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ORIGINAL ARTICLE

# Bioactive lignans from sesame (*Sesamum indicum L.*): evaluation of their antioxidant and antibacterial effects for food applications

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**Abstract** Antioxidants protect the body from various disease conditions through their ability to neutralize the effects of free radicals. Synthetic antioxidants are extensively used in processed foods for prevention of oxidation and retention of sensory quality. Consumer awareness and preference has led to a vigorous interest in the search for natural antioxidants. Sesamin and sesamolin, the major lignans present in sesame oil, are known for their antioxidative properties. Roasted sesame oil has a higher concentration of sesamol, the thermally degraded product of sesamolin, which is considered a more potent antioxidant compared to its parent molecule. The isolated lignans and sesamol were tested for their antioxidant, free radical scavenging and antibacterial properties. Sesamol is the best antioxidant and free radical scavenger amongst the molecules studied with $IC_{50}$ value of 5.44 μg / mL (DPPH radical scavenging activity). Antibacterial assays against food borne pathogens revealed sesamol to be an antimicrobial agent with minimal inhibitory concentration (MIC) of 2 mg /mL in the culture. Its activity was synergistic with γ-tocopherol, also present in sesame seeds. Inhibition of browning (60–65 %) in fruit pulps (apple, banana and potato) was observed in presence of 20 μM sesamol.

**Keywords** Sesamol · Sesamin · Free radicals · Lignans · Antioxidant · Antimicrobial activity

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## Introduction

Free radicals, or more generally, reactive oxygen species (ROS) are well recognized for playing a dual role since they can be either harmful or beneficial to living systems (Valko et al. 2006). The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by mechanisms called "redox regulation" (Kovacic and Jacintho 2001). Excess ROS can damage cellular lipids, proteins, or DNA by inhibiting their normal function and, through a series of events, deregulate cellular function resulting in various pathological conditions.

A wide variety of natural and synthetic antioxidants differing in composition, physical and chemical properties, mechanism and site of action, neutralize the free radical effects and protect the body from disease. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), synthetic antioxidants, used extensively in food preservation, are reported to be harmful for human health (Ito et al. 1983, 1986). The search for natural antioxidants from food has intensified in recent years (Guan et al. 2011, 2014).

Antioxidants are likely to influence xenobiotic-metabolizing enzymes, antioxidant enzymes and DNA repair pathways. No single assay can address all of these issues and therefore different approaches are required. Cells in culture are not always reliable models as different cell types can differ widely in their redox responses. However, this is incomplete without supporting data on their bioavailability and metabolism. If good biomarkers are chosen, experiments with human volunteers allow the evaluation of bioavailability and provide an overview of antioxidant effectiveness.

There are several methods to determine the antioxidant potential of food products. Trolox equivalent antioxidant capacity (TEAC) using ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) as an oxidant,

DPPH (2,2′-diphenyl-1-picrylhydrazyl) free radical scavenging potential, the oxygen radical absorption capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays are commonly used (Ozgen et al. 2006). Antioxidants reduce free radicals by two mechanisms—by single electron transfer or by hydrogen atom transfer. ORAC measures the hydrogen atom transfer while FRAP, ABTS and DPPH measure the electron transfer. No single universally accepted assay is adequate enough to quantitatively determine action of an antioxidant and a combination of hydrogen atom transfer, electron transfer as well as metal ion binding or chelation assay are required (Apak et al. 2013).

Sesame has long been used extensively as a traditional health food in the orient (Hirose et al. 1991). The oil from this plant is an important ingredient in Ayurvedic remedies in India and is used in Chinese medicine to increase energy and prevent aging (Namiki 2007). Sesame seeds are rich in phytochemicals called lignans, which are methylene dioxyphenyl compounds. Many of the medicinal properties of raw sesame oil are due to presence of 0.5–1.1 % sesamin, 0.2–0.6 % sesamolin and trace amounts of sesamol.

