# Antioxidant capacity of tea seed (*Camellia oleifera*) oil planted in the Northern of Thailand

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## Abstract

The oil of *Camellia oleifera* originated from China commonly known as tea seed oil. The oil has been used for cooking and applied as a medicine and cosmetic because of containing of antioxidant and other compounds. The work was conducted on the study of the antioxidant capacity of Thai virgin tea seed oil extracted by a single screw press, compared that to commercial Thai and Chinese tea seed oil samples. The antioxidant capacity were determined by the 2,2-di-phenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging (ABTS) assay. Bythyl hydroxyl toluene (BHT) and ascorbic acid were used as standard reagent for DPPH assay. Trolox was used as standard reagent for ABTS assay. The DPPH radical scavenging activity equivalent to BHT and ascorbic acid were in the range of 0.050-0.058 mg/g oil and 0.040-0.047 mg/g oil. Whereas, the ABTS radical scavenging activity equivalent to Trolox was in the range of 0.031-0.046 mg/g oil. The results demonstrate that antioxidant capacities of the tea seed oil planted in the Thailand and commercial Thai and Chinese tea seed oil are difference.

**Keywords:** tea seed oil, antioxidant capacity, DPPH, ABTS

## Introduction

Antioxidants are important compounds because they used to against free radicals produced in our body. Free radicals are compounds containing with unpaired electrons in an atom. It is unstable and highly sensitive to the reactions (Serviddio et al., 2013). If this process is not suppressed, free radicals can damage cell (Obón et al., 2005) which cause of chronic disease (Siritrakulsak et al., 2013; Alberto, 2005) such as Alzheimer's disease, cardiovascular disease, and aging disease (Chen et al., 2010; Sowndhararajan et al., 2013), including carcinoma, cataract disease and rheumatoid (Lee and Yen, 2006; Rahman et al., 2013; Sengul et al., 2009). Free radicals can be eliminated or minimized by antioxidant (Sowndhararajan et al., 2013; Alonso et al., 2003). Antioxidants were found in plants (vegetables, fruit and herbs) as form of mainly polyphenolic compounds, carotenoids (Siritrakulsak et al., 2013), tocopherols, beta-carotene (Manosroi et al., 2005; Rahman et al., 2013), glutathione and ascorbic acid (Aurelia et al., 2009). In addition, antioxidant can also be found in the oil such as alpha-tocopherol (vitamin E) which is the most potent fat soluble antioxidant (Tucker and Townsend, 2005).

There are a number of oil samples that are reported to have high antioxidant activities. Minhajuddin et al. had studied that the hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice brand oil in experimentally could induce hyperlipidemic rats. It was found that rice bran oil contains with 19-49% tocopherol and 51-81% tocotrienol (Eitenmiller, 1997). Rice bran oil has antioxidant properties to prevent oxidation of LDL-C (Minhajuddin et al., 2005). Phenolic compounds and alpha-tocopherol

were presented in olive fruit analyzed by a reversed-phase high performance liquid chromatography (HPLC) (Vinha et al., 2005; Lee and Yen, 2006).
Recently, the high content of antioxidant in tea seed (*Camellia oleifera*) oil studied by a DPPH scavenging activity and Trolox equivalent antioxidant capacity and found that the methanol extracted oil present high antioxidant activity was reported by Lee et al. (Lee and Yen, 2006) Moreover, the study of the antioxidant capacity of polyphenol compound in commercial Thai tea seed oil was performed by using an ABTS technique. The results showed that the antioxidant activity of the extracted polyphenol equals to 4.21 mM Trolox/ g extract and 0.748 mg gallic acid/g extract (Suealek et al., 2012). Therefore, the study of the antioxidant capacity and total phenolic content in tea seed oil are become important. Especially, the tea seed oil planted in the Northern Thailand because it is tend to be a new economic plant in Thailand. These works conducted the antioxidant capacity of the Thai *Camellia oleifera* seed oil and compare that to the commercial Chinese tea seed oil.

