Bioactivity-guided evaluation of *Ipomoea sepiaria* stem extract fractions for antimicrobial applications-An invitro study

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Abstract:

Background: The increasing prevalence of antimicrobial resistance has intensified the search for novel plant-derived therapeutic agents. *Ipomoea sepiaria* J. Koenig ex Roxb. is a medicinal plant traditionally used in folk medicine and is known to possess diverse bioactive constituents with potential pharmacological properties. However, the potential of its stem extracts remains largely unexplored.

Aim: This study aimed to evaluate the antimicrobial efficacy of sequential solvent extracts obtained from the stem of *Ipomoea sepiaria* against selected bacterial and fungal pathogens.

Methods: Dried stem powder was sequentially extracted using n-hexane, chloroform, and ethanol following petroleum ether defatting. The antimicrobial activity of the extracts was assessed against *Staphylococcus aureus* and *Candida albicans* using the agar well diffusion method at concentrations of 100, 200, and 500 µg/mL. Statistical analysis was performed using two-way analysis of variance (ANOVA).

Results: Among the tested extracts, the ethanol fraction exhibited the strongest concentration-dependent antimicrobial activity, producing inhibition zones of 26.5–29.2 mm against *S. aureus* and 23.5–28.6 mm against *C. albicans* at 500 µg/mL. In comparison, the n-hexane and chloroform extracts demonstrated comparatively lower antimicrobial activity. Two-way ANOVA revealed highly significant differences among extraction solvents and concentrations ($p < 0.0001$), indicating that both solvent type and dose significantly influenced antimicrobial efficacy.

Conclusion: The findings suggest that the ethanol extract of *Ipomoea sepiaria* stem possesses potent antibacterial and antifungal activity and may serve as a promising natural

source of antimicrobial agents. Further studies are warranted to isolate the active constituents and validate their therapeutic potential through mechanistic and in vivo investigations.

Keywords: *Ipomoea sepiaria*; antimicrobial activity; bioactivity-guided extraction; GC–MS; phytochemical screening; ethanol extract; *Staphylococcus aureus*; *Candida albican*

Introduction: Antimicrobial resistance (AMR) has emerged as a major global health concern, limiting the effectiveness of conventional antibiotics and antifungal agents and increasing the burden of infectious diseases. The widespread and indiscriminate use of antimicrobial drugs has accelerated the development of resistant microorganisms, creating an urgent need for novel therapeutic agents with improved efficacy and safety. In this context, medicinal plants have gained considerable attention as potential sources of bioactive compounds capable of combating microbial infections.[1]

Natural products have historically played a pivotal role in drug discovery, with many clinically important antimicrobial agents originating from plant-derived compounds. Medicinal plants possess a wide range of secondary metabolites that exhibit antibacterial, antifungal, antioxidant, and anti-inflammatory activities.[2] These phytoconstituents exert antibacterial effects through multiple mechanisms of action, including disruption of cell membranes, inhibition of biofilm formation, interference with nucleic acid and protein synthesis, and modulation of bacterial virulence, thereby reducing the likelihood of resistance development and highlighting their potential as promising candidates for pharmaceutical applications**.** [3,4] *Ipomoea sepiaria* J. Koenig ex Roxb., a member of the family Convolvulaceae, is a medicinal herb widely distributed in tropical and subtropical regions. Traditionally, it has been used in indigenous systems of medicine for the management of fever, inflammation, wounds, and reproductive disorders. Although several pharmacological properties have been reported for different parts of the plant, scientific evidence supporting the antimicrobial potential of its stem remains scarce. Exploring the stem as a source of antimicrobial agents may provide valuable insights into its therapeutic applications and contribute to the identification of novel bioactive compounds.[5]

Staphylococcus aureus and *Candida albicans* are clinically important opportunistic pathogens associated with a variety of skin, soft tissue, oral, and systemic infections. The increasing incidence of drug-resistant strains of these microorganisms has highlighted the necessity for alternative treatment strategies based on natural products.[6]

Therefore, the present study aimed to evaluate the antimicrobial activity of sequential solvent extracts obtained from the stem of *Ipomoea sepiaria* against *Staphylococcus aureus* and *Candida albicans* using the agar well diffusion method. The findings are expected to provide scientific evidence supporting the potential use of *I. sepiaria* stem extracts as promising natural antimicrobial agents and lay the foundation for future pharmacological and therapeutic investigations.

