

## Antioxidant activity of sesame coat

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### Abstract

The antioxidant activity of ethanolic extracts of sesame coat (EESC) was investigated. The antioxidant activity (91.4%) of 1.0 mg EESC was equal to 1.0 mg tocopherol (90.5%) but was weaker than 1.0 mg butylated hydroxyanisole (98.6%) on peroxidation of linoleic acid. EESC showed an inhibitory effect against the formation of thiobarbituric acid reactive substances (TBARS) in a liposome model system. EESC at 10.0 mg exhibited a 94.9% scavenging effect on  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl radicals and marked reducing power, indicating that EESC acts as a primary antioxidant. The extracts, at a dose of 1.0 mg, showed a 50.0% scavenging effect on the hydroxyl radical. EESC also exhibited a metal-binding ability. Sesamin and sesamol, the lignan substances, were found in EESC, by HPLC analysis. In addition, chromatographic analysis demonstrated that phenolic compounds and tetranortriterpenoids, which had positive reactions with  $\beta$ -carotene, indicating antioxidant activity, are present in EESC. According to these results, termination of free radical reaction, metal-binding ability and quenching of reactive oxygen are suggested to be, in part, responsible for the antioxidant activity of EESC. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Antioxidant activity; Sesame coat; Lipid peroxidation; Free radical; Metal-binding ability; Phenolic compounds; Lignan

### 1. Introduction

Lipids and lipid-soluble substances that may be susceptible to oxidation are present in almost all foods, and lipid peroxidation plays an important role in the deterioration of foods during storage. In addition, toxic substances formed by lipid peroxidation may lead to other adverse effects such as carcinogenesis, mutagenesis, and aging (Yagi, 1990). The incorporation of antioxidants in fats and oils, or in foods that contain fats and oils, is effectively helpful in retarding the oxidation of lipids. However, there is currently an interest in replacing synthetic antioxidants, which are of safety concern, (Ito, Hagiwara, Shibate, Ogiso, & Fukushima, 1982), with natural antioxidants. Some chemicals that occur naturally in plants have begun to receive much attention as safe antioxidants, as they have been consumed by people and animals for years (Namiki, 1990).

Sesame (*Sesamum indicum* L.) is one of the most important oilseed crops, cultivated in India, Sudan,

China and Burma, which are the major sesame-producing countries, contributing to 60% of the global yield (Abou-Gharbia, Shahidi, Shahata, & Youssef, 1997). Sesame is a source of edible oil and provides a nutritious food for humans. Budowski (1964) noted that sesame oil is highly stable to oxidation compared with other vegetable oils. Sesame oil is especially stable because of the presence of sesamin, sesamol, sesaminol, sesamol,  $\gamma$ -tocopherol (Fukuda, Nagata, Osawa, & Namiki, 1986; Lyon, 1972; Shahidi, Amarowicz, Abou-Gharbia, & Shehata, 1997). In addition, the oxidative stability of sesame oil is dependent on the roasting temperature (Yen & Shyu, 1989). The conventional process for preparing sesame oil involves cleaning, optional dehulling, roasting, grinding, cooking, and preparing (Fukuda & Namiki, 1988). After dehulling, the sesame coat is commonly disposed of or used as animal feed. However, Abou-Gharbia et al. (1997) reported that the sesame oil from seeds with coat were more stable than those extracted from dehulled seeds. This observation may indicate that antioxidative components may exist in sesame coat. Therefore, use of sesame coat, after dehulling, as a source of natural antioxidants, may provide a means for utilization. No studies, however, have been

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conducted to investigate the antioxidant activity of sesame coat. Thus the current research investigates the antioxidant activity of sesame coat.

## 2. Materials and methods

### 2.1. Materials

The sample of white sesame (*Sesamum indicum* L.) in this study was donated by Tainan District Agriculture Improvement Station, Taiwan, Republic of China. After harvesting, the coat was removed from sesame seeds. The sesame coat was sealed in a plastic bottle, and stored at 4 °C until used. Linoleic acid, ammonium thiocyanate, ferrous chloride, lecithin, and butylated hydroxyanisole (BHA) were purchased from E. Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Chemie AG (Switzerland).

