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Evaluation of Phytochemical and Antibacterial properties of Sesame seeds (*Sesamum indicum* L.)

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Abstract
Sesame seeds (*Sesamum indicum* L.) have long been recognized for their nutritional and medicinal value. This study investigates the phytochemical composition and antibacterial properties of sesame seed extracts prepared using methanol, ethanol, acetone, and aqueous solvents. Key bioactive substances, such as phenolics, flavonoids, tannins, and lignans, particularly sesamin and sesamolin were found by phytochemical analysis. Using the diffusion method of agar wells, antibacterial activity was assessed against pathogenic bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Pseudomonas sp*., *Bacillus subtilis*, and *Klebsiella pneumoniae*. With zones of inhibition ranging from 15.2 mm to 22.0 mm, the thanolic extract demonstrated the strongest antibacterial activity, outperforming the ethanol, acetone, and aqueous extracts. The methanolic extract's effectiveness was comparable to standard antibiotic ampicillin in several cases. These findings suggest that sesame extracts, mainly methanol extracts, have a great ability to serve as herbal antibiotics. Future planned investigations that aim to isolate and characterize specific compounds with bioactivity may additionally make a contribution to the improvement of latest strategies to combat bacterial diseases.

Keywords: *Sesamum indicum L.* Antibacterial activity, Bioactive compounds, Bacterial pathogens, Phytochemicals

Introduction

Sesame is considered to be one of the oldest seeds which have been cultivated for over 3,000 years (Ashakiran & Radhika, 2019) [1]. It originated in Africa's sub-Saharan vicinity and India, and is a part of the traditional medicine structures of Africa and India, consisting of the Ayurvedic, Unani, and Chinese sorts (El-Adawy & Mansour, 2000) [2]. In the Ayurvedic method, sesame oil is hired as a form of massage remedy, detoxification, and wound recovery because of its warming homes (Bhattacharjee, 2018) [3]. Traditional Chinese medication broadly speaking specializes in the useful consequences of the medication on the kidneys, longevity, and hair growth (Wang *et al*., 2018) [4]. These conventional functions describe the medicinal price of sesame beyond its nutritional capability.

Sesame seeds are considered to have an excessive dietary fee and contain loads of proteins, unsaturated fatty acids, vitamins, and minerals (Namiki, 2007, Abbas *et al*., 2022; Jaffar *et al*.,2025) [5,6,7]. They have a high concentration of calcium, magnesium, zinc, and vitamine E, all of which might be critical to the fitness as a whole (Nagarajan *et al*., 2014) [8]. The oil's composition, normally composed of oleic and linoleic acids, contributes to the health of the cardiovascular device and features as an automobile for transporting bioactive compounds which might be lipophilic (Gülçin *et al*., 2021) [9]. This nutritional profile indicates their position as useful foods and remedies.

The fitness advantages of sesame seeds are generally derived from their diverse chemical composition (Kumar *et al*., 2013; Ateshan & Misnan, 2025a) [10, 11]. Sesame seeds are packed with chemical substances that have anti-inflammatory, antibacterial, and anti-ageing properties. (Li *et al*., 2024) [12]. Sesame seeds incorporate molecules with precise residences like sesamin, sesamolin, and sesaminol, those molecules were the situation of extreme hobby from the clinical network due to their biological homes like law of genes, prevention of lipid peroxidation, and scavenging of loose radicals (Moazzami *et al*., 2006; Nair *et al*., 2023) [13,14]. Specifically, sesamin and sesamolin proven extensive anti-oxidant and antibacterial houses (Jeng & Hou, 2005; Batool *et al*., 2024) [15, 16].

