

## ANTIMICROBIAL ACTIVITY OF SENNA ITALICA MILL. LEAF AND *PHYLLANTHUS RETICULATUS* POIR FRUIT EXTRACTS

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Antifungal, Antifungal, Bioactive, Pathogen, *Senna italica*; *Phyllanthus reticulatus*.

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### ABSTRACT

Antimicrobial agents are substances that inhibit or eliminate pathogenic microorganisms, including bacteria and fungi, and are essential for controlling infectious diseases. The growing emergence of antimicrobial resistance has reduced the effectiveness of conventional antibiotics, creating an urgent need for alternative sources of antimicrobials. Medicinal plants represent a promising option due to their rich content of bioactive secondary metabolites. The present study evaluated the antibacterial and antifungal activity of sequential solvent extracts of *S.italica* Mill. and *P. reticulatus* Poir. Hexane, ethyl acetate, and methanolic extracts were prepared and assessed using the Kirby–Bauer disc diffusion method against selected Gram-positive and Gram-negative bacteria and fungal pathogens. The results demonstrated a solvent-dependent antimicrobial response, with methanolic extracts exhibiting significantly higher activity than non-polar extracts. The methanolic extract of *S. italica* showed strong antibacterial activity against *Pseudomonas aeruginosa* ( $19.40 \pm 0.10$  mm) and notable antifungal activity against *Malassezia furfur* ( $18.20 \pm 0.20$  mm). *P. reticulatus* extracts displayed moderate but consistent antimicrobial activity, including inhibition of *Trichophyton rubrum* ( $15.30 \pm 0.10$  mm). These findings highlight the importance of solvent polarity in extracting antimicrobial compounds and support the potential of *S. italica* and *P. reticulatus* as natural sources of antibacterial and antifungal agents. This study provides a robust foundation for future research and highlights plant-based solutions in combating antibiotic resistance.

## Introduction

The rapid emergence and global spread of antimicrobial resistance (AMR) have become a significant threat to public health, significantly reducing the effectiveness of existing antibiotics and complicating the management of infectious diseases. Multidrug-resistant bacterial pathogens, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and other clinically relevant species, are increasingly associated with treatment failures in both hospital

and community settings (Garza-González *et al.*, 2010). This alarming scenario has intensified the search for alternative antimicrobial agents with novel mechanisms of action.

Medicinal plants have long been recognized as a valuable source of bioactive compounds with antimicrobial properties. Plant-derived secondary metabolites such as phenolics, flavonoids, tannins, terpenes, and alkaloids exhibit broad-spectrum antimicrobial activity and are considered promising alternatives or complements to conventional antibiotics (Gibbons, 2012; Savoia, 2012). While synthetic antibiotics target specific bacterial functions, leading often to resistance through adaptation, plant-derived agents offer a more varied approach through multiple mechanisms. Recent studies emphasize that these phytoconstituents act through multiple mechanisms, including disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and impairment of metabolic pathways, thereby reducing the likelihood of resistance development (Calvo & Martínez-Martínez, 2009; Abass *et al.*, 2024). Their ability to serve as complementary therapies alongside conventional antibiotics positions them as vital contenders in addressing growing resistance threats.

Advancements in phytochemical research have further elucidated the antibacterial mechanisms of plant-derived compounds. Updated reviews highlight that phenolics and flavonoids exert antimicrobial effects primarily through membrane destabilisation, protein precipitation, and oxidative stress induction. At the same time, alkaloids and terpenes interfere with cellular respiration and enzyme activity (Abass *et al.*, 2024). Bibliometric and experimental analyses have confirmed the growing scientific interest in plant antimicrobial compounds, particularly against spoilage and pathogenic bacteria, reinforcing their relevance in modern antimicrobial research (Pérez-Flores *et al.*, 2025).

The extraction solvent plays a crucial role in determining the antimicrobial efficacy of plant extracts. Polar solvents such as methanol and ethanol are known to enhance the extraction of phenolics and flavonoids, leading to improved antimicrobial activity compared to non-polar solvents. Experimental studies using methanolic and aqueous extracts have demonstrated measurable antibacterial effects against Gram-positive and Gram-negative bacteria, supporting solvent-dependent variations in antimicrobial performance (Rajapaksha *et al.*, 2025). Standardised *in vitro* methods, including disc diffusion and microplate-based MIC assays, are widely used to evaluate antimicrobial activity and ensure reproducible results (Eloff, 1999).

