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Antioxidant activity of ethanolic extract of *Cortex fraxini* and use in peanut oil

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Abstract

Cortex fraxini was extracted with 95% ethanol to obtain a crude antioxidant extract. The antioxidant activity was evaluated using the linoleic acid peroxidation method and the free radical scavenging assays, namely 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. *Cortex fraxini* extract (CFE) showed high inhibition of peroxidation of linoleic acid when compared with butylated hydroxytoluene (BHT). CFE also exhibited excellent scavenging activity on DPPH and hydroxyl radicals. Total antioxidant activity was measured by the reduction of Mo(VI) to Mo(V) by the extract, and subsequent formation of a green phosphate/Mo(V) complex at acid pH. CFE had significant total antioxidant activity and the effects were increased with increasing reaction time. The total phenolic content of the sample, analyzed by using Folin–Ciocalteu's reagent, was 91.33 mg/g dry weight expressed as pyrocatechol equivalents. Then the suitability of CFE as an antioxidant was determined in peanut oil, and the decrease of lipid oxidation was monitored using thiobarbituric acid-reactive substances (TBARS) assay. CFE treatment significantly ($P < 0.05$) reduced lipid oxidation in peanut oil compared to the control. No significant differences ($P = 0.05$) in lipid oxidation were detected between CFE antioxidant and BHT antioxidant samples.

Keywords: *Cortex fraxini*; Antioxidant activity; Natural antioxidant; Peanut oil

1. Introduction

Lipid peroxidation is well known to be among the main causes of deterioration during the storage and processing of foods, because it can lead to the development of unpleasant rancid or off flavours as well as nutritional loss and formation of potentially toxic end-products. Therefore, it is obvious that the prevention of lipid peroxidation in the food is effective not only in the stability of the nutritional content but also the extension of the best-before date. Therefore, much attention has been paid to the antioxidants, which are expected to prevent food and living systems from peroxidative damage. A number of synthetic antioxidant, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), have been added to foodstuffs (Valentaõ et al., 2002). Although these synthetic antioxidants are efficient and relatively cheap, there are some disadvantages when used, because they are suspected of having some toxic properties. Therefore, search for natural antioxidants has received much attention and efforts have been made to identify natural compounds that can act as suitable antioxidants to replace synthetic ones (Pan, Liang, Wang, & Liang, 2004). In the past few years, several herbs and spices such as black papper, propolis (Dessouki, El-Dashlouty, El-Ebzary, & Heikal, 1980), rosemary (Cuvelier, Berset, & Richard, 1994) and oriental herbs (Kim, Kim, Kim, Oh, & Jung, 1994) have been reported to provide significant protection to freshly cooked

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meat and were effective in retarding lipid oxidation. Antioxidant properties of herbs and spices are apparently related to their phenolic content, suggesting that their antioxidant action is similar to that of synthetic phenolic antioxidants (Lai et al., 1991). The antioxidant activity of these phenolics is mainly due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors and free radical quenchers (Shahidi & Wanasundara, 1992).

In recent years, the mechanisms of actions of natural products from traditional Chinese herbal medicines have been the focus of interest for their antioxidant activities. What may be learned from nature could help to overcome the toxicity issues of synthetic antioxidant compounds, and to develop new antioxidants. *Cortex fraxini*, commonly used Chinese herbal medicine, has proven to be effective in the treatment of diarrhea and dysentery of intense heat type, especially for dysentery with blood stools, and lung heat syndrome with cough and dyspnea (Xiao, Li, & Yang, 2002). It is widely distributed throughout the north of china, which is officially listed in the Chinese Pharmacopoeia. Fraxin, esculin and esculetin are coumarin derivatives and are the most important bioactive components in *Cortex fraxini*. They have multiple biological activities including inhibition of xanthine oxidase activity, antiradical activity, antitumor activity and an inhibitory effect on the growth of human breast cancer cells (Li et al., 2005). However, little or no work has been done to study the effect of the *Cortex fraxini* itself as a natural antioxidant.

The present study was undertaken to investigate the antioxidant properties of CFE in order to examine its potential as a substitute for synthetic antioxidants. The antioxidant effect of CFE was evaluated by using radical scavenging method and linoleic acid peroxidation method, and quantifying the total phenolic compounds present in CFE. Subsequently, the suitability of CFE as an antioxidant was also determined in peanut oil.