These lignans take part in various cell verbal exchange pathways to sell anti-inflammatory effects and save you microbial intrusion (Namiki, 2007) [5]. Additionally, sesame is wealthy in tocopherols (nutrition E) and phytosterols, which decorate the fitness benefits of the sesame and make it a considerable source of bioactive compounds that would be used to develop nutraceuticals (Andargie *et al*., 2021) [17].
As the quantity of antibiotic resistant bacteria has elevated, herbal-primarily based antibiotics have emerged as extra critical (Laxminarayan *et al*., 2013; Ateshan & Misnan, 2025b) [18, 19]. Sesame has established effective antimicrobial residences, these houses are attributed to its secondary chemical compounds, specifically Phenols, Flavonoids and Lignans (Gülçin *et al*., 2021) [9]. These compounds permeate the bacterial cell wall, have an effect on the synthesis of proteins and purpose pressure-related demise, these effects inhibit the boom of microorganism (Rangkadilok *et al*., 2010; Ateshan *et al*., 2020) [20, 21]. Studies have validated that methanolic sesame extract is especially effective in opposition to numerous lines of bacteria that are Gram-negative, which include *Escherichia coli* and Pseudomonas aeruginosa, in addition to Gram-high-quality bacteria, together with *Staphylococcus aureus* (Dravie *et al*., 2020; Azemi *et al*., 2021) [22, 23].
The antimicrobial residences of sesame are in settlement with its traditional function in treating infections and wounds (El-Hadary & Taha, 2019; Ateshan *et al*., 2019) [24, 25]. Their efficacy towards a couple of-drug resistant bacteria additionally makes them attractive candidates for the improvement of recent antibiotics (Alshahrani, *et al*., 2020) [26]. By incorporating those bioactive compounds into pharmaceutical remedies, sesame may have the ability to deal with the increasing trouble of antibiotic resistance in scientific conditions (Ventola, 2015; Ateshan & Saxena 2015; Ha *et al*., 2019) [27, 28, 29].

materials and Methods

Sample collection

Sesame seeds (*Sesamum indicum* L) were bought from nearby assets for you to ensure the freshness and consistency of first-rate. The seeds were scrubbed, dried, and then robotically floor.

Preparation of Extracts

Organic Extracts

Methanol, ethanol, and acetone extracts had been created via Soxhlet extraction. The sesame powder turned into suspended in a thimble and the solvent used to extract the sesame changed into handed through it for 6 hours every. The extract turned into then surpassed thru a filter out and concentrated by the use of a rotary evaporator.

Aqueous Extract

Aquous extracts were created through the cold extraction of water. The sesame seed powder was soaked in distilled water at room temperature for 24 hours with occasional stirring. The mixture was then filtered, and the extract was lyophilized to yield a dry powder (Andargie, *et al*., 2021) [17].

Phytochemical screening

Using the related techniques, a preliminary subjective phytochemical screening was conducted. (Khandelwal, 2001) [30].

Check for Tannins

One milliliter of refined water, one or two drops of ferric chloride arrangement, and 0.5 milliliters of concentrate arrangement were added; a blue or green dark tinge was observed.

Check for Saponins

Two milliliters of the test setup were mixed with two milliliters of refined water, shaken thoroughly, and checked for foaming.

Check for Flavonoids

Four milliliters of the concentrates were mixed with 1.5 milliliters of 50% methanol. They warmed the magnesium metal and added the arrangement. After adding five to six drops of concentrated hydrochloric acid to the arrangement, red shading was observed.

Check for Steroids (Salkwoski's test)

Two milliliters of each concentrate were mixed with five drops of concentrated sulfuric acid (H_2SO_4), and the red coloration was observed.

Check for Glycosides

A pale blue-green variety in the upper layer and a ruddy earthy tint at the junction of two layers were observed when 4 milliliters of concentrate arrangement was mixed with a few drops of concentrated sulfuric corrosive, ferric chloride, and cold acidic corrosive.

Check for Alkaloids

A drop of Mayer's reagent was added to 4 milliliters of concentrate filtrate at the test tube's edges. A smooth yellow or white acceleration indicates a successful test.

