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Antioxidant potential of ethanolic extract of *Polygonum cuspidatum* and application in peanut oil

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Abstract

Polygonum cuspidatum was extracted with 95% ethanol to obtain a crude antioxidant extract. *P. cuspidatum* extract (PCE) had a very high content of total phenol, which was 104.83 ± 8.58 mg/g dry weight, expressed as pyrocatechol equivalent. PCE exhibited excellent antioxidant activity, as measured using α,α-diphenyl-β-picryhydrazyl (DPPH) and total antioxidant assays. It also showed a high lipid antioxidant activity and hydroxyl radicals scavenging activity. A positive correlation was found between the reducing power and the antioxidant activity of PCE, which was found to be comparable to resveratrol and butylated hydroxytoluene (BHT). Then the suitability of PCE as an antioxidant was determined in peanut oil, and the decrease of oxidation was monitored using thiobarbituric acid-reactive substances (TBARS) assay. PCE treatment significantly ($P < 0.05$) reduced lipid oxidation in peanut oil compared to resveratrol and BHT.

Keywords: *Polygonum cuspidatum*; Natural antioxidant; Lipid oxidation; Peanut oil

1. Introduction

Lipid peroxidation can lead to the development of unpleasant rancid or off flavours as well as nutritional loss and formation of potentially toxic end-products. So it is among the main causes of deterioration during the storage and processing of foods. Therefore, much attention has been paid to the antioxidants, which are expected to prevent food and living systems from peroxidative damage. Incorporation of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG) have been added to foods can retard lipid oxidation. However, the use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds (Hettiarachchy, Glenn, Gnanasambandam, & Johnson, 1996). Therefore, search for natural antioxidants that may be used in foods to replace synthetic antioxidants is necessary.

In the past few years, various plant materials containing phenolic compounds have been demonstrated to be effective antioxidants in model systems (Lai et al., 1991; Pan, Liang, Wang, & Liang, 2004; Pan et al., 2007). 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 in peanut oil and were effective in retarding lipid oxidation.

Attempts are being made in our laboratory to explore the use of novel natural antioxidants for foods, e.g. oil and meat, pharmaceutical and cosmetic industry. *Polygonum*

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cuspidatum S. et Z. belongs to the Polygonaceae perennial herb group widely distributed in China. It was traditionally used in China and Japan as a folk medicine for the treatment of atherosclerosis and for other therapeutic purposes such as scalds, burns, laxation, a diuretic, and for the treatment of suppurative dermatitis in the oriental medicine (Kim, Hwang, & Shin, 2005). We know that anthraquinones and anthraquinone derivatives from *P. cuspidatum* have been reported to possess antiviral and virucidal activities against viruses of several taxonomic groups (Andersen et al., 1991; Barnard et al., 1992), mentioned to inhibit several kinds of virus, such as HBV (HBsAg) (Chang et al., 2005), HRV 3C-protease (Singh, Graham, Reamer, & Cordingley, 2001), and used for hormone replacement therapy such as for the management of menopausal symptoms (Zhang, Wang, Zhang, Chen, & Liang, 2005). Recently, two new bacterial DNA primase inhibitors were found from *P. cuspidatum* (Vinod et al., 2004). Resveratrol, a phenolic compound, contained in the extract of *P. cuspidatum* and trivially name is 3,4′,5-trihydroxy-*trans*-stilbene, has been reported as a biologically active compound (Langcake & Pryce, 1976; Langcake, Cornford, & Pryce, 1979; Langcake & McCarthy, 1979). Protykin is an all-natural, high potency standardized extract of *trans*-resveratrol (20%) and emodin (10%). Previous studies also have demonstrated free radical scavenging and anti-inflammatory activities of reservatrol (Sato, Maulik, Bagchi, & Das, 2000). However, no work has been undergone to study the effect of PCE itself as a natural antioxidant. Therefore, the objective of this study was to study the effectiveness of PCE in preventing/minimizing lipid oxidation in peanut oil as measured by thiobarbituric acid-reactive substances (TBARS).

2. Materials and methods

2.1. Materials

Polygonum cuspidatum was obtained from the Guilin Pharmaceuticals Group of China (Zhongshan Road, Guilin City, China).

