RESEARCH PAPER

Comparative Evaluation of Antimicrobial Activity of inflorescence, stem and leaf extracts of *Strobilanthes lurida* Wight.

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Abstract
The present study evaluates the antimicrobial activity of leaf, stem, and inflorescence extracts of *Strobilanthes lurida* (Acanthaceae). Different plant parts were extracted with five solvents using the decoction method, and crude extracts were tested for their antimicrobial efficacy through agar well diffusion, minimum inhibitory concentrations (MIC), minimum bactericidal/fungicidal concentrations (MBC/MFC), and IC50 assays. All the tested samples exhibited minimum zones of inhibition against *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhimurium, and Klebsiella pneumoniae* compared with gentamicin (22 mm). Ethyl acetate stem extract exhibited the highest antibacterial activity with a 14 mm inhibition zone against *M. luteus, P. aeruginosa, and K. pneumoniae*. MIC values were 25 mg/mL for all strains, while MBC values > 25 mg/mL were observed for methanol and chloroform inflorescence extracts against M. luteus, similarly methanol, chloroform, and ethyl acetate extracts against *S. typhimurium*, and for various stem and leaf extracts against *M. luteus, K. pneumoniae, and S. aureus*. Methanol and chloroform extracts produced a mild 10 mm zone of inhibition against *Aspergillus niger and Candida albicans*, while others showed no activity. Fungal MIC values were 25 mg/mL, with some MFC values > 25 mg/mL. IC50 values varied among extracts. Overall, the results indicate moderate antimicrobial potential of *Strobilanthes lurida*, particularly in ethyl acetate stem extract, warranting further phytochemical investigation.

Keywords: *Strobilanthes lurida*; antimicrobial activity; MIC; IC50; ethyl acetate extract; *Candida albicans*.
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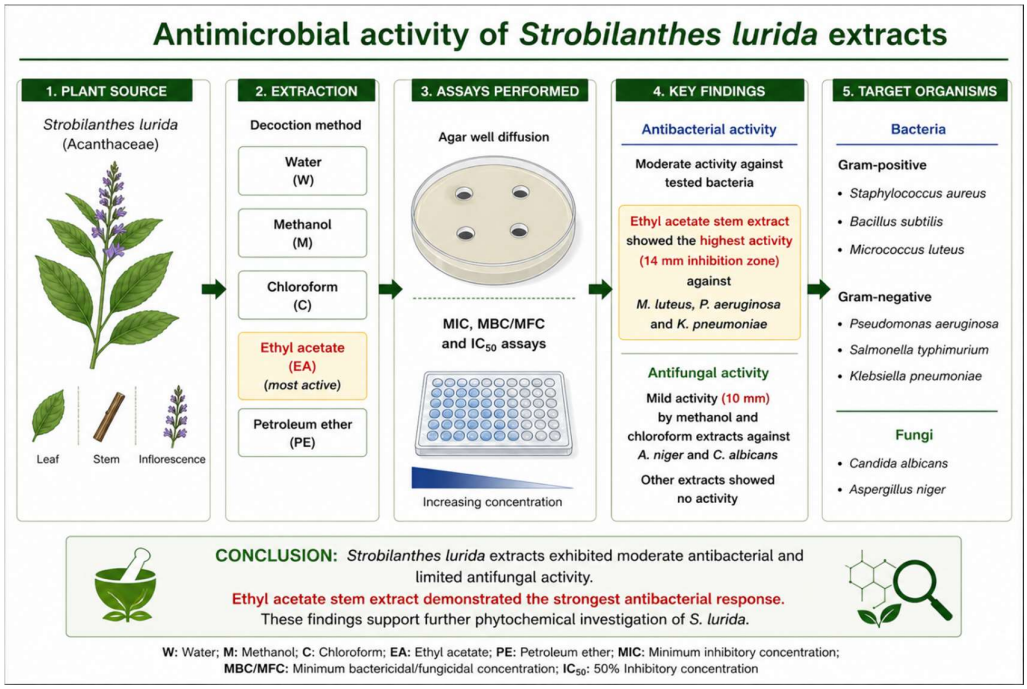

