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Comparison of active constituents, antioxidant capacity, and α-glucosidase inhibition in *Pluchea indica* leaf extracts at different maturity stages

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ABSTRACT

Pluchea indica (L.) Less. or Indian camphorweed leaves have been used as a traditional herbal medicine in addition to being consumed in various vegetable dishes throughout India, Southeast Asia, Australia, and America. The chemical composition and some biological activities have been studied using extracts of these leaves; however, the effects of the maturation stage and geographical origin have not been studied in depth. The concentrations of 6 active compounds, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaffeoylquinic acid, were determined in the leaf extracts from three different maturity stages of *P. indica* collected from three locations in Thailand using high-performance liquid chromatography. The total phenolic content and *in vitro* α-glucosidase inhibition of the leaf extracts were also studied in addition to the antioxidant properties, including the 1,1-diphenyl-2-picrylhydrazyl and 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt free radical scavenging capabilities and ferric reducing antioxidant power. Notably, the juvenile leaf shoot extracts contained significantly higher concentrations of the bioactive and phenolic compounds and showed stronger inhibition of α-glucosidase activity compared to every stage of the mature leaves. The antioxidant capacities of the juvenile leaves also surpassed those of the mature leaves. Taken together, these data suggest that juvenile *P. indica* leaves may be a more suitable choice for further development as a functional food and other nutraceutical products.

1. Introduction

Type 2 diabetes, which accounts for about 90–95% of all cases of diabetes, is a common ailment worldwide. Alpha-glucosidase inhibitors, such as acarbose and miglitol, are among the primary types of drugs used to control hyperglycaemia. However, the side effects of these drugs are sometimes severe and include abdominal distension, flatulence, and diarrhoea (He, Shi, & Mao, 2014). Oxidative stress is a known pathological feature of diabetes as well as many other diseases, such as coronary heart disease and cancer (Alam, Bristi, & Rafiquzzaman, 2013). Protection against oxidizing agents or free radicals can be boosted with adequate intake of dietary antioxidants. Indeed, epidemiological evidence has shown that consuming a diet containing antioxidants, namely antioxidant-rich fruits and vegetables, is significantly linked to disease avoidance (Boeing et al., 2012). Thus, the active compounds from such vegetables and medicinal plants are of great interest in the development of novel diabetes treatments (Jhong, Riyaphan, Lin, Chia, Weng, 2015).

Pluchea indica (L.) Less. belongs to the family Asteraceae (Compositae) and is found throughout tropical and subtropical regions such as Asia, Australia, and America. This plant is an evergreen perennial shrub that is often found in saline coastal areas (eFloras, 2008). The fresh leaves of *P. indica* are not only used in many kinds of foods, including soups, salads, and side dishes, but have also been used to alleviate dysuria, inflammation, and haemorrhoids (Suriyaphan, 2014). Moreover, the dried leaves and leaf extracts are popularly consumed as tea and food supplements in Thailand, largely for their anti-diabetic properties. Notably, the edible parts of *P. indica*, especially the leaves, have been reported to contain high amounts of calcium and β-carotene (Buapool et al., 2013; Suriyaphan, 2014). *P. indica* leaves are also a source of natural antioxidants as they contain large amounts of phenolic compounds (Andarwulan, Batari, Sandrasari, Bolling, & Wijaya, 2010; Nohd Shukri, Alan, & Site Noorzuraini, 2011). Pharmacologically, *P. indica* has been shown to have anti-inflammation, anti-ulcer, anti-alpha glucosidase, and antioxidant activity (Arsiningtyas, Gunawan-Puteri, Kato, & Kawabata, 2014; Buapool et al., 2013; Neamsuvan & Ruangrit, 2017; Srisook et al., 2012). Phenolic compounds in *P. indica* leaves, such as caffeoylquinic acid derivatives, are likely responsible for the

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plant's antioxidant and anti-alpha glucosidase activities (Arsiningtyas et al., 2014).

