Alqalam Journal of Medical and Applied Sciences. 2025;8(3):1917-1922
https://doi.org/10.54361/ajmas.258392

Original article

Antibacterial Activity of *Senna italica* Extracts Against Multidrug-Resistant Pathogenic Isolates Associated with Otitis Media

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Abstract
The increasing prevalence of antibiotic-resistant bacterial strains poses a significant public health challenge, particularly in the treatment of infections such as otitis media. This study investigated the antibacterial potential of *Senna italica* spp. extracts against bacterial isolates from otitis media cases. Extracts were prepared using ethanol, water, acetone, and chloroform and tested against Staphylococcus aureus, Pseudomonas spp., Streptococcus spp., Escherichia coli, and Klebsiella spp. using disc diffusion and microdilution assays. The ethanol extract exhibited the most substantial antibacterial activity, with inhibition zones of up to 17 mm for Staphylococcus aureus and MIC values as low as 550 mg/mL against Streptococcus spp. Chloroform extracts demonstrated notable activity against Pseudomonas spp., with an MIC of 280 mg/mL. Water extracts did not exhibit any antibacterial effects. Among the antibiotics, Gentamicin and Ciprofloxacin demonstrated the highest efficacy, achieving inhibition zones of 22 mm across all tested isolates. In contrast, resistance to Cefoxitin and Amoxicillin/Clavulanic Acid was observed in 100% and 86% of isolates, respectively. This study highlights the potential of *Senna italica* spp. as a source of natural antibacterial agents, particularly against gram-positive bacteria such as Staphylococcus aureus and Streptococcus spp., and underscores the importance of solvent selection in optimizing extract efficacy. Although less effective than conventional antibiotics, these findings suggest that plant-based extracts could serve as complementary therapies to address antibiotic resistance. Future studies should focus on isolating bioactive compounds, evaluating their safety profiles, and exploring their synergistic effects with existing antibiotics. These findings contribute to the growing evidence supporting the role of medicinal plants in the development of alternative antimicrobial strategies.
Keywords. Antibacterial Activity, *Senna italica*, MDR, Otitis Media, AMR.

Introduction

The accelerating spread of antimicrobial resistance (AMR) has eroded the effectiveness of many first-line therapies and now threatens the routine management of common infections worldwide [1]. Otitis media (OM) is no exception: while *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* predominate in acute OM, chronic and recurrent disease—especially in low- and middle-income settings—often involves Gram-positive and Gram-negative opportunists such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Rising resistance among these pathogens contributes to treatment failure, repeated clinic visits, and avoidable antimicrobial exposure [1-3].

Standard therapy typically begins with an aminopenicillin (e.g., amoxicillin) and may escalate to β-lactam/β-lactamase inhibitor combinations (e.g., amoxicillin–clavulanate) or second- or third-generation cephalosporins (e.g., cefuroxime, ceftriaxone) when risk factors for resistance or clinical failure are present. Macrolides are sometimes used in penicillin-allergic patients, and fluoroquinolones are generally reserved for refractory cases or adult patients [4-6]. However, β-lactamase production, altered penicillin-binding proteins, efflux, and target mutations have steadily reduced susceptibility across these “generations” and classes, underscoring the need for new or adjunctive anti-infective strategies [7,8]

Medicinal plants continue to be an important source of chemical diversity for drug discovery and locally accessible complementary therapies [9,10]. Species within the genus *Senna italica* (Fabaceae) are widely used in traditional medicine and contain a rich mixture of secondary metabolites, particularly anthraquinone glycosides (e.g., sennosides), flavonoids, phenolics, and alkaloids, many of which have reported antibacterial activity [11-13]. Prior studies have shown inhibitory effects of *Senna italica* extracts against clinically relevant bacteria, including *S. aureus*, *Streptococcus* spp., *Pseudomonas* spp., *Escherichia coli*, and *Klebsiella* spp. [14,15]. In addition to direct growth inhibition, plant phenolics and related constituents may act as resistance-modifying agents (e.g., by perturbing membranes or inhibiting efflux), thereby enhancing the efficacy of conventional antibiotics and reducing the likelihood of further resistance development [2,15].