Recent investigations have also highlighted the relevance of plant-derived antimicrobials in dermatological and topical applications. Studies evaluating plant-based compounds and formulations against skin-associated bacteria have demonstrated effective antibacterial activity, supporting their potential use in managing superficial infections and skin disorders (Strompfová *et al.*, 2024). Such findings are particularly relevant in the context of increasing resistance among skin pathogens. Therefore, the present study focuses on evaluating the antimicrobial activity of sequential solvent extracts of selected medicinal plants using standardised *in vitro* assays, with particular emphasis on understanding solvent-dependent efficacy and mechanistic relevance. By integrating antimicrobial screening with insights from recent phytochemical and mechanistic studies, this work aims to contribute to the growing body of evidence supporting plant-derived compounds as viable candidates for future antimicrobial development.

## Materials and Methods

### Collection and Authentication of Plant Materials

Plant materials of *Senna italica* Mill. and *Phyllanthus reticulatus* Poir. were collected from rural areas of Tirunelveli district, Tamil Nadu, India. The collected plant materials were authenticated by Dr S. Muthu Eswaran, Scientist, Xavier Research Foundation, St. Xavier's College, Palayamkottai-627002, Tamil Nadu, India. Voucher specimens were deposited for future reference as *Phyllanthus reticulatus* (Reg. No. XCM-40751) and *Senna italica* (Reg. No. XCM-40752).

### Microbial Strains

The antimicrobial activity of the plant extracts was evaluated against selected bacterial and fungal strains obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The bacterial strains included *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Staphylococcus epidermidis* (MTCC 435). The fungal strains included *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), *Malassezia furfur* (MTCC 1374), and *Trichophyton rubrum* (MTCC 296). The bacterial cultures were maintained on nutrient agar and the fungal cultures on Sabouraud dextrose agar (SDA), and stored at 4 °C until use.

### Preparation of Plant Extracts

The dried and powdered plant material was subjected to solvent extraction using hexane, ethyl acetate, and methanol following standard extraction procedures described by Eloff (1998). The extraction process involved macerating the dried plant material in methanol for 48 hours at room temperature. During this period, the mixture was occasionally stirred to ensure thorough extraction. The solvent was then evaporated under reduced pressure using a rotary evaporator to obtain a concentrated extract. The extracts were filtered and concentrated under reduced pressure, and the dried extracts were stored at 4°C until antimicrobial evaluation.

### **Preparation of Inoculum**

A loopful of actively growing microbial culture was inoculated into sterile nutrient broth (for bacteria) or Sabouraud dextrose broth (for fungi) and incubated until visible turbidity developed. The bacterial inoculum was adjusted to match a 0.5 McFarland standard (approximately  $1.5 \times 10^8$  CFU/mL) prior to inoculation, as described in standard antimicrobial susceptibility testing procedures. Sterile Whatman No. 1 filter paper discs (6 mm diameter) were impregnated with 20 µL of each plant extract at a concentration of 1 mg/mL (20 µg/disc) following the procedure described by Bauer *et al.* (1966). The discs were air-dried aseptically to remove residual solvent. Amikacin (10 µg/disc) and ketoconazole (10 µg/disc) were used as positive controls for bacterial and fungal strains, respectively, while discs containing only the extraction solvent served as negative controls.

### **Antimicrobial Activity Assay (Kirby–Bauer Disc Diffusion Method)**

Antimicrobial activity was determined using the Kirby–Bauer disc diffusion method, as initially described by Kirby and Bauer (1966). Mueller–Hinton agar was used for bacterial strains, and Sabouraud dextrose agar was used for fungal strains. The standardised inoculum (0.1 mL) was uniformly spread over the agar surface using a sterile cotton swab. The extract-impregnated discs were placed on the agar surface, and the plates were incubated at 37 °C for 24 h for bacteria and at 28 ± 2 °C for 48–72 h for fungi. After incubation, the zones of inhibition were measured in millimetres. All experiments were carried out in triplicate, and the mean zone of inhibition was calculated.