Phenolic components, including 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaffeoylquinic acid, were previously found in *P. indica* leaves obtained from different areas in Malaysia. However, the levels of these and other chemicals differed, possibly because of dissimilar environments or harvest seasons (Nohd Shukri et al., 2011). Evidently, the stage of maturity also influences the chemical composition of this and other plant species. For example, the β-cryptoxanthin and β-carotene levels in *Prunus persica* fruits were different between maturation stages (Dabbou et al., 2017). Similarly, maturation stage also affected the amounts and activity of various compounds in *Solanum tuberosum* and *Psidium guajava* (Arun et al., 2015; Nantitanon, Yotsawimonwat, & Okonogi, 2010). While the effects of maturation stage have been shown in multiple medicinal plant species, the variation in bioactive components in *P. indica* leaves at different stages of maturity is largely unknown.

In this study, the bioactive compounds of *P. indica* were quantitatively analysed. Further, the antioxidant capacity and α-glucosidase inhibitory activity were also investigated in leaf extracts from juvenile leaf shoots as well as mature leaves in the pre-flowering and flowering stage. To address the effects of the environment, the *P. indica* samples were obtained from different locations in Thailand.

2. Materials and methods

2.1. Plant materials

The *P. indica* leaf shoots, mature leaves in the pre-flowering stage and the mature leaves in the flowering stage were collected in December 2015 from three different locations. The samples from Chonburi province (Eastern Thailand) and Prachuap Khiri Khan province (Western Thailand) were grown in puddled clay, 500 m from the seashore, while the samples from Phetchaburi province (Western Thailand) were grown in a rice field, 5 km from the seashore. The specimens (BVPI151201 - BVPI151203) were deposited at the Faculty of Pharmaceutical Sciences, Burapha University, Chonburi, Thailand. The juvenile leaf shoots are defined as the first to second leaves from the apex of the branch with light green color. The mature leaves were the 7th to 12th leaves from the apex with intense green color in the pre-flowering and flowering plants. All leaf samples were cleaned with tap water and dried in a hot air-oven at 60 °C for 12 h. The dried samples were ground using a Cross beater mill (Retsch, Haan, Germany) and passed through a 20 mesh sieve. The powdered leaves were kept in sealed containers protected from light until use within 2 wk.

2.2. Plant extract preparation

The dried powders of the juvenile and mature leaves were separately decocted with distilled water (1:20, w/v) for 15 min at 80 ± 5 °C. The extract solution was then filtered through filter paper no. 1 (Whatman, Loughborough, Leicestershire, UK) and the residue was re-extracted as previously. The resulting filtrate was concentrated under reduced pressure at 50 °C using a rotary vacuum evaporator (model v-750, Buchi, St. Gallen, Switzerland). The extraction of each sample was done in triplicate and the yield of crude ethanolic extract is reported as the mean ± SD.

2.3. Determination of phenolic content

The total phenolic content of the various *P. indica* extractions was determined using the Folin-Ciocalteu procedure (Vongsak, Kongkiatpaiboon, Jaisamut, Machana, & Pattarapanich, 2015). Briefly, 200 µl of each sample was mixed thoroughly with 500 µl Folin–Ciocalteu reagent (Merck, Darmstadt, Germany) and 800 µl 7.5% (w/v) sodium bicarbonate solution. The mixture was allowed to stand for a further 30 min in the dark, and absorbance was measured at 765 nm (M965, Metertech, Taipei, Taiwan). The same process was repeated for the 6 concentrations of gallic acid (Merck, purity > 98%) and was expressed as the mean ± SD ($n = 3$) of gallic acid equivalents (GAE) in 100 g of the extract.

2.4. High performance liquid chromatography (HPLC)

The amount of bioactive compounds was measured using a validated HPLC method (Kongkiatpaiboon, Chewchinda, & Vongsak, 2018). Standard solutions of 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid, and 4,5-*O*-dicaffeoylquinic acid were prepared at a concentration of 1 mg ml^{-1} in methanol. These standard compounds, (purity > 98%) were purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, Sichuan, China). Each standard solution was diluted into 6 concentrations (150, 75, 37.5, 18.75, 9.38, and 4.69 µg ml^{-1}) to obtain a calibration curve. Quantitative analysis of the bioactive compounds was done using the validated HPLC method on an Agilent 1260 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with a 1260 ALS autosampler, 1260 TCC column thermostat, 1260 Quat pump VL quaternary pump, and 1260 diode array detector (DAD). A Hypersil® BDS C_{18} column (100 × 4.6 mm i.d., 3.5 µm) with a C_{18} guard column (Thermo Scientific, Loughborogh, UK) was also used. The elution was carried out using solvent A (0.5% acetic acid in water) and solvent B (methanol) as a mobile phase with the following gradient: 10% B to 50% B for 40 min, followed by 100% B for 10 min. The flow rate was set at 1.0 ml min^{-1} with a controlled temperature of 25 °C. Each extract was dissolved in methanol (1 mg ml^{-1}) and filtered through a 0.2 mm membrane filter (Membrane Solutions, Plano, TX, USA). The DAD detector was monitored at 326 nm and the injection volume for all samples and standards was 5 µl. Only compounds for which standards were obtained were quantitated.

