

Antioxidant and Antimicrobial Activities of *Camellia Oleifera* Seed Oils

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Abstract The antioxidant and antimicrobial activities of *Camellia oleifera* seed oil were studied. Four kinds of seed oil samples were prepared, crude oil and refined oil, extracted by cold pressing method (CPC, CPR), and organic solvent extraction (OSC, OSR). Antioxidant activity analysis was measured in 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt, ferric reducing Ability of Plasma, and 2,2-diphenyl-1-picrylhydrazyl assays. Besides, the percentage of inhibition of red blood cells hemolysis induced by 2,2'-azobis(2-amidnopropane) dihydrochlorid, the lag time of LDL conjugated dienes formation in vitro, and the inhibitors of loss in tryptophan fluorescence were all used to estimate the antioxidant activity of the samples. The total phenolic contents (TPC) were determined by Folin-Ciocalteu method. The TPC of the *C. oleifera* seed oils can be arranged in descending order: CPC (1.9172 µg/mL) > OSC (1.5218 µg/mL) > CPR (1.0611 µg/mL) > OSR (0.6782 µg/mL). And the oils were investigated for activity against *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus niger*. The results showed the antioxidant activity of crude oil by cold pressing method was stronger than others, and all oils did inhibit activity of the top three bacteria expert *A. niger*. The further significance of the study contributes to measure the antioxidant and antimicrobial activity of the potential health benefits by the different methods of preparation and the oil of *C. oleifera* seeds acting as free radical scavenger, pharmaceuticals and preservatives may offer some information in

medicine and cosmetic not just in food field.

Keywords antimicrobial · antioxidant activity · *Camellia oleifera* seed oil · comparison · polyphenols

Introduction

Camellia oleifera Abel. is one of four big woody edible oil source of tree species, others are oil palm, olive and coconut. *C. oleifera* seed oil, the unique woody oil in China, has many functional components and broad market prospect in food, cosmetic and medicine industry (Li et al., 2010). The oil which is rich in monounsaturated fatty acids (MUFA) could postpone sclerosis of arterial congee appearance, prevent or treat cardiovascular diseases, enhance immune system, so that it could act as a kind of antioxidant and functional care oil (Zhou and Wang, 2004). *C. oleifera* seed oil was comparable with the olive oil called the king of the oil. Therefore, it is honoured as Oriental Olive Oil (Long and Wang, 2008).

C. oleifera seed oil contains large amounts of active compounds so that it can act as natural antioxidant. The value of malondialdehyde was significantly lower, and the activity of superoxide dismutase was significantly higher in it in carrageenan-induced rat paw edema model (Lin and Cheng, 2011); it also can scavenge the free radical in rat liver tissue and inhibit lipid peroxidation effectively. The food containing amounts of the MUFA can reduce the blood fat (Liu et al., 2007). Compared with the lard, the antioxidant activity of *C. oleifera* seed oil has better effects in rat (Yin et al., 2009). The seeds contained saponin that could lessen the cholesterols, triglycerides, and low density-lipoprotein (LDL) in the blood of rat (Deng et al., 2012). However, the active compounds of natural sources will display the different antioxidant activities from various kinds of oil seeds. Several compounds had been isolated, such as kaemferol, kaemferol glycosides from *C. oleifera* (Li and Luo, 2003), and triterpene saponins and flavanone glycosides from *C. sinensis* (Yoshikawa et al., 2005; Li et al.,

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2007). Besides, the major fatty acids, oleic and linoleic acid of *C. oleifera* seed oil are very similar to those in olive oil (Ma, 2007). The Mediterranean diet could affect several degenerative pathologies because of eating olive oil. There are also many kinds of phenolic compounds in olive oil (Pirisi et al., 2000). Hydroxytyrosol and tyrosol, which are major two bioactive phenolic compounds of olive oil, seem account for the protective effect in vivo (Vissers et al., 2004). Weinbrenner et al. (2004) have demonstrated that olive oil might efficaciously counteract chemical oxidation of LDL.