**Methodology**

Preparation of chemicals and reagents
DPPH (1 mM) was prepared by dissolving in methanol (99%). ABTS (7 mM) was prepared by dissolving in water. Potassium persulfate (2.45 mM) was prepared by dissolving in water. ABTS radical cation solution was produced by reacting ABTS solution with potassium persulfate solution and incubating the mixture in the dark for 12 hours at room temperature before use. BHT (5 mM), L(+)-ascorbic acid (1 mM), and Trolox (1 mM) solutions were prepared by dissolving that chemicals in methanol (99%).

Preparation of samples
The ten samples of *Camellia oleifera* tea seed oil samples were collected from the Northern Thailand (Chiang Rai and Chiang Mai provinces). The commercial tea seed oil samples were purchased from supermarkets in Thailand and China. The tea seed samples were kept in sealed plastic bags, while the oil samples were kept in glass bottles and stored at -20$^{o}$C until use. The tea seed samples were dried overnight at 65$^{o}$C by a hot air oven (LDO-061SF, Labtech engineering, Samutprakarn, Thailand), then crude tea seed oils were extracted by using a homemade single screw press. The crude tea seed oils were filtered through a filter paper (No.4, Whatman®) to separate oil tea seed cake. The extracted oil samples were kept in glass bottles at -20$^{o}$C until analysis.

Antioxidant activity assays
DPPH radical scavenging activity, The antioxidant activity of the tea seed oil samples equivalent to ascorbic acid and Butylated hydroxytoluene (BHT) were analysed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to Chia-Pu et al. (2006) with minor modifications. The appropriate volume of 1 mM ascorbic acid (0.00-0.020 mM) and 5 mM BHT standard solutions (0.00-0.10 mM) or oil samples (100 µl) were added into 400 µl of 1.0 mM DPPH radical solution. It was then diluted with ethanol to 4300 µl and mixed with a vortex shaker and kept in the dark for 30 minutes at room temperature. Decolourization of the DPPH radical solution after react with the antioxidant compounds was measured at 517 nm by a visible spectrophotometer.

ABTS radical scavenging activity
The antioxidant activity of the tea seed oil samples equivalent to Trolox was also measured by the ABTS radical cation decolourization assay according to Chen et al. (Chen et al. 2010) with minor modifications. The appropriate volume of 1 mM Trolox (0.000-0.010 mM) or 60

µl of the oil sample was added into the 3000 µl of $ABTS^{\bullet+}$ radical solution. Then, the mixture was diluted with ethanol to a total volume of 3060 µl and allowed to react in the dark for 25 seconds at room temperature. Finally, the deceasing of absorbance of the $ABTS^{\bullet+}$ radical solution was measured at 734 nm by a visible spectrophotometer (Visible spectrophotometer, Genesis 20, Thermo scientific, USA) against a methanol blank.

**Results and Discussions**

DPPH scavenging activity

The DPPH scavenging activities in terms of milligram antioxidant per gram of oil sample were evaluated by using the standard calibration curves of BHT and ascorbic acid. The R-square ($r^2$) values obtained from the graph were 0.9917 and 0.9917 (Figure 1) for BHT (0.00-0.10 mM) and ascorbic acid (0.00-0.020 mM) standard, respectively. The antioxidant capacities (n=10) of tea seed oil samples planted in the Northern Thailand showed in Table 1 as BHT equivalent were ranged from 11.01 to 12.80 mg/g oil sample, whereas the antioxidant capacity presented in commercial Chinese oil samples were ranged from 13.52 to 14.75 mg/g oil and commercial Thai oil samples were ranged from 13.77 to 15.36 mg/g oil. The antioxidant capacities of the oil samples as ascorbic acid equivalence were ranged from 7.01 to 8.34 mg/g oil, whereas that obtained from commercial Chinese oil samples were ranged from 9.87 to 10.82 mg/g oil and commercial Thai oil samples were ranged from 10.03 to 11.28 mg/g oil.