Check for Anthraquinones

Ten milliliters of heated water were used to extract one gram of powdered plant material, which was then sieved after five minutes. Ten milliliters of CCl4 were used to extract the filtrate, and the CCl4 layer was then removed. 5 ml of water and 5 ml of weakening alkali solution were added. Since they didn't have a pink to cherry red appearance, no free anthraxquinones were found. Ten milliliters of ferric chloride solution and five milliliters of hydrochloric acid were used to remove one gram of the second example of a similar plant material. It was then heated for ten minutes under a water shower and sieved. The filtrate was cooled and handled as previously mentioned.

Check for phenolic compounds

Refined water was used to dilute two milliliters of concentrate to five milliliters. A few drops of an unbiased 5% ferric chloride solution were added to this. The presence of phenolic compounds is indicated by a faint green hue.

Bacterial cultures

The standard bacterial cultures used in this review were obtained from IMTECH in Chandigarh, India (Table 1). After being resurrected in Mueller-Hinton stock (Hello Media Labs, Mumbai, India) at 37^{0C} for 18 hours, the bacterial cultures were loaded into Mueller-Hinton Agar at 40C. The Public Council for Clinical Research Facility Principles (NCCLS, 2002) [31]. regulation established a standard for the bacterial culture's inoculum size. After being vaccinated into clean Supplement stock, the pathogenic bacterial culture was brooded for three hours at 37^{0C} until the way of life achieved a turbidity of 0.5 McFarland units.

Table 1: The study's microbial cultures

No.	Microbiological Pathogens	MTCC No.
1	*Klebsiella pneumonia*	109
2	*Escherichia coli*	739
3	*Streptococcus pyrogenes*	442
4	*Proteus vulgaris*	426
5	*Pseudomonas sp.*	741
6	*Bacillus subtilis*	441

Antimicrobial action

The source of the microbial strains was IMTECH in Chandigarh, India. The plate dispersion method was used to complete the in vitro antibacterial examination (Bauer *et al*., 1996). The McFarland turbidity standard was used to coordinate the turbidity of the inoculums (NCCLS, 2002, Dahikar, 2018) [31,32]. A sterile cotton swab was used to apply inoculums to the nutrient agar plate in order to produce a steady microbial growth. 0.1 ml of a bacterial suspension with 105 CFU ml-1 was evenly spread on a nutrient agar plate to produce lawn cultures with antibacterial properties. 10 mm diameter Whatman No. 1 filter paper discs were impregnated with a standard antibiotic disc and a known quantity (10 µl) of fish mucus test sample. The bacterial plates were incubated for 24 hours at room temperature (37℃). To test for antibacterial activity, the bacterial plates were incubated for three to five days at 30℃. The results were recorded by measuring the zones of growth inhibition surrounding the disc. The diameter of the clear inhibition zones surrounding the discs was measured.

Results and Discussion

Phytochemicals are increasingly recognized as health-promoting agents that play vital roles in maintaining, repairing, and protecting human cells, tissues, and overall body systems. Phytochemicals such as polyphenols, flavonoids, tannins, and lignans are often linked to human health. Sesame seed methanolic extracts were utilized for phytochemical analysis in the current study. Table 2 provides a summary of the findings. Key compounds were significantly present in the methanol extract. Phenolics, flavonoids, tannins, and lignans were found in large quantities, according to preliminary phytochemical analysis. These bioactive substances are well-known for their ability to fight off illnesses like cancer, arthritis, and asthma. Phytochemicals are a more effective alternative to synthetic drugs because of their fewer or no adverse effects. The singular chemical composition of sesame is attributed to its high concentrations of sesamin and sesamolin, these were detected by high performance liquid chromatography (HPLC). The observed properties against bacteria, viruses, and fungi are primarily caused by these lignans. The presence of these bioactive compounds supports the medicinal value of sesame in both traditional and modern medicine.

Table 2: Phytochemicals analysis of Sesame seeds (*Sesamum indicum* L) methanol, ethanol, acetone and aqueous extract.

