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Short Communication

Antibacterial potential of selected medicinal plant leaf extracts against some pathogens

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This study investigates the antimicrobial activity of leaf extracts from selected medicinal plants, *Cinnamomum tamala* (bay leaf), *Polyalthia longifolia* (Ashoka), *Cymbopogon citratus* (lemongrass), *Amomum subulatum* (black cardamom), and *Andrographis paniculata* (bhui neem) against pathogenic bacterial strains: *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Escherichia coli*. Using the agar well diffusion assay, different extract concentrations (100–250 µL) were tested for antimicrobial efficacy. Results revealed that *A. subulatum* exhibited the strongest activity against *P. mirabilis* (Zone of inhibition: 20 mm at 150 µL), while *C. citratus* demonstrated remarkable inhibition against *E. coli* (39 mm at 250 µL) and *E. faecalis* (25 mm). *A. paniculata* showed potent activity against *P. aeruginosa* (26 mm at 250 µL), and *P. longifolia* was highly effective against *S. aureus* (30 mm). These findings suggest the potential of these plant extracts as promising natural antimicrobials and support further pharmacological investigations for clinical application in managing bacterial infections.

Keywords: Antibacterial assay, Disc diffusion method, Medicinal plants, Phytochemical constituents, Zone of inhibition

IPC code; Int. cl. (2021.01) – A61K 36/00, A61P 31/00

Introduction

Traditional medicine has played a crucial role in healthcare systems worldwide for millennia. Even today, approximately 80% of the global population relies on medicinal plants to address various health conditions[1]. These plants, long regarded as a rich source of therapeutic compounds, have contributed significantly to drug discovery and continue to form the basis of many modern pharmaceuticals[2,3]. In particular, many plant-based preparations have been widely used in traditional systems such as Ayurveda, Siddha, and Unani for their curative properties[4.]

In recent years, the rise of multidrug-resistant (MDR) microbial strains has become a pressing public health concern worldwide. Conventional antibiotics are increasingly losing their effectiveness, making it crucial to identify and develop alternative antimicrobial strategies. This challenge has renewed scientific interest in medicinal plants as natural sources of antimicrobial agents. Their phytochemical diversity offers promising alternatives to synthetic drugs, especially in treating infections caused by resistant pathogens[5]. Although many medicinal plants have been documented in traditional knowledge systems, many remain underexplored in modern pharmacological research[6]. While some prior studies have investigated the antimicrobial potential of individual plants, there is limited comparative evaluation of multiple indigenous species using standardised protocols against clinically relevant pathogens[7]. Thus, a clear scientific gap exists in validating the effectiveness of traditional herbs, particularly those native to specific regions, against resistant microbial strains encountered in clinical settings[8].

In this context, the present study focuses on five traditionally used medicinal plants: *Cinnamomum tamala* (Buch. -Ham.) T. Nees & Eberm. (*Indian bay leaf*), *Polyalthia longifolia* (Sonn.) Thwaites (*false Ashoka*), *Cymbopogon citratus* (DC.) Stapf (*lemongrass*), *Amomum subulatum* Roxb. (*black cardamom*), and *Andrographis paniculata* (Burm. f.) Nees (*king of bitters*). These plants are well known in Indian ethnomedicine and possess a broad range of therapeutic properties. For instance, *A. paniculata* is known for its immune-boosting[9], hepatoprotective[10], antiviral[11], antioxidant[12], and anti-inflammatory[13] effects. Similarly, the leaves of *C. tamala* are traditionally used for their antihelminthic, diuretic, anti-inflammatory, and carminative properties and have been employed to treat conditions related to the spleen, heart, and gastrointestinal system[14]. *P. longifolia* exhibits notable antimicrobial, cytotoxic, and anti-inflammatory activities, supporting its traditional use in the treatment of fever, skin diseases, and infections[15]. *C. citratus* is widely recognized for its antioxidant, antimicrobial, and anti-inflammatory effects attributed mainly to its citral-rich essential oil[16]. *A. subulatum* demonstrates antioxidant,

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gastroprotective, and antimicrobial activities and is traditionally used for digestive and respiratory ailments[17].