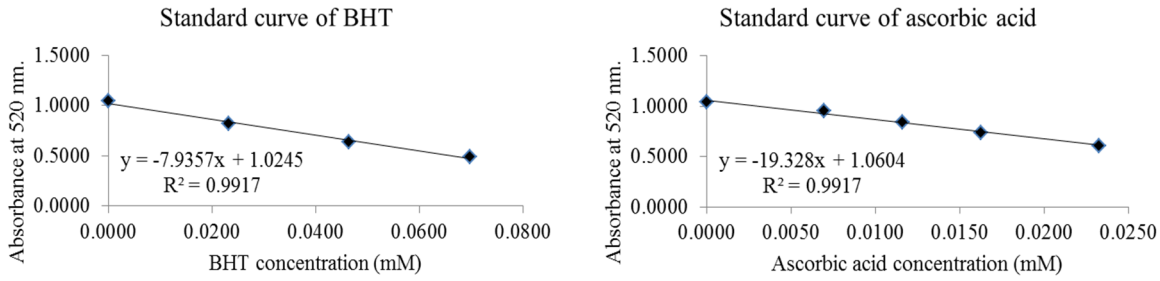


**Figure 1** Standard calibration curve for DPPH assay; BHT standard (0.00-0.10 mM) with *R*-square ($r^2$) of 0.9917 (left), ascorbic acid standard (0.00-0.02 mM) with *R*-square ($r^2$) of 0.9917 (right)

ABTS radical scavenging activity

A standard calibration curve of Trolox (0.000-0.010 mM) used as standard solution for the ABTS radical scavenging assay was shown in Figure 2. It shows good relation with *R*-square ($r^2$) of 0.9995. The Trolox-equivalent antioxidant capacities (TEAC) of oils planted in Thailand were in the range of 5.52 to 8.12 mg/g oil, whereas the antioxidant capacity presented in commercial Chinese oil samples were ranged from 8.33 to 9.42 mg/g oil and commercial Thai oil samples were ranged from 8.00 to 9.37 mg/g oil.

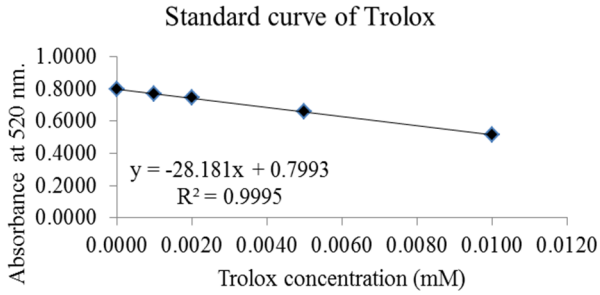


**Figure 2** Standard calibration curve for Trolox scavenging activity (0.00-0.010 mM) with *R*-square ($r^2$) of 0.9995

As shown in Table 1, antioxidant capacity of the tea seed oils planted in the Northern Thailand and commercial Chinese and Thai tea seed oil obtained from each technique were compared and evaluated by one-way analysis of variance, ANOVA at α = .05. Data were reported as the mean and standard deviation. The results were shown in Table 2-4.

**Table 1** Antioxidant capacity of tea seed oil samples obtained from the DPPH scavenging activity assay, ABTS radical scavenging activity assay and the reported fruits antioxidant

| Samples | DPPH assay | | | | ABTS assay | |
|---|---|---|---|---|---|---|
| | BHT equivalence (mg/g oil) | | Ascorbic acid equivalence (mg/g oil) | | Trolox-equivalence (mg/g oil) | |
| | Average | SD | Average | SD | Average | SD |
| S1 | 11.70 | 0.40 | 7.67 | 0.27 | 7.41 | 0.04 |
| S2 | 12.80 | 0.20 | 8.34 | 0.13 | 8.11 | 0.98 |
| S 3 | 12.19 | 0.35 | 7.88 | 0.22 | 5.92 | 0.56 |
| S 4 | 11.59 | 0.61 | 7.43 | 0.41 | 5.52 | 0.01 |
| S 5 | 12.54 | 0.23 | 8.20 | 0.13 | 7.09 | 0.45 |
| S 6 | 12.61 | 1.11 | 8.25 | 0.72 | 6.76 | 0.61 |
| S 7 | 11.01 | 0.34 | 7.01 | 0.22 | 5.89 | 1.22 |
| S 8 | 11.28 | 1.20 | 7.23 | 0.76 | 6.29 | 0.52 |
| S 9 | 12.20 | 0.63 | 7.92 | 0.37 | 6.62 | 0.37 |
| S 10 | 11.62 | 1.13 | 7.46 | 0.73 | 6.22 | 0.80 |
| S 11 | 14.16 | 1.63 | 10.39 | 1.19 | 9.10 | 0.39 |
| S 12 | 14.75 | 1.68 | 10.84 | 1.25 | 9.44 | 0.28 |
| S 13 | 13.52 | 1.92 | 9.87 | 1.41 | 8.33 | 0.48 |
| S 14 | 14.37 | 0.99 | 10.51 | 0.72 | 8.60 | 0.02 |
| S 15 | 13.77 | 1.71 | 10.03 | 1.30 | 8.00 | 1.23 |
| S 16 | 15.36 | 0.89 | 11.28 | 0.66 | 9.37 | 0.32 |