Methodology

Study Design: The present study was designed as an in vitro experimental investigation to evaluate the antimicrobial potential of sequential solvent extracts obtained from the stem of *Ipomoea sepiaria* J. Koenig ex Roxb. against selected bacterial and fungal pathogens. The current study was conducted at the Indira Gandhi Institute of Dental Sciences, School of Pharmacy, Mahatma Gandhi Medical and Advanced Research Institute, Sri Balaji Vidyapeeth, Pillarkuppam, Puducherry**.**

Plant material collection and authentication: Fresh stems of *Ipomoea sepiaria* J. Koenig ex Roxb. were collected from their natural habitat and authenticated by a qualified taxonomist. A voucher specimen was prepared and preserved in the herbarium for future reference. After authentication, the stems were washed thoroughly with running tap water, shade-dried at room temperature, and pulverized into a coarse powder using a mechanical grinder. The powdered material was passed through a 60-mesh sieve and stored in airtight containers until extraction.

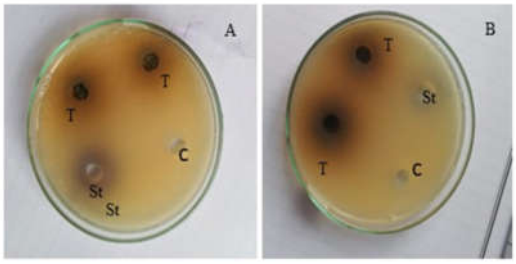
Preparation of plant extracts: The powdered stem material was initially defatted using petroleum ether to remove lipids and other non-polar impurities. The defatted residue was then subjected to sequential cold maceration using solvents of increasing polarity, namely n-hexane, chloroform, and ethanol. For each extraction, 200 g of powdered material was mixed with 600 mL of the respective solvent and maintained at room temperature for 24 hours with intermittent mechanical agitation using a rotary flask shaker. The extracts were filtered through Whatman No. 1 filter paper, and the extraction process was repeated two to three times to ensure maximum recovery of bioactive constituents. The pooled filtrates were concentrated under reduced temperature using a heating mantle, and the dried extracts were stored in sterile containers at 4°C until further use.[7,8]

Antimicrobial Assay: The antimicrobial activity of the prepared extracts was evaluated against *Staphylococcus aureus* and *Candida albicans* using the agar well diffusion method.[9] Mueller–Hinton agar (Hi-Media Laboratories, India) was prepared according to the manufacturer's instructions and sterilized at 121°C under 15 psi pressure for 15 minutes. Approximately 30 mL of sterile molten medium was poured into sterile 90-mm Petri plates and allowed to solidify under aseptic conditions. Standardized microbial suspensions were uniformly inoculated onto the agar surface using sterile cotton swabs.

Four wells of 6 mm diameter were punched into each agar plate using a sterile stainless-steel cork borer. Plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and tested at concentrations of 100, 200, and 500 µg/mL**.** A volume of 100 µL of each extract solution was dispensed into the respective wells. Chlorhexidine (100 µg/mL) served as the positive control, while 1% DMSO was used as the negative control.**[Figure-1]**

Figure 1: Representative agar well diffusion plates showing the antimicrobial activity of ethanolic extracts of *Ipomoea sepiaria* stem against A. *Staphylococcus aureus* and B

[T-test : Ethanolic extract (500µg/ml) St –Standard: Chlorheidine (100µg/ml C-Negative Control: 1% DMSO]



The plates were allowed to stand at room temperature for one hour to facilitate diffusion of the extracts and were subsequently incubated in an inverted position at 37°C for 18–24 hours. Following incubation, the antimicrobial activity was determined by measuring the diameter of the zone of inhibition (ZOI) surrounding each well using a calibrated ruler. All experiments were performed in triplicate, and the mean values were recorded in millimeters.

Statistical Analysis: The results were expressed as mean ± standard deviation (SD) of three independent experiments. Statistical analysis was carried out using two-way analysis of variance (ANOVA) to evaluate the effects of extraction solvent and concentration on antimicrobial activity. A p-value < 0.05 was considered statistically significant.

