Phytochemical and In-Vitro Evaluation of Antioxidant Activity of Sesamum Indicum Leaves

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Abstract

Objective: The aim of the present study was to evaluate preliminary phytochemicals and in-vitro evaluation of antioxidant activity of Sesamum indicum leaves.

Methods: Antioxidant activity was evaluated by using in-vitro antioxidant assay models like hydrogen peroxide scavenging activity, reducing power assay and phosphomolybdenum method. All the antioxidant activities were compared with standard antioxidant such as ascorbic acid.

Results: The ethanolic extract of Sesamum indicum leaves was found to contain flavonoids. Additionally, the capacity to scavenge hydrogen peroxide, the reducing power (capacity to reduce Fe^{3+} to Fe^{2+}) and formation of phosphomolybdenum complex were evaluated. From this study it was concluded that the extract exhibited a concentration dependent scavenging activity.

Keywords: Sesamum indicum, Flavonoids, Hydrogen peroxide scavenging, Reducing power, Phosphomolybdenum complex

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Introduction

Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against predation or infection[1]. Medicinal plants have been identified and used throughout human history[2]. Living cells generate free radicals and other reactive oxygen species (ROS) as by-products as a result of physiological and biochemical processes. The potentially reactive derivatives of oxygen are $O^{2\cdot-}$, H_2O_2 and $^{\cdot}OH$, are continuously generated inside the human body as a consequence of exposure to a plethora of exogenous chemicals in our ambient environment or a number of exogenous metabolic processes involving redox enzymes and bioenergetics electron transfer. Under normal circumstances, the free radicals are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and detoxified by the antioxidants present. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to lipids, proteins and DNA which may eventually lead to many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases in humans[3].

In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer adverse reactions but their antioxidant activities are lower than the synthetic antioxidant butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens[4]. Therefore, there is considerable interest in finding safer antioxidants from natural sources to replace the synthetic ones.

Sesame[5] (*Sesamum indicum* L.), also called beniseed belongs to the Pedaliaceae family. Sesame has been valued as a healthy food additive preventing diseases and promoting well-being. Sesame seed consumption appears to increase plasma γ-tocopherol[6] and enhanced a vitamin E activity which is believed to prevent cancer and heart disease. The plant roots and leaves are used in treating migraine, hypertension, ulcers, constipation, chicken pox and piles. The seeds are used as a demulcent in respiratory infections, infantile cholera, diarrhoea, dysentery, bowel infections and bladder diseases. The seed powder is useful in amenorrhoea, dysmenorrhoea, ulcers and bleeding piles[7]. Therefore, our present study is to investigate the phytochemical composition, *in-vitro* antioxidant and free radical scavenging potential of the plant *Sesamum indicum*.

Materials and Methods

Identification & collection of plant material: The whole plant of *Sesamum indicum* was collected from surrounding area of Kankipadu, Andhra Pradesh, India. These plants were identified and authenticated in the Department of Botany, Hindu College, Machilipatnam. The leaves were sorted, cleaned and air dried at room temperature for one week. Then the leaves were ground to powder. Powdered sample was collected and stored in air and water proof containers protected from direct sunlight and heat until used for extraction.

Preparation of extracts: The powdered material of *Sesamum indicum* (leaves) were extracted for 18 hrs with ethanol in soxhlet apparatus[8,9]. The extracts were concentrated to dryness till free from the solvents.

Phytochemical analysis: Phytochemical analysis[10] of extract was carried out for the presence of saponins, tannins, flavanoids, alkaloids, glycosides, steroids, carbohydrates, proteins and phenols by different methods.

Animals: Wistar albino rats of either sex were obtained from animal house of the department. They were housed in an environmentally regulated room on a 12 hrs light: 12 hrs dark cycle with 25 ± 2^0C and had free access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments were conducted according to the CPCSEA guidelines on the use and care of experimental animals.