2. Materials and methods

2.1. Materials

Folin–Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), synthetic antioxidant butylated hydroxytoluene (BHT), linoleic acid, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and deoxyribose (DR) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Ferrous chloride, ascorbic acid, ammonium thiocyanate, polyoxyethylenesorbitan monolaurate (Tween-20), lithium sulphate, ammonium molybdate, hydrogen peroxide (H_2O_2), ethylenediaminetetraacetic acid (EDTA) and potassium ferricyanide were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade.

The peanut oil, which was stripped, was bought from Beijing Chemical Company (Beijing, China). It contained very low α-tocopherol (2.0 mg kg^{-1}) and no synthetic antioxidants.

2.2. Equipment and apparatus

The following instruments were used: UV–1100 spectrophotometer (Beijing Rayleigh Analytical Instrument Corporation, Beijing, China); RE-52AA rotavapour (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China); DZF-1B vacuum drier (Shanghai Yuejin Medical Instrument Co., Ltd., Shanghai, China); SHB-βA water-circulation multifunction vacuum pump (Zhengzhou Great Wall Scientific Industry and Trade Co., Ltd., Zhengzhou, China).

2.3. Preparation of Cortex fraxini extract (CFE)

Cortex fraxini was obtained from Guilin Pharmaceuticals Group of China (Zhongshan Road, Guilin City, China). *Cortex fraxini* was ground (max particle size 0.4 mm) and 50 g of ground material were extracted with 500 ml 95% ethanol in a Soxhlet apparatus over a 2 h period. Solvent was evaporated using a RE-52AA rotavapour at 60 °C and a SHB-βA water-circulation multifunction vacuum pump. *Cortex fraxini* extract (CFE) was finally dried in a DZF-1B vacuum drier at 30 °C and 0.07 MPa. Dry extract was stored in a freezer until use. The yield of CFE was 5.61%.

2.4. Linoleic acid emulsion system–thiocyanate assay

The antioxidant activity of CFE on inhibition of linoleic acid peroxidation was assayed by using the thiocyanate method (Haraguchi, Hashimoto, & Yagi, 1992; Osawa & Namiki, 1981; Yen & Hsieh, 1998). CFE solution (0.1 ml) in 95% ethanol at different concentrations (0.05, 0.10, 0.15, 0.20, and 0.25 mg/ml) was mixed with 2.5 ml of linoleic acid emulsion (0.2 M, pH 7.0) and 2 ml of phosphate buffer (0.2 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween-20 as emulsifier and 50 ml phosphate buffer, and then the mixture was homogenized. The reaction mixture was incubated at 37 °C to accelerate the oxidation process and used each 24 h for assessing antioxidation activity. The mixture without added extract was used as a control. The mixture (0.1 ml) was taken and mixed with 5.0 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature. Precisely 3 min after the addition of ferrous chloride to the reaction mixture, the absorbance at 500 nm was read. The antioxidant activity was expressed as a percentage of inhibition of peroxidation (IP%): $IP\% = [1-(\text{absorbance of sample at } 500\text{ nm})/(\text{absorbance of control at } 500\text{ nm})] \times 100$. The antioxidant activity of BHT (0.15 mg/ml) was also assayed for comparison purposes. All tests were performed in triplicate and mean were centred.

2.5. Scavenging activity on DPPH radical

To evaluate the free radical scavenging activity, CFE was allowed to react with a stable free radical, 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) (Blois, 1958; Brand, Cuvelier, & Berset, 1995; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998). CFE solution (0.2 ml) in 95% ethanol at different concentrations (0.2, 0.5, 0.8, and 1.2 mg/ml) was added to 8 ml 0.004% (w/v) solution of DPPH in 95% ethanol. The reaction mixture was incubated at 28 °C. The scavenging activity on DPPH radical was determined by measuring the absorbance at 515 nm each 10 min until the reaction reached the steady state. The antioxidant activity was expressed as a percentage of scavenging activity on DPPH radical: SC% = [1−(absorbance of sample)/(absorbance of control)] × 100%. The control contains all reagents except the extract. The DPPH radical scavenging activity of BHT (0.5 mg/ml) was also assayed for comparison. All tests were performed in triplicate and mean were centred.