Sesamol (3, 4-methylene dioxyphenol) is a simple phenolic constituent present in roasted sesame oil with reported antioxidant activity. A wide range of phenolic compounds such as phenolic acids, flavonoids, anthocyanins, tannins, lignans, catechin, and others are also known to possess antioxidant activities (Kahkonen et al. 1999). These phenolics provide protection against harmful free-radicals and have been known to reduce the risk of certain types of cancer, cardiovascular disease (CVD), coronary heart disease (CHD), stroke, atherosclerosis, and other degenerative diseases associated with oxidative stress (Shahidi and Naczk 2004). Phenolic antioxidants can react directly with free radicals and convert them into stable products. These are primary or chain-breaking antioxidants and act by donating a hydrogen atom. Secondary antioxidants lower the rate of oxidation by several mechanisms. They may act by binding metal ions that catalyze oxidative processes or by inhibiting enzymes, or by scavenging free radicals. Natural phenolic compounds can function as both primary and secondary antioxidants (Decker et al. 2005).

Various studies have shown that sesamol, in particular, is an inhibitor of several steps in the generation of neoplasia (Soliman and Mazzio 1998). Sesamin acts as anti-carcinogen (Hirose et al. 1992), helps in lowering blood pressure (Noguchi et al. 2001), reducing serum lipid (Ogawa et al. 1995) and inhibits absorption and synthesis of cholesterol (Hirose et al. 1991) in rats. There are studies that show defatted sesame extracts and their hulls, which mainly contain lignan glucosides, possess good antioxidant activity both in black and white varieties (Shahidi et al. 2006).

This manuscript attempts to study the antioxidant activities associated with sesame lignans and their major products to give a scientific basis to their traditional use and understand their protective role.

## Materials and methods

Sesame seeds of *Sesamum indicum* L. (commercial variety) were acquired from the local market in Mysore, India. Sesamol, BHT, Trolox (6-hydroxy-2, 5, 8-tetramethylchroman-2-carboxylic acid), Tween 20 (Polyoxyethylene sorbitan monolaurate) emulsifier, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azobis (2-amidinopropane) dihydrochloride, (AAPH), 2,4,6-tripyridyl-S-triazin (TPTZ), $FeCl_2$, $FeCl_3$, gallic acid were purchased from Sigma–Aldrich (St Louis, MO) and employed as received. All solvents used were of ACS or HPLC grade, unless otherwise specified.

### Purification of sesame lignans by preparative HPLC

The extraction of sesamin and sesamolin were standardized by modification of reported methods (Amarowicz et al. 2001). Commercial white seeds of sesame were used for recovery of sesamin and sesamolin. Sesame seeds were oven-dried at 60 °C for 4 h, cooled to room temperature and then ground in a commercial coffee mill. The lipophilic constituents were extracted from the seeds, with hexane, for 10 h, using a Soxhlet apparatus. The organic solvent was removed from the extract, under vacuum, at 35 °C, using a Buchi Rotavapor/Water bath (Models EL 120 and 461, respectively). The recovered oil was mixed with acetone in 1: 10 ratio and stored overnight at −40 °C to precipitate the lipids. Unsaponifiable matter was recovered, extracted with ether and finally dissolved in methanol. The lignans in the unsaponifiable matter obtained from sesame oil were further purified by LC- 8A Shimadzu Preparative Liquid Chromatograph HPLC system equipped with a $C_5$ column (250×21 mm). The mobile phase consisted of methanol and water with gradient elution of 0–60 % of solvent B (methanol) flow rate was 5 mL/ min for 60 min. The lignans were detected at 290 nm and the peak fractions collected. Preparative HPLC of the unsaponifiable material gave two well resolved peaks for sesamin and sesamolin with retention times of 45 and 48 min, respectively. The mass balance indicated that 16.3 mg of sesamin and 10.5 mg of sesamolin were obtained from 100 mg of injected material. Rechromatography of sesamin and sesamolin on an analytical HPLC column confirmed that the purity of the isolated compounds to be >98 %. Mass spectrum further confirmed the purity and molecular mass of the lignans isolated.