2.5. Evaluation of biological activity

2.5.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) scavenging assay

The free radical scavenging activity of each extract and standard solution was measured using the DPPH and ABTS radical scavenging methods described by Vongsak, Kongkiatpaiboon et al. (2015). In brief, for the DPPH assay, 100 µl of methanolic DPPH solution (Sigma Aldrich, St. Louis, MO, USA) (152 µM) was added to 100 µl of the standard/extracts. After incubation in the dark for 20 min, the absorbance was measured at 517 nm. For ABTS radical cation decolorization assay, the solution of 14 mM ABTS and 4.9 mM potassium persulfate (Sigma Aldrich) were mixed together and after that 1 ml of the solution was diluted with 40 ml ethanol to yield a working solution (absorbance of 0.70 ± 0.02 at 734 nm). Test samples: 100 µl were mixed with 100 µl ABTS working solution for 6 min. Ascorbic acid (Sigma Aldrich, purity > 98%) was used as the positive control. The absorbance was measured at 734 nm. Each determination was done in triplicate, and the average IC_{50} value was calculated as follow.

$$\text{Percent inhibition} = ((I_1 - I_2)/I_1) \times 100$$

Where I_1 was the control absorbance, and I_2 was the sample absorbance. Each evaluation was done in triplicate. The IC_{50} value was calculated as the concentration of the sample required to inhibit 50%.

2.5.2. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of the *P. indica* extracts was determined using the method of Vongsak, Kongkiatpaiboon et al. (2015). In brief, 150 µl of working FRAP solution (300 mM acetate buffer pH 3.6:10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ):20 mM $FeCl_3$ (10:1:1)) was added to 50 µl of each sample and incubated at 37 °C for 8 min. $FeSO_4$ was used as the reference standard. Measurements were made at 600 nm.

The Fe^{2+} content is expressed as the mean ± SD ($n = 3$) of $FeSO_4$ equivalents in 100 g of the extract.

2.5.3. α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity was determined using *p*-nitrophenyl *β*-D-glucopyranoside (pNPG) using the method of Vongsak, Kongkiatpaiboon et al. (2015). Briefly, 50 µl of the standard solutions, samples, and acarbose (Sigma Aldrich, purity ≥95%) at varying concentrations were mixed with 0.1 M sodium phosphate buffer (50 µl, pH 7.0) and α-glucosidase (Sigma Aldrich) (50 µl, 2 unit ml^{-1}) and pre-incubated at 37 °C for 10 min. Then, pNPG (50 µl, 20 mM) was added to start the reaction. After incubation at 37 °C for 30 min, the absorbance was measured at 405 nm. Each determination was done in triplicate, and the average IC_{50} value was calculated as follow.

$$\text{Percent inhibition} = ((I_1 - I_2)/I_1) \times 100$$

Where I_1 was the control absorbance, and I_2 was the sample absorbance. Each evaluation was done in triplicate. The IC_{50} value was calculated as the concentration of the sample required to inhibit 50%.

2.6. Statistical analysis

All results are shown as the means ± SD of three replicated determinations. The statistical differences among treatments were determined using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test and calculation of the Pearson correlation coefficient. All statistical analyses were done using SPSS for Windows 22.0 (IBM Corp., Armonk, NY, USA). A statistical probability of $p < 0.05$ indicated a statistically significant difference between groups. For Pearson correlation coefficients $p < 0.01$ was also determined.

