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Evaluation of *in vitro* study of antioxidant and antibacterial activities of methanolic seed extract of *Sesamum indicum*

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Abstract

Development of antibiotic resistance in diverse bacterial pathogens has adversely affected antibiotics ability to successfully treat patients and therefore researchers shifted their attention towards herbal products. Therefore, the present study is aimed at identification of secondary metabolites and demonstration of antibacterial and antioxidative activities of methanolic seed extract of black sesame or til (*S. indicum*). The phytochemical analyses of *S. indicum* showed the presence of glycosides, flavonoids, alkaloids, tannins and phenols, phytosterols, amino acids and proteins. Total phenolic contents and antioxidant activity were measured in the seed extract was found 19.48 ± 0.98 mg gallic acid per gram of sample and $98.55 \pm 3.51\%$ inhibition, respectively. Further, antibacterial activity has been checked in terms of zone of inhibition by disc diffusion method against microorganisms like *E. coli* and *S. aureus* for different concentrations of methanolic seed extract which were compared with positive control gentamicin. Sesame seeds showed increasing antibacterial property with an increase in the extract concentration. Methanolic seed extract of *S. indicum* at concentration 500 mg/ml showed a maximum diameter of inhibition zone for *S. aureus* 13 ± 0.871 mm, while for *E. coli* maximum zone of inhibition observed was 10.17 ± 0.946 mm.

These results suggest that methanolic seed extract of black sesame contained varied types of pharmacologically active compounds with antioxidant and antimicrobial activities.

Keywords: sesame seed, total phenolic contents, FRAP, phytochemicals, antibacterial activity.

1. Introduction

Free radicals are well known for playing a dual role since they can be either harmful or beneficial to living organisms [1]. The balance between beneficial and harmful effects of free radicals is an important for development of living organisms and is achieved by mechanisms called redox regulation [2, 3]. However, excess free radicals can damage cellular proteins, lipids and DNA by inhibiting their normal functions through a series of events and resulting in various pathological conditions [4]. Wide variety of natural and synthetic antioxidants counterbalance the effects of free radicals and protect the body from diseases [5].

Human beings are often infected by diverse microorganisms like bacteria, viruses, yeasts and molds present in the environment. Recently, an uncontrolled increase of pathogens with antibiotic resistant or even multi-drug resistant, including several gram-negative bacteria such as *Escherichia coli*, *Klebsiella* sp., and gram-positive bacteria such as *Staphylococcus aureus*, is the has become a major problem. These lethal infections have led to resurgence of new antimicrobial agents which have broad-spectrum activity and considerably lower predisposition to induce microbial resistance, compared to the antibiotics to control these infectious agents [6, 7].

At present, it has been estimated that about 80% of the world population rely on botanical preparations as medicine to meet the needs as they are considered safe and effective against certain ailments [8, 9]. For over thousands of years, natural plants have been used as a valuable source of medicinal agents with proven potential for treating infectious diseases and with lesser side effects compared to synthetic drug agents. However, the problem posed by the high cost and increasing side effects of synthetic drugs, researchers are being more emphasized on herbal drugs which have little or no side effects [8, 10, 11].

Sesame (*Sesamum indicum*) is one of the oldest cultivated plants in the world, mainly grown for extraction of oil from seeds. Sesame belongs to the family Pedaliaceae and genus Sesamum. This genus consists of about 36 species, out of this 19 species are native to Africa [12].

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The oil from sesame plant is an important ingredient in Ayurvedic remedies in India and is used in Chinese medicine to increase energy and prevent aging^[13] due to the presence of bioactive components present in the seed including polyunsaturated fatty acids, phytosterols, tocopherols, vital minerals and unique class of phenylpropanoid compounds namely lignans such as sesamin, sesamol and sesamol^[14]. These phytochemicals provide defense mechanism against reactive oxygen species and increases keeping quality of oil by preventing oxidative rancidity^[15, 16].

Sesame lignans have various pharmacological properties including antioxidant activity^[17, 18], antimicrobial activity^[19], antiproliferative activity^[20], lowering cholesterol levels^[21], increasing hepatic fatty acid oxidation enzymes^[22] and show antihypertensive effects^[10, 23].

