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Evaluation of the Antibacterial Potential of *Salvia hispanica* (Chia Seeds) Against Selected Bacteria

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ABSTRACT: Medicinal plants have long been explored for their therapeutic potential, especially in the search for new antimicrobial agents to address rising antibiotic resistance. *Salvia hispanica* (chia seeds) has attracted scientific interest due to its rich bioactive profile and associated health benefits. This study investigates the phytochemical composition and antimicrobial activity of aqueous and ethanolic seed extracts. Antibacterial effects were evaluated using the agar well diffusion method against six bacterial isolates: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Salmonella* sp., *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids and cardiac glycosides, with higher concentrations generally observed in the ethanolic extract. The ethanolic extract demonstrated stronger antibacterial activity, with inhibition zones ranging from 16.2 to 22.8 mm and *Staphylococcus aureus* being most susceptible. The aqueous extract showed weaker effects, with no activity against *Pseudomonas aeruginosa* at lower concentrations. Minimum inhibitory concentration values for the ethanolic extract were as low as 6.25 mg/mL, while the aqueous extract ranged up to 50 mg/mL. Minimum bactericidal concentration results indicated bactericidal activity of the ethanolic extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Overall, ethanolic extracts showed greater antimicrobial effectiveness.

Keywords: *Salvia hispanica*, Phytochemicals, Bacteria, Antibacterial activity.

Introduction

The increasing global challenge of antimicrobial resistance has intensified the search for alternative therapeutic agents, particularly those derived from natural sources such as medicinal plants (Gupta and Sharma, 2022). The rapid emergence of resistant bacterial strains has reduced the efficacy of conventional antibiotics, posing a serious threat to global health systems. Consequently, attention has shifted toward plant-derived compounds, which are often safer, biodegradable and possess multiple mechanisms of antimicrobial action (Angelini, 2024). Medicinal plants have long been recognized as important sources of bioactive compounds, including alkaloids, flavonoids, tannins and phenolics, which exhibit potent antibacterial activities (El-Saadony *et al*., 2025). Among such promising plant resources, *Salvia hispanica* (chia) has gained increasing recognition due to its rich phytochemical composition and therapeutic potential (Abdel-Aty *et al*., 2024).

Chia seeds, obtained from *Salvia hispanica L.*, a member of the Lamiaceae family, are native to Central America, particularly Mexico and Guatemala (Motyka *et al*., 2022). Historically, chia seeds were widely consumed by ancient civilizations such as the Aztecs and Mayans, who valued them for their energy-boosting and medicinal properties. In modern times, chia has been rediscovered and classified as a functional food due to its exceptional nutritional profile. It is rich in omega-3 fatty acids, high-quality proteins, dietary fiber, vitamins and essential minerals (Hrnčič *et al*., 2020). The increasing global consumption of chia seeds has prompted extensive scientific investigations into their health benefits, including their antimicrobial, antioxidant and anti-inflammatory properties (Abdel-Aty *et al*., 2024).

The phytochemical composition of chia seeds is a key factor underlying their biological activities. Chia seeds contain significant amounts of phenolic compounds such as caffeic acid, chlorogenic acid, rosmarinic acid, quercetin, and kaempferol, all of which are known for their strong antioxidant and antimicrobial properties (Motyka *et al*., 2023). In addition, chia seeds are rich in tocopherols, phytosterols and carotenoids, which contribute to their protective effects against oxidative stress and microbial invasion (Hrnčič *et al*., 2020). These phytochemicals function by disrupting microbial cell walls, inhibiting enzyme activity, and interfering with nucleic acid synthesis, thereby suppressing bacterial growth. The presence of these diverse bioactive compounds highlights the potential of chia seeds as a natural source of antibacterial agents.

Several studies have reported the antibacterial activity of chia seed extracts against a wide range of pathogenic microorganisms. Extracts of *Salvia hispanica* have demonstrated inhibitory effects against both Gram-positive and Gram-negative bacteria (Motyka *et al*., 2023). The antimicrobial activity of chia is largely attributed to the synergistic effects of its phenolic compounds and fatty acids, which alter cell membrane permeability and lead to leakage of intracellular components. Additionally, studies have shown that chia mucilage exhibits antimicrobial and stabilizing properties, further enhancing its applicability in food preservation and pharmaceutical formulations (Chiang *et al*., 2021).