Many studies showed that the active compounds of olive oil could be used as a potential antimicrobial agent (Tripoli et al., 2005). One of the most important beneficial effects is the antioxidant activity of the polyphenol components. Therefore, we suppose that *C. oleifera* seeds oils and olive oil has the similar function. In general, in the production of edible oil, natural antioxidants often been greatly damaged, and polar antioxidants are more rare. Oil extraction process on the polar antioxidants in fat content has a decisive impact (Liu and Zhao, 2000). The research reported about it is also not uncommon, for example, sesame oil extraction with a polar extractant receive more favorable oxidation stability (Kamal-Eldin and Appelqvist, 1995) and reducing the water-to-oil process can increase the amount of fat content in polyphenols (Di Giovacchino et al., 1994).

And to date, no attempt has been made to compare antioxidant and antimicrobial activities of *C. oleifera* seeds oils prepared from different extract methods. The objectives of this study were to determine the total phenolics content (TPC), antioxidant and antimicrobial activities of *C. oleifera* seeds oils extracted by cold pressing method and organic solvent extraction. The goal was to compare and try to find the further significance on human healthy.

Materials and Methods

Chemicals. The chemicals and solvents were used to extract and test antioxidant activity, including *n*-hexane, sodium hydroxide, dehydrated ethanol, potassium bromide, sodium chloride, potassium bromide, ethylene diamine tetraacetic acid (EDTA), and other reagents were also analytical grade. 2,2-azobis(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS), ferric reducing Ability of Plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2'-azobis(2-amidnopropane) dihydrochlorid (AAPH) were obtained from Sigma Chemical Co. (USA). *Escherichia coli* BL21, *Bacillus subtilis* B2, *Saccharomyces cerevisiae* AH109 and *Aspergillus niger* XD-1 were obtained from microbiological lab of Sichuan Agricultural University.

Extraction of oils. The seeds of *C. oleifera* from Yáan Sichuan Province, China, were identified by Prof. Chunbang Ding (Sichuan Agricultural University, China). Oils were extracted by cold pressing method and organic solvent extraction process from dried seeds. The squeezer (China) and rotary evaporator (China) were used to extract crude oils. The procedures of deacidification, wash, heat up and filtrate were used to refine oils. There, four

kinds of *C. oleifera* seeds oil extracts including crude oil and refined oil which were extracted by cold pressing method (CPC, CPR), and organic solvent extraction (OSC, OSR).

Determination of TPC. After that, 4 mL of tea oil mixed with 80% ethanol (1:3), then keeping the temperature at 50°C for 30 min in the water bath. The supernatant after centrifuge for 5 min at 5,000 rpm (low temperature speeding centrifuge, China) was taken out. Repeated 3 times, gathered all supernatant and stored at -4°C in the freezer before using. The TPC of the four oil samples, determined photometrically in duplicate for triplicate samples with the Folin-Ciocalteu method (slight modification of scaled down to a 10 mL final volume), was quantified interms of gallic acid ($\mu\text{g/mL}$) measured at 765 nm (Venditii et al., 2010).

Antioxidant activity assay for ABTS. The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay, according to the method of Thaipong et al. (2006) with some modifications. ABTS^{•+} was produced by the stock solution which was dissolved in Phosphate buffered saline (PBS) (0.01 M, pH 7.4). The mixtures were obtained by reacting ABTS^{•+} (final concentration 7 mM) and potassium persulfate (final concentration 2.45 mM), then standing it in the dark for 12–6 h at about 20°C. After that it was diluted by PBS (0.01 M, pH 7.4) and was given an absorbance of 0.7 ± 0.05 at 734 nm at room temperature using the spectrophotometer (UV-1750, Shimadzu, Japan). The calibration curve of Trolox solution with various concentrations was linear between 0.15 and 1.5 mM. Appropriate solvent blanks (without a standard or sample) were also run in each assay which was prepared by PBS. After the addition of 200 μL of stock ABTS solution to 10 μL of sample or Trolox standards in 80% ethanol, absorbance was measured after the initial mixing to react for 2–6 min at 734 nm. The samples were made in triplicate determinations at each dilute standard. The Trolox equivalent antioxidant capacity (TEAC) is defined as the results of samples.

