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ANTIOXIDENT POTENTIAL AND REDUCING ACTIVITY OF SESAMOL

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Abstract: This study was carried out to evaluate the antioxidant potential of sesamol (SM) comparing it with standard antioxidant, ascorbic acid. There are numerous methods for evaluating the antioxidant activity. The antioxidant potential of SM is estimated using free radicals such as DPPH, super oxide anion, nitric oxide, hydroxyl radical, and hydrogen peroxide. The reducing capacity of SM was also estimated. The scavenging activity of SM is found to be dose dependant. The IC_{50} value of SM is 5.9 µg/ml (DPPH), 42.4 µg/ml (super oxide anion), 41.4 µg/ml (nitric oxide), 31.4 µg/ml (hydroxyl radical), 10.1 µg/ml (hydrogen peroxide), and 6.2 µg/ml (reducing capacity). When comparing with ascorbic acid, SM is less efficient in scavenging super oxide, nitric oxide, hydroxyl radical, and hydrogen peroxide, whereas it is more efficient in scavenging DPPH and has high reducing capacity. Thus, the SM is as an efficient antioxidant.

Keywords: Sesamol, Ascorbic acid, DPPH, Free radical.

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INTRODUCTION

Antioxidants are chemicals produced within the body or absorbed from the diet to neutralize the effect of free radicals. Free radicals are highly reactive and potentially very damaging molecules, synthesized by metabolism, which occur in the body due to some abnormalities. Although free radicals are short lived, they cause serious damage to the cells of the body. Short lived free radicals (e.g., oxygen radical) are involved in various diseases such as coronary disease and oxidative stress.[1, 2] Free radicals are negatively charged and they become stable by grabbing the electron from the nearby molecules. This process is called oxidation and they generate oxidative stress within the cell. Antioxidants are synthesized within the cell such as superoxide dismutase, glutathione, catalase, and so on. But they are insufficient to neutralize the free radicals generated in the cell. Thus antioxidants should be supplied through diet or medication to fulfill the need. The identification and estimation of the potential of antioxidant have become more important at this situation. When a potent antioxidant is identified, it can be used for various diseases such as cancer, neurodegenerative disorder, and so on.

Sesamol (SM) is a natural organic compound, which is a component of sesame oil. It is white crystalline solid that is a derivative of phenol. It is sparingly soluble in water. Figure 1 shows the structure of sesamol. *Sesamum indicum* is a flowering plant of the genus *Sesamum* of the Pedaliaceae family. Numerous wild relative species are found in Africa and a smaller number in India. It is widely naturalized in tropical regions around the world and is cultivated for its edible seeds. SM is a powerful antioxidant and inhibits UV- and Fe^{3+}/ascorbate-induced lipid peroxidation in rat brain.[3] It has been shown to inhibit several steps in the generation of neoplasia and mutagenesis.[4]

SM has been found to prevent the spoilage of oil.[5] SM also has antifungal activity.[6] The present study is to investigate the antioxidant potential of SM comparing it with ascorbic acid, a standard antioxidant. The free radicals such as DPPH (1,1-diphenyl-2-picrylhydrazyl), super oxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxide were used to evaluate antioxidant potential of SM and reducing activity of SM was also investigated.

MATERIALS AND METHODS

SM (98% pure) was purchased from Sigma-Aldrich. All the other chemicals used were of analytical grade.

DPPH free radical scavenging activity

Free radical scavenging activity of SM is determined using DPPH.[7] 1 ml of SM at different concentrations (2 µg–10 µg) were added to 1 ml of methanolic solution of DPPH. The reaction mixture was allowed to stand for 15 min at room temperature. The intensity was read at 517

nm. The scavenging ability of SM was calculated using the following equation. Ascorbic acid is used as standard.

Equation 1

$$\text{Percentage of inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}$$

Super oxide anion radical scavenging activity

The super oxide anion scavenging activity of SM was measured using the method described by Liu.[8] Super oxide anion radicals were generated in PMS(Phenazine methosulphate)-NADH system by oxidation of NADH and assayed by reduction of NBT(Nitroblue tetrazolium). In this study, 3 ml of tris-HCl buffer containing 1 ml of NBT (50 µM), 1 ml of NADH (78 µM), and SM in the range of 10–50 µg were added. The reaction was initiated by adding 1 ml of PMS solution (10 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min. The absorbance was read at 560 nm. The scavenging ability of SM was calculated using equation 1.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured spectrophotometrically by the method of Govindarajan et al.[9] Sodium nitroprusside (5 Mm) in PBS (phosphate buffer solution) was mixed with different concentrations of SM (10–50 µg) and incubated at 25°C for 15 min. Then, Griess reagent (1% sulphanilamide, 2% H_3PO_4, and 0.1% Naphthyl ethylenediamine dichloride) was added to the mixture. The absorbance was read at 546 nm. Ascorbic acid is used as standard. The scavenging ability of SM was calculated using equation 1.