Acute toxicity study: Different doses (5, 50, 300, 1000 and 2000 mg/kg, p.o.) of extract was used for acute toxicity in accordance to Organization for Economic Cooperation Development (OECD) guidelines 423[11]. Three female rats, each sequentially dosed at intervals of 48 hrs, were used for the test. Once daily cage side observations included changes in skin, fur, eyes, mucous membrane (nasal), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defecation) and central nervous system (drowsiness, gait, tremors and convulsions) changes. Mortality, if any, was determined over a period of 2 weeks.

Hydrogen peroxide scavenging activity: The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch[12]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (p^H 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts were added to hydrogen peroxide (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds as calculated as follows

Scavenging activity (%) =

$$[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

Reducing power assay: The reducing power was determined by the method of Oyaizu[13]. Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. One ml of test sample solution was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. Trichloroacetic acid (2.5 ml) were 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 a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Ascorbic acid (20 µg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power.

Phosphomolybdenum method: Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex[14]. Take 0.1 ml of sample solution is combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95°C for 90 min. After cooling the sample to room temperature, the absorbance of the solution is measured at 695 nm against blank in UV spectrophotometer.

Results and Discussion

Preliminary phytochemical analysis: Phytochemical analysis of ethanolic extract of *Sesamum indicum* leaves showed the presence of saponins, flavanoids, alkaloids, steroids, carbohydrates and phenolic compounds (Table 1). Since phenolic compounds and flavonoids are responsible for the antioxidant activity, the amounts present in the extract are high indicating good antioxidant activity. The scavenging ability of the phenolics and flavonoids are mainly due to the presence of hydroxyl groups. The presence of phenolic compounds[15] in the plant contributed to its antioxidant activity and thus usefulness of the plant as a medicament. Flavonoids have been shown to exhibit the actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A2.

Acute toxicity study: In the present study, single-dose oral administration of extract at different dose level (5, 50, 300, 1000 and 2000 mg/kg, p.o.) in rats had no effect on mortality, clinical signs, body weight change or gross observation. Therefore, no acute toxicity was found in rats treated with ethanolic extract. Lethal dose may be higher than 2000 mg/kg each.

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging activity of the ethanol extract and standard were given in the Table 2 and Fig. 1. *Sesamum indicum* ethanolic extract caused a strong dose-dependent inhibition of hydrogen peroxide. At a concentration of 10, 25, 50, 75 and 100 µg/ml of the extract the scavenging percentage was 26.26, 44.85, 48.94, 60.50 and 67.54 respectively.

Reducing power assay: The reducing power of the extract compared to ascorbic acid is shown in Table 3. In the reductive ability measurement, Fe^{3+}-Fe^{2+} transformation in the presence of extract sample was investigated. Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. In this investigation, Table 3 and Fig. 2 shows the reductive capabilities of ethanolic extract of *Sesamum indicum* when compared to the standard ascorbic acid. The reducing power increased significantly with increasing concentration of the extract. At 10, 25, 50, 75 and 100 µg/ml the reducing power was 30.26, 36.54, 48.09, 56.39 and 62.55 respectively.

Phosphomolybdenum method: Total antioxidant capacity assay is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The activity increased significantly with increasing concentration of the extract was given in Table 4 and Fig. 3. At 10, 25, 50, 75 and 100 µg/ml the percent inhibition was 30.26, 36.54, 48.09, 56.39 and 62.55 respectively.

Phenolic compounds are known powerful chain breaking antioxidants, important plant constituents because of their scavenging ability due to their hydroxyl groups and contribute directly to antioxidative action. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1 gm daily with a diet rich in fruits and vegetables.