Antioxidant activity assay for FRAP. The FRAP assay was based on the method of Pellegrini et al. (2003) with minor modifications. The fresh FRAP work solutions including 2,4,6-tripyridyl-s-triazine (final concentration 10 mM) solution, HCl (final concentration 40 mM) solution, FeCl₃ (final concentration 20 mM) solution and acetate buffer (final concentration 300 mM), pH 3.6, at 37°C mixed and warmed before using. 20 μL of Trolox solution was linear between 0.15 and 1.5 mM mixed with 500 μL of the FRAP work solution as the calibration curve absorbance was measured at 593 nm. 20 μL of samples was diluted by 80% ethanol to 1 mL and 500 μL of the FRAP work solution were mixed and reacted for 30 min at 37°C, then read the absorbance at 593 nm. The blank was prepared by PBS solution. The samples were made in triplicate determinations at each dilute standard. TEAC was expressed relatively to the linear range of the standard curve. In theory, the bigger FRAP value the stronger reducing capacity, which shows the stronger antioxidant activity.

Antioxidant activity assay for DPPH. The DPPH assay followed the method of Sánchez-Moreno (2002) with minor modifications. The 150 μL of the different sample extracts were added to 0.5 mL of DPPH work solution (final concentration 0.2

mM) which was prepared by methanol solution. The mixture was diluted in 2.5 mL of 80% ethanol, and that was shaken emphatically and kept for 20 min at 27°C before using; the absorbance was then measured by spectrophotometer at 515 nm. 150 µL of Trolox solution was linear between 0 and 1.0 mM mixed with 3 mL of the DPPH work solution as the calibration curve absorbance was measured at 515 nm. Based on the calibration curve, we could calculate the antioxidant capacity of all samples.

Antioxidant activity assay for hemolysis of red blood cells (RBCs) in vitro. The assay for method was described by Zhu et al. (2002) with some modifications we used. Blood was collected from healthy volunteers for sodium citrate tubes. In order to wipe off the plasma and the buffy coat from the erythrocytes, blood was centrifuged at 5,000 rpm for 30 min at 4°C, and was added the same volume of 150 mM physiological saline to wash three times at 3,000 rpm for 10 min at 4°C. After each centrifuge, the supernatant was separated from the erythrocytes should be discarded. A peroxy radical initiator called AAPH can induce oxidative hemolysis of erythrocytes. 0.5 mL of the erythrocyte suspension and 0.5 mL of different samples were mixed with 0.5 mL of AAPH solution. The reaction mixture was shaken gently while being incubated at 37°C for 1 h. After incubation, the reaction mixture was diluted with 8 volumes of distilled water or 80% ethanol, and centrifuged at 3,550 rpm for 10 min. The absorbance of the supernatant fraction was read at 540 nm. Percent inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = (A_{\text{water}} - A_{\text{ethanol}}) / A_{\text{water}}$$

Where A_{water} is the absorbance of the sample containing the distilled water and A_{ethanol} is the absorbance of the sample containing 80% ethanol.

LDL isolation and oxidation. The method described by He and Liu (2002) was used in this study with some modifications. Blood was obtained from a single healthy volunteer and collected into sodium citrate tubes. Plasma was isolated by centrifugation at 5,000 rpm for 30 min at 4°C. The serum was obtained, and was adjusted density of serum to 1.300 g/mL using the dry solid potassium bromide (KBr). The equation calculate the weight of KBr is $X = V_o (dm - d_o) / (1 - 0.312 \times dm)$ [where X is the weight of KBr, V_o is volumes of original serum, dm is 1.300 g/mL, 0.312 (mL/g) is specific volume of KBr]. After KBr dissolution completely, about 3 volumes of NaCl (density=1.006 g/mL) was added into the mixture along the tube wall slowly and carefully not confuse the interface. Then, the mixture was centrifugalized at 50,000 rpm for 5 h at 10°C. Later, the supernatant were separated into several layers which contained the LDL. In order to isolate the pure LDL, the mixture was putted into the dialysis tube, and dialyzed for 48 h at 4°C repeatedly. The dialysis fluid included NaCl (0.15 mol/L) and buffer fluid of EDTA (0.3 mM) (pH 7.4). After dialysis, the protein concentration was isolated.

