Proximate Compositions, Phenolic Compounds, Antioxidant Capacity and Antibacterial Activity of Chulta (*Dillenia indica* Linn.) Fruits: Effects of Maturity Stage and Extraction Solvent**

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Abstract

Proximate compositions, phenolic compounds, antioxidant capacity and antibacterial activity were determined for chulta *(Dillenia indica)* fruits and the effects of maturity stage and extraction solvent were also investigated. Results revealed that fruit size and color values (a*, b*, C* and h°) of immature and mature chulta fruits were significantly different. Immature fruits contained higher ash content but lower fat content compared to mature fruits. Immature chulta fruits also had higher total phenolic content in both water and ethanol extracts than mature fruits. Using HPLC-DAD/ESI-MS, 8 phenolic compounds (syringic acid *O*-hexoside, syringic acid, vanillic acid hexoside, (+)-catechin or (-)-epicatechin, procyanidin trimer type B, isoramnethin-3-*O*-hexoside, kaempferol-3,7-*O*-hexoside or luteolin-3,7-*O*-hexoside and luteolin-7-*O*-glucuronide) were tentatively identified in water extracts of chulta fruit, whereas only 3 phenolics (isoramnethin-3-*O*-hexoside, kaempferol-3,7-*O*-hexoside or luteolin-3,7-*O*-hexoside and luteolin-7-*O*-glucuronide) were found in ethanol extract. The results also showed that immature chulta fruits exhibited higher DPPH scavenging capacity and antibacterial potential against *Escherichia coli* and *Staphylococcus aureus* when compared to mature fruits. Finally, the results from this study could be used to encourage the utilization of chulta fruits as functional ingredients in healthy food or supplement production.

Keywords : Chulta Fruit, Phenolic Compound, Antioxidant Capacity, Antibacterial Activity

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Introduction

Chulta *(Dillenia indica)* is a herbal plant that originated in tropical and evergreen forests (Migliato et al., 2011). This plant is distributed in many countries in South and South East Asia including Malaysia, Vietnam, Indonesia, India, Bangladesh, Sri Lanka, China (Yunnan) and Thailand (Kviecinskia et al., 2016). Chulta has been traditionally used as medicine in Asian countries including Thailand (Kaewsorn et al., 2013; Kviecinskia et al., 2016). For example, decoction of leaves and bark has been used to relieve fever, diarrhea and malaria (Hazarika et al., 2016). Chulta leaves and barks possess anti-inflammatory and anti-diabetic properties which might be due to its phytochemicals (Talukdar et al., 2012). Moreover, unripe chulta fruits have been used for relieving abdominal pain and fever. The unripe fruits have shown a tonic laxative property, while ripe fruits have been used to treat phlegm (Khongsai et al., 2011). Additionally, chulta fruits have been applied as ingredients for cooking sour soup and curry soup since the fruits have sweet-sour and astringent tastes.

In Thailand, chulta is planted in the central, western and southern regions. Chulta fruit has a globose shape. The fruits are green when young and become yellow when they ripe (Sharma & Nath, 2014). Generally, chulta fruits grow in July-August and ripen in November- December. There is evidence that excess amounts of chulta fruits become waste in their season. To add value, food processing of chulta fruit should be encouraged. However, there is limited knowledge about chulta cultivated in Thailand. This study, for the first time, was to characterize the chemical composition (proximate compositions and phenolic compounds), antioxidant capacity and antibacterial activity of Thai chulta fruits. Regarding bioactive compounds and bioactivity of plants, maturity stage and solvent type for extraction have been reported as critical factors (Ye et al., 2015). Thus, the effects of the two factors were also investigated in this study. Finally, the information from this study will stimulate utilization of chulta fruits, especially production of health foods and supplements.

Objectives

The objective of this study was to investigate physical characteristics, proximate compositions, phenolic compounds, antioxidant capacity and antibacterial activity of chulta fruits cultivated in Thailand. Furthermore, the effects of maturity stage and extraction solvent were also studied.

Materials and Methods

1. Plant materials

Chulta fruits were manually collected from an orchard in Pathum Thani Province on October 2016. The chulta fruits were divided into two stages (immature and mature) by considering the days after full bloom (DAFB) and fruit size. The immature fruits were harvested at 30 DAFB and their diameter range was 5-7 cm, whereas mature fruits were harvested at 45 DAFB and their diameters were ranged from 8-10 cm.