### 2.2. Extraction

Sesame coat (10 g) was extracted overnight with 100 ml of methanol, ethanol or acetone, in a shaking incubator at room temperature. The extracts were filtered, and the residue was re-extracted under the same conditions. The combined filtrates were evaporated under vacuum below 40 °C using a rotary evaporator to a final volume of 5 ml.

### 2.3. Oxalic acid determination

The contents of oxalic acid in sesame coat and EESC were determined by AOAC methods 32.035 (AOAC, 1990). The test results are the average of duplicate analysis.

### 2.4. Antioxidant activity in a linoleic acid system

Antioxidant activity assay was carried out by using the linoleic acid system. Linoleic acid emulsion (0.02 M) was prepared with linoleic acid (0.2804 mg) and Tween 20 (0.2804 mg) in phosphate buffer (50 ml, 0.05 M, pH 7.4). A reaction solution, containing extracts (0.2 ml, 5.0 mg/ml), linoleic acid emulsion (2.5 ml), and phosphate buffer (2.3 ml, 0.2 M, pH 7.0) were mixed with a homogenizer. The reaction mixture was incubated at 37 °C in the dark, and the degree of oxidation was measured according to the thiocyanate method (Mitsuda, Yuasumoto, & Iwami, 1966), by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), and sample solution (0.1 ml). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the inhibition percent of linoleic acid peroxidation was calculated as (%) inhibition =  $[1 - (\text{absorbance of sample at 500 nm}) / (\text{absorbance of control at 500 nm})] \times 100$ .

All test were run in duplicate, and analysis of all samples was done in triplicate and averaged.

### 2.5. Determination of antioxidant effect on liposome oxidation

Lecithin (580 mg) was sonicated in an ultrasonic cleaner (Brranson 8210, Branson Ultrasonic Corporation, Danbury, CT) in 58 ml of 10 mM phosphate buffer (pH7.4) for 2 h. The sonicated solution (10 mg lecithin/ml), FeCl<sub>3</sub>, ascorbic acid, and extracts (0.2 ml, 0.05–50 mg/ml) were mixed to produce a final concentration of 3.12 μM FeCl<sub>3</sub>, and 125 μM ascorbic acid. The mixture was incubated for 1 h at 37 °C by the thiobarbituric acid (TBA) method (Tamura & Shibamoto, 1991). The absorbance of the sample was read at 532 nm against a blank which contained all reagents except lecithin.

### 2.6. Determination of the effects on oxidation of deoxyribose

The determination was carried out as described by Halliwell, Gutteridge, and Aruoma (1987). The reaction mixture (3.5 ml), which contained extracts (0.2 ml, 0.05–50 mg/ml), deoxyribose (6 mM), H<sub>2</sub>O<sub>2</sub> (3 mM), KH<sub>2</sub>PO<sub>4</sub>–K<sub>2</sub>HPO<sub>4</sub> buffer (20 mM, pH7.4), FeCl<sub>3</sub> (400 μM), ethylenediaminetetraacetic acid (EDTA; 400nM), and ascorbic acid (400 nM), was incubated at 37 °C for 1 h. The extent of deoxyribose degradation was tested by the TBA method. One millilitre of 1% TBA and 1 ml of 2.8% trichloroacetic acid (TCA) were added to the mixture, which was then heated in a water-bath at 90 °C for 20 min. The absorbance of the mixture was read spectrophotometrically at 532 nm. All analyses were run in three replicates and averaged.

