Antioxidant Activity and Bioactive Compounds of Tea Seed (Camellia oleifera Abel.) Oil

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The oil of tea seed (Camellia oleifera Abel.) is used extensively in China as cooking oil. The objectives of this study were to investigate the antioxidant activity of tea seed oil and its active compounds. Of the five solvent extracts, methanol extract of tea seed oil exhibited the highest yield and the strongest antioxidant activity as determined by DPPH scavenging activity and Trolox equivalent antioxidant capacity (TEAC). Two peaks separated from the methanol extract by HPLC contributed the most significant antioxidant activity. These two peaks were further identified as sesamin and a novel compound: 2,5-bis-benzo[1,5]dioxol-5-yl-tetrahydro-furo[3,4-d][1,3]dioxine (named compound B) by UV absorption and characterized by MS, IR, 1H NMR, and 13C NMR techniques. Sesamin and compound B decreased H2O2-mediated formation of reactive oxygen species in red blood cells (RBCs), inhibited RBCs hemolysis induced by AAPH, and increased the lag time of conjugated dienes formation in human low-density lipoprotein. The results indicate that both compounds isolated from tea seed oil exhibit remarkable antioxidant activity. Apart from the traditional pharmacological effects of Camellia oleifera, the oil of tea seed may also act as a prophylactic agent to prevent free radical related diseases.

KEYWORDS: Tea seed oil; antioxidant activity; sesamin; LDL; oxidative damage; comet assay

INTRODUCTION

The oil of tea seed (Camellia oleifera Abel.) is used extensively in China as cooking oil (1). It was also traditionally applied as a medicine for stomachache and burning injury in China. The seeds of plants usually contain various antioxidant factors; the naturally occurring active components in tea seed are different from other kinds of oil seeds. Chen et al. (2) indicated that the saponin in tea seed could lower the cholesterol, triglycerides, and low-density-lipoprotein in the blood of rats. Zhang and Zhou (3) found that it has the antioxidant ability to reduce liver reactive oxygen species (ROS) in rats. Some oils, such as olive oil, are rich in health beneficial compounds, except they do not supply energy for humans. The Mediterranean diet could affect several degenerative pathologies, including coronary heart diseases and cancer. In epidemiological and clinical researches, the phenolic compounds of olive oil play an antioxidative role in health (4). Phenolics and flavonoids are major materials of non-nutritive compounds and possess antioxidant, antiinflammatory, antiinflammatory, and anticarcinogenic activities, and reduce atherosclerosis (5). There are many kinds of phenolic compounds in olive oil (6). Hydroxytyrosol and tyrosol, which are major bioactive phenolic compounds of olive oil, appear to account for the important part of this protective effect in vivo (7). Several reports have demonstrated that hydroxytyrosol and tyrosol have remarkably protective effects against oxidative stress-related damages. Ramirez-
from Pharmacia Biotech Co. (Sweden). Hydrogen peroxide was purchased from Hayasahi Co. (Tokyo, Japan).

Preparation of Tea Seed Oil and Extracts. Tea seeds were roasted at 120 °C for 20 min and then pressed by twin screw extruder to obtain the tea seed oil. Tea seed oil (100 g) was extracted with 200 mL each of methanol, ethanol, acetone, ethyl acetate, and acetonitrile, respectively, for 1 h in a shaking incubator at room temperature. The extracts were filtered, and the residue was re-extracted three times under the same conditions. The combined filtrates from each solvent were evaporated under vacuum using a rotary evaporator at 40 °C, and then weighed to determine the extraction yield and stored at −20 °C until use.

DPPH Scavenging Effect of Extracts. The scavenging effect of different solvent extracts of tea seed oil on DPPH radical was estimated according to the method of Duh et al. (10). The extracts were added to a methanolic solution (0.5 mL) of DPPH radical (final concentration of DPPH was 0.2 mmol/L). The mixture was shaken vigorously and kept at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm.