### **Results and Discussion**

The antimicrobial activity of sequentially extracted hexane, ethyl acetate, and methanol fractions of *S. italica* and *P. reticulatus* was evaluated against selected bacterial and fungal pathogens at a concentration of 20 µg/disc, and the results are summarized in Table -1 and

depicted in Figure: 1. In the present study, methanolic extracts of *S. italica* and *P. reticulatus* exhibited significantly higher antimicrobial activity than ethyl acetate and hexane extracts against all tested bacterial and fungal pathogens. This enhanced activity of the methanolic extracts may be attributed to the higher extraction efficiency of polar solvents for bioactive phytochemicals such as phenolics, flavonoids, tannins, and anthraquinones, which are widely reported to possess antimicrobial properties. Solvent-dependent trends have been reported for methanolic extracts of medicinal plants, in which methanol extraction yielded higher levels of total phenolics and flavonoids, resulting in more vigorous antibacterial and antifungal activity compared to non-polar or aqueous extracts.

Patel and Pathak, (2025) reported that the methanolic leaf extracts of *Ziziphus lotus* exhibited significantly stronger antibacterial zones of inhibition and higher phenolic content than aqueous extracts. Iqbal *et al*, (2022) found their studies comparative solvent have shown that methanol extracts generally produce higher total phenolic content and greater antimicrobial efficacy than ethanol or water extracts . Adekunle *et al.*, (2025) reported that methanol extracts of several botanicals demonstrated significant activity against multidrug-resistant bacterial and fungal pathogens, consistent with solvent-dependent extraction of bioactive phytochemicals.

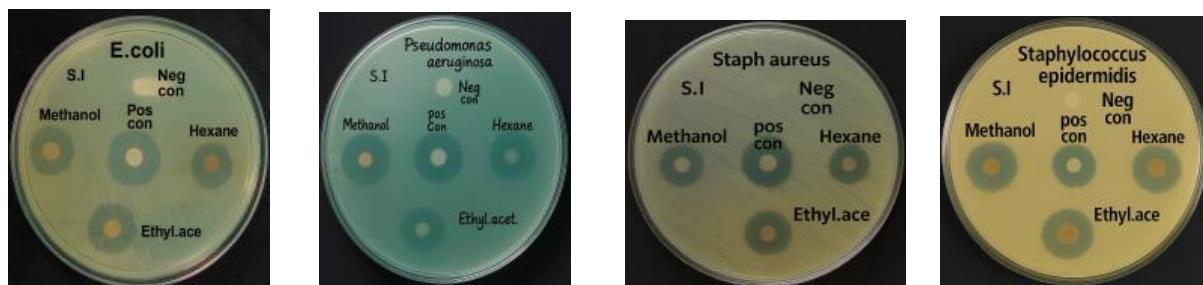
**Table 1 Inhibition Zone (mm) of *S. italica* and *P. reticulatus* Extracts Against Bacterial and Fungal Pathogens**

Inhibition Zone (mm)						
	<i>Senna italica</i>			<i>Phyllanthus reticulatus</i>		
Organism	Hexane	Ethyl Acetate	Methanol	Hexane	Ethyl Acetate	Methanol
<i>E. coli</i>	16.07 ± 0.12c	16.20 ± 0.10b	17.10 ± 0.10a	13.47 ± 0.23c	15.37 ± 0.21b	15.67 ± 0.58a
<i>P. aeruginosa</i>	17.13 ± 0.12c	17.43 ± 0.15b	19.40 ± 0.10a	14.50 ± 0.50b	14.13 ± 0.12c	17.40 ± 0.20a
<i>S. aureus</i>	13.17 ± 0.15c	18.17 ± 0.15b	18.17 ± 0.15a	13.23 ± 0.25c	16.13 ± 0.15a	15.13 ± 0.12b
<i>S. epidermidis</i>	17.17 ± 0.15b	16.30 ± 0.10c	18.37 ± 0.15a	13.70 ± 0.17b	13.33 ± 0.12c	17.17 ± 0.15a
<i>C. albicans</i>	16.10 ± 0.10c	18.30 ± 0.10b	18.33 ± 0.15a	14.50 ± 0.17 <sup>b</sup>	17.67 ± 0.58a	13.27 ± 0.31c
<i>A. niger</i>	14.17 ± 0.15b	14.10 ± 0.10c	19.17 ± 0.21a	14.17 ± 0.15b	13.20 ± 0.20c	17.50 ± 0.10a
<i>M. furfur</i>	16.23 ± 0.21c	16.60 ± 0.35b	18.20 ± 0.20a	13.87 ± 0.23b	13.07 ± 0.06c	17.07 ± 0.12a
<i>T. rubrum</i>	13.03 ± 0.06c	15.90 ± 0.10a	15.07 ± 0.12b	12.17 ± 0.15c	13.40 ± 0.20b	15.30 ± 0.10a