Table 1: Phytochemical screening of ethanolic extract of *Sesamum indicum* leaves

S. No.	Phytochemical	Result
1	Saponins	+
2	Tannins	-
3	Flavanoids	+
4	Alkaloids	+
5	Glycosides	-
6	Steroids	+
7	Carbohydrates	+
8	Proteins	-
9	Phenols	+

Table 2: Antioxidant activity of *Sesamum indicum* leaves by H_2O_2 scavenging activity

S. No.	Concentration (µg/ml)	Absorbance (mean±S.D., 230 nm)	Percent inhibition
1	Control	0.0952±0.0002	----
2	Standard	0.187±0.0006	96.43
3	10	0.1202±0.0002	26.26
4	25	0.1379±0.0002	44.85
5	50	0.1418±0.0014	48.94
6	75	0.1528±0.0004	60.5
7	100	0.1595±0.0012	67.54

Absorbance values expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test with control group. Significance represented as $P<0.01$

Table 3: Antioxidant activity of *Sesamum indicum* leaves by reducing power assay

S. No.	Concentration (µg/ml)	Absorbance (mean±S.D., 700 nm)	Percent inhibition
1	Control	0.0892±0.0002	----
2	Standard	0.1681±0.0018	88.45
3	10	0.1162±0.0002	30.26
4	25	0.1218±0.0021	36.54
5	50	0.1321±0.0013	48.09
6	75	0.1395±0.0005	56.39
7	100	0.1450±0.0011	62.55

Absorbance values expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test with control group. Significance represented as P<0.01

Table 4: Antioxidant activity of *Sesamum indicum* leaves by phosphomolybdenum method

S. No.	Concentration (µg/ml)	Absorbance (mean±S.D., 695 nm)	Percent inhibition
1	Control	0.0785±0.0012	----
2	Standard	0.1423±0.0005	81.27
3	10	0.0987±0.0025	25.73
4	25	0.1098±0.0016	39.47
5	50	0.1189±0.0014	51.46
6	75	0.1255±0.0012	59.87
7	100	0.1299±0.0002	65.47

Absorbance values expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test with control group. Significance represented as P<0.01

Table 5: Antioxidant activity of *Sesamum indicum* leaves

S. No.	Concentration (µg/ml)	Percent Inhibition		
		H_2O_2 scavenging activity	Reducing power assay	Phosphomolybdenum method
1	Standard	96.43	88.45	81.27
2	10	26.26	30.26	25.73
3	25	44.85	36.54	39.47
4	50	48.94	48.09	51.46
5	75	60.5	56.39	59.87
6	100	67.54	62.55	65.47

Absorbance values expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test with control group. Significance represented as P<0.01

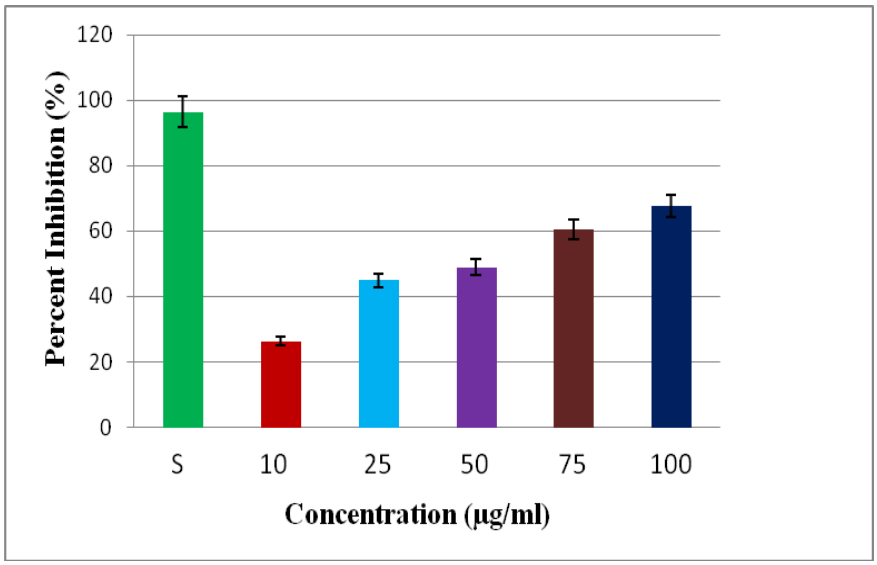

Fig. 1: Antioxidant activity of *Sesamum indicum* leaves by H_2O_2 scavenging activity. All bars are expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test. P<0.05 when the values in the group are statistically significant against the control value