### 2.7. Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Extracts (0–10 mg) in phosphate buffer (2.5ml, 0.2M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 10 mg/ml), and the mixture was incubated at 50 °C for 20min. Trichloroacetic acid (TCA) (2.5 ml, 100 mg/ml), was added to the mixture, which was then centrifuged at 650 g for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 1.0 mg/ml), and then the absorbance was read spectrophotometrically at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

### 2.8. Determination of effect on DPPH radical

The effect of extracts on the DPPH radical was estimated according to the method of Hatano, Kagawa, Yasahara, and Okuda (1988). The extracts (0.05–50

mg/ml) 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 allowed to stand at room temperature for 30 min; the absorbance of the resulting solution was then measured spectrophotometrically at 517 nm. The tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

### 2.9. Measurement of chelating activity on metal ions

The chelating activity of sample on  $\text{Fe}^{2+}$  was measured according to the method of Carter (1971). Briefly, extracts (0.05–50 mg/ml) were incubated with 0.05 ml of  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (2.0 mM). The reaction was initiated by the addition of 0.2 ml ferrozine (5.0 mM), and finally quantified to 0.8 ml with methanol. After the mixture had reached equilibrium (10 min), the absorbance at 562 nm was read. EDTA served as the positive control, and a sample without EESC or EDTA served as the negative control. Triplicate samples were run for each set and averaged.

### 2.10. High-performance liquid chromatography (HPLC) analysis

Sesamin and sesamol were analyzed by HPLC, performed with a Hitachi liquid chromatograph (Hitachi, Ltd., Tokyo) consisting of a model L-7100 pump, and a model L-7420 UV-vis detector set at 280 nm. A hypersil BDS RP-18 reversed-phase column (5  $\mu\text{m}$ , 250  $\times$  4.6 mm, i.d. Hypersil) was used for analysis. The volume injected was 10  $\mu\text{l}$ . The elution solvent was methanol. The gradient elution program was set at 0.8 ml/min, starting with 10% methanol linearly to 90% methanol in 60 min.

### 2.11. Thin-layer chromatography

An aliquot of EESC (10  $\mu\text{l}$ ) was spotted on a pre-coated silica gel plate ( $F_{254}$ , 0.25 mm, E. Merck, Darmstadt, Germany), which had been activated for 15 min at 100 °C. The plates were developed in the ascending direction for 17 cm with the solvent system chloroform:methanol [1:1, v/v]. The chromatograms were dried and subjected to further testing.

### 2.12. Test of antioxidant activity

Antioxidants on TLC plates were detected using the  $\beta$ -carotene spray according to the method of Pratt and Miller (1984). Nine milligrams of  $\beta$ -carotene were dissolved in 30 ml chloroform. Two drops of linoleic acid and 60 ml of ethanol were added to the  $\beta$ -carotene-chloroform solution. This solution was sprayed onto the chromatograms spotted with SSEC. After spraying, the background colour of chloromatograms was bleached.

Spots in which yellow color persisted possessed antioxidant activity.

### 2.13. Chromatographic spray reagents

#### 2.13.1. Ferric chloride-potassium ferricyanide

Equal volumes of aqueous 1% solutions of each salt were mixed together and sprayed. Phenols give a immediate blue colour (Pratt & Miller, 1984).

#### 2.13.2. $\text{H}_2\text{SO}_4$

The  $\text{H}_2\text{SO}_4$  (30%) was sprayed onto chromatograms spotted with EESC. After spraying, tetra-nortriterpenoids indicate a brown-black colour (Huang, 1994).

## 3. Results and discussion

From ancient times, sesame has been considered to be a valuable oil-seed not only because of its high oil content, but also because of its medical effects (Namiki, 1990). This implies that some valuable components in sesame contribute to provide a nutritional and functional food for humans. However, there are literatures reports that some adverse nutritional components exists in sesame, such as phytic acid and oxalic acid (Johnson, Suleiman, & Luusas, 1976) which are detrimental to health. Saturated oxalate in solution binds to calcium to form crystals that aggregate. In humans, high urinary oxalate increases the risk of calcium oxalate kidney stones because calcium oxalate is poorly soluble in urine (Massey, Palmer, & Horner, 2001). Therefore, it is necessary to determine the contents of oxalic acid in sesame coat. In the present study, the content of oxalic acid in sesame coat is 5.39%, which is higher than the findings of Johnson et al. (1976), who showed that the sesame coat contains 2–3% oxalic acid. In addition, EESC contains 0.49% oxalic acid. Apparently, the content of oxalic acid markedly decreased after sesame coat was extracted by ethanolic solvents.