2.6. Scavenging activity on hydroxyl radical

CFE solution (0.1 ml) in 95% ethanol at different concentrations (0.2, 0.5, 0.8, 1.2 mg/ml) was incubated with 0.1 ml deoxyribose (3.75 mM), 0.1 ml H_2O_2 (1 mM), 0.1 ml $FeCl_3$ (100 mM), 0.1 ml EDTA (100 mM) and 0.1 ml ascorbic acid (100 mM) in 0.4 ml potassium phosphate buffer (20 mM, pH7.4) for 60 min at 37 °C (Halliwell, Gutteridge, & Aruoma, 1987). Then 1 ml of TBA (1% w/v) and 1 ml of TCA (2% w/v) were added and the tubes were heated in a boiling water bath for 15 min. The content was cooled and the absorbance of the mixture was read at 535 nm against reagent blank without extract. The antioxidant activity was expressed as a percentage of scavenging activity on hydroxyl radical: SC% = [1−(absorbance of sample)/(absorbance of control)] × 100%. The hydroxyl radical scavenging activity of BHT (0.5 mg/ml) was also measured for comparison.

2.7. Determination of total antioxidant capacity

The total antioxidant capacity of CFE was assayed according to the method of Prieto, Pineda, and Aguilar (1999). CFE solution (0.1 ml) was combined with 0.3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95 °C for 150 min. After the mixture had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The readings were taken each 30 min. The antioxidant activity was expressed as the absorbance of the sample. The antioxidant activity of BHT (0.5 mg/ml) was also assayed for comparison.

2.8. Determination of total phenolic compounds

Total soluble phenolics in CFE were determined using Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard. Briefly, 1 ml of extract solution (contains 1 mg) in a volumetric flask was diluted with glass-distilled water (46 ml). Folin–Ciocalteu reagent (1 ml) was added and the contents of flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) were added, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the extract was determined as micrograms of pyrocatechol equivalents by using an equation that was obtained from standard pyrocatechol graph given as: absorbance = 0.22 μg pyrocatechol + 0.0571.

2.9. Antioxidant potential of CFE in peanut oil

Calculated amounts of CFE (0.01, 0.02, 0.05 and 0.08 mg/ml of the oil) were added to 50 ml of peanut oil. The additive was mixed into the oil with a magnetic stirrer. Synthetic antioxidant BHT was used as a reference for comparison. The oxidative deterioration of samples was studied using Schaal oven test method as described by Economou, Oreopoulou, and Thomopoulos (1991). The oil samples (50 ml each) were placed in open 100 ml beakers, and placed in 60 ± 2 °C oven for 24 h. A blank sample was prepared under the same conditions, without adding any additives. The rate of autoxidation of peanut oil was estimated according to the increase of 2-thiobarbituric acid-reactive substances (TBARS) using the classical TBA procedure. The TBARS values of untreated and treated samples were used to calculate the inhibition of lipid oxidation as follows: inhibition (%) = (control−treatment)/control × 100.

2.10. Statistical analysis

All experimental results were centred at using three parallel measurements of means ± SD. Analysis of variance was performed by ANOVA procedure. Duncan's new multiple-range test was used to determine the differences of means. P values < 0.05 were regarded as significant and P values < 0.01 as very significant.

3. Results and discussion

3.1. Linoleic acid emulsion system–thiocyanate assay

The effects of various amounts of CFE (0.05, 0.10, 0.15, 0.20, and 0.25 mg/ml) on peroxidation of linoleic acid emulsion are shown in Fig. 1. At concentrations of 0.15, 0.20 and 0.25 mg/ml, CFE showed a higher inhibition of peroxidation of linoleic acid than BHT (0.15 mg/ml). At the same concentration (0.15 mg/ml), the antioxidant activity of CFE as shown by IP% (percentage of inhibition of peroxidation) varid from 54.62% to 83.54%, while that of the BHT was 48.06–69.43%. At a concentration of 0.25 mg/ml, the inhibition of lipid peroxidation by CFE was 85.10% at 120 h.