Graphical abstract illustrating the antimicrobial activity of Strobilanthes lurida extracts

Introduction

Medicinal plants have re-emerged as credible sources of antimicrobial leads. Plants possess diverse secondary metabolites like phenolics, alkaloids, terpenoids, saponins, etc, which, through multi-target mechanisms including membrane disruption, protein/ enzyme binding, interference with intermediary metabolism and inhibition of quorum sensing and biofilms, act as adjuvants that potentiate existing antibiotics, helping to bypass resistance. This multi-component, multi-target profile offers advantages over single-target synthetic medicines. However, clinical translation still requires robust standardisation, toxicity evaluation and modern discovery frameworks to pinpoint optimal combinations and delivery strategies. Overall, leveraging plant-derived molecules alone or alongside conventional drugs represents a timely strategy to confront the antibacterial crisis (Abdallah *et al*., 2023)

Family Acanthaceae houses a diverse ethnobotanically important species, among them, one important genera is *Strobilanthes lurida,* which is a large, straggling shrub (Sundaram *et al*., 2021). *S. lurida* is endemic to shola and evergreen forests of the Southern-Western Ghats, particularly in Kerala and Tamil Nadu. Thick, virgate stems with swollen nodes characterise the plant. Leaves with an ovate-elliptic shape arranged in an opposite phyllotaxy, approximately 20 X 10 cm, which are pilose and densely hairy underneath. *S. luridus* bears compact, bristly spikes of purple, tubular-ventricose, bilabiate flowers; each bears vivid purple and white bracteoles. The inflorescence arises from older stems near ground level. Flowering is typically seen from December to April, culminating in ovoid-ellipsoid, glabrous capsules enclosed within the bracts. The genus *Strobilanthes* is known for its rich diversity of species, many of which have shown notable antimicrobial properties in traditional and modern studies. Species like *S. crispus* and *S. cusia* have been studied extensively for their antimicrobial efficacy (Suboh *et al*., 2022); the potential of *S. lurida* remains largely unexplored, making it a promising species for antimicrobial research.

Materials and Methods:

Collection of Plant Material

Strobilanthes lurida twigs with inflorescence were collected from the Tadiandamol mountain range, Kodagu district, Karnataka (Latitude: 12° 13' 2" N and Longitude: 75° 36' 30" E), reaching an elevation of 1,748meters. The Botanical Survey of India (BSI) authenticated the plant with Voucher number - BSI/SRC/5/23/2023-24/Tech - 478. Voucher specimen (twig with inflorescence) was deposited in the Herbaria, Department of Botany, Jnana Bharathi campus, Bangalore University.

Preparation of Extract by Decoction Method

Fresh and healthy leaves, stems, and inflorescence of *S. lurida* were collected and thoroughly washed with running tap water to remove dust and other debris, and the plant materials were also allowed to dry in the shade. Further, the dried materials were ground into powder separately to obtain fine powder, which was stored in an air-tight container. 15g powder of each sample was weighed individually and extracted using 150 mL of Distilled water, methanol, chloroform, ethyl acetate, and petroleum ether solvents for 4 hrs. in a water bath at 50°C by decoction method. The content was filtered through the Whatman No.1 filter paper. The extract thus obtained was allowed to evaporate the solvent in a hot air oven at 60°C for 3-4 days. The condensed extract was stored in microcentrifuge vials at 4°C for the *in vitro* antimicrobial assay.

In vitro antibacterial assay by agar well diffusion method

The antibacterial activity of the inflorescence, stem, and leaf extracts of S. lurida was evaluated by the agar well diffusion method. Six different bacterial strains were selected for in vitro assay, including three Gram-positive (*Staphylococcus aureus, Bacillus subtilis, and Micrococcus luteus*) and three Gram-negative bacteria (*Pseudomonas aeruginosa, Salmonella typhimurium, and Klebsiella pneumoniae*), which were procured from Microbial Type Culture Collection, IMTECH, Chandigarh.