| Fruits** | Total ascorbic acid (dehydroascorbic acid) (mg/g fresh wet weight) |
|---|---|
| Plum | 0.04 |
| Apple (green) | 0.06 |
| Grape (green) | 0.03 |
| Pear | 0.06 |

*S1-S10: Tea oil planted in the Northern Thailand, S11-S14: Commercial Chinese oil, S15-S16: Commercial Thai oil.

* *Total antioxidant, ascorbic acid (dehydroascorbic acid) of aqueous extracts of fruits has been reported by Szeto et al. *British Journal of Nutrition* (2002), **87**, 55-59.

**Table 2** Summary data of one way-ANOVA for DPPH free radical scavenging assay equivalence to BHT

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 21.22 | 2 | 10.62 | 25.98 | .000 |
| Within Groups | 5.31 | 13 | .409 | | |
| Total | 26.53 | 15 | | | |

**Table 3** Summary data of one way-ANOVA for DPPH free radical scavenging assay equivalence to ascorbic acid

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 28.37 | 2 | 14.18 | 58.99 | .000 |
| Within Groups | 3.13 | 13 | .240 | | |
| Total | 31.50 | 15 | | | |

**Table 4** Summary data of one way-ANOVA for ABTS radical cation scavenging assay equivalence to Trolox

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 18.54 | 2 | 9.27 | 16.64 | .000 |
| Within Groups | 7.24 | 13 | .557 | | |
| Total | 25.78 | 15 | | | |

From the Table 2-4, antioxidant capacity obtained from the tea seed oil planted in the Northern Thailand trend to be significant difference with commercial Thai and Chinese tea seed oil ($F_{value}$ *at p; 0.05, df*$_1$*; 2, df*$_2$*; 13*= 3.8). It might cause of additive chemical compounds added to preserve the commercial oils. In addition, antioxidant capacities on gram of aqueous extracts of some fruits fresh wet weight such as, plum, apple, grapes and pear are lower than gram of tea seed oil. Finally, the oil obtained from the tea seed planted in the Northern Thailand has good quality and providing health benefits and showed the antioxidant capacities higher than that some fruits such as plum and grape as reported.

**Conclusion**

The antioxidant capacity of ten tea seed oil samples were extracted from *Camellia oleifera* Abel planted in the Northern Thailand were compared to that of the commercial Chinese and Thai tea seed oils. Ascorbic acid equivalent (AAE) and Butylated hydroxytoluene (BHT) equivalent antioxidant capacity were measured by the DPPH° scavenging assays. Trolox equivalent antioxidant capacity (TEAC) was measured by the $ABTS^{o+}$ radical scavenging. The among all tea seed oil samples presented antioxidant capacity in range of 11.01 to 15.36 mg BHT/ g oil, 7.01 to 11.28 mg AAE/g oil and 5.52 to 9.42 mg TEAC/g oil, respectively. The antioxidant capacities of the oil, tea seed oil planted in the Northern Thailand and commercial Thai and Chinese tea seed oil are difference by one-way ANOVA ($\alpha$ = .05). Therefore, the Northern Thailand tea seed oil might be alternative edible oil for preventing aging disease, chronic disease and cardiovascular disease.

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