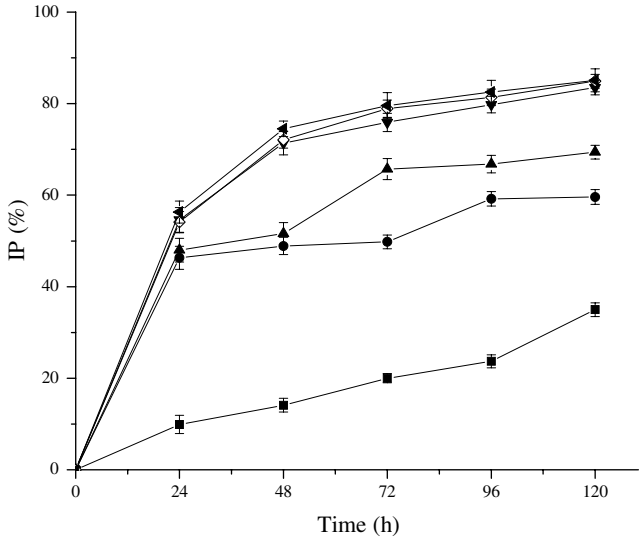

Fig. 1. Inhibition of peroxidation of linoleic acid of CFE and BHT by the thiocyanate method. 0.05 mg/ml CFE (■), 0.10 mg/ml CFE (●), 0.15 mg/ml CFE (▼), 0.20 mg/ml CFE (◇), 0.25 mg/ml CFE (◀), 0.15 mg/ml BHT (▲). IP% (percentage of inhibition of peroxidation) = [1−(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100. Results are means ± SD of three parallel measurements. $P < 0.05$, when compared to the control.

3.2. Scavenging activity on DPPH radical

A concentration-dependent assay was carried out with the extract (CFE) and the results are presented in Fig. 2. These results provide a direct comparison of the antioxidant activity with BHT. CFE possessed significant scavenging activity on the DPPH radical and acted as an antioxidant. The scavenging effect was increased with increasing concentration and reaction time. CFE showed a higher scavenging activity than BHT at concentrations of 0.8 and 1.2 mg/ml. At the same concentration (0.5 mg/ml), the scavenging activity of CFE was better than that of BHT during the first 37 min, and close to BHT after 37 min. However, scavenging activity of CFE reached a very high degree within 10 min, BHT was distinctly slower than that of CFE in scavenging DPPH radical.

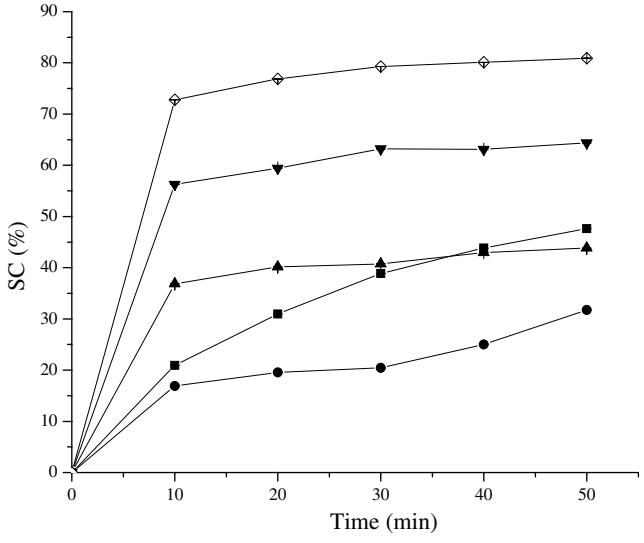

Fig. 2. DPPH free radical scavenging activity of CFE and BHT. 0.2 mg/ml CFE (●), 0.5 mg/ml CFE (▲), 0.8 mg/ml CFE (▼), 1.2 mg/ml CFE (◇), 0.5 mg/ml BHT (■). SC% (percentage of scavenging activity on DPPH radical) = [1−(absorbance of sample)/(absorbance of control)] × 100. Results are means ± SD of three parallel measurements. $P < 0.01$, when compared to the control.

3.3. Scavenging activity on hydroxyl radicals

Hydroxyl radical exhibits very high reactivity and tends to react with a wide range of molecules found in living cells. They can interact with the purine and pyrimidine bases of DNA. They can also abstract hydrogen atoms from biological molecules (e.g. thiol compounds), leading to the formation of sulphur radicals able to combine with oxygen to generate oxysulphur radicals, a number of which damage biological molecules (Halliwell et al., 1987). Due to the high reactivity, the radicals have a very short biological half-life. Thus, an effective scavenger must be present at a very high concentration or possess very high reactivity toward these radicals. Although hydroxyl radical formation can occur in several ways, by far the most important mechanism *in vivo* is the Fenton reaction where a transition metal is involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide (Stohs & Bagchi, 1995). These radicals are intermediary products of cellular respiration, phagocytic outburst and purine metabolism. Hydroxyl radical can be generated in situ by decomposition of hydrogen peroxide by high redox potential EDTA–Fe^{2+} complex (non-site specific), and in the presence of deoxyribose substrate, it forms TBARS which can be measured (Aruoma, 1994). Antioxidant activity is detected by decreased TBARS formation, which can come about by donation of hydrogen or electron from the antioxidant to the radical or by direct reaction with it. Consequently, the ability of CFE to scavenge hydroxyl radical was evaluated by the Fenton-mediated deoxyribose assay.

