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Full Length Research Paper

Antioxidation activity and total phenolic contents of various *Toona sinensis* extracts

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The purpose of this study was to analyze the antioxidation activities and total phenolic contents of *Toona sinensis*. An extractive method of the antioxidant activities of local *T. sinensis* leaf extracts and various antioxidation models were analyzed. The effects of various solvent concentrations to extract the *T. sinensis* leaf on the antioxidation activity were compared. The results showed that the *T. sinensis* leaf extracts of various solvent concentrations had good antioxidant activities. The chelating abilities of ferrous ion in the *T. sinensis* leaf extracts obtained from various solvent concentrations were above 80%. It is concluded that *T. sinensis* leaf extracts could be used as biopreservatives of food products. The total antioxidant activities, chelating abilities of ferrous ions and reducing capacities of the *T. sinensis* leaf extracts might provide a substitute for a natural antioxidant.

Key words: Antioxidative activity, bio-preservatives, extracts, *Toona sinensis*.

INTRODUCTION

Toona sinensis is widely distributed and very popular in vegetarian cuisine in Asia. It is a well-known nutritious food in Chinese society. Natural *T. sinensis* leaves have been used in Chinese medicine for a long time. The edible leaves have been applied as oriental medicine for treating enteritis and dysentery, with no irreversible side effects observed after treatment (Edmonds and Staniforth, 1998). Leaves of *T. sinensis* have anti-inflammatory and improving physical health. In addition, aqueous extracts of *T. sinensis* leaves have been used as herbal medicine for lowering blood glucose associated with diabetes (Hsu et al., 2003). Recently, the crude extracts of *T. sinensis* leaves are shown to possess anticancer and hypoglycemic activity on human tissues (Chang et al., 2002).

Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer activities. Therefore, many plants have been investigated to identify new anticancer compounds. The functions of the aqueous extracts of *T. sinensis* and gallic acid, the natural phenolic components purified from *T. sinensis* extracts, are investigated due to their interesting biological activities. The dying cells show the ultra-structural and biochemical features of characterized apoptosis (Yang et al., 2006).

The pharmaceutical characteristics of *T. sinensis* may be related to their antioxidation abilities. To further search for novel bioactive agents among Meliaceae plants, *T. sinensis* is chosen for phytochemical investigation. Fifteen known compounds including, methyl gallate, gallic acid, kaempferol, quercitin, quercitrin, rutin, catechin, epicatechin, oleic acid, palmitic acid, linoleic acid and linolenic acid, were isolated and identified from this plant (Hsieh et al., 2004). Previous phytochemical components works on *T. sinensis* have isolates of triterpenes and phenolic compounds (Edmonds and Staniforth, 1998). To further the search for novel bioactive agents, *T. sinensis*

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was chosen for phytochemical investigation (Tsai et al., 2001). Although it remains unclear which of the components of *T. sinensis* are active compounds, polyphenols have received increasing attention recently because of some interesting new biological activities. It has been demonstrated that gallic acid in these compounds possesses antioxidant and anticancer activities (López-Vélez et al., 2003). Strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities and inhibitory effects on lipid peroxidation have been demonstrated for methanol extracts of *T. sinensis* (Cho et al., 2003). Lipid oxidation may produce many reactive oxygen species such as free radicals. These free radicals may damage DNA and the membrane lipids of cells. This injury may cause cell injury and accelerate the aging processes. Free radicals may also promote the development of many chronic diseases such as cancer, cardiovascular disease and atherosclerosis (Wong et al., 1999).

Microbial growth and oxidative rancidity are the major issues causing quality deterioration in meat products during shelf life. Biopreservatives can be helpful in extending the shelf life of foods, by eliminating the survival of pathogenic bacteria and increasing the overall quality of food products through inhibition of oxidative rancidity. Some naturally occurring spices have been shown to produce antioxidant and antimicrobial activities in food products (Lambert et al., 2001). Artificial synthetic antioxidants, such as butylated hydroxyanisole (BHA), tertiary butyhydroquinone and butylated hydroxytoluene (BHT) are often utilized in food processing. However, demands for natural antioxidants have been increasing due to concerns about the safety of synthetic antioxidants (Williams et al., 1999; Zhu et al., 2011). Natural antioxidative compounds from plants have aroused more attention, and increasing efforts have been made to search for plant-derived antioxidants (Luo et al., 2002).