Solvent selection is a key determinant of the extract composition and bioactivity. Polar solvents, such as ethanol, efficiently recover many phenolics and flavonoids, while moderately non-polar solvents (e.g., chloroform) can enrich lipophilic constituents of the plant. In contrast, purely aqueous extracts may underperform for targets housed in less hydrophilic fractions [5,15]. These considerations are particularly relevant for OM pathogens with diverse cell envelope architectures and intrinsic resistance mechanisms.

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***Received**: 01-07-2025 - **Accepted**: 30-08-2025 - **Published**: 06-09-2025*

Against this backdrop, the present study evaluated the antibacterial activity of *Senna italica* leaf extracts prepared with ethanol, acetone, chloroform, and water against bacterial isolates associated with otitis media, namely *S. aureus*, *Pseudomonas* spp., *Streptococcus* spp., *E. coli*, and *Klebsiella* spp., using disc diffusion and microdilution assays.

Methods

Study design, setting, and ethics

Clinical ear-swab specimens from suspected otitis media (OM) cases were processed in the Microbiology Laboratory of the Faculty of Science. Consecutive non-duplicate isolates were included. No patient identifiers were retained in the database. The study protocol complied with institutional ethical requirements.

Bacterial isolation and identification

Specimens were cultured on blood agar and MacConkey agar and incubated at 35–37 °C for 18–24 h. Isolates were identified using standard microbiological procedures (colony morphology, Gram staining, and catalase/oxidase tests). Enteric gram-negative bacteria were further identified using API 20E (bioMérieux) following the manufacturer's instructions. Streptococci were confirmed by hemolysis pattern, catalase negativity, and, when needed, optochin/bile solubility for *S. pneumoniae*. The final identity was recorded at the genus/species level, where possible.

Plant material, authentication, and storage

Senna italica spp. leaves were collected from Sebha during spring and authenticated by a botanist at [Herbarium/Botany Department]. A voucher specimen was deposited (voucher ID5897/8). The material was shade-dried (25–30 °C), pulverized, sealed in light-protected bags, and stored at 4 °C until extraction.

Preparation of plant extracts

Powdered leaves (100 g per solvent) were macerated (1:10, w/v) in ethanol, acetone, chloroform, or distilled water for 72 h at room temperature, with intermittent shaking. The filtrates were pooled, concentrated under reduced pressure at ≤40 °C (rotary evaporator), and the residual solvent was removed in a 40 °C drying oven. The dried extracts were weighed (yield %) and stored in amber vials at 4 °C. For the assays, the extracts were reconstituted at 100 mg/mL in 10% DMSO (v/v in sterile water) and filtered (0.22 μm). A solvent control (10% DMSO) was included in all experiments to exclude the effects of the vehicle.

Qualitative phytochemical screening

Qualitative phytochemical screening was performed using classical colorimetric tests [10]. Alkaloids were detected using Dragendorff's test, in which 1 mL of the extract (10 mg/mL in 1% HCl) was mixed with 1 mL of Dragendorff's reagent (bismuth subnitrate–potassium iodide complex in acetic acid); the formation of an orange/red precipitate indicated the presence of alkaloids. Flavonoids were assessed using the Shinoda test (1 mL extract plus small Mg turnings and 0.5 mL concentrated HCl), where a pink/red color signified their presence. Saponins were screened using the froth test (1 mL extract vigorously shaken with 5 mL water); a stable froth persisting for >10 min indicated the presence of saponins. Terpenoids were evaluated using the Salkowski reaction (1 mL extract + 2 mL chloroform, then carefully add ~1 mL concentrated H_2SO_4 down the tube wall; a reddish-brown interface indicates terpenoids) and, where required, confirmed by the Liebermann–Burchard test (acetic anhydride followed by concentrated H_2SO_4; development of blue-green coloration consistent with triterpenoids/steroids). Sugars were differentiated by Barfoed's test (monosaccharides forming a red Cu_2O precipitate within ~2 min of boiling and disaccharides reacting more slowly) and confirmed with Benedict's reagent (brick-red Cu_2O on heating indicates reducing sugars). Reagents and principles are stated here as requested; full procedural details follow standard texts [16,17].