The antimicrobial activity of these plants has been previously studied by several authors[18-21]. However, a consolidated study that examines and compares the antimicrobial efficacy of these five species using a unified methodology, particularly against pathogenic strains, including those with clinical relevance, is lacking.

Therefore, this study was undertaken to fill this gap by scientifically evaluating the antibacterial and antifungal potential of methanolic leaf extracts from these five medicinal plants. The work aims to validate their traditional use and explore their potential for development into plant-based antimicrobial agents.

The selection of *C. tamala*, *P. longifolium*, *C. citratus*, *A. subulatum*, and *A. paniculata* was based on their widespread use in traditional Indian medicine for treating infections, fevers, and inflammatory conditions. These plants are also locally available, cost-effective, and environmentally sustainable. While the individual antimicrobial properties of these species have been investigated to some extent in previous studies, a systematic comparative evaluation of their leaf extracts against a broad spectrum of clinically significant bacterial strains using standardised methods has not been conducted. To the best of our knowledge, no prior study has simultaneously assessed the antimicrobial activity of all five plants under the same experimental conditions. This study, therefore, fills an important research gap and provides novel insights into their potential as alternative antibacterial agents.

Materials and Methods

Plant sample collection, identification, and extract preparation

Fresh leaves of *C. tamala*, *P. longifolia*, *C. citratus*, *A. subulatum*, and *A. paniculata* were collected from the garden premises of the CSIR-National Environmental Engineering Research Institute (NEERI), Nagpur, India. Samples were collected in December 2024. The plant materials were taxonomically authenticated by Ms. Sangita Gami, Taxonomist and Project Associate-I, CSIR–National Environmental Engineering Research Institute (NEERI), Nagpur, India. Voucher specimens were deposited in the institutional herbarium with voucher specimen numbers NEERI/BT/2024/CT-01 (*C. tamala*), NEERI/BT/2024/PL-02 (*P. longifolia*), NEERI/BT/2024/CC-03 (*C. citratus*), NEERI/BT/2024/AS-04 (*A. subulatum*), and NEERI/BT/2024/AP-05 (*A. paniculata*). The collected leaves were initially rinsed with tap water to remove surface debris, followed by thorough washing with distilled water to ensure primary sterilisation.

The cleaned leaves were subsequently air-dried at 37°C in a hot air oven until a constant weight was achieved. Once dried, the plant material was ground into a fine powder using an electric grinder and passed through a sieve to remove coarse particles.

For extraction, 10 g of the powdered leaf material was macerated in 100 mL of a methanol: acetone solvent mixture (1:1 v/v). The mixture was incubated in a rotary shaker at 120 rpm for 24 hours at room temperature to facilitate the extraction of bioactive compounds. After incubation, the solution was filtered using Whatman No. 1 filter paper. The filtrates were allowed to evaporate at ambient temperature in a shaking incubator to remove residual solvents. The resulting crude extract residue was weighed, and dimethyl sulfoxide (DMSO) was added at a 2:1 (v/w) ratio to the dry extract for reconstitution and storage.

Bacterial strains and culture maintenance

The antimicrobial activity was tested against a panel of clinically relevant bacterial strains, including *Bacillus* sp., *Proteus mirabilis*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The bacterial strains used in this study were obtained from the Microbiology Laboratory of CSIR–NEERI. The strains are authenticated reference cultures maintained in the institutional microbial repository. Strain identity was confirmed using standard morphological and biochemical characterisation prior to experimentation. Cultures were maintained on Luria–Bertani (LB) agar slants at 4°C.

For experimental use, the bacterial cultures were subcultured on freshly prepared LB agar plates. A sterile inoculating loop was used to streak each strain onto separate plates, which were then incubated at 37°C for 24 hours. These fresh subcultures served as inoculum sources for antimicrobial assays.

Determination of antimicrobial activity

The antimicrobial efficacy of the crude plant extracts was evaluated using the standard agar well diffusion technique, following the procedure described by Ginovyan *et al.*[22].

Briefly, 200 µL of standardised bacterial inoculum was aseptically spread onto sterile Mueller-Hinton Agar (MHA) plates to ensure even distribution of microbial growth. After inoculation, wells were aseptically bored into the agar surface using a sterile cork borer (6 mm in diameter). Four volumes of plant extracts (100, 150, 200, and 250 µL) were loaded into the respective wells. Each concentration was tested in triplicate to ensure reproducibility. The plates were then incubated at 37°C for 24 hours to allow for bacterial growth and diffusion of the plant extracts.