Previous studies have used water extract or methanol extractive methods to extract *T. sinensis*. In the study, various concentrations of ethanol were utilized as the extractive method for *T. sinensis* in order to get suitable extracts possessing the best antioxidation. Reduction of chemical preservatives and additives is an important topic related to food preservation. The antioxidation activities of natural *T. sinensis* were analyzed in this study.

MATERIALS AND METHODS

Sample preparation

The young leaves of *T. sinensis* were collected, picked and washed briskly with water to extract the components in summer. Samples of treated leaves were crushed and homogenized in a laboratory blade cutter and then extracted with various aqueous ethanol solutions (0, 20, 40, 60, 80 and 95%). Samples were vortexed (10 min) and then centrifuged at 3000 rpm for 10 min at room temperature. The supernatants were collected and filtered. The young leaves were lyophilized with a freeze dryer (FD-5N, Eyela, Japan) to obtain lyophilized powder.

Measurement of antioxidant activity

The antioxidant activity was measured by the method of Shimoni et al. (1998) with minor modifications. Samples of the aqueous ethanol solutions (100 µl) were added to mixtures containing 2 ml linoleic acid emulsion (pH 6.6). The mixtures were then incubated in the dark at 37°C. The samples were added into 7 ml of 80% ethanol at 0 and 15 h. The linoleic acid oxidation was assayed by absorption of conjugated dienes at 234 nm (U-2000, Hitachi, Japan). The lower absorbance was an indicator of a greater antioxidant activity. BHT was used as the positive group (1.0 mg/ml). A preparation of the linoleic acid emulsion was indicated as follows; a solution of 0.2 M phosphate buffer (pH 6.6) was mixed with 0.28% Tween-20 and 0.28% linoleic acid (Fluka, Buchs, Switzerland).

Antioxidant activity (%) = [1 - (absorbance of samples at 234 nm) / (absorbance of the control group at 234 nm)] × 100%.

Determination of reducing capacity

The reducing capacity was modified according to the method of Yen and Chen (1995). Sample solutions of the aqueous ethanol solutions (100 µl) were added to an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide. The mixed solutions were incubated in a water bath at 50°C for 20 min. An equal volume (phosphate buffer) of 10% trichloroacetic acid was mixed with the sample solutions in order to stop the reaction. The mixtures were then centrifuged at 3000 × *g* for 10 min. The supernatants were added into deionized water and 0.1% iron (III) chloride hexahydrate at a ratio of 1:1:1. The reaction was allowed to proceed for 10 min. The absorbance was measured at 700 nm by a spectrophotometer. The higher absorbance was an indication of a greater reducing capacity.

Analysis of DPPH radical scavenging activity

The analysis of DPPH scavenging activity was performed according to the method of Chung et al. (2002). An aliquot of the sample (800 µl) was added into 200 µl of 1 mM DPPH in ethanol. The mixtures were strongly shaken. These samples were then placed in a dark room for 30 min. The absorbance of the mixtures was spectrophotometrically measured at 517 nm. BHT (1.0 mg/ml) was measured as the positive treatment. The percentage of DPPH radical scavenging activity was expressed as [1 - (absorbance of samples at 517 nm) / (absorbance of the control group at 517 nm)] × 100%.

Determination of chelating ability of ferrous ion

The ferrous chelating ability was analyzed according to the method of Dinis et al. (1994). The ferrous ion was monitored by determining the formation of ferrous iron ferrozine complex. Samples were mixed with 2 mM iron (II) chloride, methanol and 5 mM ferrozine at a ratio of 1:0.1:3.7:0.2. The combination was vigorously shaken and placed at room temperature for 10 min. The absorbance of the mixture was assayed at 562 nm by a spectrophotometer. A lower absorbance was an indication of greater ferrous ion chelating capacity. Ethylenediaminetetraacetic acid (EDTA) was used as the positive group (1.0 mg/ml).