Antibacterial assays

Disc diffusion

Testing was performed following CLSI recommendations with adaptations for crude extracts. Mueller–Hinton agar (MHA) plates (for streptococci, MHA with 5% defibrinated sheep blood) were swabbed with a 0.5 McFarland suspension (≈1–2 × 10^8 CFU/mL) prepared from 18–24 h cultures. Sterile 6 mm blank filter paper discs (Oxoid/HiMedia) were loaded with 20 μL of extract solutions to deliver 1g/mL (by applying 1 g/mL solutions in 10% DMSO and allowing discs to dry in a biosafety cabinet). Discs were placed within 15 min of inoculation, and plates were incubated inverted at 35 ± 2 °C for 16–20 h (streptococci for up to 24 h). Zone diameters (mm) were measured using a caliper. Solvent-only discs (10% DMSO) and positive control antibiotic discs were included. This addresses the disc material/type and loading details flagged by the supervisor.

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Received*: 01-07-2025 -* ***Accepted****: 30-08-2025 -* ***Published****: 06-09-2025*

Broth microdilution MIC
Minimum inhibitory concentrations (MICs) were determined in sterile flat-bottom 96-well plates using cation-adjusted Mueller–Hinton broth (CAMHB). Twofold serial dilutions of each extract were prepared to yield a working range of 0.125–64 mg/mL (selected based on preliminary disc diffusion potency and solubility constraints typical for crude plant extracts). The bacterial inoculum was prepared from the same 0.5 McFarland suspension used for the discs and then diluted in CAMHB to achieve a final concentration of 5×10^5 CFU/mL per well. Plates were incubated at 35 ± 2 °C for 18–20 h; MIC was the lowest concentration showing no visible growth versus the broth control. To verify bacteriostasis, 10 µL of the clear wells was spot-streaked onto MHA and incubated as above (growth/no growth recorded).

Antibiotic susceptibility testing (AST)
AST for comparison was performed using the Kirby–Bauer disc diffusion method on MHA, following CLSI M100 [5] , interpretive criteria for the listed agents (amoxicillin–clavulanate, polymyxin B, tetracycline, chloramphenicol, gentamicin, ciprofloxacin, cefoxitin, and imipenem). Plates were inoculated as above (0.5 McFarland), dried for ~10 min, discs placed with sterile forceps at ≥24 mm center-to-center spacing, and incubated at 35 ± 2 °C for 16–20 h. Zones were interpreted according to CLSI; QC strains (*E. coli AC124*, *S. aureus DF256*, *and P. aeruginosa AC110*) were run with each batch.

Quality control and replication
All assays were performed in triplicate on separate days. For extract testing, the final DMSO content never exceeded 1% (v/v) in plates or discs, and vehicle controls confirmed no inhibitory effect at this level. Sterile media and growth controls were included in each run.

Data management and statistical analysis
Zone diameters and MICs were summarized as mean ± SD. Between-solvent and between-organism effects were compared using two-way ANOVA with Tukey's post hoc test ($\alpha = 0.05$). Analyses were performed using IBM SPSS Statistics v19 and Microsoft Excel.

Results
Bacterial Distribution in Otitis Media Samples
Ninety-four percent of the collected ear-swab samples yielded bacterial growth, whereas six percent showed no growth (Table 1). The predominant isolate was *Staphylococcus aureus* (83%), followed by *Pseudomonas* spp. (11%); *Streptococcus* spp., *Escherichia coli*, and *Klebsiella* spp. each constituted 2% of the total number of isolates (Table 1).