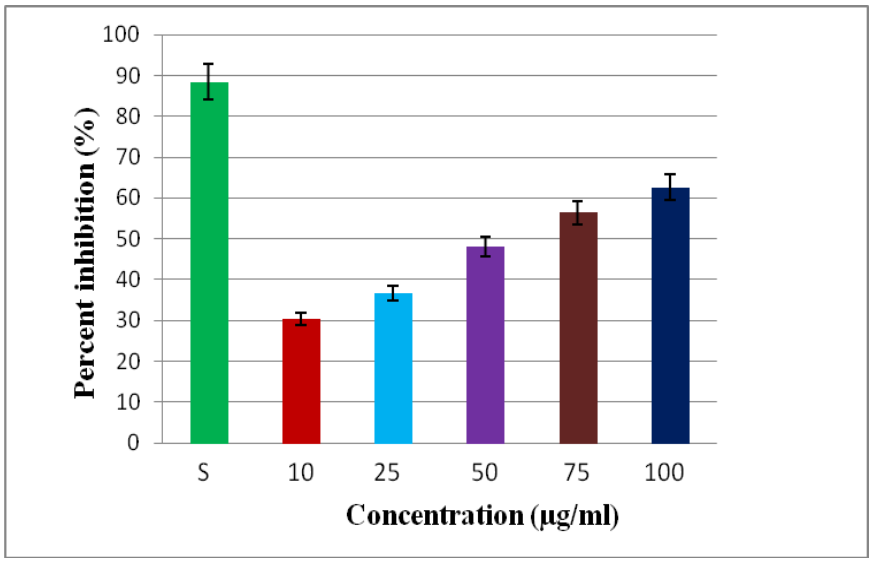

Fig. 2: Antioxidant activity of *Sesamum indicum* leaves by reducing power assay. All bars are expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test. P<0.05 when the values in the group are statistically significant against the control value

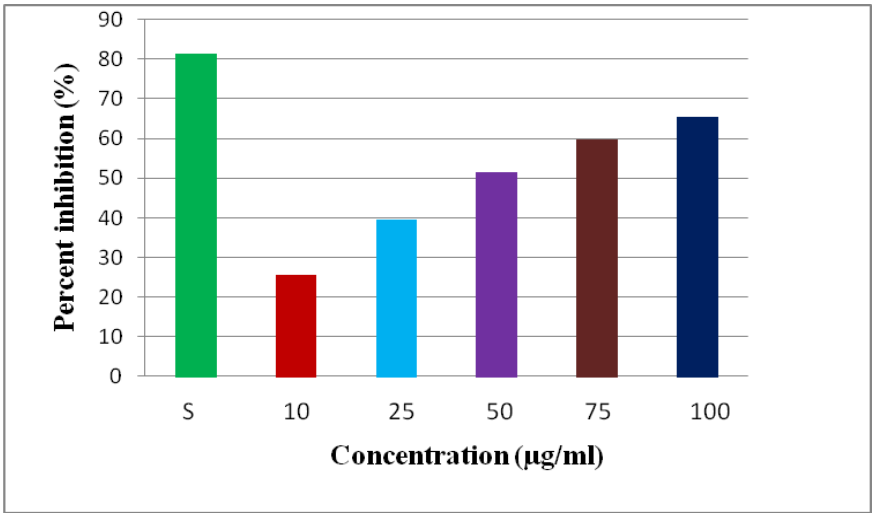

Fig. 3: Antioxidant activity of *Sesamum indicum* leaves by phosphomolybdenum method. All bars are expressed as mean±S.D. (n=6). ANOVA followed by Dunnett's test. P<0.05 when the values in the group are statistically significant against the control value

Conclusion

Based upon the results obtained in the present study, it is concluded that ethanolic extract of *Sesamum indicum* contains considerable amount of flavonoids and phenolic[15] compounds, exhibits high antioxidant activity (Table 5). It also chelates iron and has reducing power. These indicate that the plant is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and treating wound infections. However, further isolation of bioactive compounds would assist to ascertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

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