Results: The antimicrobial activity of sequential solvent extracts of *Ipomoea sepiaria* stem was evaluated against *Staphylococcus aureus* and *Candida albicans* using the agar well diffusion method at concentrations of 100, 200, and 500 µg/mL**[Table-1 & Figure -1].**Among the three extracts tested, the ethanol extract exhibited the highest antimicrobial activity against both microorganisms. The zone of inhibition increased with increasing concentration, indicating a dose-dependent effect**.** At 500 µg/mL, the ethanol extract produced the maximum inhibition zone against *Staphylococcus aureus* (26.5–29.2 mm) and *Candida albicans* (23.5–28.6 mm).[Figure 2]

The n-hexane extract showed the lowest antimicrobial activity, with smaller zones of inhibition at all tested concentrations. The chloroform extract demonstrated moderate activity, but its inhibitory effect was lower than that of the ethanol extract.

The positive control (chlorhexidine) showed greater antimicrobial activity than all plant extracts, confirming the reliability of the assay. Statistical analysis using two-way ANOVA revealed a highly significant difference in antimicrobial activity among the different extracts and concentrations **(**$p < 0.0001$**).**

Overall, the results demonstrate that the ethanol stem extract of *Ipomoea sepiaria* possesses superior antibacterial and antifungal activity, followed by the chloroform and n-hexane extracts, with antimicrobial efficacy increasing as the extract concentration increased.

Figure 2: Zone of inhibition (mm) of different solvent extracts (ethanol, n-hexane, and chloroform) from *Ipomoea sepiaria* plant material against *Staphylococcus aureus (S.a) and Candida albicans(C.a)* at concentrations of 100, 200, and 500 µg/ml. values are expressed as means ± SD from triplicate experiments. Statistical analysis was performed by two-way ANOVA; a highly significant difference was observed among treatments ($p < 0.0001$)

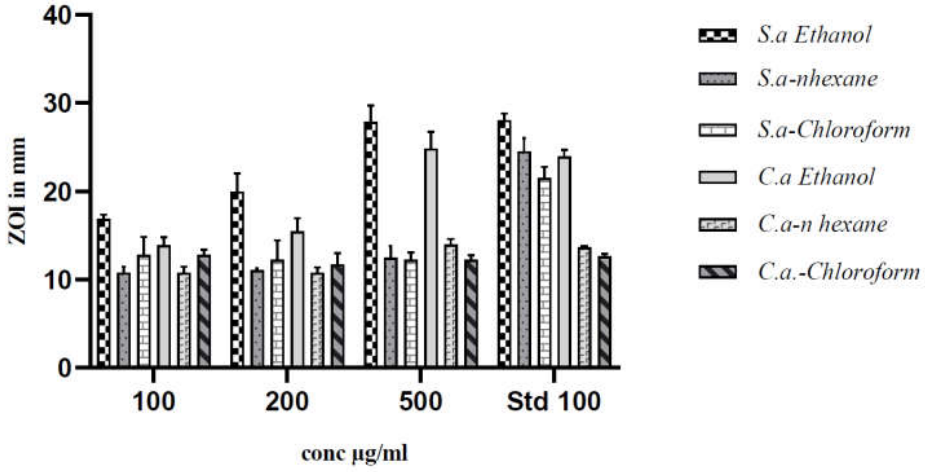


Table 1.Comparative Antimicrobial Efficacy of Sequential Solvent Extracts of *Ipomoea sepiaria* Stem Against Selected Microbial Pathogens

Extraction	Ethanol			n-hexane			Chloroform		
100	16.5	17.2	16.8	10.23	11.24	9.4	14.2	11.3	13.2
200	18.5	21.4	20.2	11.2	10.9	8.9	13.8	10.6	12.1
500	26.5	29.2	28.6	13.4	11.5	9.1	12.8	11.6	11.9
Standard 100	27.5	28.6	29.8	23.4	25.6	23.8	22.4	20.6	21.9
µg/ml	*Candida albicans*								
Extraction	Ethanol			n-hexane			Chloroform		
100	14.5	13.2	12.8	10.23	11.24	9.4	13.2	12.3	14.1
200	16.5	14.4	15.2	11.2	10.3	9.7	12.6	10.8	12.6
500	23.5	26.2	28.6	14.4	13.5	10.9	11.8	12.6	10.9
Standard 100	23.4	24.5	24.8	13.5	13.7	14.9	12.4	12.8	13.9

Discussion

The present study demonstrated that sequential solvent extracts of *Ipomoea sepiaria* stem possess antimicrobial activity against *Staphylococcus aureus* and *Candida albicans,* with the ethanol extract exhibiting the highest efficacy among the tested fractions. The antimicrobial activity increased progressively with increasing extract concentration, indicating a dose-dependent response. These findings are consistent with previous reports [10,11]showing that extraction solvent polarity strongly influences the recovery of

antimicrobial phytochemicals and that ethanolic extracts frequently exhibit superior antimicrobial activity due to their ability to solubilize phenolic and flavonoid compounds.