### Confirmation of purity of sesamin and sesamolin by HPLC and GC-MS Analysis

Purity of sesame lignans were determined from the collected fractions of preparative HPLC by an analytical Waters® HPLC system equipped with a $C_{18}$ column (250×4.6 mm, 5 μ, Waters®) protected by a 1-cm guard column (Waters® ODS)

and a photodiode array detector (Waters®) as reported earlier (Amarowicz et al. 2001). The mobile phase was methanol–water (HPLC grade), 70: 30 v/v, at a flow rate 1 mL/ min. Samples (20 μL) were injected into the column and peaks were detected at 290 nm.

The chemical structure of purified sesamin and sesamolin standards were confirmed by gas chromatography-mass spectrometry using a Perkin Elmer Autosystem XL Gas chromatograph coupled to Turbomass Gold mass spectrometer (Perkin Elmer instruments, Norwalk, CT. USA) with a NIST library/ data system. An Elite 1 fused-silica capillary column (30 m× 0.25 mm id, 0.25 μm thickness) was used and analysis were carried out with helium as the carrier gas; helium flow rate was set at 2 mL/ min. the column temperature was set to 150 °C initially, for one min and gradually increased to 290 °C with 10 °C rise per min. Finally the temperature was held at 290 °C for 15 min. EI mass spectra were recorded at electron energy of 70 eV with the source temperature at 250 °C and inter phase temperature at 180 °C. Purified lignans were prepared in the concentration of 2 mg / mL and 2 μL was injected to the column.

Scavenging of DPPH radical

DPPH solution (0.1 mM in methanol) was incubated with varying concentrations of test compounds made in threefold dilution (Espin et al. 2000). The reaction mixture was incubated for 20 min at room temperature (in dark) and the absorbance was read at 517 nm against a blank using a spectrophotometer (Shimadzu model UV1601, Japan). The scavenging of DPPH radical was calculated according to the following equation:

$$\text{DPPH radical scavenging activity}(\%) = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\left(\text{Abs}_{\text{control}}\right)\right] \times 100$$

Where Abs $_{\text{control}}$ is the absorbance of DPPH radical + methanol; Abs $_{\text{sample}}$ is the absorbance of DPPH radical + sesame lignans / standard. BHT was used as standard and all the analyses were carried out in triplicate.

For synergistic antioxidant effects, sesamol (2 μg/ mL) and sesamin (10 μg/ mL) were taken along with different concentrations (0–10 μg/ mL) of γ-tocopherol and the DPPH radical scavenging activity assayed as given above.

Antioxidant assay using β-carotene -linoleate model system

The antioxidant activity of sesame lignans was evaluated by the β-carotene -linoleate model system (Hidalgo et al. 1994). Beta-carotene (0.2 mg), 20 mg of linoleic acid and 200 mg of Tween-20 were mixed in 0.5 mL of chloroform. The chloroform was removed at 40 °C under vacuum using a rotary evaporator. The resultant mixture was immediately diluted with 10 mL of triple distilled water and mixed well. The emulsion was further made up to 50 mL with oxygenated water. Aliquots (4 mL) of this emulsion were transferred into different test tubes containing 0.2 mL of sesame lignans (2 mg) in ethanol. BHT, used for comparison, was also taken in the same concentration. A control containing 0.2 mL ethanol and 4 mL, of the above emulsion, was prepared. The tubes were placed at 50 °C in a water bath. Absorbance of all the samples was taken at zero time ($t$=0). Measurement of absorbance was continued until the color of β-carotene disappeared in the control reaction tube ($t$=180 at 15 min intervals). A mixture, prepared as above, without β-carotene, served as blank. All determinations were carried out in triplicates. Antioxidant activity was calculated using the formula

$$\text{AA} = 100[1-(A_0-A_t)/(\mathring{A}_0 - \mathring{A}_t]$$

Where $A_0$ and $\mathring{A}_0$ are the absorbance values measured at zero time of the incubation for test sample and control respectively. $A_t$ and $\mathring{A}_t$ are the absorbance measured in the test sample and control, respectively after incubation for 180 min.