Following incubation, the plates were observed for zones of inhibition around the wells, indicating antibacterial activity. The diameter of the clear zones was measured in millimetres using a calibrated ruler, from the edge of the well to the outer margin of the inhibition zone. Measurements were recorded only for distinct, measurable zones; the absence of a visible inhibition zone was noted as no activity. Sterile DMSO (used as the extract solvent) served as the negative control to confirm that observed effects were solely due to plant extracts.

Result and Discussion

The antibacterial activity of methanol/acetone leaf extracts from *C. tamala*, *P. longifolia*, *A. subulatum*, *C. citratus*, and *A. paniculata* was assessed using the agar well diffusion method against five clinically relevant bacterial strains: *Proteus mirabilis*, *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Zones of inhibition (ZI) were recorded at four volumes: 100, 150, 200, and 250 µL.

Leaf extracts from *Andrographis paniculata*, *Cinnamomum tamala* (bay leaf), *Polyalthia longifolia* (Ashoka), *Cymbopogon citratus* (lemongrass), and *Amomum subulatum* (black cardamom) were evaluated for their antibacterial activity against *Proteus mirabilis*. As shown in Table 1, the extracts were tested at four different concentrations: 100, 150, 200, and 250 µL. Among the tested species, *C. tamala* demonstrated a zone of inhibition measuring 15, 17, 16, and 18 mm, respectively. Similarly, *A. subulatum* exhibited notable antimicrobial activity with inhibition zones of 15.6, 20, 18, and 18 mm, respectively. *P. longifolia* showed moderate inhibition zones of 19, 15, 16, and 10 mm at the respective concentrations. In contrast, *C. citratus* did not exhibit any measurable antimicrobial activity against *Proteus mirabilis*. Data for *A. paniculata* were not determinable under the tested conditions and are thus not reported (Fig. 1 and Table 1).

According to the study's findings (Table 2), all tested plant extracts exhibited varying degrees of antibacterial activity against *E. coli*. Notably, *C. tamala* extract, which demonstrated a consistent antimicrobial efficacy by producing zones of inhibition measuring 17, 17, 16, and 15 mm at extract volumes of 100, 150, 200, and 250 µL, respectively. *A. subulatum* showed an increasing trend in activity with inhibition zones of 17, 17, 18, and 19 mm at the same concentrations. *C. citratus* extract exhibited strong but variable bacteriostatic effects, with inhibition zones of 20, 19, 10, and 39 mm. In contrast, *P. longifolia* and *A. paniculata* showed comparatively lower antibacterial activity, each exhibiting inhibition zones of 12, 12.5, 12.6, and 12.5 mm across the four tested concentrations.

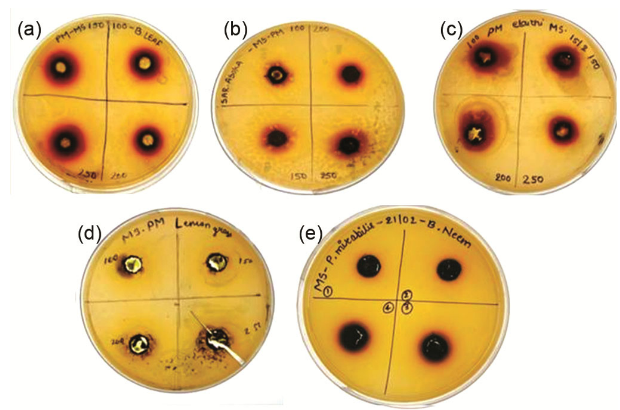

Fig. 1 — Antibacterial activity of (a) C. tamala, (b) *Polyalthia longifolium*, (c) *Amomum subulatum*, (d) *Cymbopogon citratus*, and (e) *Andrographis paniculata* against *Proteus mirabilis.*

Table 1 — Antibacterial activity of selected plant species against *Proteus mirabilis*

Conc. (µL)	Zone of inhibition diameter (mm) A	B	C	D	E
100	18	19	15	No zone	
150	16	15	20		No zone
200	17	16	18		
250	15	10	18		

