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Comparative antioxidant activity of Sesame root and leaf extract - An In-vitro study

Research Article

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Abstract

Universe has millions and millions of plants, but not all the plants have medicinal values. One such gift from nature is *Sesame* and is crowned as the "Queen of oilseed crops" due to its various medical properties like anti-inflammatory, antioxidant, anti- microbial etc. This research paper aims to compare the antioxidant activity of *sesame* leaf extract and *sesame* root extract with the standard ascorbic acid using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H2O2) assay. Different concentrations of the extracts and ascorbic acid were prepared (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL), and the antioxidant activity was measured. The results indicate that both *sesame* leaf extract and *sesame* root extract exhibit antioxidant activity, with *sesame* leaf extract showing slightly higher activity than *sesame* root extract. The findings suggest that *sesame* leaf extract has potential as a natural antioxidant source and could be used as an alternative to synthetic antioxidants. Overall, this study provides valuable information on the potential health benefits of *sesame* extracts and highlights the importance of natural antioxidants in preventing free radical damage and associated diseases.

Keywords: Antioxidant, *Sesame* leaf, *Sesame* root, *Sesamum Indicum*.

Introduction

Free radicals are highly reactive and unstable molecules that are naturally produced in the body as a by-product of normal metabolism or exposure to toxins in the environment such as tobacco smoke and ultraviolet (UV) light(1,2). These molecules lack an electron from their outer shell, making them unstable and causing them to steal electrons from other molecules in the body, leading to damage to surrounding cells (3). This damage can contribute to ageing and various diseases(4). Antioxidants, on the other hand, are molecules that can neutralise free radicals by donating an electron to them, thus preventing them from causing damage to cells (5). Therefore, consuming foods and supplements that are rich in antioxidants can help protect the body from free radical damage and potentially reduce the risk of various diseases.

Numerous studies have shown that *sesame* possesses antioxidant activity. For instance, a study investigated the effects of *sesame* seed extract as a natural antioxidant on the quality of refined olive oil and found that it exhibited a higher antioxidant activity than the synthetic antioxidant Butylated hydroxyl toluene (1). Another study evaluated the antioxidant activity of *sesame* cake extract using the thiocyanate method and found that it had significant antioxidant activity (6). Furthermore, a study examined the phytochemical profile and antioxidant activity of *sesame* seed extract and discovered that it had a high yield of antioxidant activity. Another study investigated the in vitro antioxidant and anti-colon cancer efficacy of ethanol extract of *sesame* leaves and its major bioactive component and found that they contained significant amounts of major antioxidants (7). Collectively, these studies suggest that *sesame* has antioxidant activity and could serve as a natural antioxidant.

There is a lack of extensive research on the antioxidant activity of *sesame* leaf and root extracts. However, some studies have investigated the potential antioxidant properties of sesame extracts. For example, one study examined the in vitro antioxidant and anti-colon cancer efficacy of ethanol extract of *sesame* leaves and its major bioactive component (8). Another study evaluated the antioxidant activity of ethanolic extracts of *sesame* coat (9,10). Furthermore, *sesame* seed extracts have been found to have potential as natural antioxidants to improve the stability and shelf life of refined olive oil (11). The antioxidant activity of *sesame* cake extract has also been evaluated using the thiocyanate method (12). Despite the limited research on *sesame* leaf and root extracts, these findings suggest

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that *sesame* extracts may possess antioxidant properties, which could offer potential health benefits.

In this present study, *sesame* leaf and *sesame* root aqueous extract was prepared and tested for its antioxidant activity by performing 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and hydrogen peroxide *(H_2O_2)* assay.

Materials and Methods

Collection of Sesame leaf and sesame root

The *sesame* plant was collected near Vellore, Tamilnadu, and was then subjected to a thorough washing under tap water to remove any debris. The plant was then separated into its individual parts, namely leaves and roots, and these parts were shade-dried for a period of 5-6 days. After the drying process, the leaves and roots were then ground into a fine powder using a mixer grinder. This step helps to break down the plant material into smaller particles, which can facilitate better extraction of its constituents. The resulting fine powder was then stored in an airtight container to prevent any moisture or air from entering and degrading the quality of the powder. Proper storage helps to maintain the shelf life and potency of the plant material.