3. Results and discussion

3.1. Total phenolic content

Dried *P. indica* leaves were extracted with hot water to mirror how it is typically consumed as a health-promoting drink prepared by boiling with hot water (Srisook et al., 2012; Suriyaphan, 2014). Crude extract yields from the young and mature *P. indica* leaves as well as their total phenolic content and biological activity are shown in Tables 1 and 2, and Fig. 1. The juvenile leaf shoots from all locations had higher extract yields and higher phenolic content than the mature leaves at all stages. Interestingly, the total phenolic content in the leaf shoot extracts from different areas were all higher than those from the mature leaves. These results indicated that leaf maturity stage likely influences the phenolic content in *P. indica*, an observation that is supported by a previous report investigating a similar phenomenon in guava leaf extract (Nantitanon et al., 2010). Furthermore, these data also support a former study on the variation of leaf phenolic compounds, in which high concentrations were reported during the initial growth stage (Bai et al., 2017).

Table 2
The antioxidant properties of juvenile leaf shoots (1), mature leaves in the pre-flowering stage (2), and mature leaves in the flowering stage (3) of *P. indica* from different locations in Thailand using DPPH, ABTS, and FRAP assays, α-glucosidase inhibition.

Stages of maturity in different provinces/ Standard compounds	DPPH assay IC_{50} (µg.ml^{-1})*	ABTS assay IC_{50} (µg.ml^{-1})*	FRAP Assay (g $FeSO_4$ equivalent. 100 g^{-1} extract)*	α-glucosidase inhibition assay IC_{50} (µg.ml^{-1})*
1	17.8 ± 0.3 ^{a}	9.1 ± 1.7 ^{a}	4.0 ± 0.4 ^{a}	51 ± 4 ^{a}
2	21.7 ± 0.2 ^{b}	17 ± 4 ^{b}	2.3 ± 0.3 ^{b}	99 ± 5 ^{b}
3	23 ± 2 ^{b}	17 ± 1 ^{b}	2.1 ± 0.2 ^{b}	104 ± 1 ^{b}
From Chonburi				
1	16.1 ± 0.3 ^{a}	6.4 ± 2.3 ^{a}	3.8 ± 0.3 ^{a}	25 ± 4 ^{a}
2	21 ± 1 ^{b}	13 ± 3 ^{b}	2.1 ± 0.5 ^{b}	61 ± 1 ^{b}
3	22 ± 1 ^{b}	15 ± 2 ^{b}	2.8 ± 0.3 ^{b}	104 ± 2 ^{c}
From Phetchaburi				
1	21 ± 1 ^{a}	6.2 ± 0.6 ^{a}	3.6 ± 0.8 ^{a}	42 ± 2 ^{a}
2	28 ± 1 ^{b}	15 ± 2 ^{b}	1.8 ± 0.1 ^{b}	82 ± 8 ^{b}
3	25.4 ± 0.4 ^{c}	15 ± 1 ^{b}	2.4 ± 0.1 ^{c}	79 ± 6 ^{b}
From Prachuap Khiri Khan				
3 CQ	9.2 ± 0.2 ^{a}	10.0 ± 0.3 ^{a}	270 ± 20 ^{a}	11.5 ± 0.2 ^{a}
4 CQ	8.7 ± 0.2 ^{b}	11 ± 1 ^{b}	190 ± 20 ^{b}	11.3 ± 0.1 ^{a}
5 CQ	5.0 ± 0.2 ^{c}	9.2 ± 0.8 ^{a}	230 ± 30 ^{b}	7.1 ± 0.1 ^{b}
3,4 diCQ	9.4 ± 0.3 ^{a}	4.7 ± 0.2 ^{c}	310 ± 30 ^{c}	6.6 ± 0.2 ^{c}
3,5 diCQ	7.9 ± 0.4 ^{d}	4.4 ± 0.3 ^{c}	250 ± 10 ^{ab}	10.9 ± 0.1 ^{d}
4,5 diCQ	9.1 ± 0.3 ^{ab}	4.1 ± 0.7 ^{c}	310 ± 30 ^{ac}	7.7 ± 0.1 ^{e}
Ascorbic acid	8.8 ± 0.1 ^{b}	7.3 ± 0.3 ^{d}	500 ± 50 ^{d}	-
Acarbose	-	-	-	170 ± 20 ^{f}

* Shown as mean ± SD ($n = 3$). CQ = *O*-caffeoylquinic acid. Different letters for the same measurement from the same location (leaf shoots and mature leaves) and standard compounds indicate $p < 0.05$ using one-way ANOVA.