In addition to its antibacterial potential, the use of chia seeds aligns with the growing demand for natural and sustainable alternatives in food and drug industries. Synthetic preservatives and antibiotics are increasingly associated with adverse health effects and the development of resistant microbial strains. As a result, there is a shift toward plant-based antimicrobials that are safer and environmentally friendly. Chia seeds offer a unique advantage due to their dual functionality as both a nutrient-dense food and a therapeutic agent. Their incorporation into functional foods, nutraceuticals and antimicrobial formulations could significantly contribute to improved health outcomes (Hernández-Pérez *et al*., 2020).

The antibacterial activity and phytochemical composition of *Salvia hispanica* make it a promising candidate for the development of natural antimicrobial agents. The presence of diverse bioactive compounds supports its traditional use and provides a scientific basis for its application in combating bacterial infections. Therefore, this study is designed to investigate the phytochemical constituents and antibacterial potentials of chia seeds against some selected bacterial isolates, with the aim of contributing to the growing body of knowledge on plant-based antimicrobials and their potential applications in medicine and industry.

Materials and methods

Plant material and preparation: Commercially available chia seeds (*Salvia hispanica* L.) were obtained from a certified health food supplier. The seeds were authenticated at the Department of Plant Biology and Biotechnology, University of Benin; and a voucher specimen was deposited in the departmental herbarium. Seeds were cleaned to remove debris, air-dried at 25 ± 2 °C away from direct sunlight to constant weight, then ground using a mechanical blender and sieved through a 40-mesh sieve. The resulting fine powder was stored in airtight amber bottles at 4 °C until extraction.

Preparation of extracts: For the aqueous and ethanolic extract, 50 g of seed powder was suspended in 500 mL of distilled water, stirred at 60 °C for 2 h, cooled and filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 60 °C and freeze-dried to obtain the crude extract. For the ethanolic extract, 50 g of powder was macerated in 500 mL of 95% ethanol with intermittent shaking at room temperature for 72 h, filtered as above, and concentrated at 40 °C under reduced pressure. Both dried extracts were stored at 4 °C. Stock solutions of 200 mg/mL were prepared in 10% dimethyl sulfoxide (DMSO) and serially diluted to yield working concentrations of 100, 50, 25, and 12.5 mg/mL.

Qualitative phytochemical screening: Qualitative phytochemical screening of the extracts was carried out using standard procedures as described by Harborne (1998) and Trease and Evans (2002). Alkaloids were detected using Dragendorff's and Mayer's reagents, while flavonoids were identified by the Shinoda test. Tannins and phenolic compounds were determined using ferric chloride reagent, producing a characteristic colour change. Saponins were identified by the persistent frothing test, indicating the presence of surface-active glycosides. Terpenoids were detected using the Salkowski test, steroids by the Liebermann–Burchard reaction, and cardiac glycosides using the Keller–Kiliani test. The results were recorded qualitatively as present (+) or absent (−), with the intensity of reactions noted semi-quantitatively.

Quantitative phytochemical analysis: Quantitative determination of phytochemicals was performed following established protocols. Total alkaloid content was determined using the method described by Harborne (1973). Flavonoid content was estimated using the aluminium chloride colorimetric method with quercetin as the standard. Tannin content was determined using the Folin–Denis method with tannic acid as reference, while saponin content was evaluated using the method of Obadoni and Ochuko (2001). Total phenolic content was determined using the Folin–Ciocalteu reagent with gallic acid as standard. Steroid content was determined gravimetrically following extraction with petroleum ether. All analyses were conducted in triplicate to ensure accuracy and reproducibility.

Test microorganisms: Six bacterial isolates were used: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Streptococcus* sp., *Salmonella* sp., *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 700603). All isolates were maintained on nutrient agar at 4°C and sub-cultured on Mueller-Hinton agar at 37 °C for 18–24 h before use. Bacterial suspensions were adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL).

Agar well diffusion assay: Antibacterial activity was evaluated by the agar well diffusion method (Valgas *et al*., 2007). Mueller-Hinton agar plates were inoculated by uniform swabbing of bacterial suspensions. Sterile 6-mm wells were bored into the agar and filled with 100 µL of extract at each concentration. Ciprofloxacin (10 µg/disc) served as positive control; 10% DMSO as negative control. After 30 min pre-diffusion at room temperature, plates were incubated at 37 °C for 24 h. Zones of inhibition (in mm) including the well diameter were measured with a digital calliper. All experiments were conducted in triplicate.

