



Antibacterial Activity of Ethanol and Benzene Extracts of Groundnut (*Arachis hypogaea* L.) and Sesame (*Sesamum indicum* L.) Seeds against Selected Pathogenic Bacteria

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Abstract – The increasing emergence of antibiotic-resistant microorganisms has intensified interest in plant-derived antimicrobial agents. In the present study, the antibacterial activity of ethanol and benzene extracts of groundnut (*Arachis hypogaea* L.) and sesame (*Sesamum indicum* L.) seeds was evaluated against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the disc/agar diffusion (Kirby–Bauer) method, with Amikacin as the standard control. Groundnut benzene extract produced the largest zone of inhibition against *E. coli* (20 mm), exceeding the standard control (16 mm), while groundnut ethanol extract showed the highest activity against *Pseudomonas aeruginosa* (18.6 mm) and *Staphylococcus aureus* (18 mm). Sesame extracts also showed appreciable activity, particularly against *E. coli* (19.0–19.4 mm), though generally lower than groundnut extracts. *Bacillus subtilis* showed moderate inhibition (14–15 mm) across all extracts. Overall, groundnut extracts, particularly the ethanol extract, exhibited comparatively higher antibacterial activity than sesame extracts, and both seed extracts were effective against Gram-negative as well as Gram-positive bacteria. These findings support the potential of groundnut and sesame seed extracts as natural antibacterial agents and provide a basis for further isolation and evaluation of their active constituents.

Keywords – Antibacterial activity; *Arachis hypogaea*; *Sesamum indicum*; agar diffusion method; zone of inhibition; plant-based antimicrobials.

I. INTRODUCTION

The global rise in antibiotic-resistant pathogenic bacteria poses a serious public health challenge and has renewed scientific interest in plant-derived antimicrobial agents as alternative or complementary therapeutic options. Traditionally, crude plant extracts have been used as herbal medicine for the treatment of infectious diseases, and a wide range of phytochemicals such as alkaloids, flavonoids, tannins, saponins, terpenoids and phenolic compounds that have been shown *in vitro* to possess antimicrobial properties. Groundnut (*Arachis hypogaea* L., family Fabaceae) and sesame (*Sesamum indicum* L., family Pedaliaceae) are two widely cultivated oilseed crops.

They are valued not only for their nutritional importance but also for their traditional medicinal use. Information on the seeds of wild species is relatively scanty (Nial and Mahalik, 2020). Groundnut seeds contain bioactive compounds such as resveratrol, phenolic acids, flavonoids and phytosterols, while sesame seeds are distinguished by unique lignans such as sesamin and sesamol along with phenolics and tocopherols. These are the constituents reported to confer antioxidant, antimicrobial and hepatoprotective activity. Bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* are responsible for a range of human infections.

The present study was undertaken to evaluate and compare the antibacterial activity of ethanol (polar) and benzene (non-polar) extracts of groundnut and sesame seeds against

two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), using Amikacin as a reference standard.

II. MATERIALS AND METHODS

Collection and identification of plant material

Seeds of *Arachis hypogaea* L. and *Sesamum indicum* L. were collected from the College of Agriculture, Vellayani, Thiruvananthapuram district, Kerala, India.

Preparation of extracts

The collected seeds were shade-dried for approximately one month and ground into a fine powder using a mechanical grinder. For extraction, 20 g of dried powdered seed material of each species was separately macerated in 40 mL of solvent such as ethanol (polar) and benzene (non-polar) in conical flasks, giving four extract combinations: sesame-benzene, sesame-ethanol, groundnut-benzene and groundnut-ethanol. Flasks were shaken thoroughly, sealed with cotton plugs and stored under refrigeration until analysis.

Test bacterial strains

Antibacterial activity was evaluated against two Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. All test organisms were obtained from the Scudder Laboratory, Nagercoil, and maintained on nutrient agar medium.



Antibacterial activity assay (Kirby–Bauer disc/agar diffusion method)

Antibacterial activity was assessed using the Kirby–Bauer disc diffusion method, with Amikacin used as the standard antibiotic control for all four test organisms.

Preparation of plates: Nutrient agar medium was prepared and sterilised, then poured into Petri dishes on a level surface to a depth of approximately 4 mm (25 mL for an 85 mm dish; 60 mL for a 135 mm dish). Poured plates were stored at 4 °C and used within one week. Before inoculation, plates were dried with lids ajar to remove surface moisture, and the medium pH was confirmed to be within 7.2–7.4.

Preparation of inoculum: At least four morphologically similar colonies were transferred from agar medium into 1.5 mL of sterile broth and incubated for 2 hours at 35–37 °C to obtain a moderately turbid bacterial suspension. Suspension density was standardised to match the 0.5 McFarland turbidity standard.

Inoculation: Plates were inoculated within 15 minutes of preparing the suspension. A sterile cotton-wool swab was dipped in the suspension, excess fluid removed against the tube wall, and the medium evenly streaked over the entire agar surface in three directions.

Application of extract discs: After the inoculum had dried, discs impregnated with the respective plant extract were applied to the agar surface with sterile forceps and pressed gently for even contact, with care taken to avoid cross-contamination between discs.

Incubation: Inoculated plates were incubated for 16–18 hours at 35–37 °C under aerobic conditions.

Reading of zones of inhibition: Zone diameters were measured to the nearest millimetre using a vernier calliper or a transparent millimetre scale, with the point of abrupt diminution of growth taken as the zone edge.

