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Short Communication

Bioactive compounds and antioxidant activities of *Camellia sinensis* var. *assamica* in different leave maturity from Northern Thailand

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Abstract

The aim of this study was to compare the bioactive compounds [total phenolic content (TPC), total flavonoid content (TFC), and proanthocyanidin content] and antioxidant activities [DPPH, ABTS and FRAP activities] of 3 different maturity of tea leaves, shoot (first 1-3 leaves), young leaves (4-6 leaves) and mature leaves (1-3 from bottom tea shrub) by using different solvents [hot DI water (80OC), DI water, 70% ethanol and 70% acetone] for extraction. Results showed that the highest extractable yields were found on the shoot tea with hot DI water extraction (21.33 ± 9.15% dry basis). Shoot tea with ethanol extraction composed of high amount of TPC (65.26 mg trolox equivalent antioxidant capacity TEAC/mg sample). Shoot and young tea leaves in ethanol extract tended to have high proanthocyanidin content (35.94 and 32.15 mg epicatechin equivalent (ECE)/g sample, respectively) and DPPH free-radical scavenging activity (31.39 and 29.93 mg TEAC/g sample, respectively). Mature leaves by 70% acetone extracted showed obviously high amount of flavonoid content (57.39 mg quercetin equivalent (QE)/g sample) and FRAP activity (1.938 mg TEAC/mg sample). Therefore, the young and mature tea leaves that did not be used for tea processing, may be used as an alternative source of natural antioxidant for cosmetic and other products.

Introduction

In Thailand, tea has been cultivated about 97,000 hectares that is mostly found in Chiang Rai province ~45,600 hectares. Eighty five percent of total tea leaves and its products are consumed in domestic and 15 percent of them are exported to other countries such as United States of America and England that to increase revenue more than 100 million baht per year (Theppakorn, 2011).

Tea has two strains, Chinese tea (*Camellia sinensis* var.*sinensis*) and Assam tea (*Camellia sinensis* var. *assamica*). Assam tea originates from India which its leaf is larger than Chinese tea but it has less popular to consume than another tea (Roy, 2011). It mostly used to produce "Miang" or "fermented tea leaf" which is considered a lifestyle staple of northern people in Thailand, particularly hill tribes. Miang traditionally consists of steamed tea leaves and rolled into a ball. Miang used for welcoming house guests or ceremonies in northern people (Phromrukachat *et al*., 2010). The value of "Miang" is cheap, although its tea leaves may have many bioactive compound and bioactivities potential as Chinese tea. Previous studies showed that tea has many bioactive compounds mainly composed of flavonoid, caffeine and fluoride. Flavonoid are abundant in various types of tea, most attention has been paid on their constituents in tea. Catechins is a group of flavonol monomers mainly found in tea and largely include epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechingallate (EGCG) (Baibado, 2011). Furthermore, in the tea consumption and tea harvesting process, the shoot or bud (first 1-3 leaves) of tea leaves are usually used only. Other tea leaves young (2-6 leaves) and mature (last 2-3 leaves from bottom tea shrub) are not used in tea preparation and still be on its shrub. Moreover, the study of bioactive compounds and their activities of different leaf maturity (shoot, young and mature leaves) of assam tea leaves have been little investigated.

Therefore, this study aimed to compare the bioactive compounds and antioxidant activity of 3 different maturity of tea leaves: shoot (leaf bud and two youngest leaves; yellowish green), young leaves (fourth to sixth leaves from the top; light green) and mature (last 1-3 leaves from bottom shrub) by using different solvents extraction [hot DI water (80°C), DI

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water (RT), 70% ethanol, 70% acetone)].

Materials and Methods

Sample preparation

Assam tea leaves (*Camellia sinensis* var. *assamica*) were collected from tea garden of Tea Institute, Mae Fah Luang University. Tea leaves maturity were separated into three parts that were (1) shoot part (1-3 youngest leaves) (2) young part (4-6 leaves) and (3) mature part (3 leaves from base of tea shrub). Samples were air dried and then dried by hot air oven at 45°C until constant sample weight. Then, dried samples were milled as powder and kept at -20°C until used.

Chemicals and reagents

Solvents were analytical grade. Ethanol (95%), DMSO, Acetone, Acetic acid, Hydrochloric (37%), and Vanillin were purchased from Merck. Methanol (95%) and Hexane were purchased from Lab-Scan. Sodium bicarbonate (Na_2CO_3), Aluminum chloride ($AlCl_3$), Dibasic phosphate and monobasic phosphate were purchased from Univar; Potassium acetate (CH_3COOK), Potassium persulphate ($K_2S_2O_8$) and Sodium acetate (CH_3COONa) were purchased from Unilab. ABTS, DPPH, Epicatechin, Folin-Ciocalteu, Gallic acid, Quercetin, TPTZ and Trolox were purchased from Sigma.