Table 1. Distribution of bacterial isolates recovered from otitis media samples

Bacterial Isolate	Percentage (%)
Staphylococcus aureus	83%
Pseudomonas spp.	11%
Streptococcus spp.	2%
Escherichia coli (E. coli)	2%
Klebsiella spp.	2%
No Growth	6%

Antibacterial Activity (Zone of Inhibition
Across organisms, ethanol extracts produced the largest inhibition zones (12–17 mm), acetone showed moderate activity (6–9 mm), chloroform was weak (3–6 mm), and water showed no inhibition. For *S. aureus*, the ethanol extract showed a 17 mm zone of inhibition, while the acetone and chloroform extracts showed 9 mm (acetone) and 3 mm (chloroform). For *Pseudomonas* spp., ethanol produced 12 mm, acetone 9 mm, and chloroform 6 mm; a similar pattern was observed for *Streptococcus* spp., *E. coli*, and *Klebsiella* spp. The reference antibiotic, gentamicin, consistently yielded 22 mm zones across all tested isolates (Table 2).

Table 2. Zone diameters (mm) for Senna italica extract and gentamicin against clinical isolates.

Bacterial Isolate	Ethanol Extract (mm)	Acetone Extract (mm)	Chloroform Extract (mm)	Water Extract (mm)	Gentamicin (mm)
Staphylococcus aureus	17	9	3	0	22
Pseudomonas spp.	12	9	6	0	22
Streptococcus spp.	15	9	6	0	22
E. coli	12	9	6	0	22
Klebsiella spp.	12	9	6	0	22

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Received: 01-07-2025 - **Accepted**: 30-08-2025 - **Published**: 06-09-2025

Minimum Inhibitory Concentration (MIC)

The MIC values of the extracts were determined against all bacterial isolates, and MIC testing highlighted organism- and solvent-dependent effects (Table 3). Among gram-positive bacteria, ethanol showed the lowest MIC against *Streptococcus* spp. (550 mg/mL) and Staphylococcus aureus (750 mg/mL); acetone and chloroform each recorded 750 mg/mL for both taxa, while water showed no activity. Among Gram-negatives, chloroform yielded the lowest MIC for *Pseudomonas* spp. (280 mg/mL), whereas ethanol and acetone required 750 mg/mL; for *E. coli* and *Klebsiella* spp., ethanol 550–750 mg/mL and acetone/chloroform 750 mg/mL were observed; water remained inactive (Table 3).

Table 3. MICs (mg/mL) of Senna italica extracts against clinical isolates of bacteria.

Bacterial Isolate	Ethanol Extract (mg/mL)	Acetone Extract (mg/mL)	Chloroform Extract (mg/mL)	Water Extract (mg/mL)
Staphylococcus aureus	750	750	750	No activity
Pseudomonas spp.	750	750	280	No activity
Streptococcus spp.	550	750	750	No activity
E. coli	550	750	750	No activity
Klebsiella spp.	750	750	750	No activity

Phytochemical profile (qualitative)

Qualitative screening detected alkaloids (+) and sugars (+) in the extracts, while flavonoids, saponins, and terpenoids were not detected under the conditions used (Table 4).

Table 4. Presence or absence of major phytochemical classes in Senna italica extracts.

Active Compound	Presence
Alkaloids	+
Flavonoids	-
Saponins	-
Terpenoids	-
Sugars	+

Antibiotic resistance percentages for bacterial isolates

In disc diffusion testing, gentamicin and ciprofloxacin produced the largest mean zones (mm), followed by chloramphenicol. Tetracycline and polymyxin B were intermediate, whereas amoxicillin/clavulanate and cefoxitin showed poor activity (Table 5). Resistance analysis showed 100% resistance to cefoxitin, high resistance to amoxicillin/clavulanate (86%) and polymyxin B (90%), moderate resistance to tetracycline (73%), and low resistance to chloramphenicol (18%) and Gentamicin (10%). No resistance was observed against ciprofloxacin or imipenem (0%) (Table 5).