2. Chemicals

The compounds, 2,2-diphenyl-1-picrylhydrazyl (DPPH, 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), Folin-Ciocalteau's phenol reagent and gallic acid were purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade of ethanol (≥95%) acetronitrile (≥99.9%) used in HPLC analysis was purchased from Merck (Germany). All other chemicals were analytical grade and purchased from Merck (Germany). The water used in this study was distilled water or deionized water.

3. Chulta fruit preparation

The fruits were washed with clean water and air-dried after removing their peduncles. Physical characteristic including size (diameter and height) and color (L*, a*, b*, C* and h° values) of fruits were determined before removing seeds. The pulp of chulta fruits was cut into small pieces, blanched for 2 min and then dried in a hot air oven at 50 °C until the moisture content was ≤ 10% (approximately 8 hours). The dried sample was ground and sieved with 80 mesh sieve (Endecotts, England). The sample powder was packed in aluminum foil and stored at -20 °C until use.

4. Effect of maturity stage on physical characteristic and proximate compositions

In this study, the effect of maturity stage (immature and mature) on physical characteristic (diameter, height and color) and proximate compositions of chulta fruits was investigated.

4.1 Physical characteristics

4.1.1 Diameter and height measurement

Ten chulta fruits of each maturity stage (immature or mature) were selected for measuring their size in term of diameter and height. Each fruit was measured by its diameter and height using a vernier caliper (Mitutoyo, Japan). Fruit diameter was horizontally measured through the center of fruit. Fruit height was vertically measured from peduncle end to bottom end.

4.1.2 Color measurement

Ten chulta fruits of each stage (immature or mature) were measured for their skin color (external color) using Hunter Lab Mini-Scan XE (Hunter Lab, USA). The values of L*, a*, b*, C*, (Chroma) and h° (hue angle) were measured at 6 different positions on each fruit at two equatorial position, 180˚ apart.

4.2 Proximate analysis

Chulta fruit powder of each maturity stage (immature and mature) was used to determine moisture content, lipid, protein, crude fiber, ash and carbohydrate following the AOAC method (2000). The analysis was done in duplication.

5. Effect of maturity stage and extraction solvent on phenolic compounds, antioxidant capacity and antibacterial activity

In this study, the effects of maturity stage and extraction solvent on phenolic compounds, antioxidant capacity and antibacterial activity were investigated in chulta fruits.

5.1 Extraction

Sample extraction was modified from Ye et al. (2015). Briefly, one gram of sample powder was extracted with 10 mL of solvent (water or ethanol) using a sonication-assisted extraction technique for 25 min at 4 ˚C. After centrifugation at 9,000 rpm for 15 min at 4 ˚C, the supernatant was transferred into a volumetric flask (25 mL).

The residue was re-extracted with another 15 mL of solvent. After that, the second supernatant was combined with the first supernatant and then the total volume was adjusted to 25 mL. The extract was stored at -20 °C until use.

5.2 Total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu's phenol reagent. The method was modified from Ztotek et al. (2016). Briefly, 0.4 mL of extract was mixed with 2 mL of 10% (v/v) Folin-Ciocalteu's phenol reagent. After incubation for 4 min, 1.6 mL of 5% (w/v) $NaCO_3$ was added. The mixture was, then, allowed to stand for 30 min in darkness. The absorbance was measured at 765 nm using a spectrophotometer. Gallic acid (0-100 mg/L) was used as a standard. Results were expressed as mg gallic acid equivalent/g dry weight (mg GAE/mg DW). The experiment was done in duplication.