Table 1 shows the yields and antioxidant activities of different solvent extracts from sesame coat. The effects of the solvents tested on the extraction yield was not significant ( $P > 0.05$ ). Sesame coat extracts (1 mg), with various solvents, exhibited strong antioxidant activity (92.6–91.8%). No significant differences ( $P > 0.05$ ) in antioxidant activities were found among methanol extracts, ethanol extracts and acetone extracts indicating that sesame coat extracts with various solvents displayed a similarly strong antioxidant activity.

Based on the data obtained, the yields and the antioxidant activities of extracts, using different solvents, were not significantly different ( $P > 0.05$ ); however, from a toxicological point of view, ethanol, as a solvent, is safer than acetone or methanol, and therefore more

Table 1  
Yield and antioxidant activity of sesame coat with various solvents<sup>a</sup>

Solvent	Yield (g) <sup>b</sup>	Antioxidant activity (%) <sup>c</sup>
Methanol	8.19±0.17 <sup>a</sup>	92.6±4.44 <sup>a</sup>
Ethanol	8.21±2.15 <sup>a</sup>	93.9±2.49 <sup>a</sup>
Acetone	6.02±0.38 <sup>a</sup>	91.8±0.00 <sup>a</sup>

<sup>a</sup> Based on 100 g of sesame coat for each sesame coat.

<sup>b</sup> The antioxidant activity of extract (1.0 mg) was determined by the thiocyanate method.

<sup>c</sup> Values with same letter were not significantly different from mean value ( $P > 0.05$ ).

suitable for the food industry. Thus, ethanol extract was used in the following study.

Thiobarbituric acid reactive substances (TBARS) measure the formation of secondary oxidation products, mainly aldehydes (or carbonyls), which may contribute to the off-flavour of oxidized oils (Wanasundara & Shahidi, 1994). Phosphatidylcholine (lecithin), a phospholipid, is believed to be present in high amounts in cell membranes. In order to investigate EESC in biological systems, the phospholipid prepared as a liposome was used. The inhibitory effects of EESC on malondialdehyde formation in a liposome model system is plotted in Fig. 1. EESC, at 1.0 and 10.0 mg, showed 11.3 and 51.1% inhibitory effects on TBARS formation, respectively. The inhibitory effects of Toc (10 mg) and BHA (10 mg) on TBARS formation were 46.7 and 54.1%, respectively. Although the inhibitory effect of EESC on TBARS production is inferior to BHA, it is superior to Toc. EESC evidently has a good inhibitory effect on TBARS formation. Malondialdehyde (MDA) is a short-chain aldehyde, thought to belong to TBARS,

and is an intermediate product of oxidation of poly-unsaturated fatty acids. Of special note is that MDA is very reactive, and is found to take part in crosslinking reactions with DNA and proteins. Moreover, MDA can act as a catalyst in the formation of *N*-nitrosamines in foods containing secondary amines and nitrite (Eriksson, 1987). As shown in Fig. 1, EESC significantly suppresses the production of MDA but is weaker than BHA and Toc. Toc, a well-known natural antioxidant, localizes within the phospholipid bilayer of cell membranes to protect them against biological lipid peroxidation (Hafeman & Hoekstra, 1997). Although the inhibitory effect of EESC on MDA formation is less than Toc, EESC may also be expected to protect against damage to cell membranes, because it also reduces the level of lipid peroxides.