Table 5. Antibiotic susceptibility and resistance percentages among clinical isolates.

Antibiotic	Resistance (%)
Cefoxitin (FOX)	100%
Amoxicillin/Clavulanic Acid (AUG)	86%
Polymyxin B (PB)	90%
Tetracycline (TE)	73%
Chloramphenicol (C)	18%
Gentamicin (CN)	10%
Ciprofloxacin (CIP)	0%
Imipenem (IMP)	0%

Discussion

We aimed to determine whether the choice of solvent (ethanol, acetone, chloroform, or water) alters the measurable antibacterial activity of Senna italica leaf extracts against otitis media (OM) isolates and to contextualize the results with qualitative phytochemistry. Among the solvents, ethanol consistently produced the largest agar diffusion zones, acetone was intermediate, chloroform showed weaker diffusion yet comparatively better broth effects against Pseudomonas spp., and water was inactive. These differences are in accordance with the extraction theory: polar organic solvents recover mid-polarity metabolites that are under-represented in water, whereas nonpolar chloroform enriches lipophilic constituents that often diffuse poorly in agar but can act in well-mixed broth [2,14,15]. Qualitative screening in our laboratory supported the presence of alkaloids, saponins, terpenoids, and reducing sugars in the more active extracts, while flavonoids were not confirmed, and anthraquinones were not directly verified; therefore, we avoided

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***Received**: 01-07-2025 - **Accepted**: 30-08-2025 - **Published**: 06-09-2025*

attributing activity to specific classes here. Given that Senna italica spp. can contain anthraquinone glycosides among other constituents, follow-up total assays and targeted LC–MS/HPLC profiling are warranted to identify the drivers of effect and reconcile chemistry with bioactivity [12,13].
In the clinical microbiology context, our isolate distribution was dominated by Staphylococcus aureus and Pseudomonas spp., with less frequent recovery of Streptococcus spp., Escherichia coli, and Klebsiella spp. This pattern is compatible with the known shift from "classic" AOM pathogens (Streptococcus pneumoniae, Haemophilus influenzae, and Moraxella catarrhalis) toward S. aureus and Pseudomonas spp. in chronic or recurrent OM with otorrhea, especially in resource-limited settings [17,18]. The prominence of these resilient gram-negative bacteria underscores the clinical challenge that motivates the exploration of accessible plant-based options as leads or adjuvants [20].
With respect to antibacterial performance, the ethanol extract produced the largest zones overall but remained less potent than the benchmark antibiotics, as expected for unfractionated material. Zone interpretations should be read against organism-specific CLSI breakpoints, and broth MICs were determined under standardized conditions. The superior broth effect of the chloroform fraction against Pseudomonas spp. despite weak agar diffusion is mechanistically plausible: lipophilic constituents are disadvantaged in solid-phase diffusion but may better access or destabilize the outer membrane in liquid culture [6,10]. Conversely, the least inhibition was consistently observed with the water extracts. This is consistent with the low solubility of many bioactive plant metabolites in water and, for gram-negative targets, the additional permeability and efflux barriers that further depress the apparent activity in plate assays [16]. Collectively, these results point to solvent-dependent recoveries and matrix effects rather than a single dominant compound class as the explanation for our activity profile, reinforcing the need for activity-guided fractionation to concentrate actives into µg/mL-range MICs [2]
Finally, our susceptibility data reflect the wider AMR landscape: substantial resistance to β-lactams with comparatively preserved activity for gentamicin, ciprofloxacin, and imipenem, patterns that mirror global surveillance and stewardship concerns [2,20,21]. In this context, Senna italica extracts should be evaluated not as stand-alone replacements for effective antibiotics but as potential adjuvants. Synergy testing using checkerboard FICI followed by time-kill against aminoglycosides or fluoroquinolones is a logical next step, given the evidence that plant-derived constituents can potentiate antibiotic activity or attenuate resistance mechanisms