A: *C. tamala*, B: *P. longifolium*, C: *A. subulatum*, D: *C. citratus*, E: *A. paniculata*

Table 2 — Antibacterial activity of selected plant species against *E. coli*

Conc. (µL)	Zone of inhibition diameter (mm) A	B	C	D	E
100	17		17	20	12
150	17	No zone	17	19	12.5
200	16		18	10	12.6
250	15		19	39	12.5

A: *C. tamala*, B: *P. longifolium*, C: *A. subulatum*, D: *C. citratus*, E: *A. paniculata*

The aforementioned Fig. 2 demonstrates that at a dose of 250 µL, *Cymbopogon citrate* shows the highest antibacterial activity against *E. coli*. The overall zone of inhibition measures 39 mm.

The antimicrobial activity of various leaf extracts against *E. faecalis* (as detailed in Table 3) demonstrated variable inhibition profiles. *A. paniculata* exhibited strong and consistent antibacterial activity, with inhibition zones measuring 24 mm at both 100 and 150 µL, 22 mm at 200 µL, and 21 mm at 250 µL. *C. tamala* was effective only at a concentration of 150 µL, showing a zone of inhibition of 18 mm. *P. longifolia* displayed a progressive increase in antimicrobial activity with inhibition zones of 11, 13, 15, and 16 mm at 100, 150, 200, and 250 µL, respectively. *C. citratus* showed notable bacteriostatic potential with inhibition zones of 20, 22, 24, and 25 mm across the same increasing volume. *A. subulatum* was active only at 150 µL, producing a zone of inhibition of 20 mm.

The above Fig. 3 shows that *C. citratus* has the strongest antimicrobial activity against *E. faecalis* with a zone of inhibition of 25 mm at a concentration of 250 µL.

A. paniculata leaf extracts demonstrated notable antimicrobial activity against *P. aeruginosa*, with zones of inhibition measuring 13, 25, 25, and 26 mm at concentrations of 100, 150, 200, and 250 µL, respectively. In contrast, *P. aeruginosa* exhibited resistance to the extracts of *C. tamala*, *P. longifolium*, *C. citratus*, and *A. subulatum*, with no observable zones of inhibition across all tested concentrations (as shown in Table 4).

As can be seen in Fig. 4 above, at a concentration of 250 µL, *Andrographis paniculata* exhibits the highest antimicrobial activity against *Pseudomonas aeruginosa*, with a zone of inhibition of 26 mm.

The results presented in Table 5 indicate that *C. tamala* (bay leaf) did not exhibit any antimicrobial activity against *Bacillus* species across all tested concentrations. In contrast, *Amomum subulatum* (Badi elaichi) demonstrated antibacterial activity, with zones of inhibition measuring 13.5, 17, 21, and 21 mm at concentrations of 100, 150, 200, and 250 µL, respectively. *Cymbopogon citratus* (lemongrass) showed no inhibitory effect at lower concentrations but exhibited a measurable zone of inhibition only at 250 µL. *Andrographis paniculata*

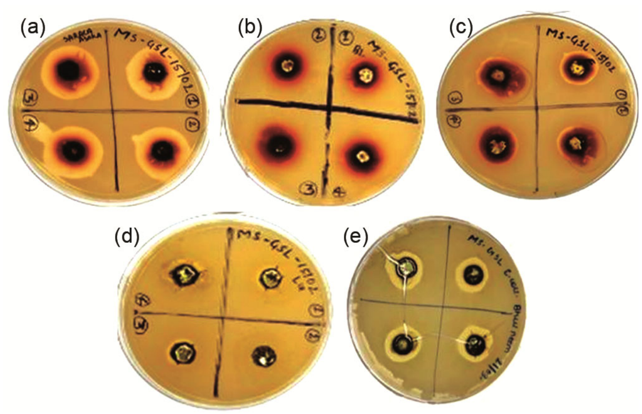

Fig. 2 — Antibacterial activity of selected plant species against *Escherichia coli*. (a) *C. tamala*, (b) *Cymbopogon citratus*, (c) *Polyalthia longifolium*, (d) *Amomum subulatum*, and (e) *Andrographis paniculata*.