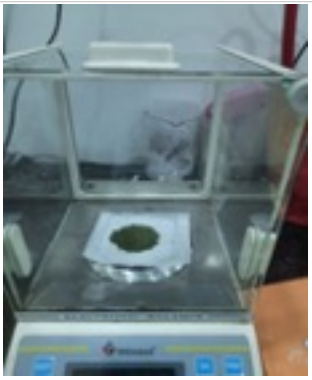
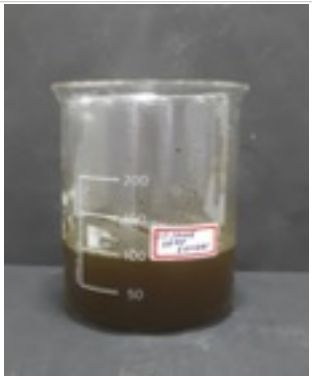
Preparation of sesame leaf extract

To prepare the *sesame* leaf extract, 1g of *sesame* leaf powder was measured and added to 100 mL of distilled water (Fig 1 and 2). The mixture was boiled using a heating mantle at 60 ^oC for 15-20 minutes. Boiling helps to break down the plant material and release the bioactive compounds into the solution.

After boiling, the mixture was filtered using Whatman No:1 filter paper to obtain a clear filtered extract. This step removes any solid particles or impurities from the extract, ensuring a clean and pure sample.

The filtered sesame leaf extract was then further boiled to condense up to 5 mL. This step is done to concentrate the active compounds in the extract, making it more potent and effective.

Finally, the condensed 5mL *sesame* leaf extract was stored in an airtight tube and kept in the refrigerator for further use. Storing the extract in the refrigerator helps to preserve its bioactive compounds and prevents any degradation due to heat or light exposure.

Fig 1: 1g of sesame leaf extract	Fig 2: Extract mixed with 100ml of distilled water
	

Preparation of sesame root extract

To prepare the sesame root extract, 1g of sesame root powder was measured and added to 100 mL of distilled water. The mixture was boiled using a heating mantle at 60 ^oC for 15-20 minutes. Boiling helps to break down the cell walls of the plant material and release the bioactive compounds into the solution.

After boiling, the mixture was filtered using Whatman No:1 filter paper to obtain a clear filtered extract. This step removes any solid particles or impurities from the extract, ensuring a clean and pure sample. (Fig 3) The filtered sesame root extract was then further boiled to get a condensed form up to 5 mL. This step is done to concentrate the active compounds in the extract, making it more potent and effective. (Fig 4)

Fig 3: Filtration using Whatman No:1 filter paper	Fig 4: Condensation of the extract
	

Finally, the condensed 5mL *sesame* root extract was stored in an airtight tube and kept in the refrigerator for further use. Storing the extract in the refrigerator helps to preserve its bioactive compounds and prevents any degradation due to heat or light exposure.

Antioxidant activity DPPH assay

To measure the antioxidant activity of *sesame* leaf and sesame root extract, two assays were performed. The first was the 2,2-diphenyl-1-picrylhydrazyl *(DPPH)* assay, in which a stock solution of 0.1 mM *DPPH* was diluted to a working concentration of 20 µM in methanol. Different concentrations of both the *sesame* leaf and root extracts (10-50 µg/mL) were added to 200 µL of the DPPH working solution in a 96-well plate, which was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a microplate reader, with methanol as the blank. The percentage of DPPH scavenging activity was calculated using the formula

% DPPH Scavenging Activity = [(A control – A sample) / A control] × 100,

where A control is the absorbance of the control (*DPPH* solution without the sample), and A sample is the absorbance of the sample (*DPPH* solution with the nanoparticles). Ascorbic acid was used as a positive control.

H_2O_2 assay

The second assay was the hydrogen peroxide ***(H2O2)*** assay, specifically the hydroxyl radical scavenging assay proposed by Halliwell et al. In this assay, a reaction mixture consisting of 2-deoxy-2-ribose (100 µL of 28 mM) and various concentrations of both the *sesame* leaf and *sesame* root extracts (10-50 µg/mL) was prepared. Ferric chloride (200 µL of 200 µM), Ethylenediamine Tetraacetic acid (EDTA) (200 µL), and ascorbic acid (100 µL) were added to the mixture, which was then incubated for 1 hour at 37°C. The optical density was measured at 532 nm against the blank solution, with vitamin E used as the positive control. The hydroxyl radical scavenging activity was calculated using the formula