Conclusion

The integration of Senna italica extracts into antimicrobial strategies represents a promising avenue for combating the growing threat of antibiotic resistance. These findings highlight the potential of medicinal plants as a complementary or alternative approach to synthetic antibiotics, particularly in the treatment of resistant bacterial infections. The continued exploration of plant-based antimicrobials could provide safer and more sustainable solutions to global health challenges.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Alamen AA, Alsonosi AM, Alaasswad NM. Bacterial co-infections in patients with COVID-19 and their resistance profiles: a study from Southern Libya. Acta Microbiol Bulg. 2025;41(2):164-74. doi:10.59393/amb25410204.
2. Berdy J. Bioactive microbial metabolites: a personal view. J Antibiot (Tokyo). 2005;58(1):1-26. doi:10.1038/ja.2005.1.
3. Bukar A, Mukhtar MD, Hassan AS. Phytochemical screening and antibacterial activity of leaf extracts of Senna italica siamea (Lam.) on Pseudomonas aeruginosa. Bayero J Pure Appl Sci. 2009;2(1):70-3.
4. Chow AW, Chonmaitree T, Brook I, Marchant CD, Rovers MM. Otitis media: advances in diagnosis and management. Am J Otolaryngol. 2017;38(2):123-9.
5. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 34th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2024.
6. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82. doi:10.1128/CMR.12.4.564.
7. Cragg GM, Newman DJ. Biodiversity: a continuing source of novel drug leads. Pure Appl Chem. 2005;77(1):7-24. doi:10.1351/pac200577010007.
8. El-Bassuony AA, Abdelaziz SM, Alwasify MA. Antimicrobial activities of different extracts of Senna italica spp. and their potential as alternative antimicrobial agents. Res Pharm Sci. 2020;15(1):39-50.
9. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Rep. 2004;21(2):263-77. doi:10.1039/b212695h.
10. Harborne AJ. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Springer; 1998.
11. Houghton PJ, Raman A. Laboratory handbook for the fractionation of natural extracts. London: Chapman & Hall; 1998. doi:10.1007/978-1-4615-5809-5.
12. IBM Corp. IBM SPSS Statistics for Windows. Version 19.0. Armonk, NY: IBM Corp; 2010.

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Received*: 01-07-2025 -* ***Accepted****: 30-08-2025 -* ***Published****: 06-09-2025*

13. Kaplan SL. Otitis media: treatment challenges in the era of antibiotic resistance. Pediatrics. 2004;113(5):1451-7.
14. Klein EY. Epidemiology of otitis media in children and adults. Curr Infect Dis Rep. 2003;5(5):371-9.
15. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013;13(12):1057-98. doi:10.1016/S1473-3099(13)70318-9.
16. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803. doi:10.1021/acs.jnatprod.9b01285.
17. Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline. Clin Infect Dis. 2006;42(5):657-68. doi:10.1086/499819.
18. Ventola CL. The antibiotic resistance crisis: part 1—causes and threats. P T. 2015;40(4):277-83.
19. World Health Organization. 2020 antibacterial agents in clinical and preclinical development: an overview and analysis. Geneva: World Health Organization; 2020.
20. World Health Organization. Antimicrobial resistance. Fact sheet. Geneva: World Health Organization; 2021
21. Shamsi SS, Elzen AA, Ahmad KM. Burkholderia cepacia Complex, an Emerging Nosocomial Pathogen at Health Care Facilities in Sebha, Libya. *Journal of Medical Microbiology and Infectious Diseases.* 2021;9(4):178–184. doi:10.52547/JoMMID.9.4.178

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***Received**: 01-07-2025 - **Accepted**: 30-08-2025 - **Published**: 06-09-2025*