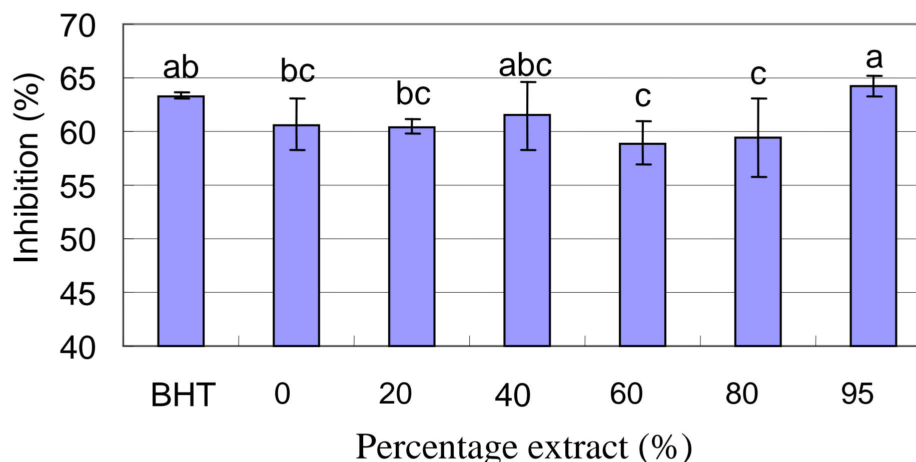

Figure 1. Antioxidative activities of *Toona sinensis* leaf extracts. Vertical bars represent SE, [a-c]: means with the same letter are not significantly different ($p < 0.05$).

Chelating capacity (%) = [1 - (absorbance of sample at 562 nm) / (absorbance of control at 562 nm)] × 100%.

Analysis of total phenol content

The analysis of the total phenol content was analyzed according to the method of Boudhrioua et al. (2009). The total phenols content was determined according to the Folin-Ciocalteu method. Samples of treated leaves were crushed and homogenized in a laboratory blade cutter and then extracted with various aqueous ethanol solutions (25:1, v/w). Samples were vortexed (10 min) and then centrifuged at 3000 rpm for 10 min at 20°C. The extraction was repeated twice and the combined supernatants were collected and filtered. The concentration of total phenols in extracts was measured by UV spectrophotometry, based on colorimetric oxidation/reduction reaction. Folin-Ciocalteu reagent was used as the oxidizing agent. To 0.5 ml of diluted extract, 2.5 ml of Folin-Ciocalteu reagent (Merck, diluted 10 times with water) was added, and 2 ml of Na_2CO_3 (75 g/l) was added. The sample was incubated for 5 min at 50°C and then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. The results were expressed in gram of caffeic acid per 100 g of dry leaves (g caffeic acid/100 g dry matter).

Statistical analysis

The study was executed in triplicate. Data were analyzed using a general linear model in the SAS system (SAS, 2006). The significant differences among samples were analyzed by using Duncan's multiple range tests. The significances are reported at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Measurement of antioxidant activity

The *T. sinensis* extracts had good antioxidant activities in the study (Figure 1). The oxidant activities of linoleic acid were obviously inhibited by the addition of the *T. sinensis* extracts after 15 h of incubation at 37°C. The *T. sinensis* extracts obtained from the various ethanol concentrations were compared with the antioxidant activities of the effective commercial antioxidant BHT. The antioxidant activities of 40 and 95% alcohol extract groups were not significantly different than those of the BHT group. The antioxidant activities among the *T. sinensis* extract groups were not significantly different except for 95% alcohol. The good antioxidant activities of the *T. sinensis* extracts might be related to the potent reducing power, DPPH scavenging ability and ferrous ion chelating abilities.

The *T. sinensis* extracts showed high antioxidant capacity (Cheng et al., 2009). As the main anti-oxidative constituents in the *T. sinensis* are gallic acid and its derivatives, gallotannins and flavonol glycosides might play an important role in the antioxidant activity of this tree vegetable (Wang et al., 2007). The results of this study clearly indicate that the *T. sinensis* extracts and gallic acid have powerful antioxidant activities against various oxidative systems *in vitro*. The various antioxidant properties of the *T. sinensis* extracts and gallic acid may be attributed to their effectiveness as scavengers of superoxide and free radicals, reductive capacity and metal chelating ability (Hseu et al., 2008).