Hydrogen peroxide scavenging activity

The scavenging of hydrogen peroxide was determined by the Ruch et al's method.[10] 0.6 ml of 4 mM hydrogen peroxide solution (prepared in PBS) was added in 4ml of SM (2–10 µg) and incubated for 10 min. The absorbance of the solution was measured at 230 nm against a blank solution. The scavenging ability of SM was calculated using equation 1.

Hydroxyl radical scavenging radical activity

Halliwell et al's[11] method was followed to measure the hydroxyl radical scavenging radical activity. The reaction mixture contained 2.8 mM deoxyribose, 0.05 M KH_2PO_4, NaOH buffer pH 7.4, 0.1 mM $FeCl_3$, 0.1 nM EDTA, 1 mM H_2O_2, 0.1 mM of ascorbic acid, and 10–50 µg of SM in final volume of 2 ml. The reaction mixture was incubated for 30 min at room temperature followed by the addition of 2 ml of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. The

reaction mixture was kept in boiling water bath for 30 min. The color developed was read at 532 nm. The scavenging ability of SM was calculated using equation1.

Estimation of reducing activity

The reducing activity of SM was assessed by the Oyaizu's method.[12] About 2 ml of SM at different concentrations (2–10 µg) was added to potassium ferricyanide (2.5, 10 g/ml) and the mixture was incubated at 50°C for 20 min. Ten percent of TCA was added to the mixture, which was then centrifuged at 650 rpm for 10 min. About 2.5 ml of the supernatant was taken and 0.5 ml of ferric chloride was added. The absorbance was read at 700 nm. Ascorbic acid was used as standard. The scavenging ability of SM was calculated using equation 1.

RESULTS

The antioxidant potential of SM is estimated using the free radical such as DPPH, super oxide anion, nitric oxide, hydroxyl radical, and hydrogen peroxide and the reducing capacity of SM was estimated. The IC_{50} value of SM and ascorbic acid is shown in Table 1.

DPPH free radical scavenging activity

DPPH free radical scavenging activity of SM is shown in the Figure 2. The scavenging activity is found to be dose dependant; as the concentration of SM increases, the scavenging activity also increases. The IC_{50} value of SM and ascorbic acid is 5.9 µg/ml and 6.45 µg/ml respectively.

Super oxide anion radical scavenging activity

Figure 3 shows the super oxide anion radical scavenging activity of SM and ascorbic acid and their IC_{50} value is found to be 42.4 µg/ml and 36.4 µg/ml, respectively.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity was measured spectrophotometrically. The nitric oxide generated in the reaction mixture was scavenged by SM in dose-dependent manner. Figure 4 shows the percentage of inhibition, and IC_{50} value of SM and ascorbic acid is found to be 41.4 µg/ml and 35.6 µg/ml, respectively.

Hydroxyl radical scavenging radical activity

Figure 5 shows hydroxyl radical scavenging radical activity of SM along with ascorbic acid. From the graph, the IC_{50} value of SM and ascorbic acid was calibrated as 31.4 µg/ml and 30.4 µg/ml, respectively.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of SM was measured by direct method. The scavenging activity was in dose-dependent manner. From Figure 6, the IC_{50} value of SM and ascorbic acid was calibrated as 10.1µg/ml and 6.9 µg/ml, respectively.

Reducing activity

Figure 7 explains that the reducing activity of SM is higher than the standard ascorbic acid. IC_{50} value of SM and ascorbic acid was found to be 6.2 µg/ml and 7.75 µg/ml, respectively.

DISCUSSION

This study was carried out to evaluate the antioxidant potential of SM comparing it with the standard antioxidant, ascorbic acid. There are numerous antioxidant methods for evaluating antioxidant activity. However, the total antioxidant activity of an antioxidant cannot be evaluated by using a single method due to oxidative process. Therefore, at least two methods should be employed.[13] DPPH is a stable free radical and accepts an electron on hydrogen radical to become a stable diamagnetic molecule.[14] The decrease in absorption of DPPH is caused by antioxidant activity of SM. Thus the SM scavenges the DPPH in dose dependant manner. Super oxide radical is generated in vivo by several oxidative enzymes, including xanthine oxidase. In the PMS-NADH-NBT system, super oxide anion derived from dissolving oxygen by PMS-NADH coupling reaction reduces NBT.[13] The decrease in absorbance at 560 nm with SM and ascorbic acid indicates the consumption of super oxide anion in the reaction mixture. H_2O_2 is highly important because of its ability to penetrate biological membrane. H_2O_2 itself is not very reactive, but can be toxic to cells because it may give rise to hydroxyl radical in the cells. The result shows that SM had an effective H_2O_2 scavenging activity in dose-dependent manner than ascorbic acid.