Minimum inhibitory concentration (MIC): Minimum Inhibitory Concentration (MIC) was determined by broth microdilution in 96-well microtitre plates per CLSI (2021) guidelines. Two-fold serial dilutions of each extract (100 to 0.195 mg/mL) were prepared in Mueller-Hinton broth and inoculated with 5×10^5 CFU/mL bacterial suspensions. Plates were incubated at 37 °C for 24 h. Resazurin (0.01% w/v) was added post-incubation; wells remaining blue indicated inhibition. MIC was defined as the lowest concentration with no visible growth.

Minimum bactericidal concentration (MBC): Following MIC determination, 10 µL from each well showing no visible growth was sub-cultured onto Mueller-Hinton agar plates and incubated at 37 °C for 24 h. MBC was defined as the lowest concentration resulting in a 99.9% reduction (≥3 $\log_{10}$) in the original inoculum, i.e., fewer than 3 colonies on the subculture plate. The MBC/MIC ratio was calculated for each organism to determine whether the extracts were bactericidal (ratio ≤ 4) or bacteriostatic (ratio > 4).

Statistical analysis: All data are expressed as mean ± standard deviation (SD) of three independent replicates. One-way ANOVA with Tukey's post-hoc test was used to compare means. Statistical significance was set at $p < 0.05$. Analyses were performed using IBM SPSS Statistics version 27.

Results

Qualitative phytochemical screening revealed the presence of diverse secondary metabolites in both extracts (Table 1). The ethanolic extract demonstrated a broader and more intense phytochemical profile, testing positive for all eight classes screened, including cardiac glycosides, which were absent from the aqueous extract. Alkaloids, flavonoids, tannins, saponins, phenols and terpenoids were detected in both extracts, while steroids appeared in trace amounts in the aqueous extract but were moderately concentrated in the ethanolic extract.

Table 1: Qualitative phytochemical screening of aqueous and ethanolic extracts of Chia seeds (*Salvia hispanica*)

Phytochemical	Aqueous Extract	Ethanolic Extract
Alkaloids	+	++
Flavonoids	+	++
Tannins	+	+
Saponins	+	++
Phenols	++	+++
Terpenoids	+	++
Steroids	+	+
Cardiac Glycosides	-	+

Key: +++ = Abundant; ++ = Moderate; + = Present; = Absent

Quantitative phytochemical analysis (Table 2; Figure 3) confirmed that the ethanolic extract consistently yielded significantly higher concentrations of all phytochemical classes compared to the aqueous extract ($p < 0.05$).

Total phenols were the most abundant constituents in both extracts (aqueous: 7.35 ± 0.42 mg/g; ethanolic: 13.20 ± 0.67 mg/g), followed by flavonoids (aqueous: 6.81 ± 0.38 mg/g; ethanolic: 11.42 ± 0.53 mg/g). Steroids had the lowest concentrations in both extracts (aqueous: 2.18 ± 0.17 mg/g; ethanolic: 4.05 ± 0.28 mg/g).

Table 2: Quantitative phytochemical composition (mg/g dry weight) of aqueous and ethanolic extracts of Chia seeds

Phytochemical	**Aqueous Extract (Mean ± SD)**	**Ethanolic Extract (Mean ± SD)**	**p-value**
Alkaloids	4.52 ± 0.31	7.93 ± 0.44	<0.05
Flavonoids	6.81 ± 0.38	11.42 ± 0.53	<0.05
Tannins	3.24 ± 0.22	5.67 ± 0.35	<0.05
Saponins	5.10 ± 0.29	8.75 ± 0.41	<0.05
Phenols	7.35 ± 0.42	13.20 ± 0.67	<0.05
Steroids	2.18 ± 0.17	4.05 ± 0.28	<0.05

Values are Mean ± SD (n = 3). p < 0.05 denotes statistically significant difference between extracts (one-way ANOVA, Tukey's post-hoc test).