Determination of reducing capacity

The reducing capacity of the *T. sinensis* extracts is given in Figure 2. The yellow color of the sample solution changed into various green and blue colors depending on the reducing capacity of the samples. The existence of reducers caused the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. The formation of the blue color was used to determine the ferrous concentration. The values of the reducing ability of the *T. sinensis* extracts were significantly higher than those of BHT (p <

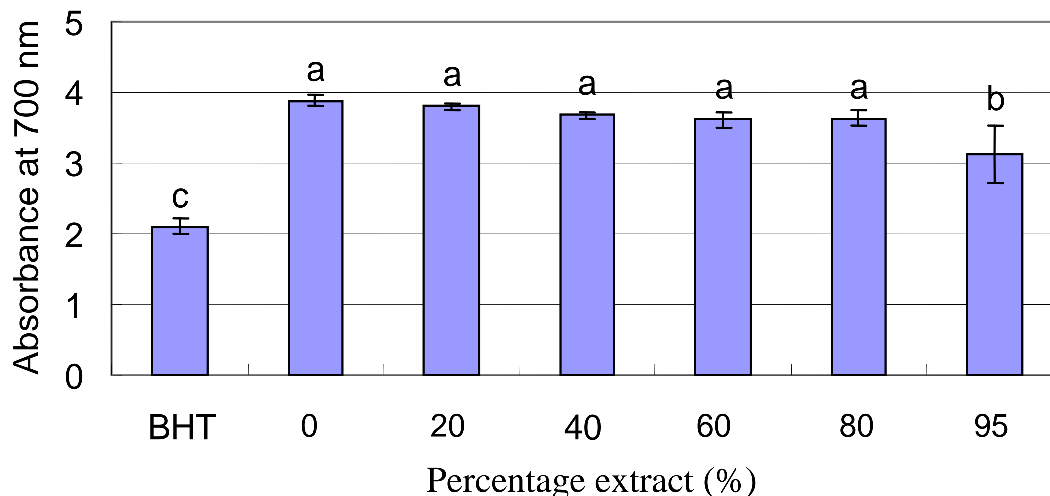

Figure 2. Reducing power of *Toona sinensis* leaf extracts. Vertical bars represent SE, [a-c]: means with the same letter are not significantly different ($p < 0.05$).

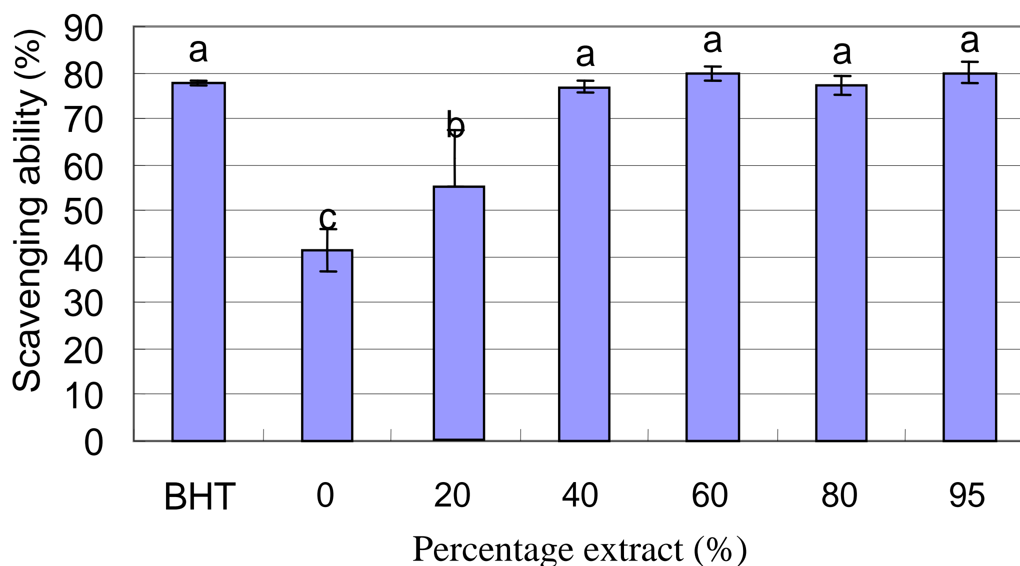

Figure 3. DPPH scavenging ability of *Toona sinensis* leaf extracts. Vertical bars represent SE, [a-c]: means with the same letter are not significantly different ($p < 0.05$).