Total Antioxidant Activity Assay (Trolox Equivalent Antioxidant Capacity, TEAC Assay). The total antioxidant activity of tea seed oil extracts was measured using the TEAC assay as described by Yeh and Yen (11). ABTS+ radical cation was generated by the interaction of ABTS (100 μM), H2O2 (50 μM), and peroxidase (4.4 unit/mL). To measure antioxidant capacity, 0.25 mL of different solvent extracts (0.2 mg/mL) of tea seed oil was mixed with an equal volume of 2,2'-azino-bis[3-ethylbenz-thiazoline-6-sulfonic acid] (ABTS), H2O2, peroxidase, and deionized water. Absorbance was monitored at 734 nm for 10 min. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. TEAC value is expressed as millimolar concentration of Trolox solution having the antioxidant equivalent to a 1000 ppm solution of the sample under investigation. The higher TEAC value of the sample means stronger antioxidant ability.

Isolation of Active Compounds from Extracts of Tea Seed Oil. Isolation of active compounds from methanol extract of tea seed oil was performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo, Japan), consisting of a model L-6200 pump, a Rhodyne model 7125 syringe-loading sample injector, a model D-2000 integrator, and a flow rate of 0.8 mL/min. The elution solvents were (A) H2O and (B) MeOH. The solvent gradient elution program used was: 75% A for 20 min; then decreasing to 50% A in 40 min; then decreasing to 10% A in 60 min; then decreasing to 0% A in 65 min, and for 20 min, followed by 5 min isocratic wash at 75% A. A monitoring UV detector (L-4200) was set at 280 nm. Two milliliters of 200 M AAPH in PBS was then added to the mixture. After its isolation, LDL was dialyzed against 0.012 M NaHCO3, mlc/370.355 (calculated for C20H18O7: 370.358). The H NMR spectrum showed the following chemical shifts: δ 6.78 (1H, d, J = 7.8 Hz). 6.84 (1H, dd, J = 1.8 and 7.8 Hz), and 6.88 (1H, d, J = 1.8 Hz); δ 3.09 (3m), 3.84 (dd, J = 4.0 and 9.0 Hz), 4.22 (m), and 4.07 (d, J = 4.0 Hz). The 13C NMR spectrum revealed 15 peaks with the following chemical shifts: δ 93.70 (C-8), 99.82 (C-6), 106.09 (C-10), 115.91 (C-50), 116.12 (C-30), 123.02 (C-10), 123.98 (C-3), 129.00 (C-60), 131.34 (C-20), 154.25 (C-2), 158.38 (C-9), 159.02 (C-1), 163.87 (C-5), 165.07 (C-7), and 181.50 (C-12). The IR spectrum of compound B showed absorptions at 1486.0, 1245.6, 1038.1, and 927.6 cm−1. The Fab-MS of compound B gave an M+1 ion peak at 370, suggesting a molecular weight of 370.3. HRMS,

Assay for Hemolysis of Red Blood Cells (RBCs). The method described by Zhu et al. (12) was used in this study. Blood was obtained from healthy volunteers and collected into heparinized tubes. Erythrocytes were separated from plasma and the buffy coat and washed three times with 5 volumes of phosphate buffered saline, pH 7.4. During each wash, the erythrocytes were centrifuged at 3000g for 10 min to obtain the cell pellet. After the last wash, the packed erythrocytes were suspended in 4 volumes of PBS solution. Oxidative hemolysis of erythrocytes was induced by AAPH, a peroxyl radical initiator. Addition of AAPH to the suspension of diluted erythrocytes induces the oxidation of membrane lipids and proteins, resulting in hemolysis. Two milliliters of the erythrocyte suspension was mixed with 2 mL of PBS solution containing different concentrations of compounds from tea seed oil. Two milliliters of 200 μM AAPH in PBS was then added to the mixture. The reaction mixture was shaken gently while being incubated at 37 °C for 3 h. After incubation, the reaction mixture was removed, diluted with 5 volumes of PBS, and centrifuged at 3000g for 5 min. The absorbance of the supernatant fraction at 540 nm was recorded in a Hitachi U-3000 spectrophotometer. Percent inhibition was calculated by the equation in ref 13.