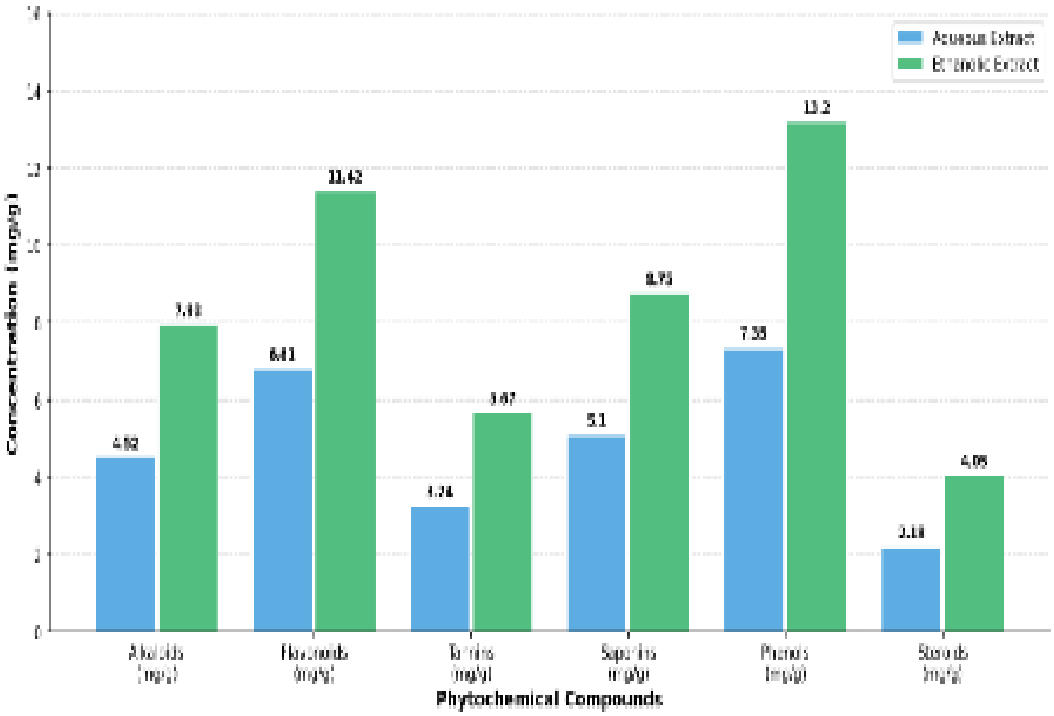

Figure 1: Quantitative phytochemical composition (mg/g dry weight) of aqueous and ethanolic extracts of *Salvia hispanica* seeds. The ethanolic extract yielded significantly higher concentrations of all phytochemical classes ($p < 0.05$).

Both extracts exhibited concentration-dependent antibacterial activity against all six bacterial isolates (Tables 3 & 4). The ethanolic extract consistently produced larger zones of inhibition than the aqueous extract at all corresponding concentrations ($p < 0.05$). No inhibition was recorded in the DMSO negative control wells. The positive control (ciprofloxacin) produced ZOI ranging from 22 to 30 mm across organisms.

In the aqueous extract (Table 3), no inhibition was observed at 12.5 mg/mL against *Escherichia coli*, *Streptococcus* sp., *Salmonella* sp., and *Pseudomonas aeruginosa* At 100 mg/mL, *Staphylococcus aureus* was most susceptible (18.4 ± 0.8 mm) and *Pseudomonas aeruginosa* least susceptible (12.0 ± 0.5 mm). In the ethanolic extract (Table 4), *Staphylococcus aureus* showed the highest inhibition at 100 mg/mL (23.0 ± 1.1 mm), followed closely by *E. coli* (21.3 ± 1.0 mm) and *Klebsiella pneumoniae* (21.0 ± 0.9 mm). *Pseudomonas aeruginosa* showed no inhibition at 12.5 mg/mL in either extract.

Table 3: Zones of inhibition (mm) of aqueous extract of Chia seeds against test organisms

Bacterial isolates	12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
Escherichia coli	0.0	8.0 ± 0.4	12.2 ± 0.5	16.5 ± 0.7
Staphylococcus aureus	7.0 ± 0.3	10.4 ± 0.5	14.0 ± 0.6	18.4 ± 0.8
Streptococcus sp.	0.0	7.2 ± 0.4	10.8 ± 0.5	15.0 ± 0.6
Salmonella sp.	0.0	6.6 ± 0.3	10.0 ± 0.5	13.8 ± 0.6
Pseudomonas aeruginosa	0.0	0.0	8.4 ± 0.4	12.0 ± 0.5
Klebsiella pneumoniae	6.0 ± 0.3	8.8 ± 0.4	13.2 ± 0.6	17.0 ± 0.7

Values presented in Mean ± SD

Table 4: Zones of inhibition (mm) of ethanolic extract of Chia seeds against test organisms