The hydrogen peroxide scavenging ability of CFE is shown in Fig. 3 and compared with that of BHT. CFE was capable of scavenging hydrogen peroxide in a concentration dependent manner. It exhibited hydrogen peroxide scavenging activity of 56.60%, 59.43%, 61.32% and 71.70% at concentrations of 0.2, 0.5, 0.8 and 1.2 mg/ml, respectively. At the same concentration (0.5 mg/ml), the hydrogen peroxide scavenging activity of CFE was closed to that of BHT (60.38%).

3.4. Total antioxidant capacity assay

The assay is based on the reduction of Mo(VI) to Mo(V) by CFE and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The total antioxidant activity was measured and compared with that of BHT and the control, which contained no antioxidant component. The high absorbance values indicated that the sample possessed

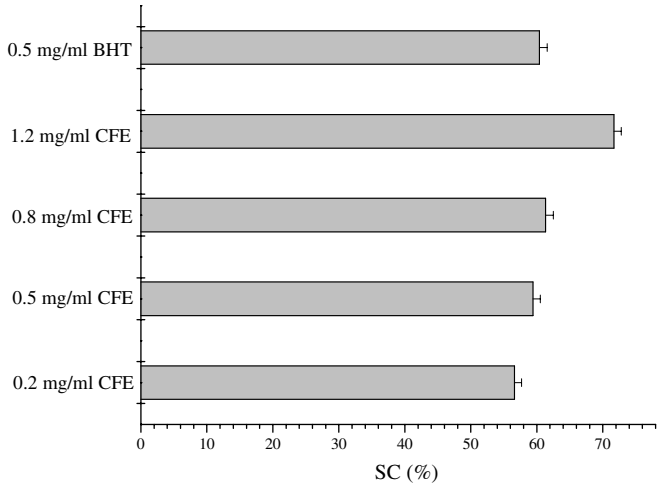

Fig. 3. Hydrogen peroxide scavenginig activity of CFE and BHT. SC% (percentage of scavenging activity on hydroxyl radical) = [1−(absorbance of sample)/(absorbance of control)] × 100. Results are means ± SD of three parallel measurements. $P < 0.05$, when compared with the control.

significant antioxidant activity. According to the results (Fig. 4), CFE had significant antioxidant activities and the effects increased with increasing reaction time.

3.5. Total phenolic content

Since the antioxidant activity of fruits and tea is derived largely from phenolic and polyphenolic compounds (Bravo, 1998; Cai, Luo, Sun, & Corke, 2004), the true antioxidant potential is often more accurately revealed by expressing antioxidant activity in terms of phenolic content. The Folin–Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in an extract. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the reagent (Singleton, Orthofer, & Lamuela-Raventos, 1999). The total phenolic content of the sample was 91.33 mg/g dry weight, expressed as pyrocatechol equivalents ($P < 0.05$).

The high antioxidant activity of CFE may be attributed to its high content of phenolic compounds and derivatives. In addition, the high amount of these phenolic compounds can also explain the high free radical scavenging activity observed in the assay performed in this study. Although the antiradical activity of phenolic compounds is already known (Li et al., 2005), it remains uncertain how a complex mixture obtained from plant extract functions against reactive oxygen species, because other compounds of the mixture may potentiate or prevent the expected activity. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components present in CFE.

3.6. Lipid peroxidation in peanut oil

The level of 2-thiobarbituric acid-reactive substances (TBARS), products of lipid peroxidation, is often measured in order to assess the extent of oxidation that occurs in biological systems. Lipid oxidation for peanut oil, which was untreated or treated with antioxidants, CFE or BHT at concentrations of 0.01, 0.02, 0.05 and 0.08 mg/ml is shown in Fig. 5. Oxidation levels decreased in all peanut oil samples as antioxidant concentration increased. The highest level of lipid oxidation occurred in untreated peanut oil samples compared with those containing the added antioxidants.