For the measurement of reductive ability, Fe^{3+} to Fe^{2+} transformation in presence of SM was investigated and found to have significant reducing ability. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. As in the case of super oxide anion scavenging activity, the SM efficiently scavenges hydroxyl radical and nitric oxide, which is generated in their respective reaction system in dose-dependent manner. The antioxidant activity of SM has been attributed to various mechanisms, among which are decomposition of peroxides, prevention of continuous hydrogen abstraction, reducing capacity, and radical scavenging. When compared with ascorbic acid, SM is less efficient on super oxide, nitric oxide, hydroxyl radical, and hydrogen peroxide scavenging activity, whereas it is more efficient on DPPH scavenging activity and high reducing capacity. Thus, SM is proven as an efficient antioxidant from the above results.

CONCLUSIONS

Free radicals play a key role in the formation of various pathogenic disorders (neurodegenerative disorder, cancer, diabetes, etc.). Our body becomes impotent to fight against them. So there is a demand for potential antioxidants. It is important to identify potential antioxidants from the natural source and its potentiality is to be estimated. It can be concluded that SM is a potential antioxidant.

Conflict of interest

Conflict of interest is declared none.

FIGURES AND TABLES:

Figure 1: Structure of sesamol ($C_7H_6O_3$)

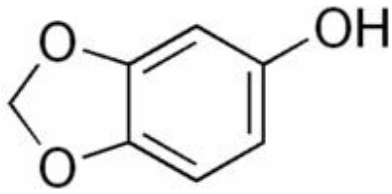

Figure 2: DPPH free radical scavenging activity

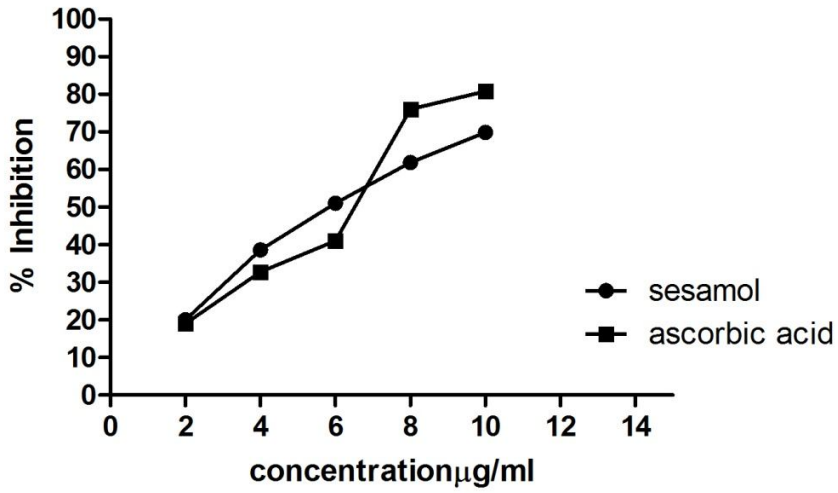

Figure 3: Super oxide anion radical scavenging activity

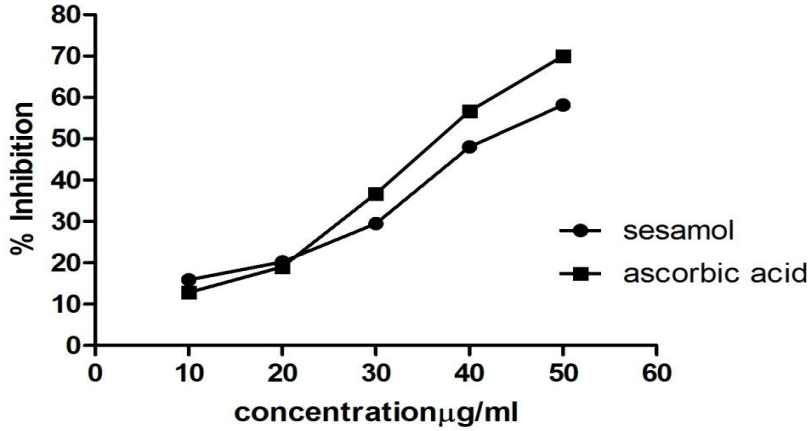

Figure 4: Nitric oxide radical scavenging activity

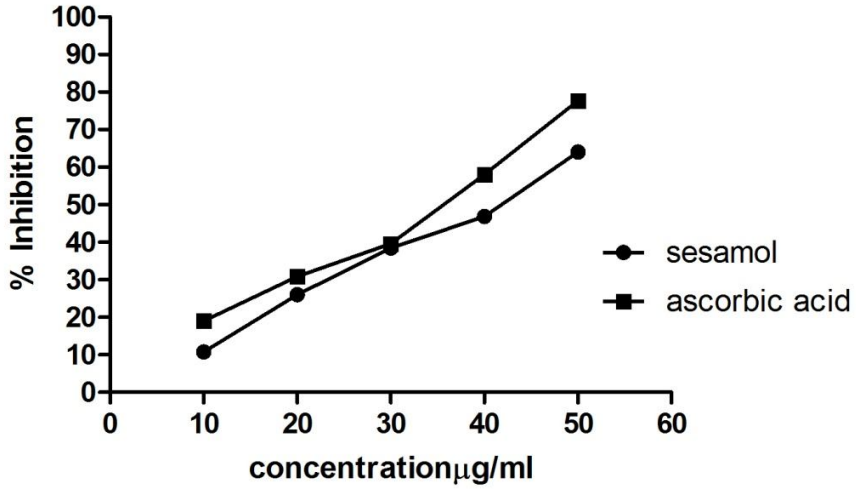

Figure 5: Hydroxyl radical scavenging radical activity

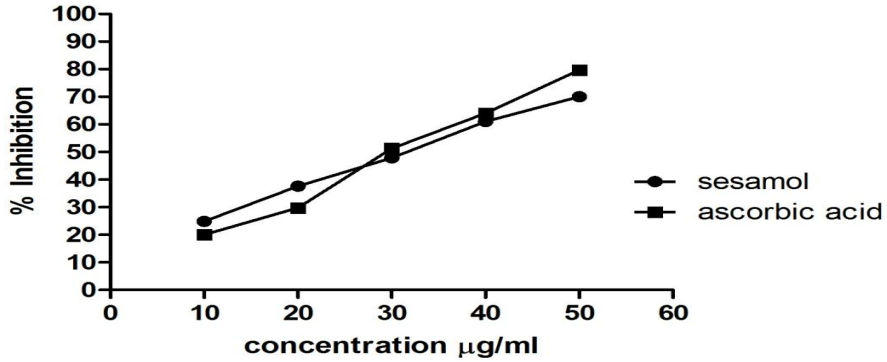

Figure 6: Hydrogen peroxide scavenging activity

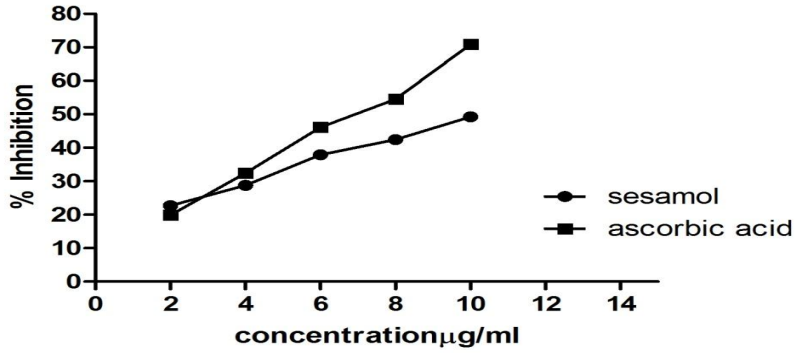

Figure 7: Reducing activity

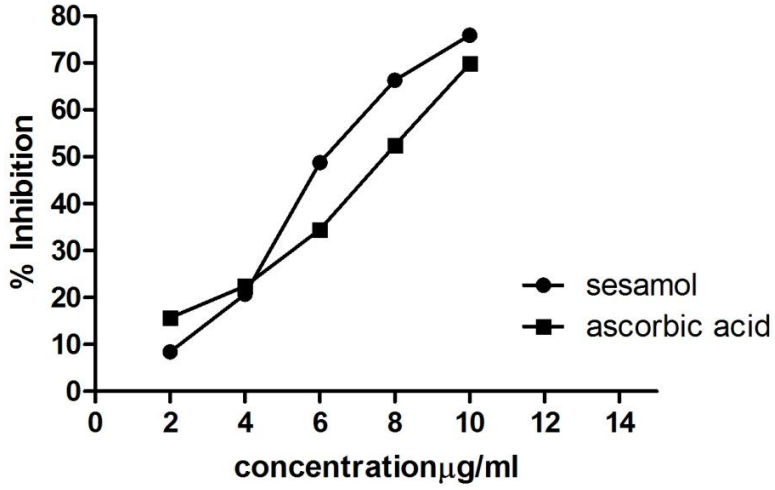

Table 1: IC_{50} values of SM and ascorbic acid

No.	Methods	Sesamol (µg/ml)	Ascorbic acid (µg/ml)
1.	DPPH	5.9	6.45
2.	Super oxide anion	42.4	36.4
3.	Nitric oxide	41.4	35.6
4.	Hydroxyl radical	31.4	30.4
5.	Hydrogen peroxide	10.1	6.9
6.	Reducing capacity	6.2	7.75

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