Intracellular ROS Measurement. Intracellular ROS was estimated by the method of Amer et al. (14) using 2’,7’-dichlorofluorescin diacetate (DCFH-DA) as a fluorescent probe. Erythrocytes were prepared as described above. Cells (106 cells/mL) were collected and resuspended with PBS and seeded in ependroff and incubated with various concentrations of compounds from tea seed oil for 30 min at 37 °C. Erythrocytes were washed with phosphate buffered saline three times again. Finally, the cell pellets were resuspended with PBS and co-incubated with 25 μM DCFH-DA for 30 min. After reaction, cells were collected and washed once with PBS, and the cells were incubated with or without freshly prepared 50 μM H2O2 for 30 min. Erythrocytes were analyzed by a FACScan flow cytometric (Becton-Dickinson Immunocytometry Systems USA, San Jose, CA). Erythrocytes were passed at a rate of about 1000 cells per second, using saline as the sheath fluid. A 488 nm argon laser beam was used for excitation. Green fluorescence of 10,000 gated erythrocytes was then measured using linear amplification.

LDL Isolation from Plasma. Blood from a single healthy volunteer was collected in tubes containing EDTA (1 mg/mL). Plasma was isolated by centrifugation at 3000g for 10 min. LDL was isolated from the plasma by density gradient ultracentrifugation (d = 1.006–1.063) at 10 °C with a Hitachi (Himac CS 120 GX) centrifuge working at 100,000g. After its isolation, LDL was dialyzed against 0.012 M shifts are reported in parts per million (ppm) from tetramethylsilane. Melting points were determined on a Buchi 535 apparatus (Haverhill, MA).

The IR spectrum of compound A showed absorptions at 1493.9, 1243.9, 1038.9, and 927.8 cm−1. The Fab-MS of compound A gave an M+1 ion peak at 354, suggesting a molecular weight of 354.4. The 1H NMR spectrum showed the following chemical shifts: δ 6.78 (1H, d, J = 7.8 Hz), 6.84 (1H, dd, J = 1.8 and 7.8 Hz), and 6.88 (1H, d, J = 1.8 Hz); δ 3.09 (3H), 3.84 (dd, J = 4.0 and 9.0 Hz), 4.22 (m), and 4.07 (d, J = 4.0 Hz). The 13C NMR spectrum revealed 15 peaks with the following chemical shifts: δ 93.70 (C-8), 99.82 (C-6), 106.09 (C-10), 115.91 (C-50), 116.12 (C-30), 123.02 (C-10), 123.98 (C-3), 129.00 (C-60), 131.34 (C-20), 154.25 (C-2), 158.38 (C-9), 159.02 (C-1), 163.87 (C-5), 165.07 (C-7), and 181.50 (C-12). The IR spectrum of compound B showed absorptions at 1486.0, 1245.6, 1038.1, and 927.6 cm−1. The Fab-MS of compound B gave an M+1 ion peak at 370, suggesting a molecular weight of 370.3. HRMS,

CDCl3 and methanol-
phosphate buffered saline (10 mM, pH 7.4) and freshened with N2 at 4 °C. A Bio Rad protein assay solution was used for protein determinations, employing bovine serum albumin as the standard.

**Detection of Conjugated Dienes.** The formation of conjugated dienes was measured by determining the absorbance increase at 234 nm of the solution of LDL (100 μg protein/mL) in PBS incubated with 2.4 mM CuSO4 in the absence or presence of compounds from tea seed oil. The absorbance was measured every 5 min for 250 min using a Hitachi U-3000 UV-vis spectrophotometer, and the results were expressed as relative absorbance at 234 nm. The duration of the lag phase was defined as time (min) to the intercept of the tangent of the absorbance curve in the propagation phase with baseline.