5.3 Identification of phenolic compounds

Phenolic compounds in chulta fruits were identified using HPLC-DAD/ESI-MS (Agilent Technologie model 1100 Series system, HP, USA) as described in Spinola et al. (2015) with some modification. The HPLC system was connected with a C-18 column (5 µm, 3.9 mm x 150 mm, Symmetry®, Waters, USA). The mobile phase used was 1% formic acid in deionized water (A) and 1% formic acid in acetonitrile (B). The solvent gradient was performed as follows: 0-5 min with 8-11% B; 5-10 min with 11-13% B; 10-20 min with 13-18% B; 20-25 min with 18-21% B; 25-30 min with 21-24% B; 30-40 min with 24-30% B; 40-50 min with 30-8% B; 50-60 min with 8% B. Flow rate of mobile phease and injection volume were 1.0 mL/min and 20 µL, respectively. The column was maintained at 35 °C during the analysis. Detection was accomplished with a diode array detector and chromatograms were recorded at 350 nm. Mass spectral analysis was done in both negative and positive modes. Full scan mass ranged from 100 m/z to 1000 m/z with speed of 13,000 Da/s. The nebulizer pressure was 50 psi, and the flow rate of nitrogen gas was 12 L/min. The ionization condition was set up at 350 °C and 3.5 keV for capillary temperature and voltage, respectively. Individual phenolic compounds in chulta fruits were identified by comparisons of the maximum wavelength and mass spectra with references.

5.4 Antioxidant capacity by DPPH radical scavenging assay

The method of DPPH radical scavenging capacity was modified from Alvarez-Suarez et al. (2010). Briefly, 2 mL of extract was mixed with 2 mL of DPPH solution (200 µM in 50% (v/v) ethanol). After incubation for 30 min in darkness, the absorbance was measured at 515 nm using spectrophotometer. Trolox (0-70 µmol) was used as standard. Results were expressed as mg Trolox equivalent/g dry weight (µmol TE/g DW). The experiment was done in duplication.

5.5 Antibacterial activity by agar well diffusion method

The chulta extract (250 mL) was filtrated through 0.45 µm nylon filter and evaporated using rotary evaporator (BUCHI, Switzerland) under a temperature range of 35-40 °C. After that, dried extract was re-dissolved with sterilized deionized water and the volume was adjusted to 3 mL. The obtained extract was diluted to 2,000,000, 1,000,000, 800,000, 600,000, 400,000 and 200,000 mg/L and then stored in -20 °C until use.

Antibacterial activity of chulta fruit extract against *Escherichia coli* and *Staphylococcus aureus* was investigated using the agar well diffusion method which was modified from Genskowsky et al. (2015). Briefly, cell suspensions were prepared to a final concentration of 10^8 CFU/mL. *Escherichia coli or Staphylococcus aureus* was swabbed on the surface of trypticase soy agar (TSA) using a sterilized cotton bud. When the surface of the TSA was dry, six wells with diameters of 6 mm were punched using a cork borer and then 100 mL of each concentration of extract was transferred into each well. Sterilized distilled water was used as the control. The plate was allowed to stand at room temperature for 5 hours and then incubated at 37 °C for 24 hrs. The zone of inhibition or clear zone was observed and expressed as millimeters (mm).

6. Statistical analysis

The experimental design of this study was completely randomized design (CRD) or factorial in CRD. Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed by using t-test or analysis of variance (ANOVA) followed by Ducan's new multiple range test which statistical difference was considered significant at $p < 0.05$.

Results and Discussion

1. Effect of maturity stage on physical characteristic and proximate compositions of chulta fruits

1.1 Physical characteristic of chulta fruits

Physical characteristic (diameter, height and color) of immature and mature chulta fruits have been shown in Table 1 and Fig. 1. Significant differences of diameter, height and color values of a*, b*, C* and h° were observed between immature and mature chulta fruits. Result showed that diameter and height of mature fruits were greater than immature fruits. For fruit color, a* values increased as the maturity increased, while b* values of chulta fruit decreased. The value of C* (color intensity) and h° also decreased as maturity increased. The results indicated that color appearance of immature fruits was lighter than mature chulta fruits, or the color of immature fruits was more green-yellow than mature fruits. However, L* values of immature and mature stage were not significantly different.