Bioactive compound extraction

Four various solvents were hot DI water (80°C), DI water, 70% ethanol and 70% acetone. A 4 g of sample powder were extracted with 200 ml of solvent (1 g: 50 ml ratio w/v) using shaking method at 150 rpm for 3 hours at room temperature. The mixtures were filtrated through Whatman no.5 filter paper. And then, organic solvents (ethanol and acetone) were removed by rotary evaporator. Extracts were freeze-dried and kept at -20°C. Extractions were done in triplicate. Yield was calculated as percentage (%) dry basis.

Total phenolic content (TPC)

One milligram of extracts was dissolved in 1 ml of DMSO. TPC method was modified from Singleton and Rossi (1965). Then 20 µl of sample, 100 µl of Folin-Ciocalteu reagent and 80 µl of 7.5% (w/v) Na_2CO_3 were added respectively. The reaction was performed in dark at room temperature (RT) for 30 min and the absorbance was measured at 765 nm. Gallic acid was used as standard. Results of all samples were reported in mg gallic acid equivalent (GAE) per mg sample.

Total flavonoid content (TFC)

TFC of extracts was determined by modified method from Chang *et al.* (2002). A 25 µl of sample, 75 µl of 95% ethanol, 140 µl of DI water, 5 µl of 10% (w/v) $AlCl_3$ and 5 µl of 1 M CH_3COOK were added respectively. The reaction was performed in dark at RT for 30 min and the absorbance was measured at 415 nm. Quercetin was used as standard. Samples were calculated and reported as mg Quercetin equivalent (QE) per mg sample.

Proanthocyanidin determination

Total proanthocyanidin content in each samples was determined following Singleton and Rossi (1965) with slightly modification. A 20 µl of samples were added into 50 µl of 1% vanillin in methanol and 50 µl of 25% (v/v) sulphuric acid. Mixtures were incubated 15 min in the dark at RT. The absorbance of reaction was measured at 500 nm using microplate reader. The total proanthocyanidin content of sample was expressed as epicatechin equivalents (mg ECE/ g sample).

DPPH radical scavenging assay

DPPH free-radical scavenging assay was modified from method of Prior *et al.* (2005). A 190 µl of 0.1 mM DPPH in ethanol was added to 10 µl of sample (1mg/ml concentration). The reaction was performed in the dark at RT for 30 min and the absorbance was measured at 515 nm. Trolox was used as standard.

$$\%\text{DPPH inhibition} = [(A_{control}-A_{sample})/ A_{control}] *100$$

A control = the absorbence of the control solution without antioxidant

A sample = the absorbence of sample or ascorbic to be tested

Samples were calculated and reported as mg Trolox equivalent antioxidant capacity (TEAC) per mg sample.

ABTS scavenging assay

ABTS free-radical decolorization assay was modified from Thaipong *et al*. (2006). ABTS radical was generated by mixing 1 (v/v) of 7 mM ABTS with 2.45 mM $K_2S_2O_8$ and kept in dark for 15 hrs. Then, the solution was diluted with phosphate buffer (pH 7, 50mM) in ratio 1:70 (v/v) before used. A 10 µl of sample was added 190 µl of diluted ABTS radical solution. The reaction was performed in dark at RT for 15 min and the absorbance was measured at 734 nm. Trolox was used as standard.

$$\%\text{ABTS inhibition} = [(A_{control}-A_{sample})/A_{control}]*100$$

Table 1. Extractable yield and bioactive compounds of Assam tea in different tea leave maturity (shoot, young and mature leaves)