Antioxidant activity in linoleic acid emulsion

The total antioxidant activity of sesame lignans was measured by use of a linoleic acid system (Yen and Hsieh 1998). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 emulsifier and 50 mL of phosphate buffer (0.2 M, pH 7.0). The reaction mixture was prepared by taking different concentration of sesame lignans in 0.5 mL of ethanol, 2.5 mL of emulsion and 2 mL of phosphate buffer (pH 7.0, 0.05 M). The reaction mixture was incubated at 37 °C, in the dark, to accelerate the peroxidation process. Aliquot of 0.1 mL were taken, at different intervals of time, during incubation. The levels of peroxidation were determined according to the thiocyanate method by sequentially adding ethanol (5 mL, 75 %), ammonium thiocyanate (0.1 mL, 30 %), sample solution (0.1 mL), and ferrous chloride (0.1 mL, 20 mM in 3.5 % HCl). After 3 min, the peroxide value was determined by reading the absorbance at 500 nm. Gallic acid was used as positive control.

Ferrous reducing antioxidant power (FRAP)

Antioxidant capacity was determined using modified FRAP assay (Loo et al. 2007). FRAP reagent was prepared from 0.3 M acetate buffer (pH 3.6), 20 mM ferric chloride and

10 mM TPTZ made up in 40 mM hydrochloric acid. All the reagents were mixed in 10: 1: 1 (v/v/v) and the assay performed using the above reagent (preheated to 38 °C). Before analysis, the initial absorbance of the reagent and 3 mL acetate buffer (blank) were measured at 593 nm. Ferric-reducing ability power was ascertained as described (Benzie and Strain 1996). $Fe^{3+}$ to $Fe^{2+}$ ion reduction at low pH causes the formation of a colored ferrous–TPTZ complex, resulting in an increase in absorbance at 593 nm. Sesame lignans at different concentration (final concentration of the tested compound in the reaction mixture was 2 μM, 4 μM, 6 μM, 8 μM and 10 μM) were added to 900 μL of FRAP reagent, shaken vigorously for 15 s and absorption was measured after 90 min using a UV- visible spectrophotometer. Additional control experiment to exclude any influence of test compounds alone or with TPTZ were also carried out. The results were expressed as Trolox equivalence reducing power of the compound.

### Oxygen radical absorbance capacity

Antioxidant capacity was assessed, in triplicate, by the ORAC-fluorescein assay with slight modification (Moore et al. 2005). The assay mixture contained 0.067 μM of fluorescein, 60 mM of AAPH radical initiator, 300 μL of sesame lignans or methanol (reagent blank). Fluorescence of the assay mixture was recorded (λ excitation=493 nm, λ emission= 512 nm) every minute at 37 °C. The area under the curve of fluorescence vs time plot was calculated and compared against a standard curve prepared with Trolox. The ORAC value was expressed as Trolox equivalents (TE) in micromoles of pure compound for lignans.

### Inhibition of browning in fruit pulps

Apple (red delicious), banana (Cavendish) and potato were peeled and pulped. Sesamol (0–20 μM) was dissolved in water and mixed with the pulp as described (Eissa et al. 2006).

### Bacterial cultures

Bacterial cultures, namely, *Bacillus cereus* (F4810, Public Health Laboratory, London), *Staphylococcus aureus* (FRI 722, Public Health Laboratory, Netherlands) and *Pseudomonas aeruginosa* (CFR 1734, CFTRI Mysore) were obtained from the Department of Food Microbiology, CFTRI, Mysore. The above cultures were grown on nutrient agar media (HiMedia, Mumbai, India) at 37 °C. Each bacterial strain were transferred from stored slants at 4–5 °C to 10 mL nutrient broth and cultivated at 37 °C for 24 h. Pre-culture was prepared by transferring 1 mL of above to 9 mL nutrient broth and cultivated for 48 h. The bacterial cells were harvested by centrifugation (1,200 g, 5 min), followed by washing with saline and finally suspended in 9.9 mL of sterilized saline.