To determine the effects of oil samples on the inhibition of LDL oxidation, the formation of conjugated dienes (a measure of lipid oxidation) was measured by determining the absorbance increase and tryptophan fluorescence (a measure of protein oxidation) were

monitored in the presence and absence of oil samples. 0.02 mL of the solution of LDL (100 µL protein/ml), 0.01 mL of oil samples, and 0.16 mL of 80% ethanol were mixed. The final volume was adjusted to reach 0.2 mL, 0.01 mL of 0.1 mM Cu^{2+} (final concentration 5 µM) was added and initiated the oxidation reaction. Conjugated dienes were monitored for 225 min at 37°C at 234 nm by spectrophotometer. The duration of the lag phase was then determined graphically. For monitoring tryptophan fluorescence, the method described of Ivanov et al. (2001) was used. To 0.21 mL of the solution of LDL (100 µL protein/mL), 0.3 mL of oil samples, and 2.34 mL of 80% ethanol was added. The final volume was adjusted to reach 3 mL, 0.15 mL of 0.1 mM Cu^{2+} (final concentration 5 µM) was added and initiated the oxidation reaction. Tryptophan fluorescence (280 nm excitation/331 nm emission) was monitored for 1 h at 37°C by fluorophotometer (RF-5301 PC, Shimadzu). The unexposed control's initial fluorescence as a percentage expressed the residual tryptophan fluorescence.

Assessment of inhibition of bacterial growth. The four bacterial strains, *E. coli*, *B. subtilis*, *S. cerevisiae* and *A. niger* were used to assess the antibacterial properties of the oils, using an agar dilution method. Each kind of oil was diluted by ethanol to five levels (0.1 mL mixed with 0 mL ethanol, 0.2 mL ethanol, 0.4 mL ethanol, 0.6 mL ethanol, 0.9 mL ethanol, respectively). The spread plate and filter paper methods were applied in the study. After training in specific time and temperature, zone of inhibition was measured for assessment of inhibition of bacterial growth, namely the evaluation of antimicrobial activity of *C. oleifera* seed oils.

Statistical analysis. The data of antioxidant activity by the ABTS, FRAP, and DPPH assays, were given as mean \pm standard deviation (SD). One-way analysis of variance with Duncan's multiple range tests are used to examine statistical significance by SPSS, where p values are <0.05 .

Results and Discussion

Antioxidant activity and total phenolic content. The results of TPC and Duncan for antioxidant activity by the ABTS, FRAP, and DPPH assays based on different oil samples were showed in Table 1. There were good linear relations with Trolox in the three assays. There was a wide variation in the TPC of *C. oleifera* seed oils ranging from 0.6782–1.9172 µg/mL. The TPC of *C. oleifera* seed oils under investigation can be arranged in descending order: CPC (1.9172 µg/mL) > OSC (1.5218 µg/mL) > CPR (1.0611 µg/mL) > OSR (0.6782 µg/mL). In the study, the relationship between antioxidant activity and TPC were investigated.