All chemicals and solvents used were of analytical grade. Folin-Ciocalteu reagent, α,α-diphenyl-β-picryhydrazyl (DPPH), resveratrol, linoleic acid, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and deoxyribose were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Butylated hydroxytoluene (BHT), ferrous chloride, ammonium thiocyanate, tween 20, ammonium molybdate, and potassium ferricyanide were ferricyanides were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China).

The peanut oil, which was stripped, was bought from Beijing Chemical Company (Beijing, China). It contained very low α-tocopherol (2.0 mg/kg) 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 *P. cuspidatum* extract (PCE)

Roots of *P. cuspidatum* were ground (max particle size 0.4 mm) and 50 g of ground material were extracted with 500 ml of 95% ethanol in a Soxhlet apparatus during 2 h. Solvent was evaporated using a RE-52AA rotavapour at 60 °C and a SHB-βA water-circulation multifunction vacuum pump. *P. cuspidatum* extract (PCE) 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 PCE was 15.8 ± 2.1%.

2.4. Determination of total phenolic compounds

Total phenolic compounds in the PCE were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using pyrocatechol as a standard. Briefly, 1 ml of PCE solution (contains 1 mg PCE) in a flask diluted 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%) was added, then the mixture was allowed to stand for 2 h with intermittent shaking. Absorption at 760 nm was measured using a spectrophotometer. The concentration of total phenolic compounds in PCE was determined as microgram of pyrocatechol equivalent.

2.5. Linoleic acid emulsion system–thiocyanate assay

Lipid antioxidant activity of PCE was evaluated using the thiocyanate method (Haraguchi, Hashimoto, & Yagi, 1992). 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. PCE solution (0.5 ml) in 95% ethanol at different concentrations (0.5, 0.8, 1.2 and 1.6 mg/ml) was added to 2.5 ml of the above linoleic acid emulsion, and 2.5 ml of phosphate buffer (0.2 M, pH 7.0) and the reaction mixture incubated at 37 °C to accelerate the oxidation process. The mixture prepared as above, without extract was the control. The readings were taken after each 24 h. 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 measured. The antioxidant activity was expressed as a percentage of inhibiting the oxidant of lipid: IP% = [1 − (absorbance of sample)/(absorbance of control)] × 100%. The antioxidant activity of resveratrol (0.5 mg/ml) and BHT (0.5 mg/ml) were also assayed for comparison purposes. All tests were performed in triplicate and mean were centred.

2.6. Scavenging activity on DPPH radical

The scavenging effect of PCE on the DPPH radical was measured using a modified version of the method described by Hatano, Kagawa, Yasuhara, and Okuta (1988). In brief, 0.2 ml of PCE solution in 95% ethanol at different concentration (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 mixtures were incubated at 28 °C. The scavenging activities on DPPH radical were determined by measuring the absorbance at 515 nm every 10 min until the reaction reached the steady state. The antioxidant activity was expressed as a percentage of scavenging of DPPH: SC%= [1 − (absorbance of sample)/(absorbance of control)] × 100%. The control contains all reagents except the extract. The DPPH radical scavenging activity of resveratrol (0.2, 0.5, 0.8 and 1.2 mg/ml) and BHT (0.5 mg/ml) were also assayed for comparison. All tests were performed in triplicate and mean were centred.

2.7. Scavenging activity on hydroxyl radical

The scavenging activity for hydroxyl radical was measured according to the method described by Halliwell and Gutteridge (1981). The reaction mixture contained PCE solution in 95% ethanol (0.2, 0.5, 0.8 and 1.2 mg/ml), was incubated with deoxyribose (3.75 mM), H_2O_2 (1 mM), $FeCl_3$ (100 μM), EDTA (100 μM) and ascorbic acid (100 μM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37 °C. The reaction was terminated by adding 1 ml of TBA (1% w/v) and 1 ml of TCA (2% w/v) and then heating the tubes in a boiling water bath for 15 min. The contents were cooled and the absorbance of the mixture was measured at 535 nm against reagent blank. Decreased absorbance of the reaction mixture indicated decreased oxidation of deoxyribose. Scavenging effects (%) was calculated from the absorbance data using the equation: SC% = [1 − (absorbance of sample)/(absorbance of control)] × 100%. The hydroxyl radical scavenging activity of resveratrol (0.2, 0.5, 0.8 and 1.2 mg/ml) and BHT (0.5 mg/ml) were also measured for comparison.