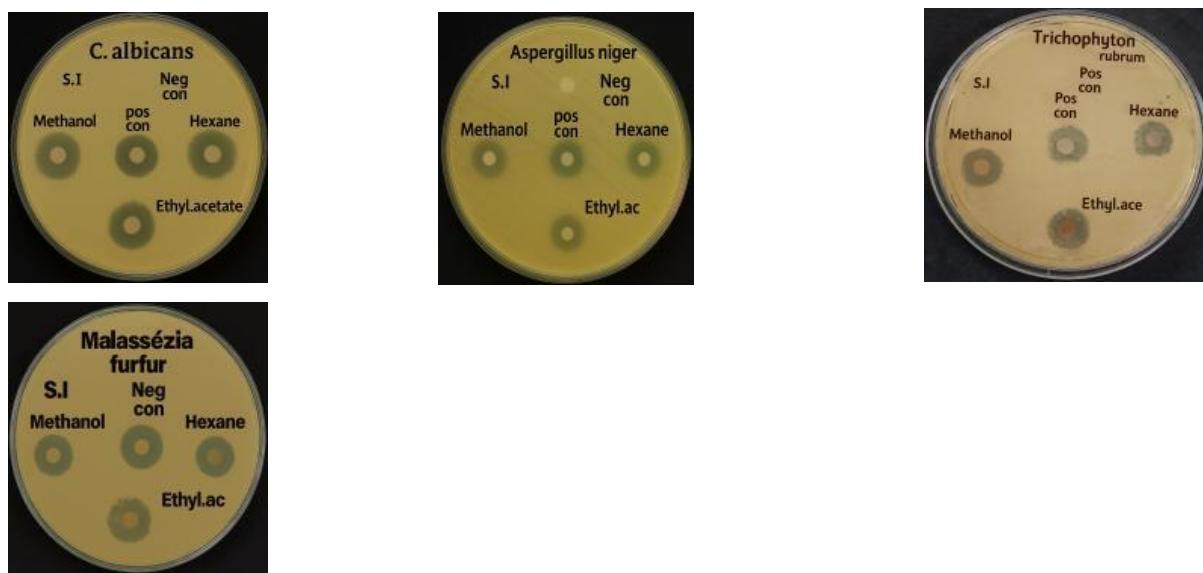
Note: Error bars represent mean ± SD (n = 3). Different letters (a-c) in each column indicate statistically significant differences (p < 0.05, one-way ANOVA followed by Tukey's HSD).

**Fig.1 Disc diffusion test (Zone of inhibition) of Hexane, Ethyl Acetate, and Methanol Extracts of *S. italica* and *P. reticulatus* Against Test Microorganisms**

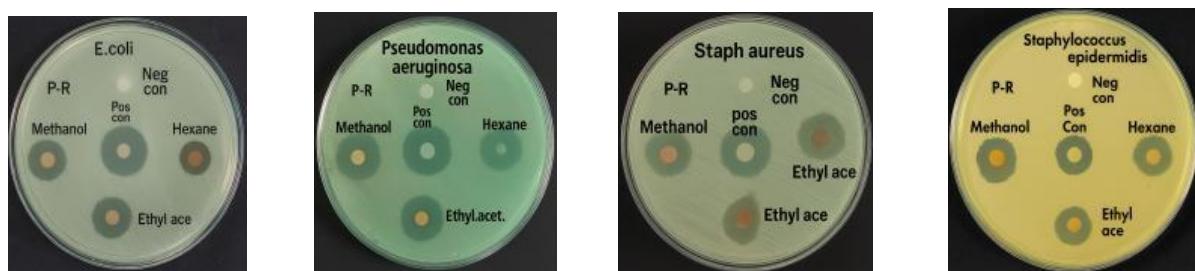
***Senna italica* (Bacterial pathogen)**