Protocol:

The six selected bacterial strains were grown on BHI (Brain Heart Infusion) media for 24h. at 37°C. BHI media (1.5% w/v agar), pre-inoculated with bacterial pathogens (1% v/v) were poured into sterile petri-dishes and solidified for a while. The plant stock solution was prepared in DMSO (50 mg/mL). Using a sterile cork borer, 4 mm wells were bored on the agar plate and inoculated with 10µl of plant samples. The plates were kept for proper diffusion at 4°C for 30 min. Then the plates were incubated at 37°C for 24h. Antibiotic gentamicin (1mg/mL) was used as a positive control, and the antibacterial activity was determined by measuring the diameter (in mm) of the zone of inhibition (ZOI) around the well using the antibiotic zone scale (Brantner *et al*. 1994).

Determination of MIC against bacteria (Minimum inhibitory concentration)

Further, the broth dilution method determined MIC for methanol, chloroform and ethyl acetate plant extracts against all selected bacterial pathogens. Briefly, overnight broth cultures of the pathogen were suspended in BHI broth with turbidity adjusted to 0.5 McFarland, resulting in a suspension containing approximately 8 CFU/ml. 100 µl of the respective broth was poured into 8 wells of a 96-well microtiter plate. In the first well, 100 µl of the sample stock solution was added. A two-fold dilution was then made to obtain different concentrations in each well. Then, 100 µl of the microbial suspension was added to each well and the microplate was then incubated at 37°C for 24 hrs. The sample concentration in the well without visible growth of the bacterial cells was considered for MIC. OD was measured at 600 nm (Andrews, 2001).

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Determination of MBC (Minimum bactericidal concentration)

To determine the MBC, 100 µl of broth was taken from all the wells of the MIC plate that showed no visible signs of growth/turbidity (MIC and higher dilutions) and spread on respective agar plates. The plates were then incubated at 37°C for 24 - 48 hrs. The MBC is defined as the lowest sample concentration that prevents the tested microbe's growth (Andrews, 2001).

Determination of IC_{50} concentration

To determine the IC_{50}, different sample concentrations were taken in the wells of 96-well plates. The volume was made up to 100 µl using the respective broth. Later, 100 µl of pathogen broth suspension was added, and the plates were incubated at 37°C for 24 hrs. A positive control was with the pathogen, but without the extract. The negative sample was only broth. Chloramphenicol was used as a standard. After incubation, absorbance was read at 600 nm. IC_{50} is defined as the concentration of the sample required to inhibit 50% of the pathogenic strain (Andrews, 2001)

***In vitro* antifungal assay by agar well diffusion method**

The agar well diffusion method tested the antifungal activity of the inflorescence, stem, and leaf extracts of *S. lurida*. *Aspergillus niger* (MTCC1344) and *Candida albicans* (ATCC90028) were procured from Microbial Type Culture Collection, IMTECH, Chandigarh.

Protocol:

The selected fungal strains were grown on potato dextrose broth for 2-3 days at 30°C under constant shaking (150 rpm). Potato dextrose broth media pre-inoculated with fungal pathogens (1%) was poured into a sterile petri-dish and allowed to solidify for a while, and then 50mg/mL plant stock solution in DMSO was prepared. Wells of 4mm were bored using sterile cork borers and inoculated with 100µL of plant samples. The plates were kept for proper diffusion at room temperature for 30 min. A standard antifungal drug, fluconazole (1mg/mL), was used as a positive control. Then the plates were incubated at 30°C for 2-3 days, and the antifungal activity was determined by measuring the diameter (in mm) of the zone of inhibition (ZOI) around the well using the antibiotic zone scale (Shubha and Hiremath, 2010).