Keeping this in view, the present study was aimed to evaluate antibacterial potential against gram positive and gram negative bacteria *S. aureus* and *E. coli*, respectively as well as phytochemical analysis and antioxidant activity of methanolic extract of *S. indicum* seeds.

2. Materials and Methods

2.1 Chemicals

All the chemicals used in this study were of analytical grade. Folin–ciocalteu, gallic acid, potassium ferricyanide, sodium carbonate, ferric chloride, methanol (HPLC grade), etc were purchased from Merck (Germany) and Sigma-Aldrich. (St. Louis, Mo, USA). Antibiotics and reagents for culture media were procured from Hi Media, Mumbai, India.

2.2 Instrument

Important equipments used in the present study were spectrophotometer (Electronics Corporation of India Limited), centrifuge (Remi, India), autoclave, hot air oven (Scientific equipment works), electronic analytical balance (Sartorius, Germany), laminar flow (Swastik India), incubator (Toshiba), deep freezer, micropipettes (Eppendorf, Germany), binocular Microscope 100 X (Nikon), soxhlet extractor, rotary evaporator (Heidolph), pH meter, etc.

2.3 Isolation and Culture of Bacteria

E. coli and *S. aureus* strains were isolated from soil. Isolated bacteria were identified by Gram's stain and standard biochemical tests^[24]. The cultures were grown on nutrient agar media (HiMedia, Mumbai, India) at 37 °C. Each bacterial strain was 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.

2.4 Collection of Black Sesame Seeds

Black sesame (*Sesamum indicum*) seeds were procured from the National Seed Corporation, Agra (U.P.). They were identified according to their taxonomical classification by the botanists at IRIS institute, Agra.

2.5 Preparation of the Seed Extract

Sesame seeds were washed thoroughly with tap water and finally with distilled water. Then seeds were dried in the air and were crushed in an electric grinder to coarse powder for the extraction. Sesame seed extract was prepared by soxhlet extraction method described by Okeke, *et al.*^[25]. About 45 g

of *S. indicum* seeds powder material were uniformly packed into a thimble and run in soxhlet extractor separately. It was exhaustible extracted with 70% methanol for the period of about 48 hours or 22 cycles or till the solvent in the siphon tube of an extractor become colorless. After that extracts were filtered with the help of filter paper and solvent evaporate from extract in rotary evaporator to get the syrupy consistency. The residue was dried over anhydrous sodium sulphate to remove trace of methanol. Then extract was kept in refrigerator at 4 °C for biochemical and antibacterial analyses.

2.6 Phytochemical Screening

Various phytochemicals such as steroid, tannins, phenols, flavonoids, alkaloids, glycosides, saponins, carbohydrates and proteins were detected in the extracts according to the procedures followed by Debela^[26].

2.7 Determination of Total Phenolic Content (TPC)

The total phenolic content of seeds extract was determined spectrometrically as described by Singleton and Rossi^[27] with little modification. According to this, 1ml of methanolic seed extract was mixed with 5 ml of folin-ciocalteu reagent (diluted to ten folds) and 4 ml of Na₂CO₃ (75g/l) and 10 ml of distilled water. The mixture was allowed to stand at 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 minutes and the absorbance of supernatant was taken at 760 nm using UV-VIS spectrometer. Different concentration ranges from 20, 30, 40, 60, 80, 100 ug/ml methanolic gallic acid solution were used as standard. Results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight of sample. All determinations were performed in triplicate.

2.8 Ferric Reducing Antioxidant Assay Power (FRAP)

The reducing power of methanolic sesame seed extract was determined by the method of Oyaizu^[28]. According to this, 1ml of seeds extract was mixed with 1 ml of methanol and then added phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and FeCl₃ (0.5 ml, 0.1%) and then absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer and compared with ascorbic acid as standard. Results were expressed as percent inhibition which was calculated using the following expression:

% inhibition = $(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$, where A_{blank} and A_{sample} stand for absorption of blank sample and absorption of the tested extract solution, respectively. All determinations were performed in triplicate.