0.05). The *T. sinensis* extracts had higher contents of total phenol compounds, which might be related to the better reducing capacity because total phenol compounds might be proton donors. The proton donors might stop the reaction chain of free radicals from forming stable products. The reducing power of samples may be due to the hydrogen donating ability (Shimada et al., 1992). The greater reducing power of the *T. sinensis* extracts and gallic acid correlates well with their marked antioxidant action, indicating the possible contribution of reducing power to this activity (Hseu et al., 2008).

Analysis of DPPH radical scavenging activity

The results of the *T. sinensis* extracts ranged from 41.0 to 80.0% of the scavenging activity of the DPPH radicals (Figure 3). The DPPH radical scavenging activities of the *T. sinensis* extracts, 40 to 95% alcohol, were significantly higher than those of 0 and 20% alcohol ($p < 0.05$). The DPPH radical scavenging activities of the *T. sinensis* extracts were alcohol concentration dependent. The DPPH radical scavenging activities of the *T. sinensis* extracts, 40 to 95% alcohol, were as high as those of BHT.

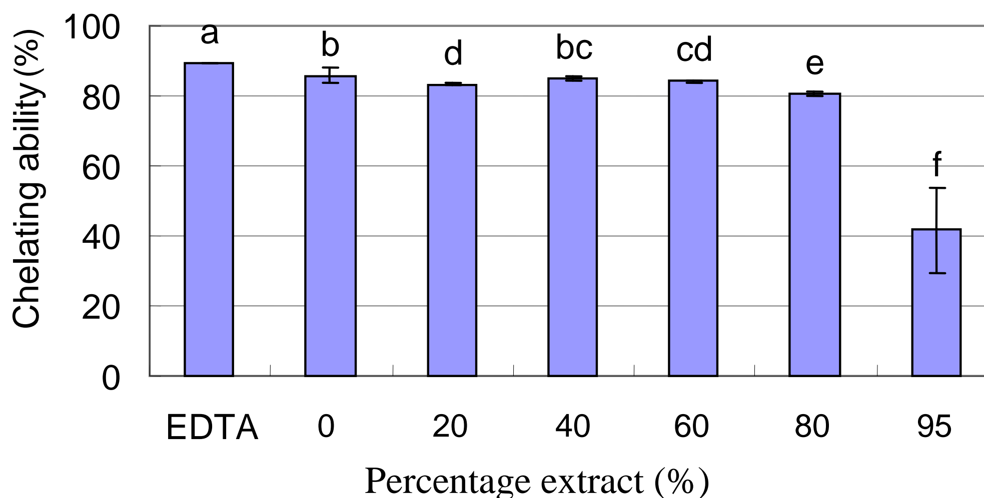

Figure 4. Ferrous ion chelating of *Toona sinensis* leaf extracts. Vertical bars represent SE, [a-f]: means with the same letter are not significantly different ($p < 0.05$).

The radical scavenging activity of the stable radical DPPH has been used before because the method is very uncomplicated, sensitive and fast. DPPH radical scavenging activity offers a very convenient sample screening (Koleva et al., 2000). The antioxidant activities of compounds isolated from *T. sinensis* are determined by DPPH radical-scavenging assay. Most of the isolated compounds exhibited considerable scavenging activity in the DPPH assay. Gallic acid and its derivatives display obvious antioxidant activity in the DPPH assay. Gallic acid is a well-known natural antioxidant found widely in plants (Wang et al., 2007). The DPPH assay of phenolic compounds is known to be related to the number of hydroxyl groups on the aromatic rings and the presence of a second hydroxyl group on the ortho or para position which enhances such activity. The purified compounds show strong activity in the DPPH radical scavenging assay, and thus contribute to the high antioxidant activity of the *T. sinensis* leaf extracts (Cheng et al., 2009).

Chelating ability of ferrous ions

The chelating ability of ferrous ions of the *T. sinensis* extracts showed good chelating abilities of ferrous ion (Figure 4). The results of the chelating abilities of ferrous ions of the *T. sinensis* extracts, 0 to 80% alcohol, ranged from 80.5 to 85.9%. The chelating abilities of ferrous ions of the *T. sinensis* extracts were as high as those of EDTA. The results of this study showed that the total phenol content might play an important role in the chelating ability of ferrous ions. Ferrous ion chelating ability measures the ability of secondary antioxidants to chelate metal ions. Primary antioxidants prevent oxidative damage from directly scavenging free radicals, while secondary antioxidants act indirectly by preventing the formation of free radicals (Chan et al., 2010). Formation of the Fe^{2+}-ferrozine complex is not completed in the presence of the *T. sinensis* extracts which might have higher chelating activities and capture ferrous ions. The absorbance of the Fe^{2+}-ferrozine complex decreases linearly in a dose-dependent fashion (Hseu et al., 2008).