Determination of MIC against the fungus *C. albicans* (Minimum inhibitory concentration)

MIC of all plant extracts against the selected fungus *C. albicans* was performed using the broth dilution method. Briefly, the fungal spore suspension was suspended in PDB broth. To measure the MIC, 100 µl of the respective broth was poured into 8 wells of a 96-well microtiter plate. In the first well, 100 µl of the sample stock solution was added. A two-fold dilution was then made to obtain different concentrations in each well. Later, 100 µl of the fungal suspension was added to each well. The microplate was then incubated at 30°C for 2 days. The sample concentration in the well without visible growth was considered as MIC. A positive control containing medium with the pathogen and a negative control containing only broth were used in the study. Sample control was also kept with the same dilution without the pathogen. The MIC is defined as the lowest concentration of the extract that inhibits fungal growth after 2 days of incubation. The MIC was reported by observing the visual turbidity. The experiment was performed in triplicate (Espinel-Ingroff *et al*., 2002).

Determination of MFC (Minimum fungicidal concentration)

To determine the MFC, 100 µl of broth was taken from all wells of the MIC plate that showed no visible signs of growth/turbidity (MIC and higher dilutions) and spread on respective agar plates. The plates were then incubated at 30°C for 2-3 days. The MFC was defined as the lowest sample concentration that prevented the tested fungi's growth (Espinel-Ingroff *et al*., 2002).

Determination of IC_{50} concentration

To determine the IC_{50}, different sample concentrations were taken in the wells of 96-well plates. The volume was made up to 100 µl using the respective broth. Later, 100 µl of pathogen broth suspension was added, and the plates were incubated at 37°C for 24 hrs. A positive control was with the pathogen, but without the extract. The negative sample was only broth. Chloramphenicol was used as a standard. After incubation, absorbance was read at 600 nm. IC_{50} is defined as the sample concentration required to inhibit 50% of the pathogenic strain (Espinel-Ingroff *et al*., 2002).

Results and Discussion: Antibacterial Assay by Agar Well Diffusion Method.

The antimicrobial activity of the inflorescence, stem, and leaf extracts of *Strobilanthes lurida* was tested by the agar well diffusion method against six different bacterial strains. All the extracts from three different plant samples (inflorescence, stem, and leaf) showed a considerable zone of inhibition against the tested bacterial strains when compared to the standard used (Gentamicin - 22mm). Aqueous extracts failed to inhibit the growth of bacterial strains, except aqueous leaf extract, which inhibited the growth of a gram-positive bacterium, *Micrococcus luteus*, with a zone of inhibition of 10 mm. Petroleum ether inflorescence extract also exhibited a zone of inhibition of 10 mm against the gram-positive bacterium *M. luteus*. Still, it could not inhibit the growth of the rest of the bacterial strains. The methanol inflorescence extract revealed a negligible zone of inhibition of 10 mm against *M. luteus* and *Salmonella typhimurium*; meanwhile, no inhibitory action was observed against the remaining bacterial strains studied. For the stem sample, the methanol extract revealed a negligible zone of inhibition of 10 mm, contrary to the *Staphylococcus aureus*, *M. luteus, Pseudomonas aeruginosa, and Klebsiella pneumoniae*. In leaf samples, the methanol extract showed an insignificant zone of inhibition of 10 mm for *Bacillus*