2.8. Determination of total antioxidant capacity

The total antioxidant capacity of PCE was measured using a modified version of the method described by Prieto, Pineda, and Aguilar (1999). The assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. PCE solution (0.3 ml) in 95% ethanol was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95 °C. The total antioxidant capacity was determined using measuring the absorbance at 695 nm each 30 min after the mixture had cooled to room temperature. The mixture prepared as above, without PCE was the control. The antioxidant activity is expressed as the absorbance of samples measured at 695 nm. Increased absorbance of the reaction mixture indicated increased total antioxidant capacity. The total antioxidant activity of resveratrol (0.5 mg/ml) and BHT (0.5 mg/ml) were also assayed for comparison.

2.9. Reducing power

The reducing power of PCE was determined according to the method of Oyaizu (1986). PCE solution (1 ml) in 95% ethanol (0.2, 0.5, 0.8 and 1.2 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The reducing power of resveratrol (0.2, 0.5, 0.8 and 1.2 mg/ml) and BHT (0.2, 0.5, 0.8 and 1.2 mg/ml) were also assayed for comparison.

2.10. Antioxidant potential of PCE in peanut oil

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

2.11. Statistical analysis

All experimental results were centred at using three parallel measurements of mean ± 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. Total phenolic content

Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Duh, Tu, & Yen, 1999; Yen, Duh, & Tsai, 1993). Most antioxidant activities from plant sources are derived from phenolic-type compounds (Bravo, 1998). In our work, the total phenolic content of the samples was 104.83 ± 8.58 mg/g dry weight, expressed as pyrocatechol equivalent.

3.2. Linoleic acid emulsion system–thiocyanate method

Lipid oxidation is an important chemical change that lowers the nutritional quality of food. The primary and secondary products of lipid oxidation are detrimental to health. In the body, excessive production of free radicals affects lipid cell membranes to produce lipid peroxides. Lipid peroxides are likely involved in numerous pathological events; including inflammation, metabolic disorders and cellular aging (Wiseman & Halliwell, 1996). The reason is membrane lipids are rich in unsaturated fatty acids that are most sensitive to oxidative processes. Specially, linoleic acid and arachidonic acid are targets of lipid peroxidation (Yu, 2001).

The antioxidant activity of PCE was determined by the thiocyanate methods. The amount of peroxides formed in emulsion during incubation was determined spectrophotometrically by measuring absorbance at 500 nm. The effects of various amounts of PCE (0.5, 0.8, 1.2 and 1.6 mg/ml) on peroxidation of linoleic acid emulsion are shown in Fig. 1. The antioxidant activity of PCE increased with the increasing concentration and time dependently. When reactive time was 120 h, all of concentration of PCE showed higher antioxidant activities than resveratrol and BHT. At concentrations of 0.5, 0.8, 1.2 and 1.6 mg/ml, PCE had 80.30%, 80.71%, 82.26% and 84.38% inhibition on peroxidation of linoleic acid, respectively, but that of resveratrol (0.5 mg/ml) and BHT (0.5 mg/ml) were 72.5% and 80.23%. At the same concentration (0.5 mg/ml), the lipid antioxidant activity of PCE was closed to that of BHT, but was superior to that of resveratrol ($P < 0.05$).

3.3. Scavenging capability on α,α-diphenyl-β-picryhydrazyl (DPPH) free radicals

The DPPH (α,α-diphenyl-β-picryhydrazyl) free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants (Kim, Guo, & Packer, 2002; Leong & Shui, 2002). In order to evaluate the antioxidant potency

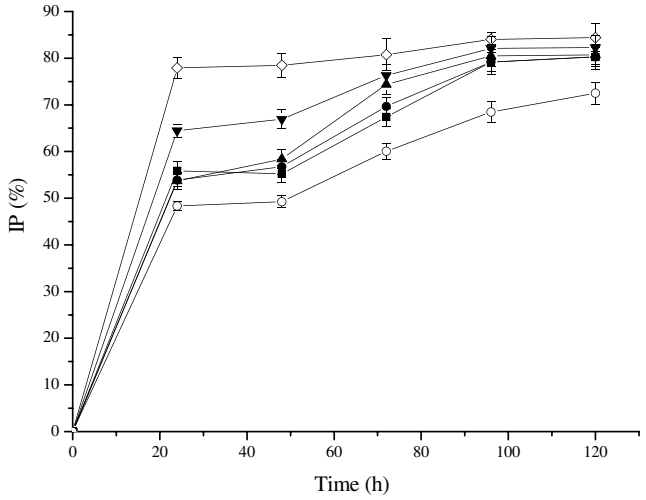

Fig. 1. Inhibition on peroxidation of linoleic acid of PCE, resveratrol and BHT by the thiocyanate method. 0.5 mg/ml PCE (●), 0.8 mg/ml PCE (▲), 1.2 mg/ml PCE (▼), 1.6 mg/ml PCE (◇), 0.5 mg/ml resveratrol (○), 0.5 mg/ml BHT (■). IP% (percentage of inhibition of peroxidation) = [1 − (absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100. Results are mean ± SD of three parallel measurements. $P < 0.05$, when compared to the control.