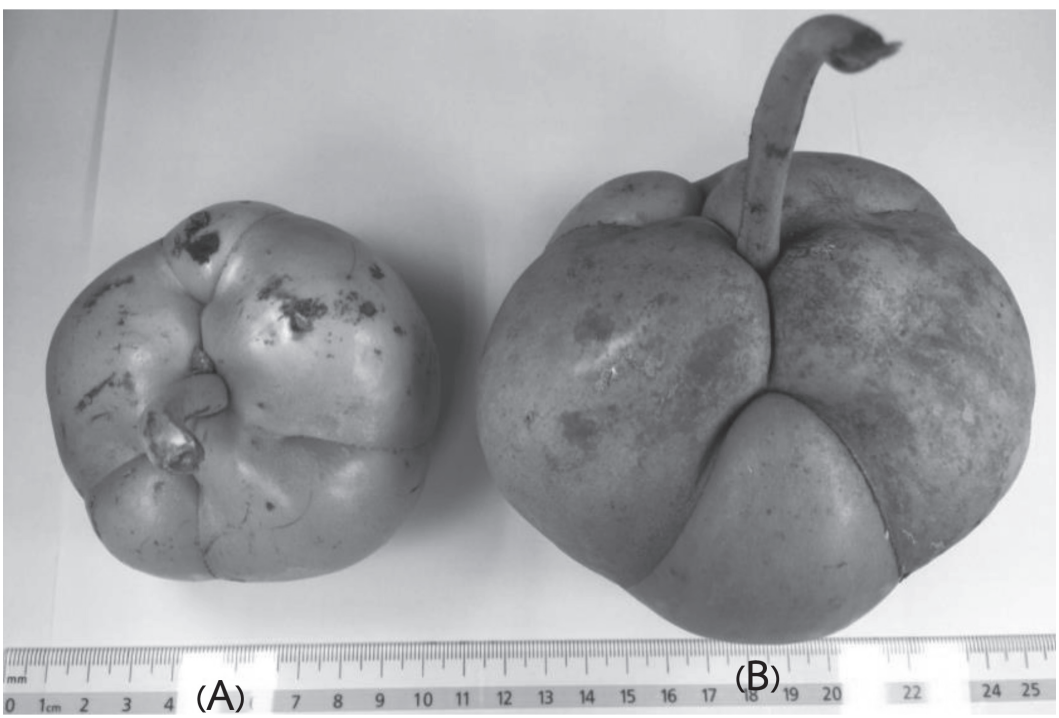

Fig. 1 Physical characteristic of immature (A) and mature (B) chulta fruits.

Table 1 Physical characteristics and proximate compositions of chulta fruits at different maturity stages

Parameter	Maturity stage	
	Immature	Mature
Physical characteristics		
Diameter (cm)	$6.10^{b} \pm 0.26$	$8.34^{a} \pm 0.37$
Height (cm)	$8.17^{b} \pm 0.21$	$11.19^{a} \pm 0.40$
Color values		
$L^{*\,ns}$	49.58 ± 2.56	47.62 ± 4.78
a*	$-7.97^{b} \pm 1.84$	$-4.64^{a} \pm 0.85$
b*	$31.18^{a} \pm 3.05$	$28.63^{b} \pm 2.13$
C*	$32.32^{a} \pm 3.05$	$29.09^{b} \pm 2.09$
h°	$106.34^{a} \pm 4.45$	$99.33^{b} \pm 2.12$
Proximate compositions		
Moisture (%)^{ns}	4.81 ± 0.13	4.98 ± 0.38
Ash (%)	$5.73^{a} \pm 0.01$	$5.18^{b} \pm 0.04$
Crude fiber (%)^{ns}	18.38 ± 0.07	18.41 ± 0.05
Fat (%)	$1.09^{b} \pm 0.00$	$1.26^{a} \pm 0.09$
Protein (%)^{ns}	9.71 ± 0.03	9.36 ± 0.03
Carbohydrate (%)^{ns}	60.28 ± 0.21	60.55 ± 0.35

Remark: Values are expressed as mean ± standard deviation (S.D.) (n=10 for physical characteristic; n = 2 for proximate composition).
Different letters indicate significant differences between matuity stages at $p < 0.05$.
ns: no significant difference between maturity stages at $p>0.05$

1.2 Proximate compositions in chulta fruits

Proximate compositions of immature and mature chulta fruits have been shown in Table 1. Results showed that there were significant differences in fat and ash contents between immature and mature chulta fruits. Higher fat content was found in mature fruits, while ash content was higher in immature fruits. Similar results were found in banana *(Musa sp. var. 'Robusta')* (Tapre & Jain, 2012). However, there were no significant differences in moisture, crude fiber, protein and carbohydrate contents between

immature and mature fruits. Interestingly, average contents of proteins (9.67%) and crude fibers (18.40%) of chulta fruits in this study were greater than avocado fruits which is a source of fruit protein (Maitera et al., 2014).