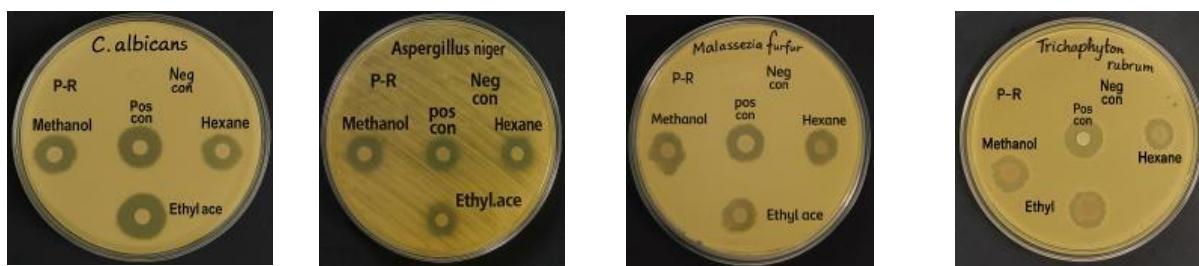


***Senna italica* (Fungal pathogen)**



***Pyllanthus reticulatus* (Bacterial Pathogen)**



***Pyllanthus reticulatus*(Fungal Pathogen)**

The methanolic extract of *S.italica* exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria. Among the tested organisms, the highest antibacterial activity was observed against *Pseudomonas aeruginosa*, with a zone of inhibition of  $19.40 \pm 0.10$  mm, indicating pronounced antibacterial efficacy. When compared qualitatively with standard antibiotics used as positive controls, the inhibition zones produced by *S.italica* methanolic extract indicate substantial antibacterial efficacy. All experiments were conducted using extract-impregnated discs prepared from a stock solution corresponding to the tested concentration, and assays were performed in triplicate to ensure reproducibility. Standard antibiotics were used as positive controls, while methanol served as the negative control.

Comparable antibacterial activity against *P. aeruginosa* and *S. aureus* has been reported for leaf extracts of *Mentha piperita*, supporting the ability of plant-derived bioactive compounds to inhibit clinically relevant bacterial pathogens (Afrin *et al.*, 2025). The antibacterial effects observed in the present study may be attributed to phenolic and flavonoid constituents, which are known to disrupt bacterial membrane integrity and inhibit essential metabolic enzymes, thereby impairing bacterial growth (Górniak *et al.*, 2019).

Supportive evidence is provided by Adil *et al.* (2024), who reported strong antimicrobial and antioxidant activities in methanolic extracts of *Euphorbia parviflora*. Their phytochemical screening and HPLC analysis confirmed the presence of high levels of phenolics and

flavonoids, which correlated with effective inhibition of Gram-positive, Gram-negative, and fungal species. The inhibition zones reported in their study (15.00–19.00 mm) are comparable to those observed in the present study, particularly against *P. aeruginosa* ( $19.40 \pm 0.10$  mm), suggesting similar levels of antimicrobial efficacy. The authors attributed these effects to membrane disruption, enzyme inhibition, and oxidative stress induction in microbial cells, mechanisms consistent with the antimicrobial patterns observed here, particularly the enhanced activity of methanolic extracts relative to non-polar extracts.

Solvent polarity plays a critical role in antimicrobial screening. The moderate-to-strong antimicrobial effects observed against clinically relevant bacteria and skin-associated fungi in the present study further emphasise the importance of solvent selection, as polar solvents such as methanol enhance the extraction of bioactive phytochemicals with antimicrobial potential. The antifungal evaluation revealed that methanolic extracts of *S. italicica* exerted strong inhibitory effects against both yeast and filamentous fungi. Notably, significant inhibition was observed against the skin-associated yeast *Malassezia furfur*, with a zone of inhibition measuring  $18.20 \pm 0.20$  mm. This result suggests that plant-derived phenolic compounds may interact effectively with lipid-dependent fungal membranes, thereby impairing fungal growth. Phenolic acids such as gallic acid disrupt membrane sterols and interfere with mitochondrial function in fungi, supporting the antifungal mechanisms proposed in the present study.

Comparable antifungal activity against *Candida albicans* and *Aspergillus niger* has been demonstrated for methanolic extracts of *Lawsonia inermis*, where bioactive constituents such as phenolics and flavonoids contribute to the disruption of fungal cell membranes and inhibition of growth (Saif *et al.*, 2025; Moutawalli *et al.*, 2023). Mahmoud, 2011 found that the *Azadirachta indica* leaf and seed extracts have also shown significant antifungal effects against dermatophytes, including *Trichophyton rubrum* and *Candida* spp., corroborating the susceptibility of filamentous fungi and yeasts to plant secondary metabolites. . Similarly, ethanol extracts of *Aloe vera* leaves and roots have been reported to inhibit the growth of *A. niger* and *C. albicans* *in vitro*, further validating the broad-spectrum antifungal potential of medicinal plants rich in phenolic and other bioactive compounds (Danish *et al.*, 2020). In the present study, inhibition zones of  $17.50 \pm 0.15$  mm for *C. albicans*,  $16.80 \pm 0.12$  mm for *A. niger*, and  $15.30 \pm 0.10$  mm for *T. rubrum* further demonstrate the relative efficacy of the methanolic extracts across different fungal taxa.