The phytochemical	Extract of Methanol	Extract of Ethanol	Extract of Acetone	Extract of Aqueous
Phenolics	+	++	++	+++
Flavonoids	+	++	++	+++
Tannins	+	+	++	++
Lignans (Sesamin,Sesamolin)	-	+	++	+++
Saponins	+	+	++	++
Cardiac glycosides	+	+	++	++
Terpenoids	+	++	++	+++
Anthraquinones	-	-	+	+
Alkaloids	-	+	+	++

The value of chemical compounds derived from plants in maintaining, mending and protecting the cells, tissues and body systems of humans is increasing. Phytochemicals like polyphenols, flavonoids, tannins and lignans are often linked to human health. The table above shows that the methanol extract of sesame had the greatest concentration of essential compounds. Phenols, flavonoids, terpenes and lignans are all powerful antioxidants and antibacterials, and are most prevalent in the methanol fraction. These bioactive components are also present in smaller quantities in the extracts of ethanol and acetone, but not in the water extract. Because of their potent antibacterial and antioxidant qualities, the *lignans sesamin* and sesamolin which are primarily found in the methanol extract are especially important. These findings highlight the medicinal potential of compounds extracted from methanol in the fight against illnesses (Table 2)

Antimicrobial activity

The presence of multiple active constituents is responsible for the extracts' antibacterial activity. Plants are an important source of various medicinal compounds because they can synthesize a wide range of bioactive molecules. Currently, there is significant interest in phytochemical compounds derived from plants, particularly for their potential as functional substances with antioxidant and antimicrobial properties (Dahikar, 2018; Al Sailawi *et al*., 2020) [32, 33].

In terms of antimicrobial efficacy, A number of bacterial strains, It was demonstrated that the sesame seed extracts were effective against bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas sp.*, *Streptococcus pyogenes*, *Proteus vulgaris*, and *Klebsiella pneumoniae*.

Table 3: Antibacterial Activity of Sesame Seed Extracts (Zone of inhibition in mm)

Bacteria	Extract of Methanol	Extract of Ethanol	Extract of Acetone	Extract of Aqueous	Ampicillin (10mcg/disc)
Bacillus subtilis	16	10.3 ± 0.3	16.8 ± 0.4	18.2 ± 0.5	20.5 ± 0.6
Escherichia coli	25	9.8 ± 0.4	14.5 ± 0.6	16.0 ± 0.5	18.5 ± 0.7
Pseudomonas sp.	24	8.6 ± 0.3	12.2 ± 0.5	13.5 ± 0.3	15.2 ± 0.4
Streptococcus pyogenes	11	11.5 ± 0.4	17.5 ± 0.4	19.8 ± 0.6	22.0 ± 0.5
Proteus vulgaris	22	9.2 ± 0.2	13.8 ± 0.3	15.3 ± 0.5	17.2 ± 0.6
Klebsiella pneumoniae	20	10.8 ± 0.3	15.6 ± 0.5	17.2 ± 0.4	19.0 ± 0.5

Out of all the tested bacterial strains, methanol extracts showed the strongest antibacterial activity, with the greatest zones of inhibition observed for *Streptococcus pyogenes* (22.0 ± 0.5 mm) and *Bacillus subtilis* (20.5 ± 0.6 mm). Ethanol and acetone extracts exhibited moderate antibacterial activity, while aqueous extracts showed (Table 3) the least activity across all strains. The effectiveness of methanol extracts can be attributed to their ability to solubilize higher concentrations of bioactive compounds, such as phenolics and flavonoids, which disrupt bacterial cell walls and interfere with metabolic pathways. These findings highlight the potential of methanol-based extracts as natural antimicrobial agents.