Overall differences in the inhibition rate of lipid oxidation between CFE and BHT in all peanut oil samples examined are also presented in Fig. 5. Both CFE and BHT significantly ($P < 0.05$) improved oxidative stability of peanut oil. Addition of BHT at a concentration of 0.01, 0.02, 0.05 and 0.08 mg/ml reduced lipid oxidation by 25.30%, 38.78%, 43.97% and 55.78% for peanut oil samples, respectively. However, addition of CFE at the same concentration inhibited lipid oxidation by 33.56%,

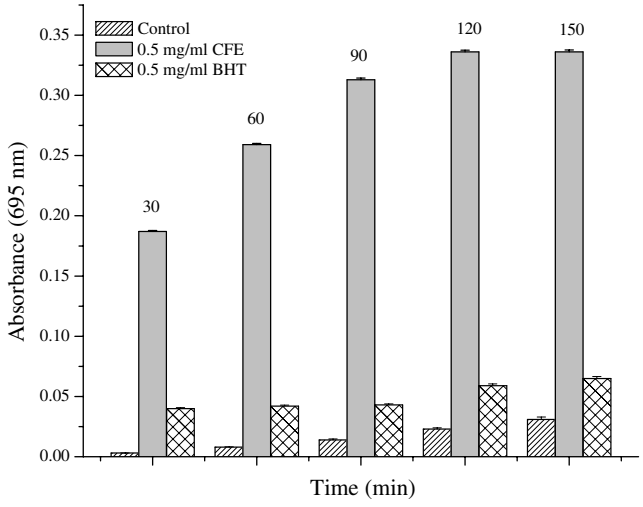

Fig. 4. Total antioxidant activity of CFE and BHT. Results are means ± SD of three parallel measurements. $P < 0.01$, when compared with the control.

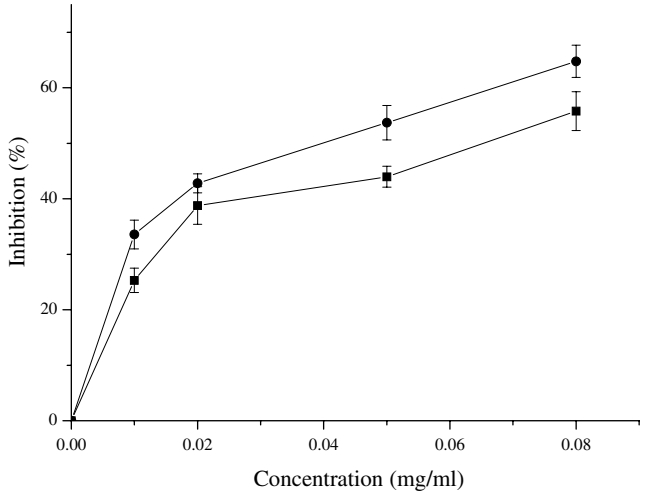

Fig. 5. Lipid oxidation inhibited by CFE and BHT in peanut oil. CFE (●), BHT (■). Results are means ± SD of three parallel measurements. $P < 0.01$, when compared with the control.

42.79%, 53.70% and 64.76% for peanut oil samples, respectively. No significant differences ($P = 0.05$) in lipid oxidation were detected between CFE and BHT samples of peanut oil. The results indicated that CFE had strong antioxidant activity in peanut oil.

4. Conclusions

The antioxidant activity of 95% ethanolic extract of *Cortex fraxini* was evaluated in five different testing systems. The results revealed that CFE had significant antioxidant and free radical scavenging activities on DPPH and hydroxyl radicals. The free radical-scavenging property might be one of the mechanisms by which this herbal medicine exhibits higher antioxidant activity, and total phenol content was also very rich in CFE. The suitability of CFE as an antioxidant was also determined in peanut oil. CFE treatment significantly ($P < 0.01$) reduced lipid oxidation in peanut oil compared to the control. No significant differences ($P = 0.05$) in lipid oxidation were detected between CFE and BHT samples in peanut oil. Thus, this study gives a strong impact for expanding the investigations of natural antioxidants for use in the food industry. It also provides useful information on pharmacological activities associated with free radicals of this traditional folk remedy. Even though the antioxidant activity of the compounds present in the extract is strong, the overall antioxidant effect could be higher by the combined and synergistic effects of other compounds. Therefore, isolation and identification of individual active compounds, their in vivo antioxidant activities as well as different antioxidant mechanisms in vitro are still needed.

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