**Effect of Isolated Compounds from Tea Seed Oil on H2O2-Induced DNA Damage.** To evaluate the effect of isolated compounds from tea seed oil on modulating DNA damage in lymphocytes induced by H2O2, the DNA damage was determined using comet assay as described by Yen and Hsieh (15). Human lymphocytes were isolated from fresh whole blood by adding blood to the RPMI 1640 medium, before underlaying it with Histopaque 1077 and centrifuging at 1600 rpm for 10–15 min. Lymphocytes were separated as a pink layer at the top of the Histopaque 1077. The lymphocytes were washed in the RPMI 1640 medium. The cell number and viability (Trypan blue exclusion) were determined with a Neubauer improved haemocytometer before treatment. Human lymphocytes were incubated at a density of 5 × 10⁶/mL, the viability being over 90%.

The cell suspension was incubated with compounds (10–50 μM) and H2O2 (50 μM) at 37 °C for 30 min. After treatment with compounds, the cells were centrifuged and resuspended in preheated 1% low melting point agarose. The cell suspension was then added to Dakin fully frosted microscope slides (Menzel-Glaser, Germany), precoated with 1% normal melting agarose. After application of a third layer of 1% low melting point agarose, the slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauryl sarcosinate, 1% Triton X-100, 10% DMSO, pH 10) for at least 1 h at 4 °C. The microscope slides were then placed in an electrophoresis tank, and the DNA was allowed to unwind for 20 min in freshly prepared alkaline electrophoresis buffer (1 mM EDTA, 0.3 N NaOH, pH 13). Electrophoresis was conducted at 4 °C for 20 min at 25 V and 300 mA. The slides were then neutralized with Tris buffer (0.4 M, pH 7.5) and stained with ethidium bromide. All of the steps were performed under yellow light to prevent additional damage. The slides were observed using a fluorescent microscope attached to a CCD camera connected to a personal computer-based image analysis system (Komet 3.0; Kinetic Imaging Ltd., UK). For each analysis, 30 individual cells were calculated, and, in most cases, three separate experiments were conducted for each series. Single cells were analyzed under the fluorescent microscope as desired. The DNA damage was expressed as % Tail DNA, where % Tail DNA = Tail DNA/(Head DNA + Tail DNA) × 100. A higher % Tail DNA means a higher level of DNA damage.

**Statistical Analysis.** Data presented are given as mean ± SD. Statistical significance was examined through one-way analysis of variance and Duncan’s multiple range tests. Significant differences were accepted at p < 0.05.

**RESULTS AND DISCUSSION**

**Antioxidant Activity of Extracts from Tea Seed Oil.** Comparison of antioxidant activity of tea seed oil extracts from various solvents is shown in Table 1. The yields of solvents extracts from 100 g of oil were in the order of methanol (1.65%) > ethyl acetate (0.73%) > acetone (0.52%) > acetonitrile (0.16%). At the same concentration of 200 μg/mL, the scavenging effects of different extracts on DPPH radical were in the order of methanol (66.50%) > ethyl acetate (59.21%) > acetone (5.92%) > acetonitrile (4.46%). The similar antioxidant activity was also obtained by the TEAC assay. The TEAC value of methanol extract of tea seed oil at 200 μg/mL was stronger than other extracts; it was the equal of 59 μM Trolox. Of the four solvent extracts, the methanol extract exhibited the highest yield and the strongest antioxidant activity; asignificant difference (p < 0.05) was found in antioxidant activity among the four extracts. Therefore, the methanol extract of tea seed oil was used for the following isolation and identification of active compounds. The result was similar to the other studies on olive oil; methanol was usually used to extract bioactive compounds of olive oil (16).

**Isolation of Active Compounds in Tea Seed Oil.** To isolate and identify the active compounds, the methanol extract was separated by means of HPLC. The results showed that there were two major peaks (A and B) in the HPLC profile of METSO with retention times of 66 and 69 min, respectively. Diode array detector set at 280 nm.