subtilis, M. luteus, P. aeruginosa, and *K. pneumoniae*. Chloroform extracts (stem, leaf and inflorescence) exhibited a lower zone of inhibition of 10 mm against *S. aureus and M. luteus*. The inflorescence and leaf samples were found to possess antibacterial efficacy with a zone of inhibition of 10mm against the gram-negative bacterium *S. typhimurium*. Further stem and leaf samples noted the zone of inhibition of 10 mm against *K. pneumoniae*, and the chloroform stem extract showed the zone of inhibition of 10 mm against *P. aeruginosa*. Ethyl acetate inflorescence extract exhibited a zone of inhibition of 10 mm against *M. luteus and K. pneumoniae* and 8 mm against *S. typhimurium*. Researchers reported similar observations for the genus *Strobilanthes'* antibacterial properties against various bacterial strains. Sundaram *et al*. (2021) reported that the methanol root extract of *S. heyneanus* subjected to a well diffusion assay showed the highest zone of inhibition of 24 mm for the gram-negative bacterium *S. typhimurium* at 500 µg/mL concentration. Ethyl acetate stem extract presented a moderate zone of inhibition of 10 mm against *S. aureus* and *a* significant zone of inhibition of 14 mm against *M. luteus, P. aeruginosa,* and *K. pneumoniae.* Ban and Fong (2022) made a similar observation: ethanol, acetone, and chloroform leaves extract of *Strobilanthes crispus* were superior to *Clinacanthus nutans* extracts against *P. aeruginosa.* Interestingly, ethyl acetate leaf extract displayed a clear zone of inhibition of 10 mm against all the bacterial strains, excluding *Salmonella typhimurium* **(Fig. 1- 6, a,b,c).** Another study was conducted by Arum *et al*. (2023), where they reported that the ethanolic leaf extract of *S. crispus* demonstrated an average zone of inhibition of 10 mm against *Aggregatibacter actinomycetemcomitans* bacterium, corresponding to the zone of inhibition of the positive control (16.33 mm). The present study is correlated with the previous work performed by Raghavendra *et al*. (2017) in *S. sessilis.* Leaves, inflorescence, and stem extracts obtained from the methanol solvent of this plant were assessed for the well diffusion assay. Leaf and inflorescence extract inhibited *K. pneumoniae* to a greater extent, while the stem extract caused higher inhibition against B. *subtilis* and *S. aureus. B. subtilis* and *K. pneumoniae* were inhibited to a greater extent among Gram-positive and Gram-negative bacteria, respectively. Overall, the stem extract displayed the least inhibitory activity. Compared with the current study, the above reviews clearly corroborate in *S. lurida* that all the extracts from three different plant parts (inflorescence, stem, and leaf) exhibited moderate inhibition zones against all the tested bacterial strains.

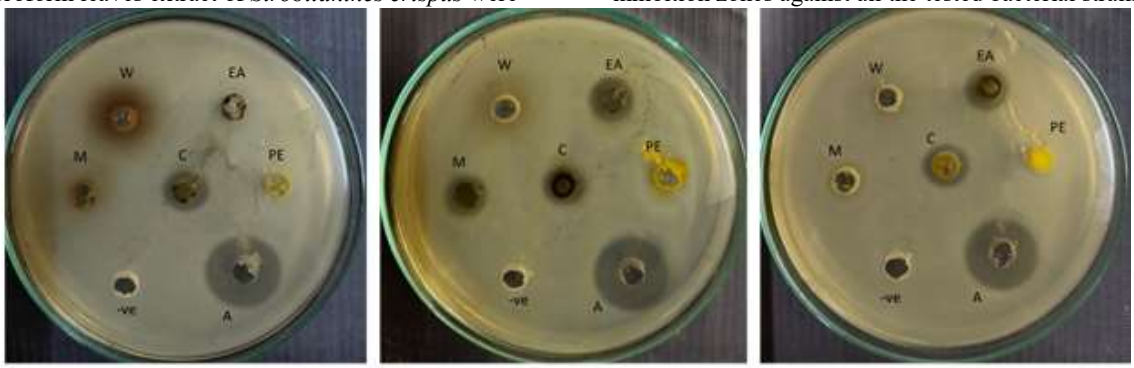


Fig. 1: Antibacterial activity of *S. lurida* against *S. aureus.* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