Tea leave maturity	Solvents	% Yield	Bioactive compound		
			Total Phenolic content (mg GAE/ g sample)	Total Flavonoid content (mg QE/ g sample)	Proanthocyanidin (mg ECE/ g sample)
Shoot	Hot DI water	21.33 ± 9.15	59.65 ± 4.10^{b}	13.10 ± 6.77	22.87 ± 1.20^{b}
	DI water	15.58 ± 4.27	29.55 ± 0.82^{d}	26.20 ± 7.44	17.60 ± 0.47^{c}
	70%, EtOH	17.33 ± 1.01	65.26 ± 0.92^{a}	36.36 ± 19.28	35.94 ± 1.55^{a}
	70%, Acetone	20.75 ± 6.80	53.54 ± 2.69^{c}	26.70 ± 18.46	33.88 ± 3.73^{a}
Young	Hot DI water	14.75 ± 5.27	46.49 ± 0.22^{b}	26.27 ± 10.44	29.60 ± 0.89^{b}
	DI water	12.75 ± 2.41	21.89 ± 0.42^{c}	9.00 ± 4.97	16.12 ± 0.75^{c}
	70%, EtOH	14.25 ± 1.80	48.96 ± 1.02^{a}	34.30 ± 29.88	32.15 ± 0.82^{a}
	70%, Acetone	16.67 ± 5.20	49.89 ± 0.67^{a}	31.17 ± 20.60	31.17 ± 0.34^{a}
Mature	Hot DI water	17.50 ± 5.65	29.10 ± 0.62^{b}	6.18 ± 9.19	18.50 ± 0.82^{c}
	DI water	13.25 ± 3.61	25.18 ± 1.06^{c}	45.19 ± 53.69	19.49 ± 0.62^{c}
	70%, EtOH	13.17 ± 2.75	37.50 ± 0.61^{a}	36.01 ± 23.89	26.32 ± 0.61^{b}
	70%, Acetone	13.17 ± 3.75	39.35 ± 2.13^{a}	57.37 ± 16.68	28.45 ± 0.90^{a}

Mean ± S.D. (n=7). The different of superscript letter indicated statistically different between extract in each tea maturity leave ($p<0.05$)

Samples were calculated and reported as mg TEAC per mg sample.

FRAP assay

Ferric-reducing antioxidant power (FRAP) of extracts was determined by modified method from Benzie and Stain (1996) and Prior *et al.* (2005). FRAP solution was prepared by mixing 3 ml of TPTZ (10 mM in 40 mM HCl), 3 ml of 3.2 mM $FeCl_3$ solution and 30 ml of sodium acetate buffer (pH 3.6, 300 mM), respectively. A 10 µl of sample was added 190 µl of FRAP solution. The reaction was performed in the dark at RT for 15 min and then measured the absorbance at 593 nm. Trolox was used as standard. Samples were calculated and reported as mg TEAC per mg sample.

Statistical analysis

Obtained data were statistically analyzed using SPSS program (IBM, version 21). The comparison on bioactive contents and activities of each samples were investigated by ANOVA (Tukey HSD) for parametric analysis and Kruskal-Wallis for non-parametric analysis at $p<0.05$ level.

Results and Discussion

Extractable yield

The extractable yields of three maturities of tea leaves were shown in Table 1. There were no significant different in each tea maturities. However, the yield ranged from 12.75 to 21.33% dry basis that the highest yields were found on the shoot tea with hot DI water extraction. Moreover, hot DI water extraction of mature tea leaves also gave higher yield than others solvent (17.50%). However, young leaves were more extracted by both acetone and hot DI water (16.67% and 14.75%, respectively). In the study of Perva-Uzunalic *et al*. (2006), they also found that the best condition of tea extraction efficiency was hot DI water (80°C) similar to this study. Moreover, shoot leaves tended to has more extractable yield than young and mature leaves. The yield decreased with increased age of tea leaves because of leaf physiological changes during the growth period (Farhoosh *et al*., 2007).

Determination of bioactive compounds

The bioactive compounds (phenolic, flavonoid and proanthocyanidin contents) of various three maturities of tea leaves were shown in Table 1. The

Table 2. Antioxidant activity of Assam tea in different tea leaves maturity (shoot, young and mature leaves)