3.2. Principal bioactive components

Caffeoylquinic acid (CQ) derivatives were previously reported as the major constituents in *P. indica* leaves (Nohd Shukri et al., 2011). The amounts of the CQ derivatives, 3-*O*-caffeoylquinic acid (3-CQ), 4-*O*-caffeoylquinic acid (4-CQ), 5-*O*-caffeoylquinic acid (5-CQ), 3,4-*O*-

Table 1
Yields of crude extracts, total phenolics, and bioactive components in juvenile leaf shoots (1), mature leaves in the pre-flowering stage (2), and mature leaves in the flowering stage (3) of *P. indica* from different locations in Thailand.

Stages of maturity in different provinces	Percent yield*	Total phenolics (g GAE.100g^{-1} extract)*	Percentage of caffeoylquinic acid derivatives in aqueous extracts (% w/w)*					
			3 CQ	**4 CQ**	**5 CQ**	**3,4 diCQ**	**3,5 diCQ**	**4,5 diCQ**
1	45 ± 2 ^{a}	7.4 ± 0.2 ^{a}	3.4 ± 0.1 ^{a}	1.7 ± 0.1 ^{a}	0.7 ± 0.0 ^{a}	1.9 ± 0.1 ^{a}	6.3 ± 0.7 ^{a}	19 ± 1 ^{a}
2	42 ± 3 ^{a}	6.1 ± 0.1 ^{b}	1.0 ± 0.1 ^{b}	0.5 ± 0.0 ^{b}	0.2 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	1.0 ± 0.1 ^{b}	4.4 ± 0.3 ^{b}
3	38 ± 2 ^{ab}	5.8 ± 0.2 ^{b}	2.2 ± 0.1 ^{c}	0.9 ± 0.0 ^{c}	0.3 ± 0.0 ^{c}	0.8 ± 0.0 ^{c}	1.9 ± 0.1 ^{c}	7.8 ± 0.4 ^{c}
from Chonburi								
1	47 ± 4 ^{a}	8.1 ± 0.7 ^{a}	7.3 ± 0.5 ^{a}	3.1 ± 0.2 ^{a}	1.6 ± 0.0 ^{a}	2.1 ± 0.1 ^{a}	11.7 ± 0.0 ^{a}	28 ± 1 ^{a}
2	42 ± 2 ^{b}	5.7 ± 1.0 ^{b}	2.9 ± 0.4 ^{b}	0.8 ± 0.2 ^{b}	0.3 ± 0.1 ^{b}	0.8 ± 0.1 ^{b}	2.9 ± 0.3 ^{b}	8.0 ± 0.6 ^{b}
3	39 ± 2 ^{b}	6.3 ± 0.2 ^{b}	3.1 ± 0.0 ^{b}	0.9 ± 0.0 ^{b}	0.3 ± 0.0 ^{b}	0.8 ± 0.0 ^{b}	2.6 ± 0.0 ^{c}	9.4 ± 0.2 ^{c}
from Phetchaburi								
1	45 ± 3 ^{a}	8.0 ± 0.9 ^{a}	3.8 ± 0.3 ^{a}	2.0 ± 0.1 ^{a}	0.9 ± 0.1 ^{a}	1.9 ± 0.1 ^{a}	6.2 ± 0.9 ^{a}	22 ± 1 ^{a}
2	40 ± 2 ^{b}	5.1 ± 0.2 ^{b}	1.4 ± 0.3 ^{b}	0.7 ± 0.2 ^{b}	0.2 ± 0.1 ^{b}	0.6 ± 0.1 ^{b}	1.6 ± 0.3 ^{b}	5.6 ± 1.1 ^{b}
3	35 ± 2 ^{c}	6.2 ± 0.7 ^{c}	1.2 ± 0.2 ^{b}	0.6 ± 0.1 ^{b}	0.2 ± 0.0 ^{b}	0.5 ± 0.0 ^{b}	1.5 ± 0.1 ^{b}	4.9 ± 0.4 ^{b}
from Prachuap Khiri Khan								

* Shown as mean ± SD (n = 3), GAE = gallic acid equivalents, CQ = *O*-caffeoylquinic acid. Different letters for the same measurement from the same location (leaf shoots, mature leaves in the pre-flowering and the flowering stage) indicated $p < 0.05$.

