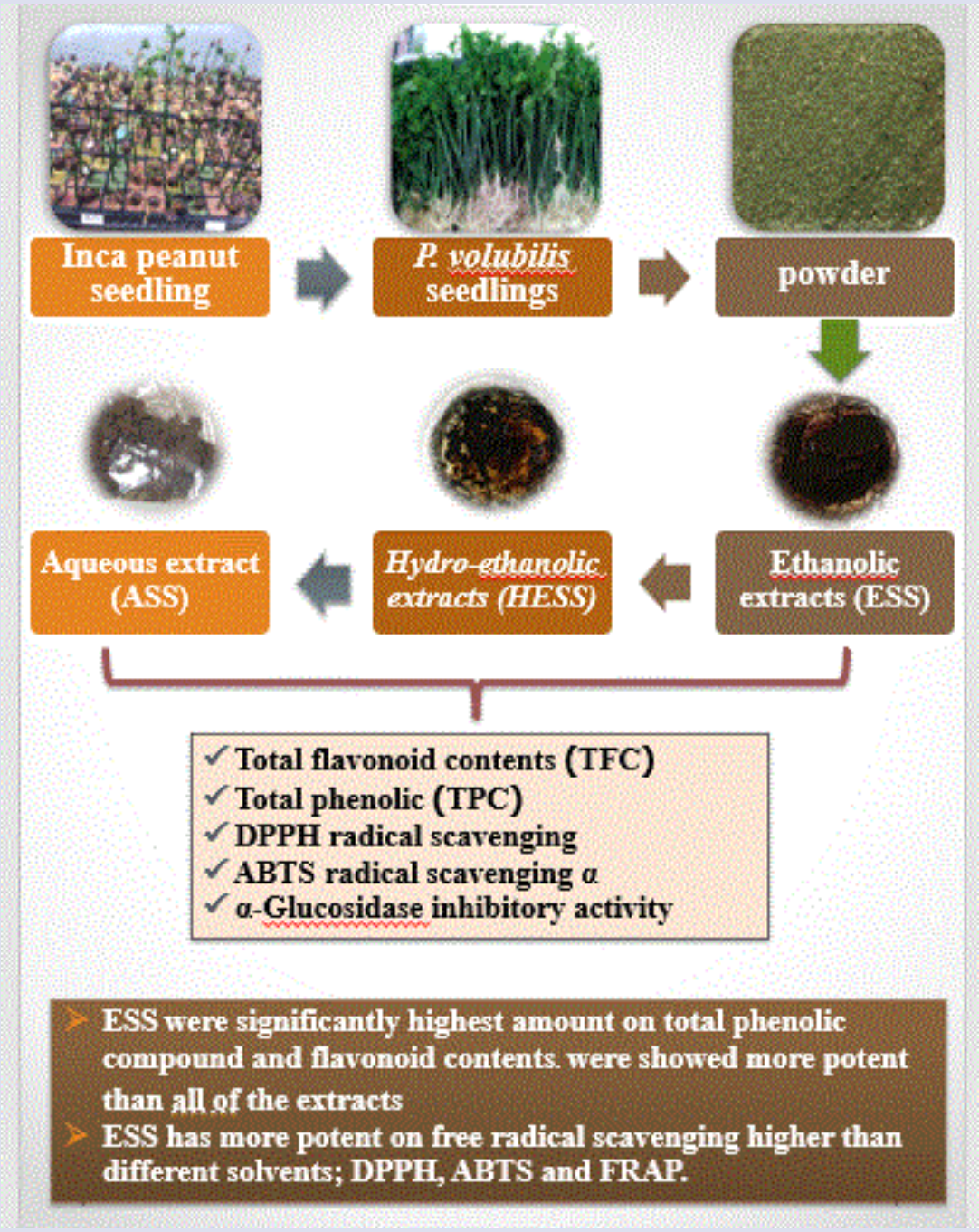
GRAPHICAL ABSTRACT



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Cite this article: Puangpronpitag D, Tankitjanon P, Sumalee A, Konsue A. Phytochemical Screening and Antioxidant Activities of the Seedling Extracts from Inca Peanut *Plukenetia volubilis*. Pharmacog J. 2021;13(1): 52-8.