

## Comparison of the Antioxidant Activity of Pickled Tea (*Camellia sinensis* var. *assamica*) Extract with that of Other Teas

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### ABSTRACT

Mieng (*Camellia sinensis* var. *assamica*), widely distributed in the northern part of Thailand, has been consumed as chewing tea. Mieng or pickled Assam tea leaves collected from the markets in Chiang Mai were oven dried at 50°C and extracted by reflux extraction for 120 min. The extracts were evaluated for antioxidant activities by DPPH radical and ABTS cation radical assays. Their total phenolics contents were determined by Folin-Ciocalteu's reagent-reactive compounds (FRC). Antioxidant activities and total phenolic contents were compared with Chinese green tea, Oolong and black tea. Correlations of ABTS, DPPH and FRC tests were studied. Inhibition concentration at 50% of DPPH radical scavenging activity and ABTS<sup>+</sup> radical cation decolorization were found in the range from 12.63 to 36.59 ppm and from 3.48 to 21.61 ppm, respectively. Linear correlation between DPPH and ABTS radical scavenging activity ( $R^2 = 0.9980$ ) was observed. Total phenolic contents were ranged from 124.4 to 383.8 mg of gallic acid equivalent per gram extract. The pickled Assam tea contained higher radical scavenging assay than those of black tea and lower than Chinese green tea and Oolong. The results showed that pickled tea had the high of DPPH<sup>+</sup> and ABTS<sup>+</sup> radical scavenging activity and comparable total phenolic content among other teas.

**Keywords:** pickled Assam Tea, *Camellia sinensis* var. *assamica*, DPPH, ABTS, Folin-Ciocalteu

### 1. INTRODUCTION

Tea is the most widely consumed beverage and well known to be rich in polyphenolic compound, which plays important roles as antioxidants (Worthy, 1991). Antioxidant compounds like phenolic acids, polyphenols and flavonoids (catechins) can prevent or postpone oxidation caused by free radicals and reactive oxygen species emerges in food or in biological systems, therefore it may help the body to protect itself from various types of oxidative damage which are linked to diseases such as cancer, diabetes, cardiovascular disorders and aging (Halliwell, 1991).

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Catechins, a type of polyphenolic compound, constitute up to 30% of dry weight in fresh tea leaves (Graham, 1992). Tea and its constituent catechins have high antioxidant and free radical scavenger activity, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS) (Ito *et al.*, 1992; Fujiki *et al.*, 1992).

There are different kinds of tea products available in the markets, in general, there are three main methods of processing and each produces a different type of tea. These main types are unfermented green tea, partially fermented Oolong tea and fully fermented black tea. Oxidizing enzymes occurred during fermentation, that changes the color, aroma and taste in tea leaves. (Roberts & Chandradasa, 1982). The degree of fermentation intensely affects the content of polyphenols, particularly the catechins (Lin *et al.*, 1998). In fermented tea, the total catechins content is decreased and originated new products, such as theaflavins and thearubigins (Lin, C. C. and Liang, J. H., 2002). Therefore, may also affect their antioxidant activities. Many reports have been studied on the beneficial effects of tea on antioxidant oxidative and total phenolic content (Gadow *et al.*, 1997; Atoui *et al.*, 2005; Chan *et al.*, 2007; Rusak *et al.*, 2008).

Assam Tea, *Camellia sinensis* var. *assamica*, can be consumed in fresh, tea and pickled. Pickled tea leaves have been popular food and snack for the people in the North of Thailand for a long time. Chemical components and free radical scavenging of fresh Assam tea leaves has been reported while those of pickled tea have not been much studied comprehensively.

The objective of this study was to evaluate the antioxidant activity and estimate the phenolic content of the extracts of pickled Assam tea. Different artificial specie of free radicals have been used such as 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1'-diphenyl-2-picrylhydrazyl (DPPH) in order to evaluate the radical scavenging potentials compare to gallic acids. Its total phenolic contents were determined by Folin-Ciocalteu's reagent (FCR). Furthermore, the antioxidant activities and total phenolic contents of three typical commercial teas (Chinese green tea, Oolong and black teas) were tested and compared.

## 2. METHODOLOGY

### 2.1. Materials

Pickled Assam tea leaves (*Camellia sinensis* var. *assamica*) were purchased from markets in Chiang Mai Province, Thailand. It was dried in a hot air oven (50°C) for 3 days. Chinese green tea, Oolong and black tea were purchased at local supermarket. The samples were powdered and kept refrigerated at 4°C in darkness until ready for extraction.