III. RESULTS

The antibacterial activity of sesame and groundnut extracts, prepared in ethanol and benzene, was evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* using the agar/disc diffusion method, with Amikacin as the reference standard. The zones of inhibition (mm) recorded for each organism and extract are summarised in Table 1.

Escherichia coli showed the greatest inhibition with groundnut benzene extract (20 mm), followed by sesame benzene (19.4 mm) and sesame ethanol (19 mm). All exceeded the standard antibiotic control (16 mm). *Pseudomonas aeruginosa* was most strongly inhibited by groundnut ethanol extract (18.6 mm), followed by groundnut benzene (18.2 mm), both above the control value of 17 mm. *Bacillus subtilis* showed moderate inhibition across all extracts, ranging narrowly from 14 mm to 15 mm,

comparable to the control (14 mm). *Staphylococcus aureus* was most strongly inhibited by groundnut ethanol extract (18 mm), followed by groundnut benzene (17.3 mm), both exceeding the control (16 mm).

Across all four test organisms, the ethanol extract of groundnut generally produced larger inhibition zones than the corresponding sesame extracts. It indicates comparatively higher antibacterial activity. Both extracts of each seed type were active against the Gram-negative organisms (*E. coli* and *P. aeruginosa*) as well as the Gram-positive organisms (*B. subtilis* and *S. aureus*), indicating broad-spectrum antibacterial activity.

Table 1. Zone of inhibition (mm) of groundnut and sesame seed extracts against selected bacterial strains, compared with the standard antibiotic Amikacin.

Test Organism	Control (Amikacin)	Sesame Benzene	Sesame Ethanol	Groundnut Benzene	Groundnut Ethanol
<i>E. coli</i>	16	19.4	19	20	19
<i>Pseudomonas aeruginosa</i>	17	16.5	16.2	18.2	18.6
<i>Bacillus subtilis</i>	14	14	15	14.8	14.6
<i>Staphylococcus aureus</i>	16	15	14.3	17.3	18

IV. DISCUSSION

The present study demonstrates that both sesame and groundnut extracts possess antibacterial activity against Gram-positive and Gram-negative bacteria. The higher activity observed in groundnut ethanol extract may be attributed to the presence of bioactive compounds such as fatty acids, phenolics and phytosterols. Ethanol is known to extract polar phytochemicals more effectively, which may explain the larger inhibition zones observed for ethanol extracts relative to benzene extracts. Ethanol is widely recognised as an efficient solvent for extracting phenolic compounds and flavonoids, which possess strong antimicrobial properties through mechanisms such as cell membrane disruption, enzyme inhibition and interference with microbial metabolism (Cowan, 1999).

The comparatively lower, still appreciable, activity observed for benzene extracts may reflect the non-polar nature of the solvent, which limits extraction of polar antimicrobial compounds. The moderate antibacterial activity retained by benzene extracts suggests that non-polar constituents such as lipids and certain fatty acids also contribute to antimicrobial effects, since fatty acids have been reported to disrupt microbial cell membranes, causing leakage of cellular contents and eventual cell death (Desbois and Smith, 2010).



Both extract types were effective against the Gram-negative organisms, *E. coli* and *P. aeruginosa*, which are generally considered more resistant to antimicrobial agents owing to their outer membrane, rich in lipopolysaccharides, which acts as a permeability barrier (Nikaido, 2003). The significant inhibition observed here, exceeding the standard antibiotic control in several cases, indicates that the active compounds in these extracts are capable of penetrating this barrier and exerting antibacterial effects, consistent with comparisons against reference antibiotics such as Amikacin (Willey et al., 2017).

In contrast, the Gram-positive organisms *B. subtilis* and *S. aureus*, which lack an outer membrane and possess a more accessible peptidoglycan cell wall, showed moderate to high inhibition. This structural distinction is consistent with the general susceptibility pattern reported for Gram-positive bacteria toward plant-derived antimicrobial agents (Tortora et al., 2014).

Phytosterols such as β -sitosterol, reported to be present in groundnut, have themselves been shown to exhibit antimicrobial and anti-inflammatory activity (Gupta et al., 2011), and the synergistic interaction between multiple phytochemicals within a single extract may further enhance overall antimicrobial efficacy. This is the pattern noted broadly in studies of plant-derived antimicrobial agents as potential alternatives to synthetic antibiotics (Burt, 2004). These findings are consistent with previous reports of antibacterial activity in sesame and groundnut extracts against similar bacterial panels (Pathak et al., 2014; Shittu et al., 2014; Ateshan, 2025), reinforcing the case for these oilseeds as sources of natural antimicrobial agents.

V. CONCLUSION

The present study confirms that ethanol and benzene extracts of both groundnut (*Arachis hypogaea* L.) and sesame (*Sesamum indicum* L.) seeds possess measurable antibacterial activity against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, with several extracts matching or exceeding the inhibition produced by the standard antibiotic Amikacin. Groundnut extracts, particularly the ethanol extract, showed comparatively higher and more consistent antibacterial activity than sesame extracts, likely reflecting the greater efficiency of ethanol in extracting polar antimicrobial phytoconstituents. These findings support the potential of groundnut and sesame seeds as natural, broad-spectrum antibacterial agents and provide a basis for further work isolating and characterising their specific active constituents.

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