### Growth inhibition assay

Effect of sesame lignans on the growth of bacteria was studied, as described earlier (Jayaprakasha et al. 2003). Appropriate quantities of sesame lignans were transferred into different flasks containing 20 mL of melted nutrient agar to obtain final concentrations of 0.25, 0.5, 0.75, 1 mg/mL. A control sample was prepared by transferring an equivalent amount of DMSO to 20 mL of melted nutrient agar. 100 μL (about $10^4$ cfu /mL) of each bacterium was inoculated into flasks aseptically, poured into sterilized petri-plates in quadruplet and incubated at 37 °C for 24 h. The colonies, after incubation, were counted and expressed as colony forming units per mL of culture (cfu/ mL). The inhibitory effect was calculated using the following formula, % Inhibition$=(1-T/C)\times 100$, where $T$ = cfu /mL of test sample and $C$ = cfu/mL of control. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the compound capable of inhibiting the complete growth of the bacterium tested.

### Zone of inhibition test

This test was carried out for all the lignans. The above bacterial cultures were inoculated on nutrient agar media by spread plate method. Small circular wells were dug into agar plates using the back end of the 100 μL pipette tip. 2 mg of each lignan samples were suspended in 100 μL of methanol and placed in the wells. The plates were then incubated at 37 ° C for 24 h. The diameter of the zone of inhibition was then measured after the incubation.

All results are expressed as the mean ± the standard deviation (SD) of three replicate analyses.

## Results and discussion

Sesame lignans, which play an important role in plant defense, are unique phenolic antioxidants with many health benefits (Namiki 1995). In the present study, sesamin and sesamolin were purified, from sesame oil, after saponification and preparative HPLC. The retention times for sesamin and sesamolin are similar to the retention times reported in literature (Amarowicz et al. 2001; Kamal-Eldin et al. 1994). The lignans were also characterized by GC-MS as given under "Materials and methods". Sesamin and sesamolin were obtained at purity >98 % from sesame oil (results not shown). Commercial sesamol (purity >98 %) was used for the study as only trace amounts are present in the oil.

## Antioxidant activity of lignans

In the present study, a panel of assays were employed to evaluate the antioxidant potency of pure lignans individually and in comparison with known standards. We also selected FRAP and ORAC-FL methods to evaluate radical quenching ability of sesame lignans along with lipid peroxidation and DPPH radical scavenging assay.

The use of the DPPH free radical is advantageous in evaluating antioxidant effectiveness because it is more stable than the hydroxyl and superoxide radicals (Liyana-Pathirana et al. 2006). Sesamol scavenges DPPH radicals efficiently with an $IC_{50}$ value of 5.44 μg / mL similar to BHT ($IC_{50}$ value of 5.81 μg / mL). Both sesamin and sesamolin have very weak radical scavenging ability with only 30 and 32 % scavenging activity at as high as 250 μg / mL concentrations. The high radical scavenging capacity of sesamol is probably due to the presence of free hydroxyl group in its structure.

## Antioxidant activity by β-carotene—linoleate model system & linoleic acid emulsion

The mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from the hydroperoxides formed from linoleic acid. The linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecule. As the β-carotene molecules get oxidized, it loses its characteristic orange color. Antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical in the system. Sesamol, sesamin and sesamolin exhibit antioxidant activity of 78.5, 68 and 62.5 % respectively (Fig. 1a) when compared to standard BHT (97 %).

The antioxidant effects of sesame lignans on the peroxidation of linoleic acid are shown in Fig. 1b. The oxidative activity of linoleic acid is markedly inhibited by all 3 lignans tested. BHT is included as positive control for phenolic antioxidants. Taken together, the results show that the inhibitory potential follows the order sesamol > gallic acid > sesamolin > sesamin. Sesamol and sesamin exhibited excellent antioxidant activity with 97.8 and 96 % inhibition of linoleic acid peroxidation respectively. Sesamol is a unique phenolic compound due to its solubility in the aqueous as well as the oil phase and thermal stability. It has a benzodioxole group, which is known to scavenge hydroxyl radical to produce 1, 2-dihydroxybenzene (Kumagai et al. 1991). This makes sesamol a potent hydroperoxide scavenger compared to sesamin and sesamolin.