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## 2.2. Chemicals

Potassium persulfate was obtained from Ajax Finechem, Australia. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) were purchased from Fluka, Germany. Folin-Ciocalteu's phenol reagent was purchased from Merck, Germany. HPLC grade ethanol was purchased from Fisher Scientific UK Limited, UK. Gallic acid (GA) was purchased from Sigma-Aldrich (Steinem, Germany). All other chemicals and solvents used were of analytical grade available commercially. Ultrapure water obtained from Milli-Q water filtration system.

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## 2.3. Preparation of extracts

Five grams of each sample were extracted with 100 ml methanol in a round-bottom flask equipped with a condenser. All samples were refluxed at 78°C for 120 min, then the extract mixtures were cooled to room temperature, followed by filtration through a Whatman No. 1 filter paper and the solvent were removed under vacuum at 50°C until dryness. Crude extracts were dissolved and made up to 10 ml with methanol then filtered by Whatman No. 4 filter paper and kept at 4°C in darkness until further use.

## 2.4. Antioxidant activity of the tea extracts with different solvents and times

The picked Assam tea was extracted with water, 50% ethanol-water and ethanol and the suitable solvent was selected and determined for optimized extraction time.

## 2.5. Evaluation of antioxidant activity of the tea extracts

### 2.5.1 DPPH radical scavenging activity

The antioxidant activity of the extracts was determined in term of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method as described by Gaulejac *et al.* (1998) with a slight modification. Different dilutions (0-100 ppm) of the extract, 100 µl, were mixed with 300 µl of DPPH solution (6×10<sup>-5</sup> M in methanol). The solution was incubated in darkness at room temperature for 30 min and reduction of DPPH free-radicals was measured by reading the absorbance at 515 nm using a Hitachi U-2900 spectrophotometer, Kyoto, Japan. Methanol (100 µl) was mixed with 300 µl DPPH and this served as the control.

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This activity is given as %DPPH radical scavenging or the inhibition percentage of free radical by the sample calculated according to the following equation:

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$$\text{DPPH radical scavenging activity (\%)} = \left( \frac{A_{\text{control}} - A_{\text{absorbance}}}{A_{\text{control}}} \right) \times 100 \quad (1)$$

IC<sub>50</sub> value, the concentration of sample required for 50% inhibition of DPPH free radical, was determined from the plot between %inhibition and concentration.

### 2.5.2 ABTS cation radical scavenging activity

The total antioxidant activity of the samples was measured by ABTS cation radical decolorization assay according to the method as described by Re ~~et al~~ *et al.* (1999) with some modification. ABTS<sup>•+</sup> was produced by mixing 7 ml of ABTS aqueous solution (7 mM) with 10 ml of potassium persulfate (2.45 mM). Before use, the mixture was incubated at room temperature in darkness for 12-16 hours. Freshly-prepared ABTS<sup>•+</sup> working solution (ABTS<sup>•+</sup> stock solution diluted with aqueous to achieve an absorbance of 0.70±0.02 at 734 nm) was used. Different dilutions (0-100 ppm) of the extract, 100 µl, were mixed with 300 µl of diluted ABTS cation radical solution. The absorbance at 734 nm was measured after the solution has been allowed to stand in the darkness for 1 min at room temperature. De-ionized water (100 µl) was mixed with 300 µl ABTS<sup>•+</sup> and this served as the control.

Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample. Percentage inhibitions were calculated using equation 1 and IC<sub>50</sub> were also determined.

### 2.6. Determination of total phenolic content (TPC)

The TPC of the sample extracts was determined by Folin-Ciocalteu method according to Singleton ~~et al~~ *et al.* (1999) with some modifications. Appropriately diluted extract (1 ml) was mixed with 2.5 ml of 10 % (v/v) Folin-Ciocalteu's phenol reagent. After 8 min, 1 ml of 7.5% (w/v) aqueous Na<sub>2</sub>CO<sub>3</sub> were added. After incubation for 15 min at 50°C, the absorbance was measured at 760 nm and compared to a standard curve of gallic acid (GA) solution. TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (mg gallic acid/g dry weight). Each sample extract was done in triplicate. The calibration equation for gallic acid was  $y = 0.0066x$  ( $R^2 = 0.9996$ ).