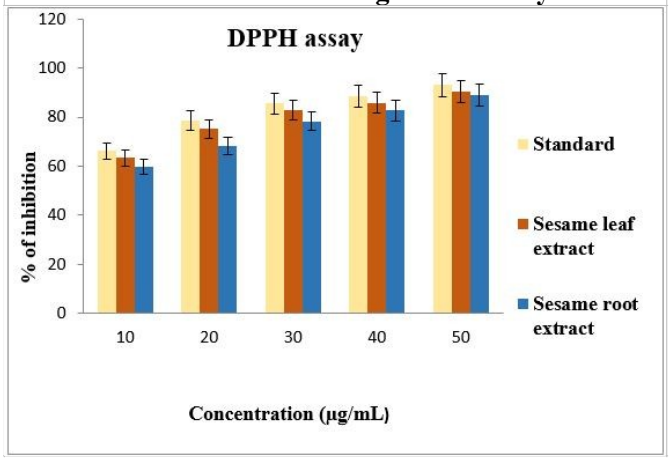
Hydroxyl radical scavenging activity (%) = [(A blank − A sample)/A blank] × 100,

where A blank is the absorbance of the control reaction (without sample), and A sample is the absorbance of the reaction with the sample.

Result and discussion DPPH assay

The antioxidant activities of sesame leaf extract and sesame root extract were compared to that of standard ascorbic acid at different concentrations (10, 20, 30, 40, and 50). (Graph 1).

Graph 1: Antioxidant activity of both sesame root and leaf extract using DPPH assay



At a concentration of 10, the antioxidant activity of standard ascorbic acid was 66.25, while that of *sesame* leaf extract and *sesame* root extract were 63.38 and 59.8, respectively. At a concentration of 20, the antioxidant activity of standard ascorbic acid was 78.52, while that of *sesame* leaf extract and *sesame* root extract were 75.27 and 68.2, respectively. At a concentration of 30, the antioxidant activity of standard ascorbic acid was 85.63, while that of *sesame* leaf extract and *sesame* root extract were 82.97 and 78.32, respectively. At a concentration of 40, the antioxidant activity of standard ascorbic acid was 88.68, while that of *sesame* leaf extract and *sesame* root extract were 85.81 and 82.65, respectively. At a concentration of 50, the antioxidant activity of standard ascorbic acid was 93.15, while that of *sesame* leaf extract and *sesame* root extract were 90.38 and 88.93, respectively.

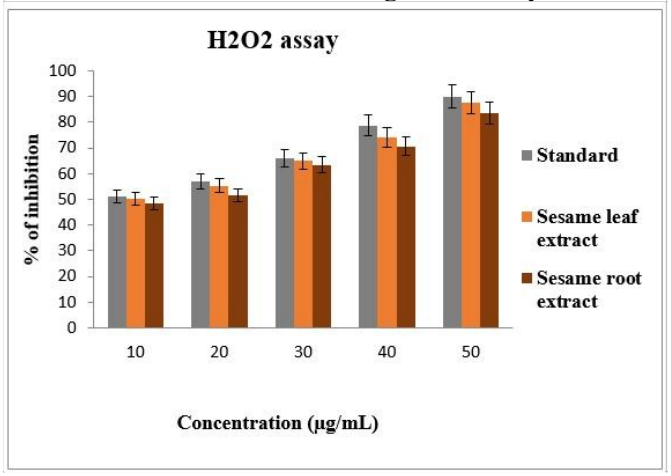
Overall, both *sesame* leaf extract and *sesame* root extract showed antioxidant activity that increased with increasing concentration. *Sesame* leaf extract consistently showed higher antioxidant activity than *sesame* root extract at all concentrations tested. At a concentration of 50, the antioxidant activity of *sesame* leaf extract was 90.38, which was 2.22% lower than that of standard ascorbic acid (93.15), while the antioxidant activity of *sesame* root extract was 4.64% lower than that of standard ascorbic acid. These findings suggest that *sesame* leaf extract has potential as a natural antioxidant source, and may be used as an alternative to synthetic antioxidants.

H_2O_2 assay

The aim of this study was to compare the antioxidant activity of *Sesame* leaf extract and *Sesame* root extract with the standard ascorbic acid. The antioxidant activity was measured using the H_2O_2 assay.