Numerous methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, the DPPH assay, the reducing ability, metal ion chelating, and active oxygen species quenching assay are most commonly used for the evaluation of antioxidant activities of extracts (Amarowicz, Naczka, & Shahidi, 2000; Duh, Tu, & Yen, 1999). Fig. 2 shows the scavenging effect of EESC on the DPPH radical, where the scavenging effect, as the absorbance difference from the control, was calculated. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. The decrease in absorbance of the DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. As shown in Fig. 2, the scavenging effect of EESC increases with increasing amounts of EESC. As a

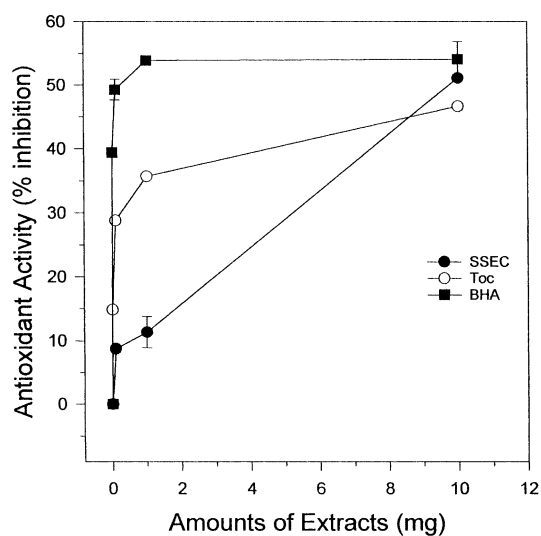


Fig. 1. The inhibitory effect of different amounts of ethanolic extracts of sesame coat (EESC) on TBARS formation.

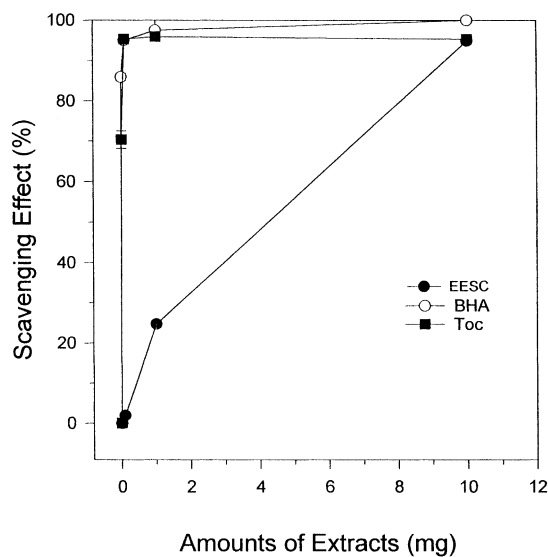


Fig. 2. The scavenging effect of different amounts of ethanolic extracts of sesame coat (EESC) on DPPH radical.

whole, the scavenging effect of EESC on the DPPH radical was less than that of Toc and BHA. EESC, at 10 mg, shows a 94.9% scavenging effect on the DPPH radical, indicating that EESC is sufficient for a noticeable effect. Fatimah, Zaiton, Jamaludin, Gapor, Nafeeza, and Khairul (1998) reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic conditions such as atherosclerosis. Based on the data obtained, EESC is a free radical inhibitor, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body.

Fig. 3 shows the reducing ability and antioxidant activity of EESC. The reducing ability of EESC increased with increasing amounts of EESC. The equation of reducing ability ( $y$ ) and amount of EESC ( $x$ ) used is  $y = 0.03380x + 0.013880851$  ( $r = 0.9975$ ,  $P < 0.05$ ), indicating that reducing ability correlated well with amount of extracts. In addition, the equation of antioxidant activity ( $y$ ) and the reducing ability ( $x$ ) is  $y = 136.18778x + 2.81814$  ( $r = 0.977$ ,  $P < 0.05$ ). This implies that a high positive correlation existed between antioxidant activity of EESC and reducing ability, revealing that the reducing ability of EESC is in part a contributor to antioxidant activity.

Fig. 4 shows the chelating effect of EESC on ferrous ions. EESC, at 0.1 mg, had a negligible effect (7.0%) on  $\text{Fe}^{2+}$  binding, and 10.0 mg of EESC showed a 79.9% chelating effect. Although this result is relatively small compared with that of EDTA, it is an iron chelator. Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation

(Halliwell, 1991). As shown in Fig. 4, the chelating effect on iron, of EESC at lower concentrations is slight, although, it may minimize the amount of iron in the Fenton reaction or in the lipid peroxidation.