**Table 1. Antioxidant Activity and Yield of Extracts from Tea Seed Oil with Various Solvents**

<table>
<thead>
<tr>
<th>solvent</th>
<th>DPPH scavenging effect (%)</th>
<th>TEAC (μM)</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>66.50 ± 1.08 a</td>
<td>59.21 ± 4.72 a</td>
<td>1.65 ± 0.22 a</td>
</tr>
<tr>
<td>acetone</td>
<td>4.46 ± 1.17 b</td>
<td>10.42 ± 3.25 b</td>
<td>0.52 ± 0.12 b</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>5.92 ± 1.62 b</td>
<td>12.53 ± 3.24 b</td>
<td>0.73 ± 0.23 b</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>0.4 ± 0.96 c</td>
<td>2.32 ± 0.75 c</td>
<td>0.16 ± 0.04 c</td>
</tr>
</tbody>
</table>

*The concentration of different solvents extracts used in antioxidant activity assays was 200 μg/mL. Each value is expressed as mean ± SD (n = 3). Values within a column followed by a different letter represent a significant difference at p < 0.05.*

![Figure 1. Typical HPLC profile of methanol extract from tea seed oils.](#)

The retention times of peaks A and B were 66.08 and 69.52 min, respectively. Diode array detector set at 280 nm.

Sesamin and sesamolin are the two major components of sesame oil lignans (18), and both of them have antioxidant and health promoting activities. Nakai et al. (19) noted that the lignans were highly correlated to the antioxidant effect of sesame. According to the chemical structure, the new compound B isolated from tea seed oil is one of the lignans. Among sesamin, sesamolin, and sesaminol, sesamin is the least effective antioxidant, but it can be converted into more antioxidative derivatives. During metabolism in liver, sesamin increased the
hydroxyl groups on the phenyl structure and increased its antioxidant activity (20). Sesamin could protect oxidation, lower cholesterols, and improve lipid metabolism both in vitro and in vivo tests (21). On the other hand, Miyahara et al. (22) found that the sesamin in unbaked sesame could suppress the growth of lymphoid leukemia Molt 4B cells and induce apoptosis.

Inhibition of Hemolysis of RBCs by Sesamin and Compound B. Red blood cells act as an oxygen carrier. They are always exposed to high oxygen tension; therefore, they are easily subjected to oxidation injury by superoxides, H₂O₂, and hydroxyl free radicals in metabolites. Mammalian RBCs are often used as a model system to test the efficacy of antioxidants. Figure 3 shows the inhibitory effect of sesamin and compound B on hemolysis of RBCs induced by AAPH. As the results in Figure 3 show, the inhibitory effect of sesamin on hemolysis showed no dose-dependence at the tested concentrations. However, compound B showed significant (p < 0.05) inhibitory effect at concentrations from 10 to 50 μM. Results also indicated that sesamin and compound B were able to suppress the oxidative injury of RBCs by free radicals up to 49% and 48%, respectively. Manna et al. (23) reported that with olive oil, both in vivo and in vitro, not only the triglycerides but also the phenolic compounds could suppress the hemolysis caused by the oxidative stress. Lipophilic polyphenols, which easily penetrate the cytoplasm of erythrocytes, seem to react with hemoglobin (24). Sesame seeds lignans could increase plasma α-tocopherol and alter plasma tocopherol ratios in humans and enhanced vitamin E bioactivity (25). Thus, sesamin and compound B isolated from tea seed oil may also bind at cell membranes and protect against damage from free radical attack.

Inhibition of the ROS Production in Intracellular RBCs by Sesamin and Compound B. Figure 4 shows the inhibition of sesamin and compound B on H₂O₂-induced ROS in RBCs. Among the test ranges of 10–50 μM, sesamin showed no significant inhibition on ROS formation. In contrast, the inhibitory effect of compound B increased significantly (p < 0.05) from 14% to 86% with increasing concentration. Apparently, the effect of compound B is much higher than that of sesamin, even up to 16 times at 50 μM.

Suppressing ROS could reduce the oxidative damage in cells. Hou et al. (26) reported that sesamin could reduce injuries of the PC12 cell through the decrease of ROS production due to the inhibition of mitochondria activated protein kinases. Zhang and Zhou (3) also found that feeding the tea seed oil to Wistar rats could increase the defense effect by increasing the superoxide dimutase activity of serum and liver. ROS could attack membranes of RBCs and induce cell membrane oxidation. In the present study, compound B showed more inhibitory effect than sesamin on H₂O₂-induced intercellular ROS formation. It is thought that compound B could potentially reduce the oxidative injury by the aerobic metabolism.