2.9 Disc Diffusion Assay

The antibacterial sensitivity testing of the extract was determined using disc diffusion method described by Mukherjee *et al.*^[29]. The MIC of the extract was also determined using a two-fold dilution method. Sesame seed extract with different concentrations were prepared by serial dilutions range from 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml and 15.625 mg/ml. The bacteria were first grown in nutrient agar for 18 hours before use. The inoculum suspensions were standardized. It was performed using an 18 h culture at 37 °C in 10 ml of Mueller Hinton Broth. The cultures were adjusted to approximately

10⁵CFU/ml with sterile saline solution. Five hundred microlitres of the suspensions were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on test plates. Sterile discs having a diameter of 6 mm were impregnated with 25 ul of each serial dilution of extract solution and were placed on inoculated surface of agar plate with the help of sterile forceps. All petridishes were sealed with sterile laboratory parafilms to avoid eventual evaporation of the test samples. These plates were incubated for 24 hours at 37 °C and measured the zone of inhibition in millimeter the plates. The zones of inhibition of the tested microorganisms by the extracts were measured using a Fisher-Lilly antibiotic zone reader model 290 (U.S.A). The effects were compared with that of the standard antibiotic gentamicin at a concentration of 1mg/ml which was used as positive control, while methanol was used as negative control. The inhibitory zone around test paper discs indicated as positive (growth inhibition observed) and absence of a zone as negative. The test was repeated thrice to ensure reliability of the results.

2.10 Statistical Analysis

All determinations were performed in triplicate. The results are reported as mean \pm standard deviation.

3. Results and Discussion

3.1 Phytochemical Screening

Data in table 1 shows the results of preliminary phytochemical analysis of methanolic extracts of black sesame seeds. These tests revealed the presence of different bioactive constituents, including, alkaloids, glycosides, flavonoids, phytosterols, tannins and proteins.

Table 1: Table showing phytochemicals present in methanolic seed extract of *S. indicum*

Phytochemicals	Methanolic Extract of <i>S. indicum</i>
Alkaloids	+
Glycosides	+
Flavonoids	+
Amino Acids & Protein	+
Phytosterols	+
Carbohydrates	-
Tannin & Phenols	+
Saponins	-

+ = presence; - = absence

Alcoholic extract (either methanol or ethanol) is more efficient in degradation of cell walls and seeds which have unpolar character and causes polyphenols to be released from the cell. Methanol or ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the seeds materials [30, 31]. Since nearly all of the identified components from seeds, active against microorganisms are aromatics or saturated organic compounds, they are most often obtained through initial ethanol or methanol extractions [32]. The higher concentrations of more bioactive compounds were detected with 70% methanol due to its higher polarity than pure methanol. Thus, extraction of phenolic compounds increased due to the relative increase in the polarity and also due to the increase in the swelling of plant tissue [33].

3.2 Total Phenolic Content and Reducing Antioxidant Power

Table 2 shows the content of total phenols that were measured

by Folin- Ciocalteau reagent in terms of gallic acid. The total phenolic content in the methanolic seeds extract of black sesame was found 19.48 \pm 0.98 mg/g of the dry weight of sample.

Table 2: Table showing total phenolic content and reducing power assay of methanolic seeds extract of *S. indicum*.

Parameters→ Sample↓	Ferric Reducing Power (% inhibition)	Total Phenolic Content (mg/g dry weight of sample)
Ascorbic acid (50 ug/ml)	99.96 \pm 3.62	-
<i>S. indicum</i> methanolic seeds extract (9000 ug/ml)	98.55 \pm 3.51	19.48 \pm 0.98

In the present investigation ferric reducing antioxidant power assay was used to evaluate the antioxidant activity of *S. indicum* seed extract. This assay is a relatively simple method frequently used in the assessment of antioxidant activity of biological samples. It is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous blue colored form in the presence of antioxidants [34]. Table 2 also presents the result of FRAP assay which was measured as percent inhibition for methanolic seed extract of black sesame was found 98.55% \pm 3.51 and for standard (ascorbic acid) was 99.96% \pm 3.62.