Bacterial isolates	12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
Escherichia coli	8.2 ± 0.4	11.8 ± 0.5	16.2 ± 0.7	21.0 ± 1.0
Staphylococcus aureus	9.4 ± 0.4	13.6 ± 0.6	17.8 ± 0.8	22.8 ± 1.1
Streptococcus sp.	7.6 ± 0.3	11.2 ± 0.5	15.0 ± 0.7	19.4 ± 0.9
Salmonella sp.	7.0 ± 0.3	10.6 ± 0.5	14.4 ± 0.6	18.6 ± 0.8
Pseudomonas aeruginosa	0.0	8.0 ± 0.4	11.8 ± 0.5	16.2 ± 0.7
Klebsiella pneumoniae	7.8 ± 0.4	12.2 ± 0.5	16.5 ± 0.7	20.8 ± 0.9

Values are Mean ± SD (n = 3). 0.0 = No zone of inhibition detected.

Table 5 presents the MIC and MBC values for both extracts against all six test organisms, together with the MBC/MIC ratio. The ethanolic extract showed lower MIC and MBC values overall, indicating higher antimicrobial potency. MIC values for the ethanolic extract ranged from 6.25 mg/mL (*Staphylococcus aureus* and *Klebsiella pneumoniae*) to 25 mg/mL (*Pseudomonas aeruginosa*), compared to 12.5–50 mg/mL for the aqueous extract. MBC values for the ethanolic extract ranged from 12.5 to 50 mg/mL, while those of the aqueous extract ranged from 25 to 100 mg/mL. The ethanolic extract showed bactericidal activity against *Staphylococcus aureus* and *Klebsiella pneumoniae.*

Table 5: Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts of Chia seeds against the bacterial pathogens

Bacterial isolates	Aqueous Extract			Ethanolic Extract		
	MIC (mg/mL)	MBC (mg/mL)	Inference	MIC (mg/mL)	MBC (mg/mL)	Inference
Escherichia coli	25	200	Bacteriostatic	12.5	100	Bacteriostatic
Staphylococcus aureus	12.5	100	Bacteriostatic	6.25	12.5	Bactericidal
Streptococcus sp.	25	200	Bacteriostatic	12.5	100	Bacteriostatic
Salmonella sp.	25	200	Bacteriostatic	12.5	100	Bacteriostatic
Pseudomonas aeruginosa	50	400	Bacteriostatic	25	200	Bacteriostatic
Klebsiella pneumoniae	12.5	100	Bacteriostatic	6.25	12.5	Bactericidal

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration

Discussion

Antibiotic resistance has become a major global health challenge, largely driven by the misuse and overuse of antimicrobial agents in human and veterinary medicine. This has facilitated the emergence of multidrug-resistant bacteria that survive conventional therapy through mechanisms such as enzymatic drug degradation, efflux pump activation, and reduced membrane permeability (Rajput *et al*., 2024). Consequently, there is growing interest in plant-derived bioactive compounds as alternative antimicrobial agents. Chia seeds (*Salvia hispanica* L.) have gained attention due to their rich phytochemical composition and reported biological activities. This study therefore evaluated the phytochemical profile and antibacterial activity of aqueous and ethanolic extracts of chia seeds against selected bacterial pathogens.

The detection of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids, and cardiac glycosides in both extracts confirms that chia seeds are rich in bioactive secondary metabolites. This aligns with previous studies reporting high levels of phenolic acids and flavonoids in chia seeds (Reyes-Caudillo *et al*., 2008; Hrnčič

et al., 2020). Phenols were the most abundant class in both aqueous (7.35 ± 0.42 mg/g) and ethanolic (13.20 ± 0.67 mg/g) extracts, consistent with reports identifying rosmarinic, caffeic, and chlorogenic acids as dominant compounds responsible for antioxidant and antimicrobial activity (Grancieri *et al*., 2019; Al-Juhaimi *et al*., 2024).

The higher phytochemical yield in ethanolic extracts compared to aqueous extracts agrees with earlier studies showing that solvent polarity strongly influences extraction efficiency (Ncube *et al*., 2008; Gyawali *et al*., 2014). Ethanol extracts both polar and moderately non-polar compounds, explaining the higher levels of phenols, flavonoids, and alkaloids observed. The exclusive presence of cardiac glycosides in the ethanolic extract further supports solvent-dependent extraction (Trease and Evans, 2002).