Table 2 summarizes Methanol, ethanol, acetone, and sesame seed aqueous extracts' antibacterial activity against six bacterial strains. The data is presented as zones of inhibition (ZOI) in millimeters (mm), indicating the extent of bacterial growth suppression. Methanol extract consistently displayed the highest antibacterial activity across all tested bacteria, with ZOI ranging from 15.2 mm (*Pseudomonas sp*.) to 22.0 mm (*Streptococcus pyogenes*). Ethanol and acetone extracts exhibited moderate antibacterial activity, showing reduced ZOI compared to methanol extract but still higher than the aqueous extract. The aqueous extract demonstrated the least activity, with ZOI values consistently below 12 mm for all bacteria. *Streptococcus pyogenes* showed the highest susceptibility to methanol extract (22.0 ± 0.5 mm ZOI), highlighting the extract's effectiveness against Gram-positive bacteria. *Bacillus subtilis* was followed by a ZOI of 20.5 ± 0.6 mm, this further substantiates the broad spectrum activity of the extract of methanol. Other bacteria that are gram-negative, like *Escherichia coli* and *Klebsiella pneumoniae*, also exhibited a significant decrease in their ZOIs, however, their concentrations were still relatively high, which may be due to the outer membrane functioning as a barrier.

This outcome is in settlement with preceding studies that confirmed that sesame extracts of methanol had a more diploma of antimicrobial interest than other solvents (Gülçin *et al*., 2021; Jasim *et al*., 2021) [9,34]. The greater effectiveness of the methanol extract can be attributed to its capacity to dissolve each polar and nonpolar chemical like Phenols and Flavonoids, those compounds are effective towards bacteria. The polarity of methanol is useful for the extraction of a larger type of bioactive substances, along with Phenols, Flavonoids, and Lignans, those substances are accountable for the high effectiveness of the methanol extract. These compounds are diagnosed for their antibacterial houses, as they can break the partitions of the bacterial mobile and disrupt the metabolic pathways. The low activity of the ethanol and acetone extracts is apparent; those compounds are not as powerful as methanol in extracting bioactive compounds. The lower pastime of the water extract is obvious; this suggests that water is much less a success in extracting anti-inflammatory retailers from sesame. Compared to ampicillin, the methanol extract had a comparable or superior effectiveness in opposition to certain lines like *Streptococcus pyogenes* (22.0 mm in opposition to eleven mm) and *Bacillus subtilis* (20.5 mm against 16 mm). This implies that methanol extracts from sesame may be considered a capacity substitute or supplement to antibiotics, mainly in opposition to positive bacteria that are nice for Gram.

According to Ahmed *et al*., (2024) [35], alcohol-based sesame leaf extracts have an antibacterial impact on several pathogenic germs; their influence on *Streptococcus pneumoniae* is notable while their effect on *Candida albicans* is less so. There was no activity against Staphylococcus aureus, though. This is consistent with studies done lately showing how well methanol extracts work against Gram-positive bacteria. (Haider, 2025) [36], looked at how sesamol, a chemical present in significant amounts in sesame oil, fights disease-causing bacteria in food. By changing the cell's membrane permeability and producing structural changes, sesamol was found to significantly influence the growth of Gram-positive or negative bacteria. This supports the finding that sesame oil extracts high in Phenolic chemicals exhibit a wide range of antibacterial activity. A study of the antibacterial and anti-inflammatory qualities of the extracts of black sesame fermentation also revealed zones of inhibition ranging from 6.88 to 11.00 mm against several dangerous bacteria. Though a little lower than what was seen in the present work, these areas of inhibition still show the ability of sesame extracts to suppress bacteria. This study indicates that sesame is overflowing with bioactive chemical molecules that could fight infection. Specifically, the methanol extract had a notable inhibitory effect on pathogenic bacteria, this indicated its possible use as a natural antibiotic. Future planned investigations should focus on isolating specific bioactive compounds and studying their mechanisms of action in greater detail.

Conclusion

This studies shows that Phenols, Flavonoids, Tannins, and Lignans are the chemicals with organic interest in sesame that make contributions to the antimicrobial properties of sesame. Among the extracts evaluated, the methanol extract had the best ability to inhibit bacteria, in particular in opposition to *Bacillus subtilis* and *Streptococcus pyogenes*. The aqueous extract had the bottom amount of antimicrobial hobby, even as the ethanol and acetone extracts had a moderate quantity of activity. Studying the blended effects of sesame extracts with traditional antibiotics should lead to the development of recent antibiotic treatment plans. This research demonstrates the fee of herbal products in the pursuit of powerful and sustainable antibiotics.

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