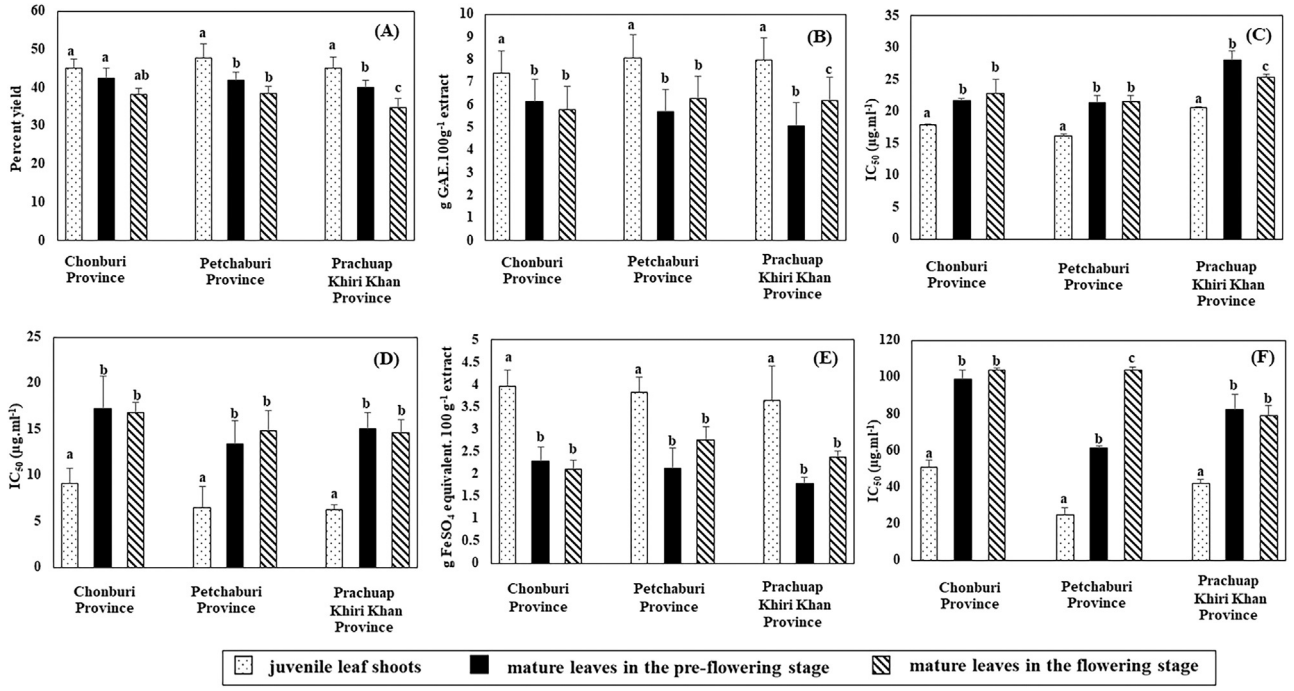

Fig. 1. Yields of crude extracts (a), total phenolics (b), antioxidant properties using DPPH (c), ABTS (d), FRAP (e) assays, and α-glucosidase inhibitory activity (f) of juvenile leaf shoots, mature leaves in the pre-flowering stage, and mature leaves in the flowering stage of *P. indica* from different locations in Thailand. ($p < 0.05$, Mean ± SD, n = 3).