The superior activity of the ethanol extract observed in the present study is consistent with previous reports demonstrating that polar solvents extract a greater diversity of antimicrobial phytochemicals than non-polar solvents. Hussain et al. investigated different solvent extracts of *Ipomoea nil* and reported that ethanol and methanol extracts exhibited significantly greater antibacterial and antifungal activity than aqueous and dichloromethane extracts against *Staphylococcus aureus* and *Candida albicans*. The authors attributed this enhanced activity to the higher extraction efficiency of polar bioactive compounds.[12]

Similarly, studies on other members of the *Ipomoea* genus have reported promising antimicrobial properties. Alam et al. demonstrated that the methanolic extract of *Ipomoea mauritiana* possesses appreciable antibacterial activity against several pathogenic microorganisms, supporting the therapeutic potential of plants belonging to the genus *Ipomoea*.[13] Likewise, studies on *Ipomoea cairica* have shown that ethanol extracts and their fractions exhibit inhibitory activity against *Staphylococcus aureus*, further highlighting the antimicrobial potential of members of the family Convolvulaceae.[14]

The concentration-dependent increase in the zone of inhibition observed in the present study is in agreement with earlier investigations of medicinal plant extracts, where higher concentrations resulted in greater microbial growth inhibition due to the increased availability of active phytochemicals. Such dose-dependent responses have been consistently reported in antimicrobial screening studies and reinforce the biological significance of plant-derived compounds.

Among the tested microorganisms, *Staphylococcus aureus* showed slightly greater susceptibility to the ethanol extract than *Candida albicans*. This difference may be explained by structural variations in microbial cell envelopes. The fungal cell wall contains chitin and β-glucans, while the plasma membrane is rich in ergosterol, features that can limit the penetration and efficacy of several phytochemicals compared with the peptidoglycan-rich cell wall of Gram-positive bacteria.[15,16] Nevertheless, the appreciable inhibition observed against *Candida albicans* suggests that the *I. sepiaria* stem extract possesses antimicrobial activity against both bacterial and fungal pathogens and warrants further investigation as a potential natural antimicrobial agent.

The statistically significant differences among extraction solvents and concentrations ($p < 0.0001$) further validate that extraction methodology strongly influences antimicrobial efficacy. Ethanol appears to be the most suitable solvent for isolating antimicrobial constituents from *I. sepiaria* stem, as evidenced by the consistently larger zones of inhibition compared with n-hexane and chloroform extracts.

Overall, the findings of this study support the traditional medicinal value of *Ipomoea sepiaria* and suggest that its ethanol stem extract may serve as a promising source of natural antimicrobial agents. However, additional studies involving determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), isolation and characterization of active compounds, mechanistic investigations, toxicity assessment, and in vivo validation are required before its clinical application can be considered.

Conclusion:

This study demonstrates that *Ipomoea sepiaria* stem possesses promising antimicrobial potential against *Staphylococcus aureus* and *Candida albicans*. Among the sequential solvent extracts evaluated, the ethanol extract exhibited the greatest inhibitory activity, with antimicrobial efficacy increasing in a concentration-dependent manner. The superior performance of the ethanol fraction suggests that it effectively extracts bioactive constituents responsible for antibacterial and antifungal activity. These findings provide scientific support for the traditional medicinal use of *I. sepiaria* and highlight its potential as a natural source of novel antimicrobial agents. Given the growing challenge of antimicrobial resistance, the plant may serve as a valuable candidate for the development of alternative phytotherapeutic formulations. Nevertheless, further investigations involving isolation and characterization of the active compounds, determination of minimum inhibitory concentrations, mechanistic studies, toxicity assessment, and in vivo validation are essential to establish its safety, efficacy, and potential for clinical application.

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