Total phenol contents

Figure 5 showed that the total phenol contents of the *T. sinensis* extracts varied within a narrow range, 6.51 to 6.84 g caffeic acid in 100 g (dry matter). The total phenol contents, and potent antioxidant substances, were easily extracted in the samples. Gallic acid, a polyhydroxyphenolic compound, is one of the major bioactive compounds isolated and purified from *T. sinensis*. It is widely distributed in various plants and fruits (Wolfe et al., 2003). Many of these total phenol compounds exhibit a wide range of biological activities, including antioxidant (Hsieh et al., 2004; Masuda et al., 2010), antibacterial activity (Vaquero et al., 2010), anti-inflammation properties (Muanda et al., 2010) and therapeutic (Chen et al., 2009; Satpathy et al., 2011) properties. Gallic acid and its derivatives display obvious antioxidant activity in the DPPH assay. Gallic acid is a well-known natural antioxidant found widely in plants, and methyl gallate from *T. sinensis* has been reported to have a protective effect against hydrogen peroxide-induced oxidative stress and DNA damage in cell culture (Hsieh et al., 2004). Methyl gallate and other gallates are often used in food products as additives to increase shelf life by delaying lipid peroxidation. Fat oxidation is one of the major problems for deterioration of food products during

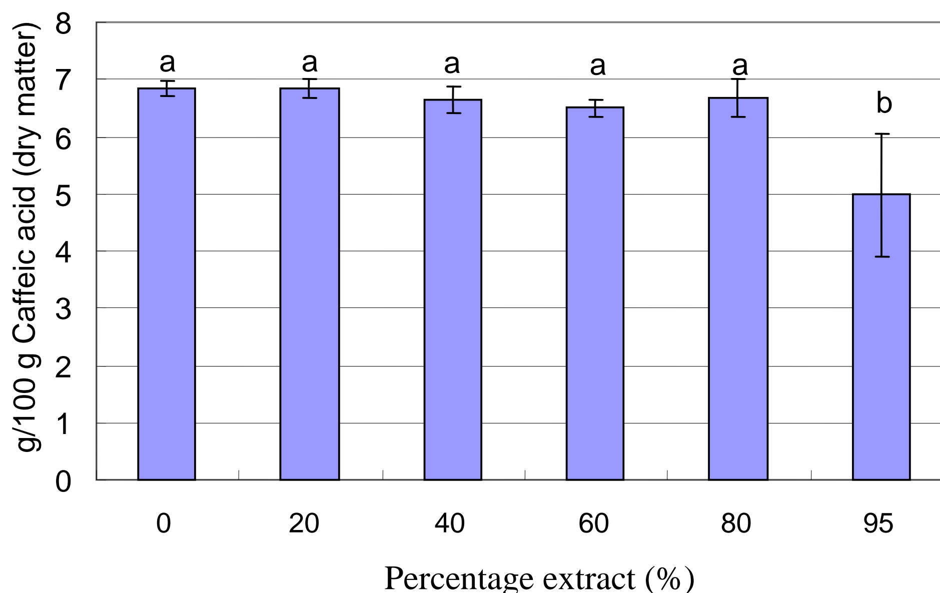

Figure 5. Total phenol content of *Toona sinensis* leaf extracts. Vertical bars represent SE, [a-b]: means with the same letter are not significantly different ($p < 0.05$).

processing and storage (Van der Heijden et al., 1986).

Conclusion

The antioxidative capacities of the *T. sinensis* extracts were assayed in this study. The *T. sinensis* extracts had good antioxidant activities and good reducing abilities as sources of electron donors to stop the reaction chain of free radicals. The scavenging activities of DPPH radicals of the *T. sinensis* extracts might depend on the solvent concentrations. The *T. sinensis* extracts showed good chelating abilities of ferrous ions. In conclusion, the *T. sinensis* extracts serve as a substitute for chemical antioxidant substances due to the good antioxidation ability. Therefore, the *T. sinensis* extracts might have the potential to serve as biopreservatives.

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