2. Effect of maturity stage and extraction solvent on phenolic compounds, antioxidant capacity and antibacterial activity of chulta fruits

2.1 Phenolic compounds of chulta fruits

Results showed that maturity stage and extraction solvent had significant effects on phenolic compounds of chulta fruits, as shown in Table 2. The highest total phenolic content was found in water extracts of immature chulta fruits. Total phenolic content in water extracts was distinctly higher when compare to ethanol extracts. Similar results were reported by Hayouni et al. (2007) who found that water had a higher efficiency for phenolic extraction. In contrast, Abdille et al. (2005) found that lower amount of total phenolic content was observed in water extract. Variation in total phenolic content might be influenced by different extraction methods and fruit species (Metrouh-Amir et al., 2015). Moreover, environment such as season, planting area, storage and post-harvest management could have also affected total phenolic content of plants (Vicente et al., 2009). The results also showed that immature chulta fruit had higher total phenolic content than mature fruits for both water and ethanol extracts.

Table 2 Effects of maturity stage and extraction solvent on total phenolic content and antioxidant capacity of chulta fruits

Extraction Solvent	Maturity Stage	Total phenolic content (mg GAE/g DW)[1]	Antioxidant capacity by DPPH scavenging assay (mg TE/g DW)[2]
Water	Immature	$42.11^{a} \pm 1.34$	$106.40^{a} \pm 1.14$
	Mature	$19.06^{b} \pm 0.41$	$90.34^{b} \pm 0.21$
Ethanol	Immature	$4.33^{c} \pm 0.05$	$10.97^{c} \pm 0.23$
	Mature	$2.11^{d} \pm 0.21$	$4.98^{d} \pm 0.22$

Remark: Values are expressed as mean ± standard deviation (S.D.) (n=2).
Different letters indicate significant differences between matuity stages at $p < 0.05$.
[1]mg GAE/g DW is mg gallic acid equivalent/g dry weight
[2]mg TE/g DW is mg Trolox equivalent/g dry weight

Phenolic compounds in chulta fruits were identified using HPLC-DAD/ESI-MS (Fig. 2 and Table 3). Thirteen peaks were detected in water extracts of chulta fruits (Fig. 2A-B), while only 8 peaks were achieved in ethanol extracts (Fig. 2C-D). Peak 1-5 was observed only in water extracts. For identification of individual phenolic compounds, maximum wavelength and mass spectra were considered, by comparisons with references. The first three peaks were in the phenolic acid group which were tentatively syringic acid-*O*-hexoside (peak 1), syringic acid (peak 2) and vanillic acid hexoside (peak 3). Peak 4 and 5 were likely to be -(+)catechin or –(-)epicatechin and procyanidin trimer type B, respectively, which are in the flavanol group. The last eight peaks were detected in both water and ethanol extracts of chulta fruits (Fig. 2A-D). Only three peaks were probably identified as flavonols, which might be isoramnethin-3-*O*-hexoside (peak 7), kaempferol-3,7-*O*-hexoside or luteolin-3,7-*O*-hexoside (peak 10) and luteolin-7-*O*-glucuronide (peak 12). According to maximum wavelength, peak 6, 8, 9, 11 and 13 were possibly flavonoids. The results clearly showed that type of extraction solvent is a critical factor that affects the existence of phenolic compounds in chulta fruits. Our results were in agreement with Pellegrini et al. (2007) and Dai & Mumper (2010) who revealed that the presence of phenolic compounds in plants depended on polarity of extraction solvent. Moreover, phenolic compounds of chulta fruit were also influenced by maturity stage. Generally, plants synthesize phenolic compounds during growing for protection from environmental stress and insects (Randhir et al., 2004). Therefore, higher concentrations of phenolics have been mostly found in young tissue.