Fig. 5 shows the scavenging activity of EESC on hydroxyl radical. The scavenging activity against hydroxyl radical for EESC (0–10mg) ranged from 0 to 62.5%, indicating that the scavenging effect increased with increasing amounts of EESC. As for BHA, the scavenging effect ranged from 0 to 66.5%. Apparently, EESC and BHA exhibited good scavenging effects on hydroxyl radicals. The hydroxyl radical is the most reactive radical known; it can attack and damage almost every molecule found in living cells (Halliwell, 1991). Aruoma, Halliwell, and Dizdaroglu (1989) reported that, when the hydroxyl radical attacks DNA, free radical

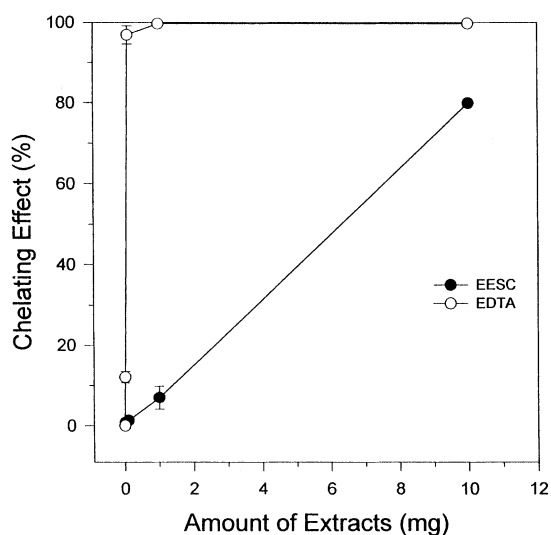


Fig. 4. The chelating effect of different amounts of ethanolic extracts of sesame coat (EESC) on ferrous ions.

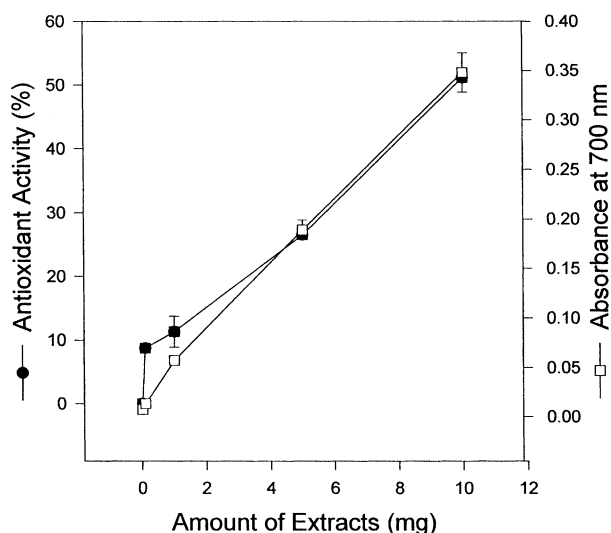


Fig. 3. Reducing ability and antioxidant activity of different amounts of ethanolic extracts of sesame coat (EESC).

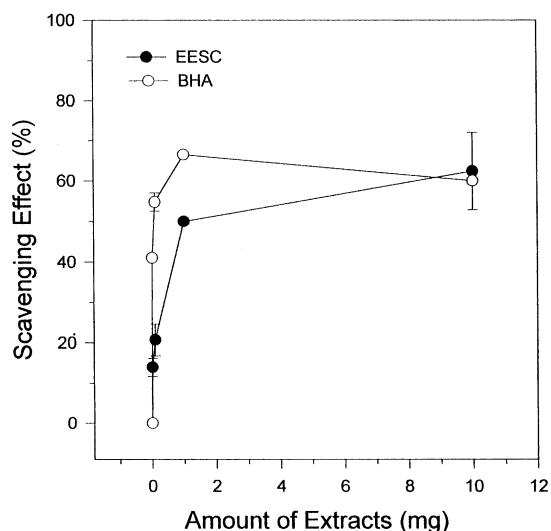


Fig. 5. The scavenging effect of different amounts of ethanolic extracts of sesame coat (EESC) on hydroxyl radical.