through free radical scavenging by PCE, the change of optical density of DPPH radicals was monitored. And resveratrol and BHT were used as standards. The percentages of DPPH remaining in the presence of PCE at different concentrations are shown in Fig. 2.

The concentrations of PCE measured were 0.2, 0.5, 0.8 and 1.2 mg/ml. As a contrast, the concentrations of resveratrol measured were 0.2, 0.5, 0.8 and 1.2 mg/ml and that of BHT was 0.5 mg/ml. According to the results, both

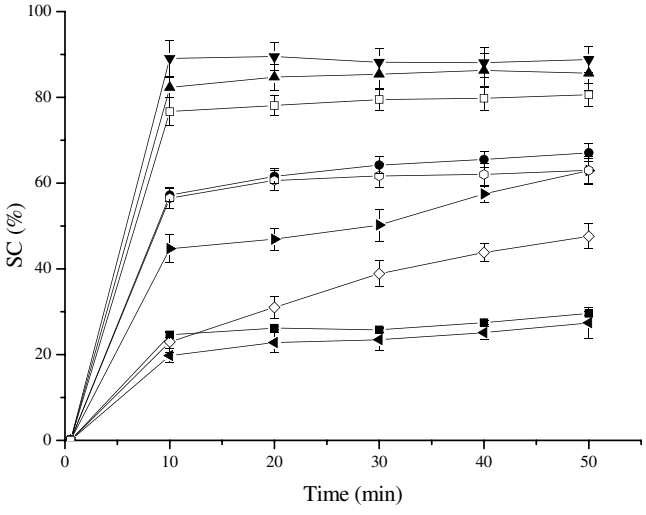

Fig. 2. DPPH free radical scavenging activity of PCE, resveratrol and BHT. 0.2 mg/ml PCE (■), 0.5 mg/ml PCE (●), 0.8 mg/ml PCE (▲), 1.2 mg/ml PCE (▼), 0.2 mg/ml resveratrol (►), 0.5 mg/ml resveratrol (◄), 0.8 mg/ml resveratrol (○), 1.2 mg/ml resveratrol (□), 0.5 mg/ml BHT (◇). SC% (percentage of scavenging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

PCE and resveratrol had significant scavenging effects on the DPPH radical and the effects increased with the increasing concentration. At the same concentration of PCE, resveratrol and BHT, the scavenging effect on the DPPH radical decreased in that order: PCE > resveratrol > BHT. The maximum scavenging effect of the different concentration of PCE and resveratrol nearly reached when the samples mixed for 10 min. But when the samples mixed for 50 min the maximum scavenging effect of BHT barely reached. In a word, PCE possessed higher DPPH free radical-scavenging capacity than resveratrol and BHT ($P < 0.01$).

3.4. Scavenging ability on hydroxyl radicals

The role of an antioxidant is to remove free radicals. Hydroxyl radical is very reactive and can be generated in biological cells such as sugars, amino acids, lipids and nucleotides through the Fenton reaction. In the reaction metals are used as a catalyst to break up the superoxide and hydrogen peroxide (Stohs & Bagchi, 1995). So reducing agents, such as ascorbic acid, can accelerate ·OH formation by reducing Fe^{3+} ions to Fe^{2+} (Puppo, 1992). In the work herein, the effect of PCE on oxidative damage, induced by Fe^{3+}/H_2O_2 on deoxyribose, as measured by the thiobarbituric acid method, is plotted in Fig. 3. According to Fig. 3, the effects of various amounts of PCE (0.2, 0.5, 0.8 and 1.2 mg/ml) had 53.77%, 57.55%, 63.20% and 68.87% inhibition on scavenging the hydroxyl radicals respectively. The hydrogen peroxide scavenging activity of resveratrol (0.2, 0.5, 0.8 and 1.2 mg/ml) were 48.79%, 54.34%, 59.11% and 68.53%, respectively, and that of BHT (0.5 mg/ml) was 60.38%. At the same concentration (0.5 mg/ml), the hydrogen peroxide scavenging activity of PCE was closed to that of BHT, but was higher than that of resveratrol ($P < 0.05$).