Boateng *et al.* (2025) demonstrated that medicinal plant extracts rich in phenolic and flavonoid compounds exhibited significant antibacterial activity against multidrug-resistant bacteria and antifungal activity against *Candida albicans*. Their findings indicate that phytochemicals extracted using polar solvents play a key role in disrupting microbial defence mechanisms. The antimicrobial activity observed in the present study aligns well with these findings, suggesting that similar classes of bioactive compounds may be responsible for inhibiting both bacterial and fungal pathogens.

Moderate antifungal activity against dermatophytes was observed in the present study. Specifically, the methanolic extract of *P. reticulatus* inhibited *Trichophyton rubrum*, with a zone of inhibition of  $15.30 \pm 0.10$  mm, indicating measurable antifungal efficacy. Havlickova *et al.* (2008) dermatophytes such as *T. rubrum* are major causative agents of dermatophytic infections and are known to exhibit moderate resistance due to their keratin-degrading ability and thick, multilayered cell walls, which restrict the penetration of antifungal agents.

The moderate inhibition observed may therefore be attributed to the action of polar phytochemicals, particularly phenolics and tannins, which are reported to interfere with fungal cell wall synthesis, inhibit keratinolytic enzymes, and disrupt membrane integrity (Abass *et al.*, 2024). Timothy *et al.*, (2002), Danish *et al.*, (2020); similar moderate antidermatophytic activity has been reported for methanolic extracts of *Cassia alata*, *Curcuma longa*, and *Aloe vera*, in which phenolic-rich extracts inhibited *Trichophyton* species by impairing cell wall formation and enzymatic activity involved in keratin degradation. The inhibition pattern observed in the present study is consistent with these reports and supports the susceptibility of dermatophytes to plant-derived phenolic compounds.

*S.italica* exhibited comparatively higher antimicrobial activity than *P. reticulatus* across most tested bacterial and fungal strains, particularly in methanolic extracts. This difference in efficacy could be attributed to differences in the phytochemical profiles of the two plants. Specifically, *S.italica* contains higher levels of anthraquinones and flavonoids, compounds often associated with strong antimicrobial properties. By contrast, *P. reticulatus* predominantly contains tannins, which generally exhibit moderate antimicrobial activity. Quantitatively, *Senna italica* exhibited inhibition zones of  $19.40 \pm 0.10$  mm against *Pseudomonas aeruginosa* and  $18.20 \pm 0.20$  mm against *Malassezia furfur*.

*P. reticulatus* exhibited a zone of inhibition of  $15.30 \pm 0.10$  mm against *Trichophyton rubrum*. These quantitative differences underscore the superior potency of *S.italica* extracts.

Nevertheless, both plants showcased consistent antibacterial and antifungal activity, highlighting their potential as natural sources of antimicrobial agents. The findings demonstrate that solvent polarity significantly influences antimicrobial efficacy and confirm that *S. italica* and *P. reticulatus*, particularly their methanolic extracts, are promising sources of plant-derived antimicrobial agents with potential applications against bacterial and fungal pathogens.

## CONCLUSION

The findings of the present investigation demonstrate that solvent polarity plays a decisive role in determining the antimicrobial efficacy of plant extracts. The enhanced activity observed in methanolic extracts suggests that polar phytochemicals contribute substantially to microbial growth inhibition. Comparative analysis revealed that *S. italica* possesses a higher antimicrobial potential than *P. reticulatus*, although both plants exhibited consistent inhibitory effects against bacterial and fungal pathogens. The antimicrobial activity of the plant extracts was lower than that of standard antimicrobial agents. The observed effects support their potential use as complementary or alternative natural antimicrobial sources. The bioactivity observed may be associated with the presence of secondary metabolites such as phenolics, flavonoids, tannins, and anthraquinones, which are known to disrupt microbial cellular processes. This study provides foundational evidence supporting the traditional relevance of these plants and establishes a basis for future investigations. Further research focusing on minimum inhibitory concentration determination, bioactive compound isolation, toxicity evaluation, and formulation studies is necessary to validate their practical applicability in the development of plant-based antimicrobial products.

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