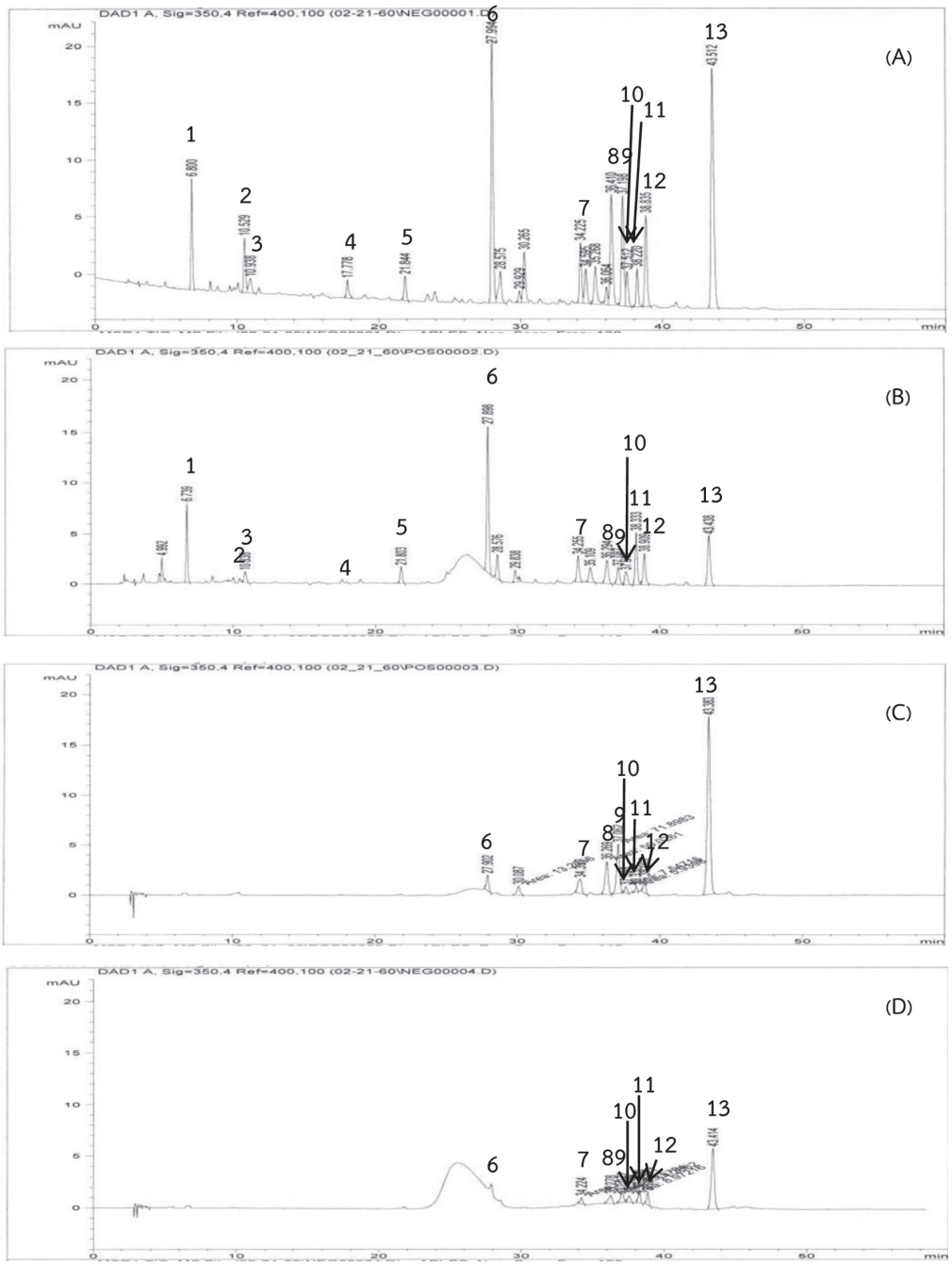

Fig. 2 Representative HPLC chromatograms of phenolic compounds detected in water extracts (A: immature; B: mature) and ethanol extracts (C: immature; D: mature) of chulta fruits. The peak numbers correspond to the UV spectrum and MS spectral data in Table 3.