chain reactions occur and lead to chemical alteration of the deoxyribose and purines and pyrimidines. This can lead to mutations and DNA strand breakage. In addition, hydroxyl radicals stimulate the free-radical chain reaction known as lipid peroxidation, which leads to the production of the toxic substances, lipid hydroperoxides and their aldehyde decomposition products, responsible for the rancidity of peroxidized food material (Halliwell, 1991). In the present investigation, EESC exhibited antioxidant activity. The ability of EESC to quench the hydroxyl radical seems to relate directly to the prevention of propagation of the process of lipid peroxidation.

Previous reports have shown that polyphenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid oxidation (Yen, Duh, & Tsai, 1993). In order to elucidate the causes for the antioxidant characteristics of EESC, it is essential to determine whether the antioxidant activity is related to phenolic compounds. The UV-visible spectra of the diluted EESC is shown in Fig. 6. Absorption maxima at 237 and 274 nm may be due to the presence of flavone/flavonol derivatives (Marcheix, 1990; Wanasundara, Amarowicz, & Shahidi, 1994), indicating that EESC may contain phenolic compounds. In addition, ascending TLC on silica gel plates developed in chloroform-methanol (9:1, v/v), yielded three spots with  $R_f$  values of 0.52, 0.62 and 0.88, which gave positive reactions to  $\beta$ -carotene and ferric chloride-potassium ferricyanide sprays. This result indicated these compounds ( $R_f=0.52$ , 0.62 and 0.88) to be phenolics. Clearly, these phenolic compounds give positive reactions to  $\beta$ -carotene, indicating that they exhibit antioxidant activity.

Fig. 7 shows the antioxidant activity and the contents of polyphenolic compounds of different amounts of EESC. The amounts of phenolic compounds depended upon the concentration of EESC, and the correlation coefficient was  $r=0.999$  ( $P<0.05$ ). In addition, there was a correlation between antioxidant activity ( $y$ ) and phenolic compounds ( $x$ ):  $y=4430.2878x+3.57878713$  ( $r=0.97678$ ,  $P<0.05$ ), indicating that the phenolic compounds in EESC seem to be in part associated with the antioxidant activity.

Sesamin, sesamol and sesamol, which belong to the lignans and contribute to the stability of sesame oil, have been proven to be present in sesame oil (Yoshida, Shigezaki, Takagi, & Kajimoto, 1995); however, whether these compounds are present in sesame coat is still unclear. The HPLC analysis of sesamin and sesamol was successfully achieved as these compounds appeared at 56.55 and 57.62 min after the injection of the sample, respectively. Identification of sesamin and sesamol was done by comparing their retention times with those of authentic standards. Clearly, sesamin and sesamol are proven to be present in EESC. In addition,  $\gamma$ -tocopherol with  $\delta$ -tocopherol were present in a very small amounts and sesamol was found in sesame oil (Fukuda, Osawa,

& Namiki, 1981; Yoshida & Takegi, 1997). Sesamol may be transformed during the bleaching process to sesaminol and sesamol, which are the major antioxidant factors in refined sesame oils (Fukuda et al., 1986). Fukuda et al. (1986) noted that sesamol and  $\gamma$ -tocopherol were present in sesame seed, but sesamol was not detected in the unroasted seed oil (Yoshida et al., 1995). In the present investigation, sesamol and  $\gamma$ -tocopherol were not detected in EESC. The fact that sesamol was not present in EESC, or was too low to be detected, may be as a result of using unroasted sesame. Although sesamin and sesamol were both present in EESC, structurally, sesamin and sesamol apparently have no potential as antioxidants. Shyu (2001) has reported that sesamin and sesamol show no antioxidant activity in scavenging DPPH radical in a model system. However, sesaminol and sesamol may be produced when sesame coat is fried or roasted.