Tea leave maturity	Solvents	Antioxidant activity		
		DPPH (mg TEAC / g sample)	ABTS (mg TEAC / g sample)	FRAP (mg TEAC/ mg sample)
Shoot	Hot DI water	30.56 ± 1.55^{a}	36.67 ± 0.07^{a}	1.262 ± 0.039^{c}
	DI water	24.45 ± 3.45^{b}	36.87 ± 0.13^{a}	1.278 ± 0.043^{c}
	70%, EtOH	31.39 ± 3.04^{a}	36.54 ± 0.13^{b}	1.552 ± 0.030^{b}
	70%, Acetone	28.27 ± 2.15^{ab}	36.60 ± 16.22^{b}	1.698 ± 0.014^{a}
Young	Hot DI water	30.18 ± 1.76^{a*}	36.93 ± 0.09^{a}	1.530 ± 0.258^{a}
	DI water	28.77 ± 2.31^{a*}	36.77 ± 0.04^{b}	0.966 ± 0.023^{b}
	70%, EtOH	29.93 ± 2.89^{a*}	36.56 ± 0.03^{c}	1.751 ± 0.065^{a}
	70%, Acetone	28.91 ± 3.03^{a*}	36.60 ± 0.12^{c}	1.533 ± 0.076^{a}
Mature	Hot DI water	19.74 ± 1.60^{b}	36.69 ± 0.06	1.190 ± 0.028^{c}
	DI water	17.79 ± 0.84^{b}	36.06 ± 1.62	1.069 ± 0.030^{b}
	70%, EtOH	23.72 ± 0.79^{a}	36.76 ± 0.05	1.758 ± 0.043^{b}
	70%, Acetone	25.40 ± 1.21^{a}	36.80 ± 0.04	1.938 ± 0.067^{a}

Mean+S.D. (n=7). The different of superscript letter indicated statistically different between extract in each tea maturity leave ($p<0.05$)

total phenolic content (TPC) of each tea part was significantly difference between four extracts (hot DI water, DI water, EtOH and Acetone) ($p<0.05$). Shoot tea composed of the higher TPC in the ethanol extract (65.26 mg GAE/ g sample) than those of hot DI water, acetone, and DI water (59.65, 53.54 and 29.55 mg GAE/ g sample, respectively). Moreover, TPC of young leaves tea was high on the acetone and ethanol extracts (49.89 and 48.96 mg GAE/ g sample), but in case of mature tea, the highest TPC was found in both acetone and ethanol samples (39.35 and 37.50 mg GAE/ g sample).

The flavonoid contents did not significantly difference between samples in each tea leaf maturity ($p>0.05$). The highest value was found in the shoot and young leave of tea part with 70% ethanol extracts (36.36 and 34.30 mg QE/ g sample); however, the acetone extract of mature tea leaves composed of the greatest flavonoid contents (57.37 mg QE/ g sample). Consistency, the tendency of proanthocyanidin content was similar to the flavonoid contents that the highest content was found in both ethanol and acetone extract of shoot (35.94 and 33.88 mg ECE/g sample) and young tea leaves (32.15 and 31.17 mg ECE/g sample) and acetone extract of mature tea leaves (28.45 mg ECE/g sample) ($p<0.05$). The tendency of bioactive content of these three maturities tea leaves may caused by the distribution or accumulation of these compounds; phenolic compound, epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechingallate (EGCG), with the age of tea leaves (Lin *et al*., 2003; Caffin *et al*., 2004).

Investigation of antioxidant activities

Antioxidant activities (DPPH radical scavenging, ABTS, and FRAP capacities) were shown in Table 2. DPPH activities were slightly similar to the amount of bioactive compounds that tended to high activity on ethanol extracts of shoot leaves (31.39 mg TEAC/ g sample) and on both hot DI water and ethanol extracts of young leaves (30.18 and 29.93 mg TEAC/ g sample) as well as the acetone extracts of mature leaves (25.40 mg TEAC/ g sample) ($p>0.05$). Results were similar to previous studies that the highest DPPH was found in 50 % ethanol extract of black mate tea from Australia (Turkmen *et al*., 2006). Furthermore, the ABTS activity was significantly difference among various extracts in each tea leaves ($p<0.05$) and its tendency was likewise the DPPH activity (Table 2). ABTS activity ranged from 36.06 to 36.93 mg TEAC/ g sample. However, FRAP capacity tended to be significantly higher in the acetone extracts of shoot and mature tea leaves (1.698 and 1.938 mg TEAC/ g sample, respectively) and the ethanolic extract of young leaves (1.751 mg TEAC/ g sample) than other extracts in the same

leaves maturity ($p<0.05$). The antioxidant activity of each maturity tea may related to the amount of their bioactive compound that phenolic may act the DPPH activity whereas proanthocyanidin may relate to the FRAP activity.

Conclusion

The highest yields were found on the shoot tea with hot DI water extraction. Shoot tea leaves also composed of high amount of phenolic content in ethanol extract. Moreover, Shoot and young tea leaves in both acetone and ethanol extract tended to have high proanthocyanidin content and great DPPH free-radical scavenging activity. Mature leaves by 70% acetone extracted showed obviously high amount of flavonoid content and FRAP activity. Therefore, the young and mature tea leaves, did not to be used for tea harvesting, may be used as alternative source of natural antioxidant for cosmetic and other products.

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