Sesame lignans are known to inhibit the course of fatty acid metabolism, in vivo in animal models, and also in microorganisms by specifically inhibiting the rate limiting enzyme Δ5 desaturase (Fujiyama-Fujiwara et al. 1992). In the present study, all the 3 lignans show inhibition of lipid peroxidation, in vitro, resulting in prevention of β-carotene bleaching and also formation of linoleate peroxyl radical. The synergistic effect of lignans on lipid peroxidation in rat liver and mitochondrial microsomes in presence of tocopherols is reported (Ghafoorunissa et al. 2004). The order of inhibitory effects of different lignans in combination with $\alpha$-tocotrienol is as follows: sesamol > sesamin > sesamolin.

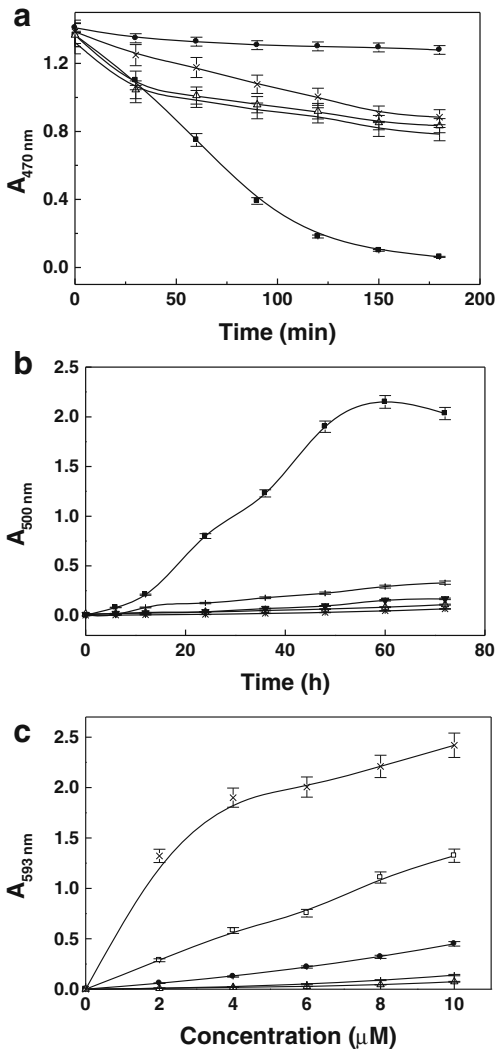


**Fig. 1** Antioxidant activities of sesame lignans by **a** β-carotene -linoleate model system. **b** Antioxidant activities of sesame lignans by thiocyanate method with gallic acid was used as standard at the same concentration. **c** Ferrous Reducing Ability Power assay for sesame lignans. All the lignans were taken at 200 μg $mL^{-1}$ concentration. Sesamol (×), sesamin (△), sesamolin (|), Gallic acid (▼), Control (■), trolox (□), BHT (●) at different concentrations

## Ferrous reducing ability power & ORAC-FL assays

Of the three compounds analyzed (Fig. 1c), sesamol has 1.83 ±0.08 μM TE (trolox equivalence) reducing power compared to 0.6±0.02 of BHT. Sesamin and sesamolin show very low