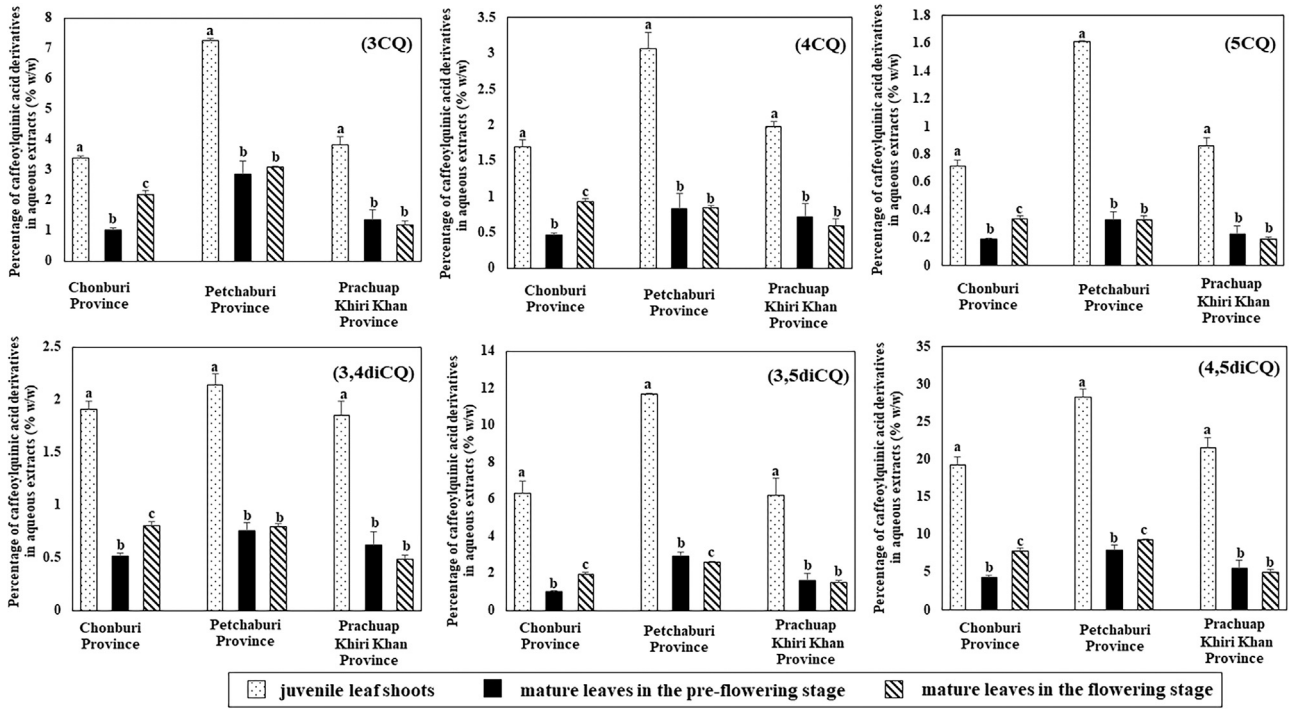

Fig. 2. Bioactive contents in juvenile leaf shoots, mature leaves in the pre-flowering stage, and mature leaves in the flowering stage of *P. indica* from different locations in Thailand. Different letters for the same measurement from the same location indicated $p < 0.05$. (Mean ± SD, n = 3, CQ = *O*-caffeoylquinic acid).

dicaffeoylquinic acid (3,4-diCQ), 3,5-*O*-dicaffeoylquinic acid (3,5-diCQ), and 4,5-*O*-dicaffeoylquinic acid (4,5-diCQ) are shown in Table 1 and Fig. 2. In the juvenile leaf shoot, the extracts varied between locations. Notably, all of these levels are significantly higher than those found for any mature leaves stage (Table 1 and Fig. 2), and higher than the samples collected in the dry season in Malaysia (Nohd Shukri et al., 2011). Phenolics, particularly CQ, are known for their health benefits and their roles as anti-oxidant, anti-diabetes, and anti-cancer agents (Arsiningtyas, et al., 2014; Palmioli et al., 2017; Spínola & Castilho, 2017; Vongsak, Mangmool, & Gritsanapan, 2015). The juvenile leaf shoots of *P. indica* could, therefore, be used as a good source of herbal dietary supplements and functional foods.

Table 3
Pearson's correlation between biological activities and chemical constituents.