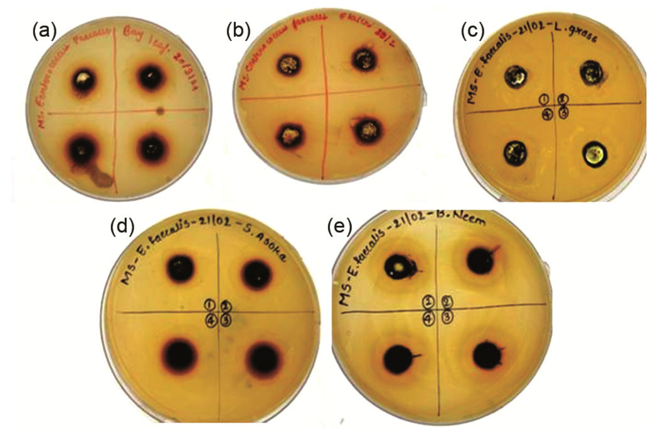

Fig. 3 — Antibacterial activity of selected plant species against *Enterococcus faecalis*. (a) *C. tamala* (b) *Amomum subulatum* (c) *Cymbopogon citrate* (d) *Polyalthia longifolium*, and (e) *Andrographis paniculate*.

Table 3 — Antibacterial activity of selected plant species against *Enterococcus faecalis*

	Zone of inhibition diameter (mm)				
Conc. (µL)	A	B	C	D	E
100	No zone	11	No zone	20	24
150	18	13	20	22	24
200	No zone	No zone	No zone	24	22
250	No zone	16	No zone	25	21

A: *C. tamala*, B: *P. longifolium*, C: *A. subulatum*, D: *C. citratus*, E: *A. paniculata*

Table 4 — Antibacterial activity of selected plant species against *Pseudomonas aeruginosa*

	Zone of Inhibition Diameter (mm)				
Conc. (µL)	A	B	C	D	E
100					13
150	No zone	No zone	No zone	No zone	25
200					25
250					26

A: *C. tamala*, B: *P. longifolium*, C: *A. subulatum*, D: *C. citratus*, E: *A. paniculata*

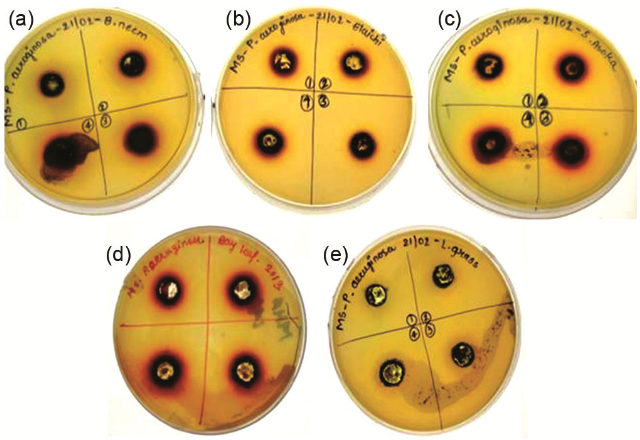

Fig. 4 — Antibacterial activity of selected plant species against *Pseudomonas aeruginosa*. (a) *Andrographis paniculata*, (b) *Amomum subulatum*, (c) *Polyalthia longifolium*, (d) *C. tamala*, and (e) *Cymbopogon citratus*.

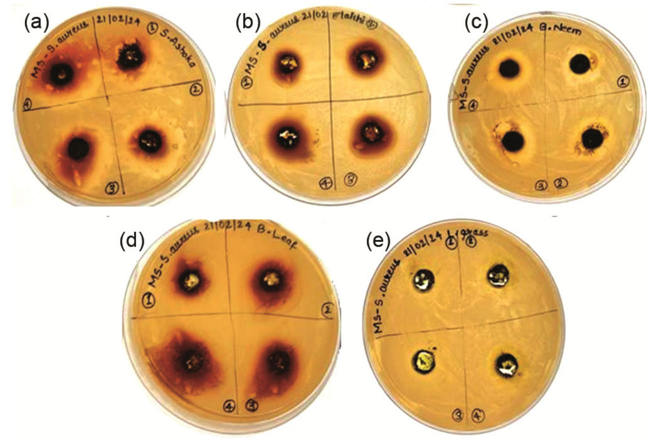

Fig. 5 — Antibacterial activity of selected plant species against *Staphylococcus aureus*. (a) *Polyalthia longifolium*, (b) *Amomum subulatum*, (c) *Andrographis paniculata*, (d) *C. tamala*, (e) *Cymbopogon citrates.*