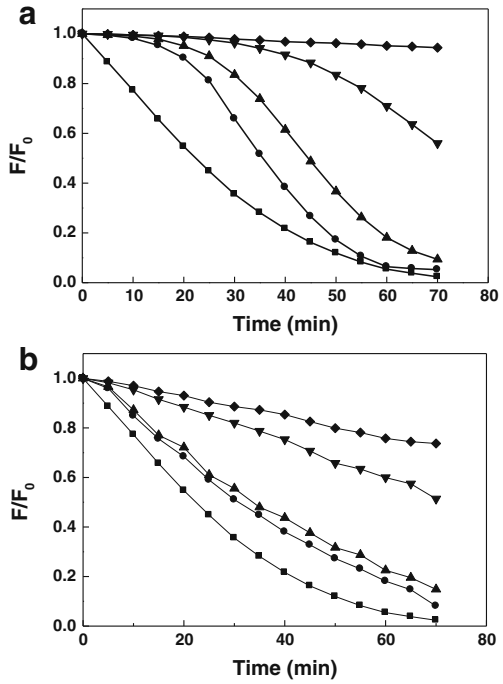


**Fig. 2** Time-course of the consumption of fluorescein (FL) (70 nM) incubated at 37 °C in the presence of 10 mM AAPH in absence and presence of **a** Trolox **b** Sesamol. FL fluorescence (493 and 515 nm, for the excitation and emission wavelengths, respectively) decay curve induced by AAPH in the presence of free radical scavenger. F is the relative fluorescence intensity obtained in presence of free radical scavenger; $F_0$ is the initial fluorescence of fluorescein in the absence of sesamol or trolox. (■) Control, (●) 2 μM (▲) 4 μM, (▼) 5 μM (◆) 10 μM

FRAP values with 0.06±0.01 and 0.12±0.05 TE. This reveals that sesamol has potent antioxidant capacity to reduce the $Fe^{3+}$/ tripyridyl-s-triazine (TPTZ) complex to the ferrous form.

The oxygen radical absorbance capacity assay (ORAC) is based on the chemical damage caused to the fluorescent (fluorescein, FL) substrate by the peroxyl radicals produced in situ. This has been quite widely used to assess the free radical antioxidant activity of pure compounds, fruit and vegetable extracts, wines, and biological fluids (Prior and Cao 1999). The concentration interval leading to a linear relationship between the net area under the curve (AUC) and the antioxidant concentration is determined for all the lignan samples (Fig. 2). Within this interval, any antioxidant

**Table 1** Synergistic antioxidant effects of sesamin and sesamol with γ tocopherol

| Compounds | $IC_{50}$ values (μg)[a] |
|---|---|
| γ tocopherol | 4.5 |
| γ tocopherol + sesamol | 1.6 |
| γ tocopherol + sesamin | 2.74 |

[a] Values are represented as mean of three different experiments

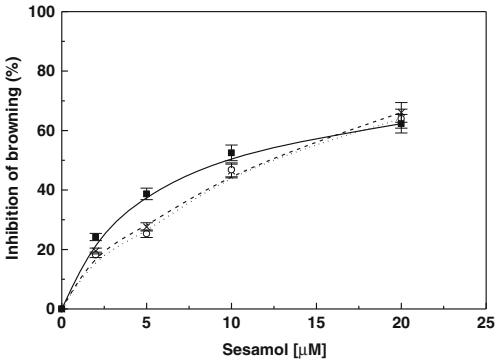


**Fig. 3** Inhibition of browning by sesamol in fruit pulps. Sesamol (0–10 μM) was added to the pulps and the browning studied after a period of 24 h at 27 °C. The pulps were stored in a petridish covered by cellulose film. (■) apple, (○) potato, (×) banana

concentration gave the same oxygen radical absorbance capacity (ORAC-FL) value. As expected, the ORAC-FL value of sesamol is 4.4 μmol of trolox equivalents/mL, which is highest among all the 3 lignans. Sesamin and sesamolin