The antibacterial activity of both extracts showed a clear concentration-dependent response against all tested organisms, with increasing concentrations producing progressively larger zones of inhibition. This dose-dependent antimicrobial effect is a well-documented characteristic of crude plant extracts (Parekh and Chanda, 2007). Similar trends have been reported in plant-based antimicrobial studies, where higher extract concentrations enhance inhibition of both Gram-positive and Gram-negative bacteria (Doughari *et al*., 2008). In chia seed studies, solvent-based extracts have also demonstrated increased antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, attributed to higher levels of bioactive phytochemicals (Ixtaina *et al*., 2011). The increasing activity is associated with the accumulation of phytochemicals at the site of action, leading to disruption of microbial cell structures and metabolic processes. The absence of inhibition at lower concentrations (12.5 mg/mL), particularly in the aqueous extract, reflects the concept of minimum effective concentration. At sub-inhibitory levels, phytochemicals are insufficient to disrupt microbial integrity or metabolism, a phenomenon widely documented in antimicrobial pharmacodynamics (Ncube *et al*., 2008; Balouiri *et al*., 2016).

The stronger performance of the ethanolic extract suggests that solvent type influences phytochemical yield and antimicrobial efficiency. This agrees with Aldughaylibi *et al*. (2022), who reported higher antimicrobial activity in organic solvent extracts of chia compared to aqueous forms. Overall, Gram-positive bacteria, especially *Staphylococcus aureus*, were more susceptible than Gram-negative organisms. This is due to structural differences in cell walls, where Gram-negative bacteria possess an outer membrane that limits antimicrobial entry (Nikaido, 2003). Farhadi *et al*. (2019) also confirmed higher sensitivity of Gram-positive bacteria to flavonoid-rich extracts.

The relatively higher susceptibility of *Klebsiella pneumoniae* may be linked to variation in outer membrane porins such as OmpK35 and OmpK36, which affect permeability (Paczosa and Mecsas, 2016). In contrast, *Pseudomonas aeruginosa* showed the highest resistance due to intrinsic mechanisms like low permeability and efflux pumps such as MexAB-OprM (Pang *et al*., 2019; Moradali *et al*., 2021). The aqueous extract showed only bacteriostatic activity, as indicated by MBC/MIC ratios of 8, meaning it inhibited growth without killing the organisms (Pankey and Sabath, 2004). This is likely due to its lower content of lipophilic antimicrobial compounds such as phenols and flavonoids (Sibanda and Okoh, 2007; Bouarab-Chibane *et al*., 2019).

The ethanolic extract exhibited bactericidal activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* (MBC/MIC = 2), likely due to higher levels of phenols (13.20 mg/g), flavonoids (11.42 mg/g), and saponins (8.75 mg/g). These compounds act synergistically by disrupting membranes and inhibiting essential enzymes (Cheeke, 2000; Cushnie *et al*., 2020). The bactericidal effect against *S. aureus* is notable due to its lack of an outer membrane, making it more vulnerable to phytochemicals. The effect against *K. pneumoniae* suggests sufficient compound concentration to partially overcome Gram-negative barriers. MIC values (6.25–50 mg/mL) fall within expected ranges for crude plant extracts, which generally show moderate antimicrobial potency (Kuete, 2010). However, crude extracts are less active than purified compounds, which often show significantly lower MIC values.

Overall, these findings highlight the antimicrobial potential of *Salvia hispanica* seeds. The aqueous extract shows broad bacteriostatic activity, while the ethanolic extract demonstrates stronger and selective bactericidal effects against clinically important pathogens such as *Staphylococcus aureus* and *Klebsiella pneumoniae* (Tacconelli *et al*., 2018). The presence of phenols, flavonoids and saponins supports further exploration for drug development, as these compounds exhibit antioxidant and antimicrobial potential with low toxicity (Al-Juhaimi *et al*., 2024).

Conclusion

This study demonstrated that *Salvia hispanica* (chia seeds) possess notable antibacterial properties, with activity varying by solvent type, concentration, and test organism. Both aqueous and ethanolic extracts showed concentration-dependent inhibition, with the ethanolic extract exhibiting stronger and broader antimicrobial

effects, including bactericidal activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The observed activity is attributed to the presence of bioactive compounds such as phenols, flavonoids, and saponins, which act individually and synergistically to disrupt microbial growth and cellular integrity. Although MIC values indicate moderate activity typical of crude plant extracts, the findings support the potential of chia seeds as a natural source of antimicrobial agents. Overall, *Salvia hispanica* shows promise for further development in pharmaceutical and functional food applications.

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