Different concentrations of *Sesame* leaf extract, *Sesame* root extract, and ascorbic acid were prepared (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL). (Graph 2)

Graph 2: Antioxidant activity of both sesame root and leaf extract using H_2O_2 assay



The results showed that both *Sesame* leaf extract and *Sesame* root extract had antioxidant activity, with the *Sesame* leaf extract having slightly higher activity compared to the *Sesame* root extract. At 10 µg/mL, the antioxidant activity of *Sesame* leaf extract was 50.2%, while that of *Sesame* root extract was 48.36%. At 20 µg/mL, the antioxidant activity of *Sesame* leaf extract was 55.3%, while that of *Sesame* root extract was 51.52%. At 30 µg/mL, the antioxidant activity of *Sesame* leaf extract was 64.9%, while that of *Sesame* root extract was 63.29%. At 40 µg/mL, the antioxidant activity of *Sesame* leaf extract was 74.1%, while that of *Sesame* root extract was 70.68%. At 50 µg/mL, the antioxidant activity of *Sesame* leaf extract was 87.5%, while that of *Sesame* root extract was 83.56%.

The standard ascorbic acid also showed antioxidant activity, with increasing activity at higher concentrations. At 10 µg/mL, the antioxidant activity of

ascorbic acid was 51.1%. At 20 µg/mL, the antioxidant activity of ascorbic acid was 56.9%. At 30 µg/mL, the antioxidant activity of ascorbic acid was 66.1%. At 40 µg/mL, the antioxidant activity of ascorbic acid was 78.8%. At 50 µg/mL, the antioxidant activity of ascorbic acid was 89.9%.

There are several studies on the antioxidant activity of *sesame* seed extract, but there is limited research on *sesame* leaf extract and *sesame* root extract. Some studies have investigated the antioxidant activity of *sesame* seed extract using different methods (13,14).

Sesame seeds are a popular ingredient in many cuisines around the world, and they are also known for their health and nutritional benefits. *Sesame* seeds contain a variety of antioxidants, including *sesamol, sesamin*, and *sesamolin*, which have been shown to have anti-inflammatory, anti-cancer, and anti-diabetic properties. Extracting these antioxidants from *sesame* seeds can provide several benefits, including improving the stability and shelf life of oils and other products, as well as providing a natural source of antioxidants for use in supplements and functional foods. There are several methods for extracting antioxidants from *sesame* seeds, including using fine powder of *sesame* seed extracted in different solvents, selective extraction techniques, and lyophilised *sesame* seed coats extract. Each method has its own advantages and limitations, and the specific method used may depend on the application and desired outcome. For example, using fine powder of *sesame* seed extracted in different solvents was found to be effective in stabilising sunflower oil during storage, while lyophilised *sesame* seed coats extract was used to improve the stability and shelf life of refined olive oil (14,15).

One of the advantages of using *sesame* seed extract as a natural antioxidant is its higher solubility of antioxidants in semi-polar solvent systems. This means that the extract can be easily incorporated into a variety of products, including oils, emulsions, and other aqueous and non-aqueous systems. This can be particularly useful in the food industry, where antioxidants are often added to products to improve their shelf life and stability.

In addition to its functional benefits, *sesame* seed extract also provides several nutritional benefits. *Sesame* seeds are a good source of fibre, protein, and healthy fats, and consuming *sesame* seed extract can help to increase the intake of these important nutrients. Furthermore, *sesame* seeds have been shown to have anti-inflammatory and antioxidant properties, which may provide additional health benefits (16).

Overall, using *sesame* seed extract as a natural antioxidant can provide several benefits, including improving the stability and shelf life of products and providing a natural source of antioxidants for use in supplements and functional foods.

Both *Sesame* leaf extract and *Sesame* root extract showed antioxidant activity, with *Sesame* leaf extract having slightly higher activity than *Sesame* root extract. The standard ascorbic acid also showed antioxidant activity, with increasing activity at higher concentrations. These findings suggest that both *Sesame* leaf and *Sesame* root extracts could be potential sources of natural antioxidants.

Conclusion

The study mentioned provides important information on the antioxidant activity of *sesame* leaf and root extracts. Antioxidants are important molecules that help to protect cells from damage caused by free radicals, which are unstable molecules that can damage cells and contribute to the development of various diseases. The study found that both *sesame* leaf and root extracts have antioxidant activity that increases with increasing concentration, with *sesame* leaf extract consistently showing higher antioxidant activity than *sesame* root extract at all concentrations tested. At a concentration of 50, the antioxidant activity of *sesame* leaf extract was 90.38%, which is only slightly lower than that of the standard ascorbic acid (93.15%). These findings are particularly interesting because they suggest that *sesame* leaf extract may be a promising natural source of antioxidants that could potentially be used as an alternative to synthetic antioxidants. This is important because synthetic antioxidants can have potential side effects, and there is a growing interest in natural sources of antioxidants.

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Conflict of interest : Nil

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