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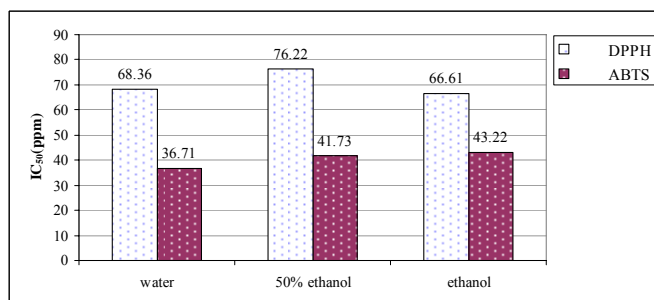
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### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of extraction solvent

The extraction efficiencies by using different solvents (water, 50% ethanol in water, ethanol) 120 min on antioxidant capacity and TPC of pickled Assam tea leaf extracts were investigated. IC<sub>50</sub> values of the tea extracts are shown in Figure 1. Depending on the solvent of extraction, IC<sub>50</sub> values of the DPPH and ABTS radical were found in the range of 66.61-76.22 ppm and 36.71- 43.22 ppm, respectively. Pure ethanol gave the lowest IC<sub>50</sub> value for DPPH radical scavenging and water gave the lowest IC<sub>50</sub> value for ABTS radical scavenging indicating the highest antioxidant activity it contained.

TPCs were ranged from 27.55-64.73 mg GAE/g DW in pickled Assam tea as shown in Figure 2. The result showed that pure ethanol extract gave the highest phenolic concentration, which was the most effective solvent for the extraction.



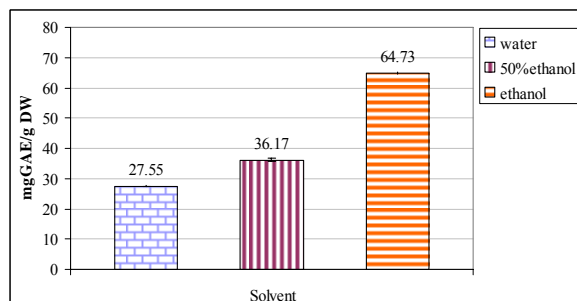
**Figure 1** IC<sub>50</sub> of the tea extracts by using various solvent Types in DPPH and ABTS reaction systems

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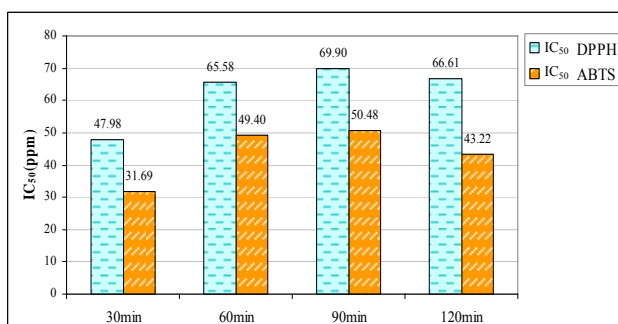
**Figure 2** Averaged total phenolic content and standard deviations of the tea extract by using various solvent types (mg equivalent of gallic acid per gram dry weight)

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### 3.2. Effect of extraction time

A Comparison of extraction time (30min, 60min, 90min and 120min) with ethanol had been made on their total antioxidant capacity of the pickled Assam tea leaves.  $IC_{50}$  values were determined from the plots between %inhibition of DPPH and ABTS radicals and concentration extracts are shown in Figure 3.  $IC_{50}$  values reached a minimum in 30 min of extraction time with DPPH and ABTS radicals scavenging, and constantly after 60 min. In this experiment, extraction time of 120 min was applied in tea extraction. TPCs were found increased as time increased and 120 min gave the highest TPC (see Figure 4).

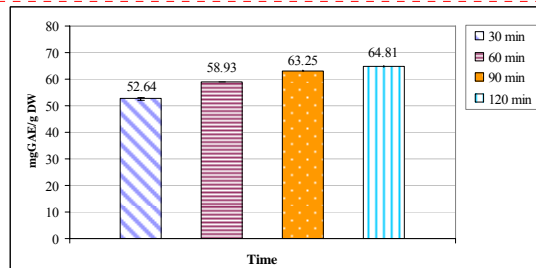
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**Figure 3**  $IC_{50}$  of the tea extract by using different extraction times in DPPH and ABTS reaction systems

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**Figure 4** Averaged total phenolic content and standard deviations of the tea extract by using different extraction times (mg equivalent of gallic acid per gram dry weight)

### 3.3. Radical scavenging activities of tea extracts

Scavenging effect of tea extracts were determined by DPPH and ABTS radical assay and detected by spectrophotometry. Percentage of radical scavenging activities of tea extracts by DPPH and ABTS radicals are shown in Figure 5.  $IC_{50}$  values of the extract teas were determine from the plots between %inhibition of DPPH and ABTS radicals and concentration of tea extracts and  $IC_{50}$  values of the extract teas are shown in Figure 6.  $IC_{50}$  calculated as the amount of sample causing a 50% inhibition of the DPPH and ABTS radical were found ranging from 12.63-36.59 ppm and 3.48-21.61 ppm, respectively. The lower  $IC_{50}$  values the higher antioxidative capacity of the samples. In general, the radical scavenging assay (RSA) of tea extract in  $ABTS^{+}$  reaction system was slightly higher comparing to DPPH reaction. Chinese green tea gave lowest  $IC_{50}$  values for DPPH and ABTS radical scavenging means the highest antioxidative power it contained. Antioxidant activity of tea extracts tested using DPPH method was tested for their correlation using Excel®, corrol function. It was found the strong correlation between  $IC_{50}$  values obtained from ABTS method and DPPH method ( $R^2=0.9980$ ). Pickled Assam tea contained lower radical scavenging assay than those of Chinese green tea and Oolong but higher than black tea.