Many researchers have reported that sesame seeds exhibited antioxidant activity due to the presence of unique phenolics, lignans present in sesame seeds, sesamin, sesamol and sesamol [11, 35, 36]. The phenolic compounds exhibit extensive free radical scavenging activities through their reactivity as hydrogen or electron-donating agents and metal ion chelating properties [37]. In addition, the reducing properties of phenolic compounds are normally due the presence of reductones, which have the capacity to donate an electron to free radicals and convert them into more stable forms. Moreover, reductones can reduce the oxidized intermediates generated from lipid peroxidation reaction [38].

3.3 Antibacterial Activity of Methanolic Seed Extract of *S. indicum*

In the present investigations, the methanolic seed extract of *S. indicum* at different concentrations showed considerable antibacterial activity against the pathogenic bacterial strains *E. coli* and *S. aureus* which were compared with positive control gentamicin.

Table 3: Table shows antibacterial activity against *S. aureus* and *E. coli* at different concentration of methanolic seed extract of *S. indicum*

Inhibitory Concentration of Methanolic Black Sesame Seed Extract (mg/ml)	Diameter of zone of inhibition (mm) <i>S. aureus</i>	Diameter of zone of inhibition (mm) <i>E. coli</i>
500 (D1)	13 \pm 0.871	10.17 \pm 0.946
250 (D2)	10.33 \pm 0.943	9.67 \pm 0.991
125 (D3)	9.83 \pm 0.505	8.47 \pm 0.470
62.5 (D4)	7.88 \pm 0.416	6.15 \pm 0.433
31.25 (D5)	-	-
15.625 (D6)	-	-
1 (Gentamicin)	36 \pm 2.07	35 \pm 1.99
Methanol	-	-

From the data of table 3, the strongest antibacterial activity was observed against *S. aureus* at 500 mg/ml concentration (D1) with 13 ± 0.871 mm zone of inhibition. 10.17 ± 0.946 mm zone of inhibition was shown against *E. coli* at 500 mg/ml concentration (D1). The antibiotic gentamicin exhibited 36 and 35 mm zone of inhibition against *E. coli* and *S. aureus*, respectively. The extract was found to be active against upto concentration 62.5 mg/ml against both the bacterial strains. The degree of sensitivity increased with increase in inhibitory concentration. Tabulated data is also expressed graphically in figure 1.

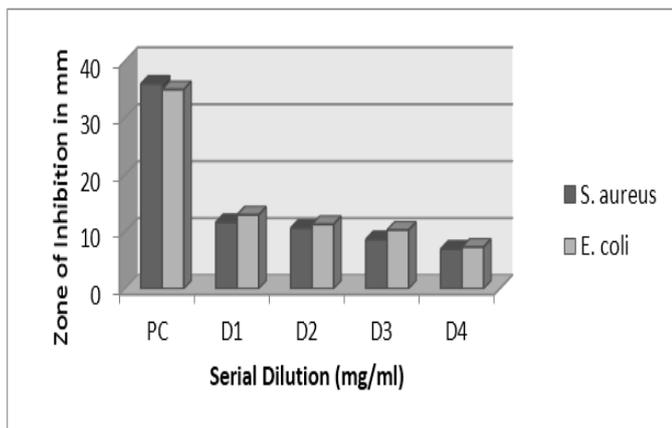


Fig 1: Graphical representation of antibacterial potential of different concentration of methanolic extract of black sesame seed against *S. aureus* and *E. coli*. D1, D2, D3 & D4 are dilutions of sesame seed extract, PC is positive control.

Phenolics of sesame seed are a major class of bioactive components which have the free radical scavenging property due to the presence of high reactivity as hydrogen or electron donors and metal chelating activity [35] and therefore, also confer resistance against microbial pathogens [11]. This explains the demonstration of antibacterial activity by the sesame seed extract used in the present study.

4. Conclusion

Based on these results, we conclude that the methanolic extract of *S. indicum* seeds showed good antibacterial and antioxidant activities which may be due to the presence of phenolic contents such as lignans and other secondary metabolites. Further research work involving more detailed *in vitro* and *in vivo* investigations to establish which component of the extract offer best antioxidant and antimicrobial activity is needed. Detailed toxicological studies are also recommended to explore the use of sesame seed extract as a natural antibacterial drug and effectiveness in clinical trials.

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