Parameters	Compounds					
	3 CQ	4 CQ	5 CQ	3,4 diCQ	3,5 diCQ	4,5 diCQ
DPPH	–0.806**	–0.767*	–0.780*	–0.803**	–0.805**	–0.808**
ABTS	–0.808**	–0.903**	–0.867**	–0.939**	–0.898**	–0.944**
FRAP	0.752*	0.836**	0.808**	0.935**	0.849**	0.912**
α- glucosidase	–0.765*	–0.851**	–0.834**	–0.843**	–0.871**	–0.856**

* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

3.3. Antioxidant capacity and α-glucosidase inhibitory activity

The antioxidant properties of the *P. indica* leaf extracts were determined on the basis of their scavenging activity of DPPH and ABTS free radicals as well as their ferric reducing antioxidant power (Table 2). The results showed that the juvenile leaf shoot extracts had greater DPPH free radical scavenging activity compared to both mature leaf stages in both the pre-flowering and flowering stages. The strong free radical scavenging activity was also found in Indonesia where *P. indica* extracts were higher than those of other indigenous vegetables (Andarwulan et al., 2010). Moreover, the CQ derivatives, the active compounds, had IC_{50} values comparable to the range of 5.0–9.4 μg ml^{-1} which made them comparable to ascorbic acid, the positive control. For ABTS radical, the juvenile and mature leaf extracts were both able to scavenge the radical, but the young leaf extracts showed higher activity compared to both types of mature leaf extracts.

The ferric reducing antioxidant power was also studied as this is the reducing property that leads to the antioxidant activity of the extracts and involves the reduction of ferric to ferrous ion (Sudan, Bhagat, Gupta, Singh, & Koul, 2014). The juvenile leaf extracts had reducing activity in the range of 3.6–4.0 g $FeSO_4$ equivalent. 100 g^{-1} extract, that were lower than the CQ derivatives but were higher than the mature leaf extracts. Thus, it seemed that the juvenile leaf shoots, regardless of origin, had the highest antioxidant potential. Furthermore, the activity of the crude extracts from *P. indica* leaves cultivated in Thailand were also comparable to the antioxidant activity of plants originating from Indonesia and Malaysia (Andarwulan et al., 2010; Nohd Shukri et al., 2011).

Finally, the α-glucosidase inhibitory activity was evaluated. Inhibition of this enzyme is a well-known approach to counteract the metabolic alterations related to type 2 diabetes (Jhong et al., 2015). The results indicated that the juvenile leaf shoots are active and were more efficient at inhibiting α-glucosidase activity, IC_{50} = 25–51 μg ml^{-1}, compared to the other stages of maturity, IC_{50} = 61–104 μg ml^{-1}, and a positive control, acarbose, IC_{50} = 170 μg ml^{-1}. Arsiningtyas et al. (2014) previously reported that CQ derivatives are very strong α- glucosidase inhibitors. Thus, it is not altogether surprising that the juvenile leaf extracts, which contain a high concentration of these compounds, had higher activity than the mature leaves.

3.4. Correlation between bioactive content and biological activity

The Pearson correlation coefficients (r) for the experiments are show in Table 3. The most significant correlations ($p < 0.01$) were observed between 3,4-diCQ, 3,5-diCQ, and 4,5- diCQ levels and biological activity. Significant correlations ($p < 0.01$, $p < 0.05$) were also obtained between 3-CQ, 4-CQ, and 5-CQ and biological activity. These strong correlations further support the likelihood that CQ derivatives are the bioactive compounds responsible for the antioxidant and anti-alpha glucosidase activities of *P. indica* leaf extracts (Andarwulan et al., 2010; Nohd Shukri et al., 2011).

4. Conclusions

The antioxidant potential of *P. indica* leaves from multiple maturity stages and geographical location was analysed, focusing on variation in total phenolic content, amount of bioactive constituents, antioxidant properties, and α-glucosidase inhibition. These parameters collectively showed that the juvenile leaf shoots had stronger activities and higher amount of bioactive components compared to the mature leaves, while different habitats had a slight effect on chemical constituents and bioactivities. Thus, *P. indica* juvenile leaves would likely provide greater health benefits than mature leaves and should be considered the better choice for consumption and starting material for nutraceutical products.

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Conflict of interest

The authors declare that they have no conflict of interest.

Human and animal rights

This article does not contain any studies with human or animal subjects.

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