ABTS radical cation scavenging activity. The TEAC assay has been criticised as ABTS which is not a physiological radical source and thus may not accurately represent in vivo effects (Prior et al., 2005). For the assay, four oil samples on ABTS radical cation scavenging activities are presented in Fig. 1. In contrast, antioxidant activity was measured following the order: CPC (0.8172) > OSC (0.4667) > CPR (0.2468) > OSR (0.1692). The extensive investigations on antioxidant and antiradical activities of small phenolics,

Table 1 The contents of total phenolic (TP) and duncan^a for antioxidant activity by the ABRS, FRAP, and DPPH assay based on different oil extracts of CPC, CPR, OSC, and OSR (mean \pm SD, $p < 0.05$, N=3)

Type	Antioxidant activity (mM TE/g FM)			TP contents ($\mu\text{g/mL}$)
	ABTS	FRAP	DPPH	
CPC	0.8172 \pm 0.4496	0.2213 \pm 0.0225	0.0930 \pm 0.0187	1.9172
CPR	0.2468 \pm 0.0434	0.1511 \pm 0.0080	0.0700 \pm 0.0567	1.0611
OSC	0.4667 \pm 0.1308	0.1935 \pm 0.0233	0.0888 \pm 0.0258	1.5218
OSR	0.1692 \pm 0.0751	0.1046 \pm 0.0340	0.0536 \pm 0.0195	0.6782
Assay mean	0.4250 \pm 0.2903 ^a	0.1676 \pm 0.0510 ^{ab}	0.0764 \pm 0.0181 ^b	

^{a-c}Means within a column followed by different letters are significantly different, $p < 0.05$.

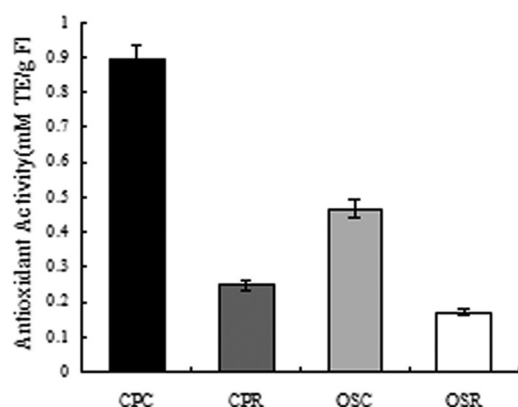


Fig. 1 Antioxidant activity (expressed as mM trolox equivalent antioxidant capacity) of CPC, CPR and OSC, OSR, determined using the ABTS assay.

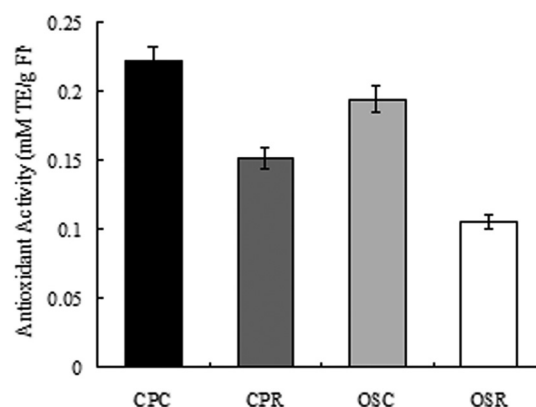


Fig. 2 Antioxidant activity (expressed as mM trolox equivalent antioxidant capacity) of CPC, CPR and OSC, OSR, determined using the FRAP assay.

including flavonoids and phenolic acids have been reported (Heim et al., 2002). Combine antioxidant activity with TPC, CPC exhibited the highest TPC and showed strongest antioxidant activity. The two orders were consistent. Apart from these, the high molecular weight phenolics (tannins) have more ability to extinguish free radicals (ABTS^{•+}) and their effectiveness rest with the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution because of the specific functional groups had been reported (Prior et al., 2005). The presence of high molecular phenolics such as pelargonin, catechins and leucopelargonin derivatives in addition to the flavonoids might influence free radical (ABTS^{•+}) scavenging activity of *C. sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L. (Manian et al., 2008). **FRAP reducing power assay.** On the basic of FRAP assay, as the concentration of the active substance in the sample extract get more concentrated, there the antioxidant capacity becoming stronger (Mao et al., 2009). And, the antioxidant activity of synthetic antioxidants measured by the FRAP assay as reported by Hossain et al. (2008), compared to the antioxidant activity of oil samples examined in this study, the results about strength of peroxy radical scavenging activity were showed in Fig. 2, following the order: CPC (0.2212) > OSC (0.1937) > CPR (0.1514) > OSR (0.1052). Combine antioxidant activity with TPC, the orders of results were showed similarly like in ABTS assay.