Inhibition of LDL Oxidation by Sesamin and Compound B. Figure 5 shows that the formation of LDL conjugated dienes under the induction of copper ion was inhibited by the functional compounds of tea seed oil. Results indicated that the addition of either sesamin or compound B could retard the LDL oxidation. During pretreatment with the isolated compounds of tea seed oil, oxidation inducing time could increase from 38 to 51–89 min with the increasing concentrations of samples. There was no conjugated dienes formation in the group without copper ion. In this test, compound B was obviously more effective than that of the sesamin. The lag time of compound B was prolonged by 9 and 27 min as compared to those of sesamin at concentrations of 10 and 30 μM, respectively.

It is well known that the Mediterranean diet could reduce the risk of cancer and arteriosclerosis, a major reason being that...
bioactive compounds of vegetables and olive oils provide antioxidant effects to protect against the lipid oxidation. In Oriental countries, sesame is considered a healthy food that is rich in sesamin. In the present study, sesamin and compound B could suppress LDL oxidative modification, which is an important marker of arteriosclerosis. Compound B had more protective efficacy than sesamin in the LDL oxidation system. Yamashita et al. (27) found that sesamin could increase the tocotrienol and tocopherol in rat liver, kidney, and blood plasma to increase the antioxidant defensive effect. Sesamin is highly related to the LDL tocopherol content and oxidation inducing time in the presence copper ion (21). In another study, sesamin in vivo could provide more antioxidant effect than in vitro (19). Sesamin and compound B have antioxidant effects in the in vitro system, and we speculate that they may exhibit potential biological and pharmacological activities.

Inhibition of Genotoxic Injury in Human Lymphocytes by Sesamin and Compound B. H$_2$O$_2$ could produce free radicals during metabolism and then attack cell DNA. Figure 6 shows the inhibitory effect of sesamin and compound B on H$_2$O$_2$-induced DNA damage of lymphocytes. The oxidative DNA damage of lymphocytes induced by H$_2$O$_2$ was effectively suppressed by these two compounds from tea seed oil. At concentrations of 10–50 μM, the % tail DNA of sesamin and compound B treated groups was significantly different (p < 0.05) from the control group. Many studies showed that antioxidants could reduce cell and DNA damage from free radicals. Some antioxidants, such as quercetin and caffeic acid, showed efficacious protection using the comet assay for DNA damage (28). A vegetarian diet can prove to have an antioxidant effect, leading to a slight decrease in oxidative DNA damage in lymphocytes (29). Many studies indicated that sesamin did not provide a strong antioxidant effect, but sesamin could inhibit lipid peroxidation in the microsomal enzyme system due to its methylenedioxy groups. Sesamin could be metabolized to monocatechol and di-catechol metabolites by liver enzymes, and changed into more antioxidant metabolites in liver (19). Sesamin can be absorbed from the intestines and reaches the liver via the portal vein where it is metabolized to catechol and enhances antioxidant. Both metabolites had antioxidant activity that means sesamin could be a pro-antioxidant (20). Sesamin also could be absorbed and affected hepatic fatty acid oxidation and synthesis (30). Furthermore, sesamin had antioxidant and anti-inflammatory effects on hepatic ischemia-reperfusion injury in rat animal testing (31). Compound B is similar to sesamin in catechol moiety structure; it may be metabolized to contain catechol structure by liver.

In the present study, our results demonstrated that methanol extract from tea seed oil showed strong DPPH scavenging effect and antioxidant ability. There are two major constituents of lignans, sesamin and compound B, isolated from tea seed oil. By using in vitro experiments, both of the compounds could reduce the oxidative DNA damage. Apart from the traditional pharmacological effects of *Camellia oleifera*, the oil of tea seed may also act as a prophylactic agent to prevent free radical related diseases.

**LITERATURE CITED**