3.5. Total antioxidant capacity

The total antioxidant activity, which reflected the ability of PCE to reduce Mo (VI) to Mo (V) was measured and compared with that of the control which contained no antioxidant component. Resveratrol and BHT were using as a contrast. The reductive capabilities of PCE, resveratrol, BHT and control are shown in Fig. 4. The total antioxidant capacity of PCE was significantly ($P < 0.01$) higher than that of resveratrol, BHT and control. And the effects were increased with increasing reaction time.

3.6. Reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir, Kanner, Akiri, & Hadas, 1995) and indicates that these compounds are electron donors. Prophase investigators have found that there is a specific relationship between antioxidant activities and reducing power (Pin-Der-Duh, 1998; Tanaka, Kuei, Nagashima, & Taguchi, 1998). So these compounds can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen & Chen, 1995).

We investigated the Fe^{3+}–Fe^{2+} transformation in the presence of PCE. The reducing power of PCE increased steadily with the increased concentrations. As a contrast, the reductive capabilities of various amounts of PCE, resveratrol and BHT (0.2, 0.5, 0.8 and 1.2 mg/ml) are shown in Fig. 5. The absorbances of PCE at 700 nm were 0.310, 0.845, 1.083 and 1.285, respectively, while those of BHT were 0.264, 0.655, 0.997 and 1.166, respectively. Both PCE and BHT had stronger reductive capability than resveratrol which were 0.217, 0.458, 0.564 and 0.805, respectively. The reductive capability of PCE was slight higher than BHT ($P < 0.05$).

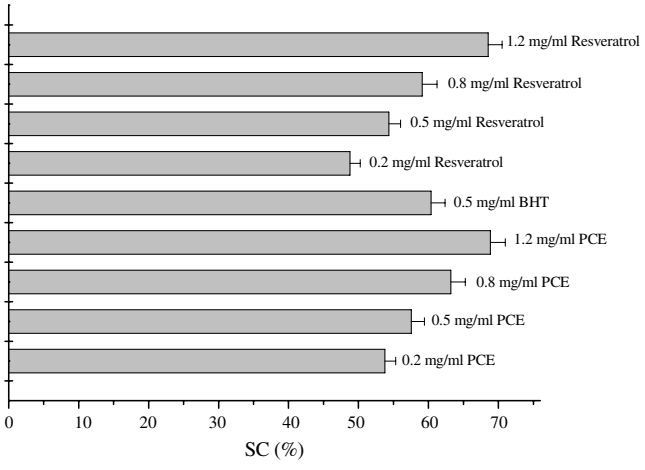

Fig. 3. Hydrogen peroxide scavenging activity of PCE, resveratrol and BHT. SC% (percentage of scavenging activity on hydroxyl radical) = [1 − (absorbance of sample)/(absorbance of control)] × 100. Results are mean ± SD of three parallel measurements. $P < 0.05$, when compared with the control.

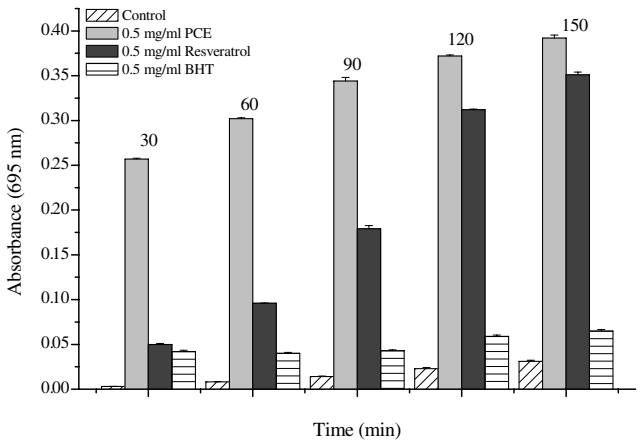

Fig. 4. Total antioxidant activity of PCE, resveratrol and BHT. Results are mean ± SD of three parallel measurements. $P < 0.01$, when compared with BHT and control.