Table 5 — Antibacterial activity of selected plant species against *Staphylococcus aureus*

	Zone of Inhibition Diameter (mm)				
Conc. (µL)	A	B	C	D	E
100	No zone	2.5	13.5	No zone	19
150	No zone	30	17	No zone	19
200	No zone	28.5	21	No zone	19
250	No zone	30	21	17	22

A: *C. tamala*, B: *P. longifolium*, C: *A. subulatum*, D: *C. citratus*, E: *A. paniculata*

(Bhuineem) consistently exhibited antibacterial activity, with inhibition zones of 19 mm at 100, 150, and 200 µL, and 22 mm at 250 µL against *S. aureus*.

The antibacterial activity *of P. longifolium* against *S. aureus* is demonstrated in Fig. 5 above, with a zone of inhibition of 30 mm at a concentration of 250 µL.

The antibacterial patterns observed in the present investigation are consistent with those reported in previously published studies on medicinal plant extracts. The broad-spectrum activity exhibited by *A. paniculata*, particularly against *P. aeruginosa* and *S. aureus*, supports earlier findings that attribute its antimicrobial efficacy to bioactive diterpenoids such as andrographolide[23]. Similarly, the pronounced inhibitory effect of *C. citratus* against *E. coli* and *E. faecalis* aligns with reports demonstrating the strong bactericidal properties of citral-rich extracts, which can disrupt bacterial cell membranes[24]. The significant activity of *P. longifolia* against *S. aureus* is consistent with earlier reports demonstrating potent antibacterial and antibiofilm effects of *P. longifolia* leaf extracts against Gram-positive organisms[25]. Furthermore, the reduced susceptibility of *P. aeruginosa* to most plant extracts observed in this study corroborates established evidence that intrinsic resistance mechanisms, such as efflux pumps and low outer membrane permeability, limit the efficacy of many herbal extracts against Gram-negative bacteria[26]. Overall, the present findings reinforce the growing scientific evidence supporting medicinal plants as promising sources of antibacterial agents.

Conclusion

The study highlight the promising antimicrobial potential of selected medicinal plant leaf extracts, *A. paniculata*, *C. tamala*, *P. longifolia*, *C. citratus*, and *A. subulatum* against a range of clinically significant bacterial strains, including *Proteus mirabilis*, *E. coli*, *E. faecalis*, *P. aeruginosa*, and *Bacillus* spp. The agar well diffusion assay revealed that the antimicrobial efficacy varied considerably across plant species, concentrations (100–250 µL), and target microorganisms. Among the tested botanicals, *A. paniculata* and *C. citratus* demonstrated the most consistent and significant inhibitory activity across multiple bacterial strains and concentrations, particularly against *P. aeruginosa* and *E. faecalis*, respectively. These results suggest the presence of potent phytochemicals with broad-spectrum bacteriostatic or bactericidal effects. *A. subulatum* also exhibited selective antibacterial activity, indicating its potential as a complementary antimicrobial agent. Conversely, *C. tamala* and *P. longifolia* showed limited or concentration-specific effects, suggesting a narrower spectrum or weaker potency of their active constituents. The concentration-dependent inhibition patterns observed further support the notion that dosage optimisation is critical for the efficacy of plant-based antimicrobials. The absence of activity in certain extracts and concentrations also emphasises the need for targeted phytochemical profiling to identify and isolate specific bioactive compounds responsible for the observed effects. Overall, this study supports the ethnomedicinal relevance of these plants and provides a scientific basis for their potential development as alternative or adjunct antimicrobial agents. In light of increasing antimicrobial resistance, such natural products offer valuable avenues for novel drug discovery. Future research should aim to (i) isolate and characterise the active phytochemicals responsible for antimicrobial activity, (ii) evaluate cytotoxicity and safety profiles, and (iii) explore synergistic effects with standard antibiotics to enhance therapeutic efficacy.

Conflict of interest

The authors declare no conflict of interest related to the publication of this paper

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