DPPH radical scavenging activity. The DPPH radical has been

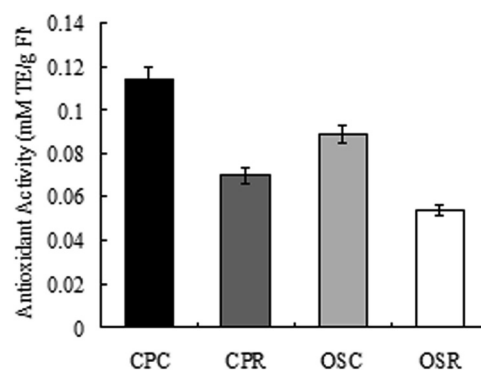


Fig. 3 Antioxidant activity (expressed as mM trolox equivalent antioxidant capacity) of CPC, CPR and OSC, OSR, determined using the DPPH assay.

widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods. Stronger scavenging effects were observed with increasing concentrations used in the test (Chen et al., 2011). In this study, the order of scavenging activity of samples is CPC (0.1138) > OSC (0.08882) > CPR (0.07005) > OSR (0.05379) showing in Fig. 3. Interesting, the sample with higher TPC showed the stronger antioxidant activity like the forenamed assays. The strong DPPH scavenging activity of tea

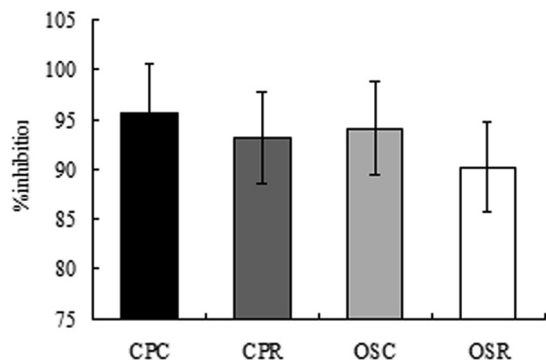


Fig. 4 Effects of CPC, CPR and OSC, OSR on AAPH-induced hemolysis of human erythrocytes.

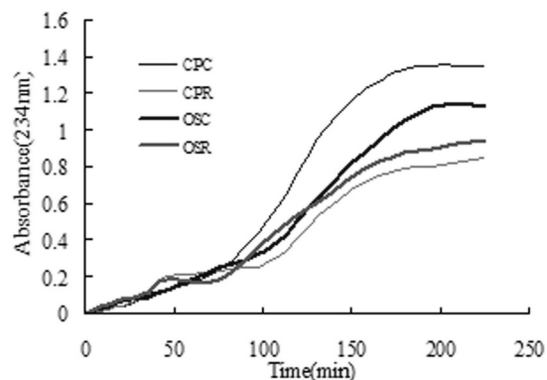


Fig. 5 Effects of the sample CPC, CPR and OSC, OSR on Cu²⁺-mediated conjugated dienes formation in LDL.

could be ascribed to part of the tea catechins and some low molecular polyphenols (Zhu et al., 2002). In the present study and the theory, this radical scavenging activity of oil samples could be related to the nature of phenolics, hence contributing to their electron transfer (hydrogen donating) ability.