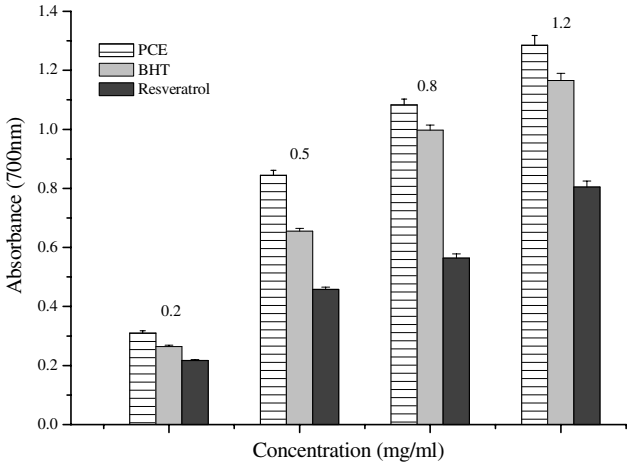

Fig. 5. The reducing capability of PCE, resveratrol and BHT. Results are mean ± SD of three parallel measurements. $P < 0.05$, when compared with control.

3.7. Lipid peroxidation in peanut oil

The level of malonaldehyde (TBARS), a product of lipid peroxidation, is often measured in order to assess the extent of oxidation that has occurred in biological systems. Lipid oxidation for peanut oil, which was untreated or treated with antioxidants, PCE or resveratrol or BHT, at concentrations of 0.01, 0.02, 0.05 and 0.08 mg/ml are shown in Fig. 6. All of PCE, resveratrol and BHT significantly ($P < 0.05$) improved oxidative stability of peanut oil. Oxidation levels in all peanut oil samples accompanied with antioxidant concentration were 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 of PCE, resveratrol and BHT in all peanut oil samples examined are also presented in Fig. 6. Addition of BHT at a concentration of 0.01, 0.02, 0.05 and 0.08 mg/ml reduced lipid oxidation by 25.3%, 38.78%, 43.97% and 55.78%, respectively, and that of resveratrol were 23.04%, 35.74%, 57.04% and 60.11%, respectively. However, addition of PCE inhibited lipid oxidation by 30.8%, 41.01%, 63.68% and 75.97%, respectively, for peanut oil samples. PCE treatment significantly ($P < 0.05$) reduced lipid oxidation in peanut oil compared to resveratrol and BHT. The results indicated that PCE had demonstrated strong antioxidant activity in peanut oil.

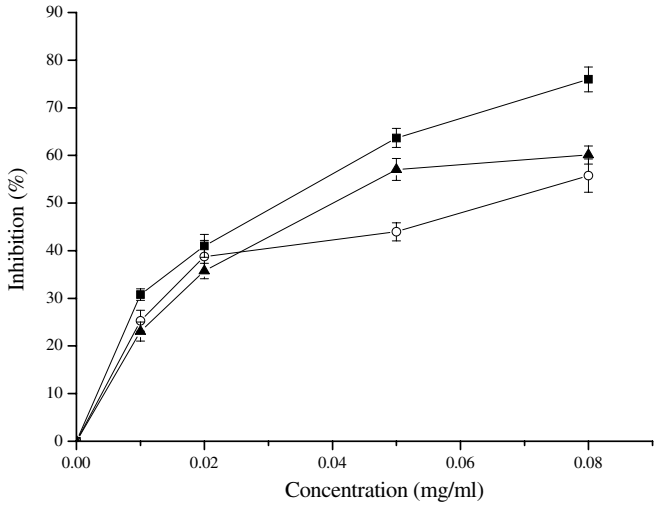

Fig. 6. Lipid oxidation inhibited by PCE and BHT in peanut oil. PCE (■), resveratrol (▲) and BHT (○). Inhibition (%) = [(control − treatment)/control] × 100. Results are mean ± SD of three parallel measurements. $P < 0.01$, when compared with the control.

The antioxidant activity of natural compounds is now intensively studied due to the current growing demand from the pharmaceutical and food industries, which are interested in natural bioactive compounds that possess health benefits. Plant sources may bring new natural products into the food industry with safer and better antioxidants that provide good protection against oxidative damage, which occurs both in the body and in the processed food. Addition of PCE to peanut oil may be able to prevent lipid peroxidation. This treatment could have an important economic impact as it might reduce losses attributed to spoilage and also allow the products to reach new and distant markets.

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