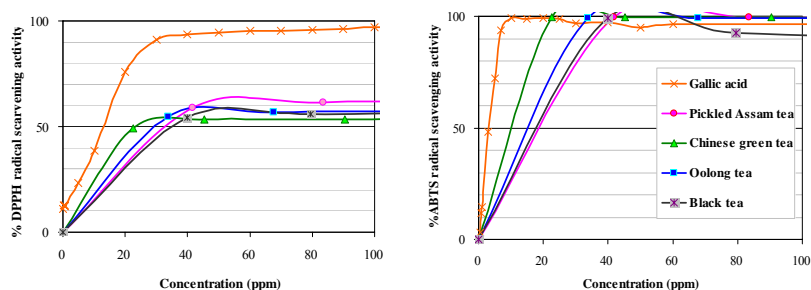
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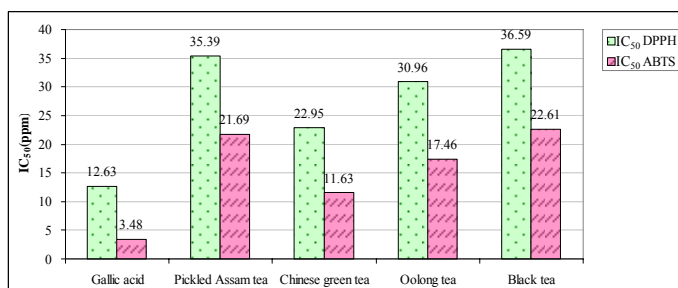
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**Figure 5** Percentage of radical scavenging activity of the tea extracts using DPPH assay (A) and ABTS assay (B)

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**Figure 6** IC<sub>50</sub> of tea extracts in DPPH and ABTS reaction systems

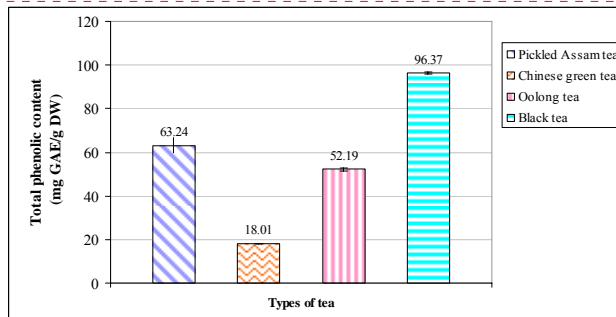
#### 3.4. Total phenolic contents

TPCs were found in the range of 18.01-96.37 mg GAE/g DW (see Figure 7). Black tea exhibited the highest phenolic content. TPC of tea extracts were decreased in the following order: Black tea > Oolong tea > pickled Assam tea > Chinese green tea. We obtained positive and strong correlation of TPC and IC<sub>50</sub> values using ABTS ( $R^2 = 0.9368$ ) and DPPH assays ( $R^2 = 0.9366$ ).

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**Figure 7** Averaged total phenolic content and standard deviations of the tea extracts (mg gallic acid equivalent per gram dry weight)

#### 4. CONCLUSION

Antioxidant activities of pickled Assam tea were similar to black tea and slightly lower than Oolong. Chinese green tea extract showed significantly higher antioxidant activities than Oolong, pickled Assam tea and black tea. TPC of pickled Assam tea leaves was lower than black tea but higher than Oolong and green tea. Hence, pickled Assam tea was contained antioxidant power and total phenolic content between Oolong and black tea but lower than that of green tea which caused of the degree of fermentation (Atoui *et al.*, 2005).

Pickled Assam tea leaves could be consider to consume for health according to the knowledge on its high potential on antioxidant activity that could help to increase in the consumption.

The hydrolyzed phenolic compounds are the biological active compounds which may be not corresponded to the scavenging properties, hence the major catechins (EC, ECG, EGC, and EGCG), some minor catechins (GC, C) and caffeine (CAF) in pickled Assam tea leaves could be quantified by high performance liquid chromatography technique.

#### 5. ACKNOWLEDGMENTS

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