Inhibition of hemolysis of RBCs. RBCs act as an oxygen carrier. Especially in mammalian, their RBCs were used to test the efficacy of antioxidants. They are easily subjected to oxidation injury in metabolites because their high oxygen tension. Lipophilic polyphenols, which easily penetrated the cytoplasm of erythrocytes, seemed to react with hemoglobin (Kitagawa et al., 2004). Also, sesamin and compound B were isolated from tea seed oil may also bind at cell membranes and protect against damage from free radical attack (Lee and Yen, 2006). Therefore, here, the inhibitory effect of oil samples on hemolysis of RBCs induced by AAPH is showed in Fig. 4. As the results, the inhibitory effect flows the order: CPC (95.66%) > OSC (94.07%) > CPR (93.13%) > OSR (90.18%). However, these results showed high inhibitory effect but no significant different among the four samples. Well, CPC got the champion again from the TPC and inhibition of hemolysis of RBCs. There were similar situations like that three fractions of oolong tea were more effective object to lipid oxidation in the erythrocytes membranes and fractions were also proved to have dose-dependent inhibition effects toward RBCs hemolysis were reported by Zhu et al. (2002). Antioxidant properties of the phytochemicals were evaluated by is a more sensitive system based on more over the RBCs hemolysis. In likewise, activities were nearly similar to caffeic acid of *Oudneya africana*, *Artemisia arboresens* and *Globularia alpyum* whose also observed to inhibit radical-induced RBCs hemolysis in a highly significant efficiency (Djeridane et al., 2007). Lipophilic polyphenols could easily penetrate the cytoplasm of erythrocytes, appear to react with hemoglobin (Kitagawa et al., 2004). Thus, the *C. oleifera* seeds oils may protect against damage from free radical attack.

Inhibition of LDL oxidation. Under the induction of copper ion, the inhibition of formation of LDL conjugated dienes by the oil samples is showed in Fig. 5. Oxidation inducing time could be increased over 225 min with the attention of samples. In this test, CPC was more effective than the other samples obviously. In the

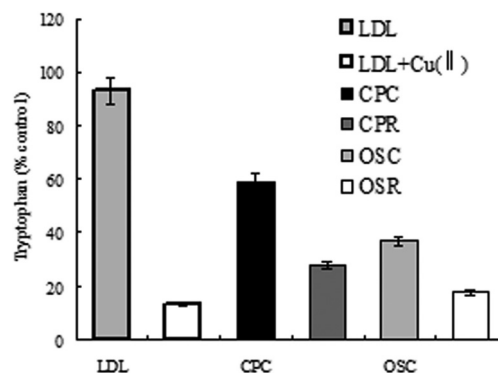


Fig. 6 Effects of CPC, CPR and OSC, OSR, shown as percentage of residual tryptophan fluorescence in LDL incubated in vitro in relation to the initial fluorescence of the control.

presence of antioxidants, LDL oxidation is delayed, as characterised by the increasing in lag time (Venditti et al., 2010). The lag times were obtained from monitoring conjugated diene formation in the oil samples. The lag time of CPC and CPR was prolonged by 16.98 and 8.64 min, OSC and OSR was compared as 15.47 and 7.80 min, respectively.

Furthermore, with regard to the absence of tryptophan fluorescence because of protein oxidation, one can be observed in Fig. 6. There was a prominent decrease in tryptophan fluorescence in oxidised LDL in the loss of oil sample compared to the control, and LDL was oxidised with copper. All oil samples significantly suppressed this loss in fluorescence with respect to the oxidised LDL. The CPC (59%) suppressed the significant differences of loss in tryptophan fluorescence to a greatest extent. Interesting, order of the tryptophan (% control) showed similarly in the all forenamed assays. We should carry on furthermore study.

When measuring antioxidant activity in this way in vitro, the interesting finding may have some important implications as if it were to hold true for LDL oxidation in vivo, since one of the steps involved in atherogenesis thought to be oxidation of LDL in the vessel wall (Steinberg, 2009). Furthermore, the presence of tea components would likely protect the protein portion when in the

Table 2 Comparison of antioxidant activity of *C. oleifera* seed oils

Sample	ABTS	FRAP	DPPH	hemolysis of RBCs	lag time of LDL oxidised	tryptophan (%)
CPC vs. OSC	>	>	>	>	>	>
CPR vs. OSR	>	>	>	>	>	>
CPC vs. CPR	>	>	>	>	>	>
OSC vs. OSR	>	>	>	>	>	>

> the former sample has stronger antioxidant activity, < the later sample has stronger antioxidant activity.