Table 3 Identification of phenolic compounds of chulta fruit extracts using HPLC-DAD/ESI-MS

Peak	RT (min)	$^{1}\lambda_{max}$ (nm)	$^{2}\lambda_{max}$ (nm)	$[M+H]^+/[M-H]^-$ (m/z)	MW	^{1}Fragment ions (m/z)	^{2}Fragment Ions (m/z)	Tentative compound	References
1	6.8	280	280	-/359	360	153	153	Syringic acid-*O*-hexoside	4
2	10.5	280	276	199/197	198	197	197	Syringic acid	1
3	10.9	260	264	331/329	330	-	-	Vanillic acid hexoside	3
4	17.8	280	280	291/289	290	289	289	-(+)Catechin	1
4	17.8	280	280 or 270	291/289	290	289	289	-(-)Epicatechin	1
5	21.8	280	280	867/865	866	289	289	Procyanidin trimer type B	5
6	27.9	280, 340	-	543/541	542	-	-	Unknown	-
7	34.3	265, 350	265, 354	479/477	478	315	315	Isoramnethin-3-*O*-hexoside	6
8	36.3	270, 350	-	453/-	452	-	-	Unknown	-
9	37.1	260, 350	-	397/395	396	-	-	Unknown	-
10	37.6	275, 335	348	449/447	448	285	285	Kaempferol-3,7-*O*-hexoside	7
10	37.6	275, 335	264, 345 or 348	449/447	448	285	285	Luteolin-3,7-*O*-hexoside	2
11	38.3	260, 355	-	493/491	492	-	-	Unknown	-
12	38.9	275, 340	254, 268, 346	463/461	462	285	285	Luteolin-7-*O*-glucuronide	4
13	43.4	260, 350	-	409/411	410	-	-	Unknown	-

Remark: RT: retention time; MW: molecular weight

[1]The data that obtained from this experiment;

[2]The data that obtained from references: 1) Sun et al. (2007); 2) Plazonic et al. (2009); 3) Inbaraj et al. (2010); 4) Abu-Reidah et al. (2012); 5) Barros et al. (2013); 6) Simirgiotis et al. (2013); 7) Spinola et al. (2015)

2.2 Antioxidant capacity of chulta fruits

Antioxidant capacity by DPPH scavenging capacity was determined in chulta fruits. The results showed that maturity stage and extraction solvent had significant effect on antioxidant capacity (Table 2). The highest antioxidant capacity was found in water extracts of immature chulta fruits. Antioxidant capacity in water extracts was obviously higher when compared to ethanol extracts. Furthermore, immature fruit had greater antioxidant capacity than mature fruits in both water and ethanol extracts. It is possible that antioxidant capacity of chulta fruits might have resulted from their phenolic compounds. The water extract of chulta fruits in this study exhibited higher DPPH radical scavenging capacity than purple star apple, yellow cashew and red cashew (Moo-Huchin et al., 2015). However, the maximum antioxidant capacity of chulta fruits was lower than guava (*Psidium guajava* Linn.), mango (*Mangifera indica*), litchi (*Litchi chinensis* Sonn.) and banana (*Musa basjoo* Sieb. et Zucc. (ba jiao) (Chen et al., 2017).

2.3 Antibacterial activity of chulta fruits

The effect of maturity stage and extraction solvent on antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* of chulta fruits were determined using six different concentrations (200,000, 400,000, 600,000, 800,000, 1,000,000 and 2,000,000 mg/L) of water and ethanol extracts from immature and mature chulta fruits. Results of antibacterial activity of the chulta fruit extracts against *Escherichia coli and Staphylococcus aureus* have been summarized in Table 4. The antibacterial activity of chulta fruits was significant different depending on maturity stage and extraction solvent. The minimum concentrations of water extracts that inhibited growth of *Escherichia coli* and *Staphylococcus aureus* were 400,000 mg/L for immature chulta fruit and 800,000 mg/L for mature chulta fruit (Fig. 3-4 A and B). Ethanol extracts of chulta fruits showed an inhibitory effect on the bacterial growth at concentrations ≥ 600,000 mg/L for immature chulta fruit and ≥ 2,000,000 mg/L for mature chulta fruit (Fig. 3-4 C and D). The water extract of immature chulta fruits had the highest potency of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* when compared to ethanol extracts. It is possible that water extracts contained more phenolic compounds than ethanol extracts, resulting to higher potency of antibacterial activity. In literature, plant flavonols in both aglycone and glycoside forms possessed antimicrobial activity (Balasundram et al., 2006).