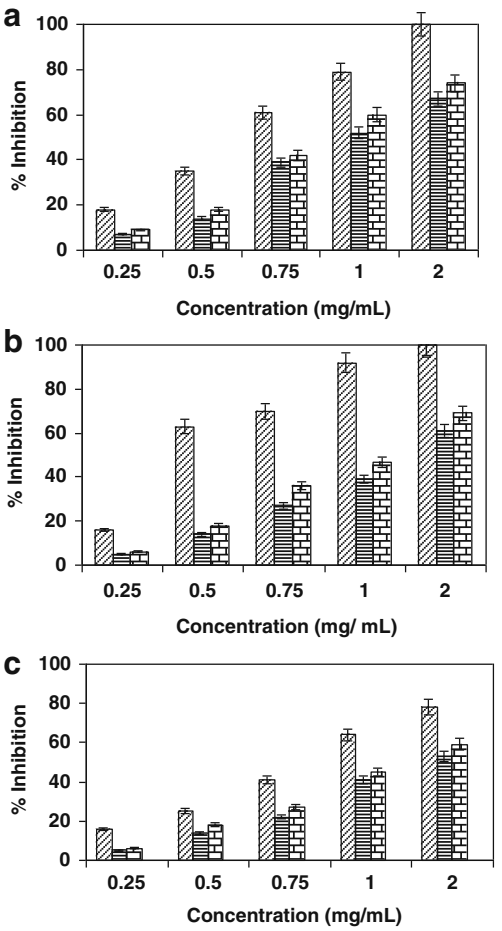


**Fig. 4** Effect of sesame seed lignans on growth of different bacteria at different concentrations **a** *Bacillus cereus*, **b** *Staphylococcus aureus* and **c** *Pseudomonas aeruginosa*. Sesamin (▭), Sesamolin (▭) Sesamol (▨). Data are presented as means ± SE ($n$=4)

showed 0.8 and 1.52 μmol of trolox equivalents/mL of ORAC- FL value. Positive control BHT shows a TE value of 2.36 μmol of trolox equivalents, which is lesser than sesamol. To the best of our knowledge and from the cited literature, this is first time the antioxidant activity of sesame lignans by FRAP and ORAC- fluorescein are reported.

### Synergistic antioxidant effects of sesamol and sesamin with γ-tocopherol

The synergistic effect of γ-tocopherol with sesamol and sesamin were studied by DPPH radical scavenging. The radical scavenging ability of γ-tocopherol is enhanced by three times in presence of sesamol or sesamin. γ-tocopherol alone has the $IC_{50}$ value of 4.5 μg/ mL and in presence of 10 μg of sesamin $IC_{50}$ value decreases to 2.74. In presence of 2 μg sesamol, $IC_{50}$ value was found to be 1.6 μg which is more than three times potent compared to γ-tocopherol alone (Table 1). Sesamin very less antioxidant activity in vitro, But it enhances the antioxidant potency of γ-tocopherol.

### Inhibition of browning in fruit pulps

Many of the molecules that are antioxidant in nature find application as inhibitors of browning reactions. In the food industry, browning reactions lead to great economic loss due to the deterioration in sensory characteristics. We studied the effect of sesamol in inhibition of browning in apple, banana and potato pulps. Sesamol inhibits browning reactions to the extent of 60–65 % (Fig. 3).

### Antibacterial activity

The minimum inhibition concentration (MIC) is calculated as given under "materials and methods". Viability test is conducted on three foodborne pathogens: *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Figure 4 shows the results of lignan concentration against % inhibition bacterial growth which is carried out to determine MIC value. The results reveal that, as the concentration of the lignans increase, the viability of the organisms decrease gradually in dose dependent manner. The minimum inhibitory concentration for the sesamol is 2 mg/mL against *Bacillus cereus* and *Staphylococcus aureus*, but it inhibits only 80 % of growth of *Pseudomonas aeruginosa* at 2 mg/mL concentrations. Sesamin and sesamolin show relatively less antimicrobial activity. Sesamin inhibited 69, 69 and 59 % of growth at as high as 2 mg/mL concentration while sesamolin shows 61, 62 and 53 % of growth inhibition at that same concentration against *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively (Fig. 4). Investigations were further carried out to determine antibacterial activity using agar well diffusion assay. A clear zone of inhibition by the sesame lignans, particularly sesamol, against *Bacillus cereus* is observed (results not shown). Sesamol is a potent inhibitor of *Mucor circinelloides* and inhibits microbial growth upon assimilation and subsequent metabolism to catechol-like compounds (Wynn et al. 1997).

## Conclusion

The biological activities of sesame lignans and sesamol were studied by comparing their antioxidant properties as well as antimicrobial activity. The order of radical scavenging, reducing power ability as well as antimicrobial activity of the three compounds was sesamol > sesamin > sesamolin. Our results reveal that sesamol acts as both primary and secondary antioxidant. The presence of hydroxyl group in sesamol, its ability to bind metal ions and its hydrophilic as well as lipophilic nature makes it more effective than the lignans-sesamolin and sesamin. The present investigation reveals the potential of sesame lignans as antioxidants and antimicrobial preservatives. They could be used in cosmetics and pharmaceuticals as effective stabilizers for prolonging the shelf life of oils and oil based products.

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