Ascending TLC on silica gel plates, developed in a chloroform-methanol (9:1, v/v) solvent, and observation of the chromatograms with visible and UV light, before

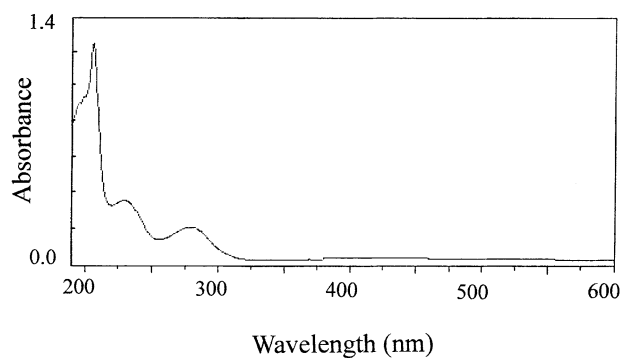


Fig. 6. UV-Vis spectra of ethanolic extracts of sesame coat (EESC).

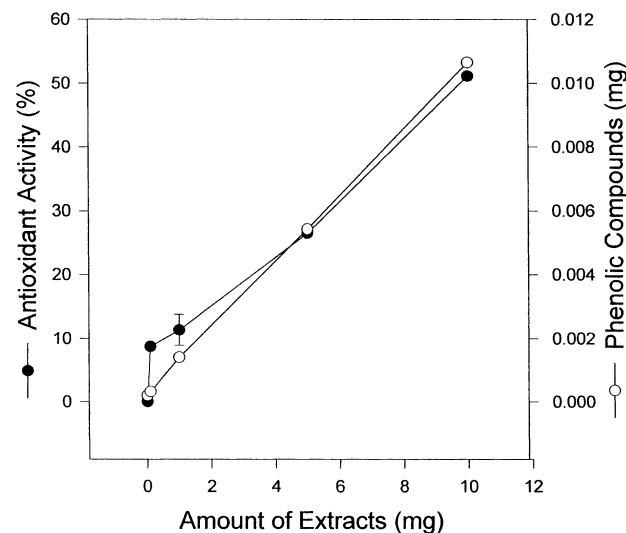


Fig. 7. Antioxidant activity and contents of phenolic compounds of different amounts of ethanolic extracts of sesame coat (EESC).

and after exposure to H<sub>2</sub>SO<sub>4</sub> fumes, yielded two brown-black spots with *R<sub>f</sub>* values of 0.82 and 0.93. Positive reactions with H<sub>2</sub>SO<sub>4</sub> indicated these compounds to be tetranortriterpenoids (Huang, 1994). In addition, a positive response to the β-carotene spray indicated that the spots with *R<sub>f</sub>* 0.82 and 0.93 possessed antioxidant activity. The chemical nature of tetranortriterpenoids, which belong to the triterpenoids, has been reported (Govindachari & Kumari, 1998; Govindachari & Gopalakrishnan, 1997). In addition, the antioxidant activity of triterpenoids has been investigated. For example, ursolic acid and oleanolic acid, which are triterpenoids, exhibited an inhibitory effect on liver microsome lipid peroxidation due their metal-chelating ability and scavenging of free radicals (Balanehru & Nagarajan, 1991, 1992; Chen, Shi, & Ho, 1992). In the present research, tetranortriterpenoids are proven, using TLC analysis, to be present in sesame coat, and these compounds show antioxidant activity in the β-carotene spray model system. This finding may indicate that these compounds contribute to the antioxidant activity of EESC.

On the basis of the results of this study, it is clearly indicated that sesame coat has significant antioxidant activity against various lipid peroxidation systems in vitro. The various antioxidant mechanisms of sesame coat may be attributed to a strong hydrogen-donating ability, a metal-chelating ability and their effectiveness as good scavengers of hydroxyl radicals. Sesamin and sesamol, as found in EESC, seem to make no contribution to the antioxidant activity of EESC. The phenolic compounds and tetranortriterpenoids appear to be responsible for the antioxidant activity of EESC, although further studies are required to reveal whether they contain other antioxidative constituents. In addition, in vivo evidence and isolation of antioxidant components in sesame coat merit further investigation.

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