As shown in Table 4, the chulta extracts had more potential of antibacterial activity against *Staphylococcus aureus* (gram-positive bacteria) than *Escherichia coli* (gram-negative bacteria). Therefore, the chulta extracts might be potent in inhibition of gram-positive bacteria (Table 4). Previous research also reported that herbal extract had higher antimicrobial activity against gram-positive bacteria than gram-negative bacteria because the cell wall of gram-positive bacteria does not consist of outer membrane (Parekh & Chanda, 2007). Therefore, antibacterial agents can easily penetrate into cells, resulting in inhibition of bacterial growth.

Table 4 Antibacterial activity of chulta fruits on *Escherichia coli* and *Staphylococcus aureus* at different maturity stages and extraction solvents

Extraction solvent	Maturity stage	Diameter of clear zone (mm) at different extract concentration						
		control	2,000,000 mg/L	1,000,000 mg/L	800,000 mg/L	600,000 mg/L	400,000 mg/L	200,000 mg/L
Escherichia coli								
Water	Immature	-	$20.58^{a} \pm 0.08$	$16.58^{a} \pm 0.05$	$15.21^{a} \pm 0.02$	$13.63^{a} \pm 0.02$	$11.25^{a} \pm 0.03$	-
	Mature	-	$16.63^{b} \pm 0.01$	$12.08^{b} \pm 0.08$	$11.13^{b} \pm 0.03$	-	-	-
Ethanol	Immature	-	$14.38^{c} \pm 0.15$	$8.96^{c} \pm 0.06$	$8.06^{c} \pm 0.09$	$7.42^{b} \pm 0.02$	-	-
	Mature	-	$9.29^{d} \pm 0.08$	-	-	-	-	-
Staphylococcus aureus								
Water	Immature	-	$21.33^{a} \pm 0.06$	$16.83^{a} \pm 0.06$	$15.50^{a} \pm 0.04$	$13.50^{a} \pm 0.03$	$11.42^{a} \pm 0.04$	-
	Mature	-	$17.13^{b} \pm 0.11$	$11.79^{b} \pm 0.04$	$10.13^{b} \pm 0.01$	-	-	-
Ethanol	Immature	-	$17.31^{b} \pm 0.36$	$7.80^{c} \pm 0.04$	$7.77^{c} \pm 0.11$	$6.96^{b} \pm 0.01$	-	-
	Mature	-	$10.13^{c} \pm 0.13$	-	-	-	-	-

Remark: Values are expressed as mean ± standard deviation (S.D.) (n=2).
Different letters indicate significant differences between matuity stages at $p < 0.05$.

Fig. 3 Inhibition zone of chulta extracts at different concentrations on *Escherichia coli* by agar well diffusion method: water extracts of immature chulta fruits (A) and mature chulta fruits (B); ethanol extracts of immature chulta fruits (C) and mature chulta fruits (D). Concentrations of each extract were 2,000,000 mg/L (1), 1,000,000 mg/L (2), 800,000 mg/L (3), 600,000 mg/L (4), 400,000 mg/L (5) and 200,000 mg/L (6). Sterilized distilled water was used as control (C)

Fig. 4 Inhibition zone of chulta extracts at different concentrations on *Staphylococcus aureus* by agar well diffusion method: water extracts of immature chulta fruits (A) and mature chulta fruits (B); ethanol extracts of immature chulta fruits (C) and mature chulta fruits (D). Concentrations of each extract were 2,000,000 mg/L (1), 1,000,000 mg/L (2), 800,000 mg/L (3), 600,000 mg/L (4), 400,000 mg/L (5) and 200,000 mg/L (6). Sterilized distilled water was used as control (C)

Conclusion

Maturity stage and extraction solvent significantly influenced phenolic compounds, antioxidant capacity, and antibacterial activity. The physical characteristics (fruit size and color) of chulta fruit were affected by maturity stage. For proximate compositions, maturity stage was also influenced on fat and ash contents of chulta fruits. Moreover, water extract of immature chulta fruits contained higher phenolic compounds which are responsible for higher antioxidant capacity and antibacterial activity.

Suggestion

Antimicrobial activity of chulta extracts on pathogenic bacterial and fungi should be determined in future studies.

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