vicinity of an LDL particle probably more efficiently than the lipid portion (Venditti et al., 2010). Besides, the presence of free metal ions mediated LDL oxidation in the arterial wall and flavonoids are strong chelators of free metal ions was evidenced (Kaiserová et al., 2007). For this reason, it was interested to determine whether tryptophan (% control) of the different oils could be affected by cold pressing or organic solvent extraction. **Comparison of antioxidant activity of *C. oleifera* seed oils.** The two extraction methods avoided high temperature to damage the oil quality, because heat can be the radical initiator, promoting free radicals producing, speeding up the oxidation reaction, and

decomposing or polymerizing the hydroperoxide (Wu et al., 2008). Cold pressing method can keep the natural characteristics and something like VE, γ -linolenic acid physiological activators in oil, also the oil with some antioxidant activities from the cold pressing contained the a bit of phosphorus and free aliphatic acid (Liu and Chen, 2011). Organic solvent extraction process extract oil thoroughly in contrast with the cold pressing, therefore it is rich in active substance, too. In this study, comparison the antioxidant activity of oil extracts between cold pressing method and organic solvent extraction process which is the stronger. The data is shown in Table 2. Here, from the CPC vs. OSC, CPR vs. OSR, combine the six results with the TPC, we could judge out the strongest antioxidant activity of oil extracted by cold pressing method.

However, before refined, the oil is called crude oil. There is much more substance in the crude oil. Refining techniques contain physical method and chemical method. In this experiment, the latter was used. The main purpose of refining is removing something like glue, acid, stink crude oil, and promoting character of the oil (Li et al., 2010). Though the refined oil became more stable and tasty, it also had less substance which maybe influences its antioxidant activity (Liu and Zhu, 2011). The purpose was comparison of antioxidant activity of two oils is the same or not from the same extraction method. The data is shown in Table 2. Here, from the CPC vs. CPR, OSC vs. OSR, in contrast, it is obviously that the antioxidant activity of crude oil is higher than refined oil, no matter the oil from cold pressing or organic solvent extraction.

Comparison of antimicrobial activity of *C. oleifera* seed oils. The data is showed in Table 3. Comparing the four bacteria studied, it is clear that the strain *S. cerevisiae* is most sensitive, showing the most inhibition in the presence of the oils. The worst sensitive strain was *A. niger*, just the undiluted oil had an inhibitory effect against this strain. In contrast *E. coli* and *B. subtilis* were only inhibited for some concentrations of the oil samples studied. In these studies, the CPC and OSR had more effects to inhibit bacteria. The further comparison of the data obtained in this study with results is problematic. The minimum inhibitory concentrations could not be ensured in a wide of concentrations levels. It is not clear what compounds of oil have such a wide antimicrobial activity, not just the polyphenolic compounds (Tripoli et al., 2005). We should make much further studies to indicate the phenomenon.

Table 3 Effect of oil samples on antimicrobial activity

Sample	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. cerevisiae</i>	<i>A. niger</i>
CPC				
0.1 mL CPC	+	+	+	+
+0.2 mL ethanol	+	+	+	-
+0.4 mL ethanol	+	+	+	-
+0.6 mL ethanol	+	+	+	-
+0.9 mL ethanol	+	+	+	-
CPR				
0.1 mL CPC	+	+	+	+
+0.2 mL ethanol	+	+	+	-
+0.4 mL ethanol	+	+	+	-
+0.6 mL ethanol	+	-	+	-
+0.9 mL ethanol	+	-	+	-
OSC				
0.1 mL CPC	+	+	+	+
+0.2 mL ethanol	+	+	+	-
+0.4 mL ethanol	+	+	+	-
+0.6 mL ethanol	-	+	+	-
+0.9 mL ethanol	-	+	+	-
OSR				
0.1 mL CPC	+	+	+	+
+0.2 mL ethanol	+	+	+	-
+0.4 mL ethanol	+	+	+	-
+0.6 mL ethanol	+	+	+	-
+0.9 mL ethanol	+	+	+	-
Control	-	-	-	-

Where the + is inhibition effect on antimicrobial, — is no inhibition effect on antimicrobial.

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