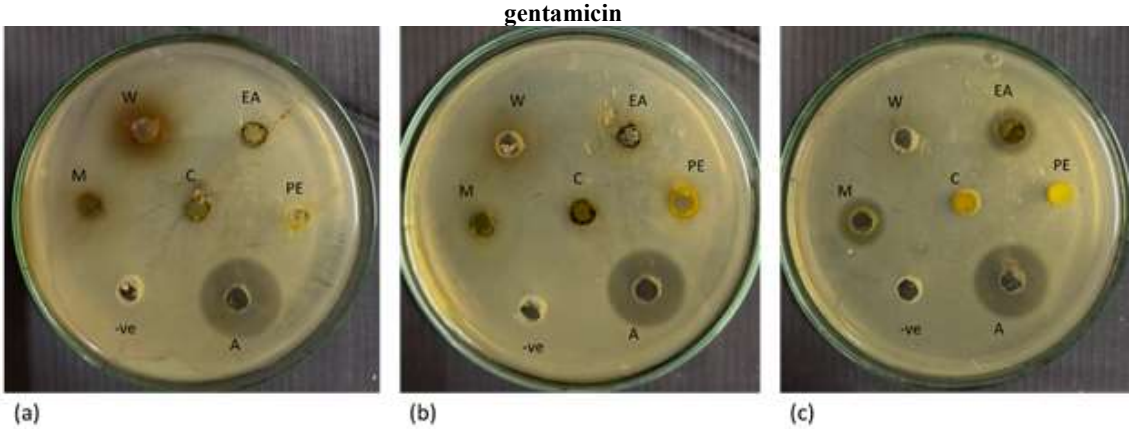


Fig. 2: Antibacterial activity of *S. lurida* against *B. subtilis.* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

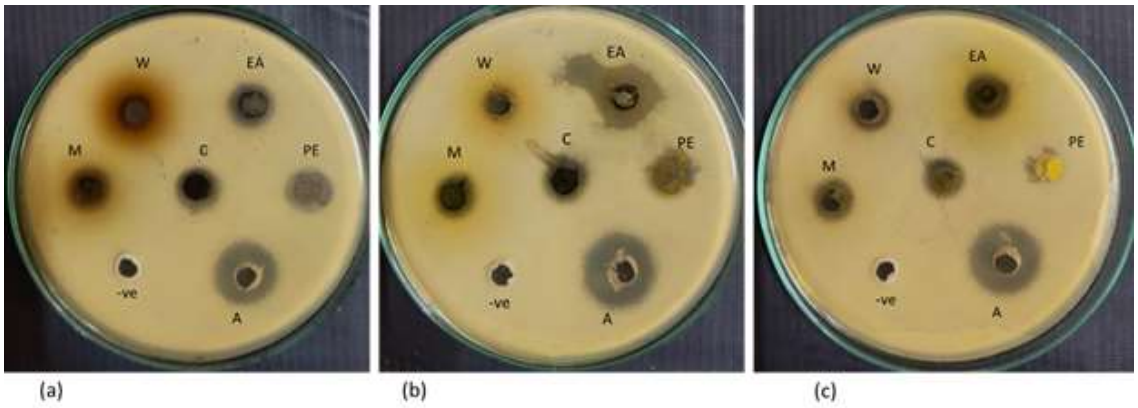


Fig. 3: Antibacterial activity of *S. lurida* against *M. luteus.* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

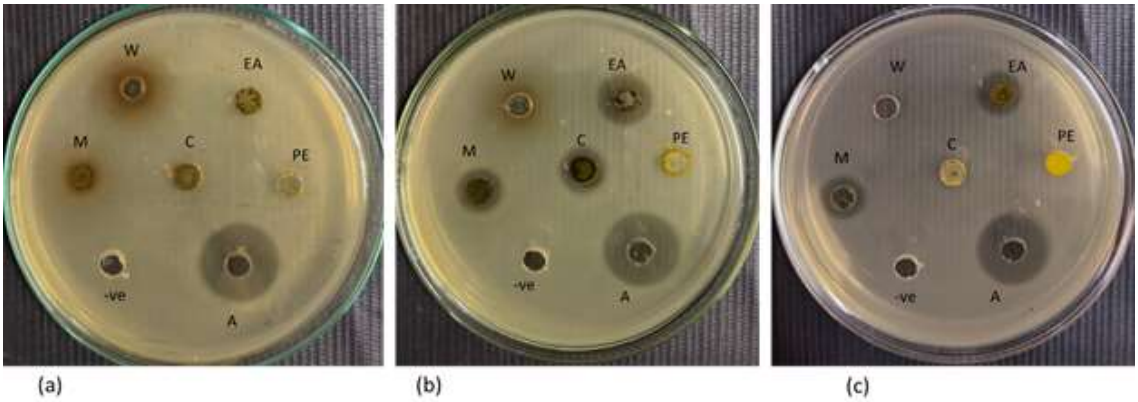


Fig. 4: Antibacterial activity of S. lurida against P. aeruginosa. (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

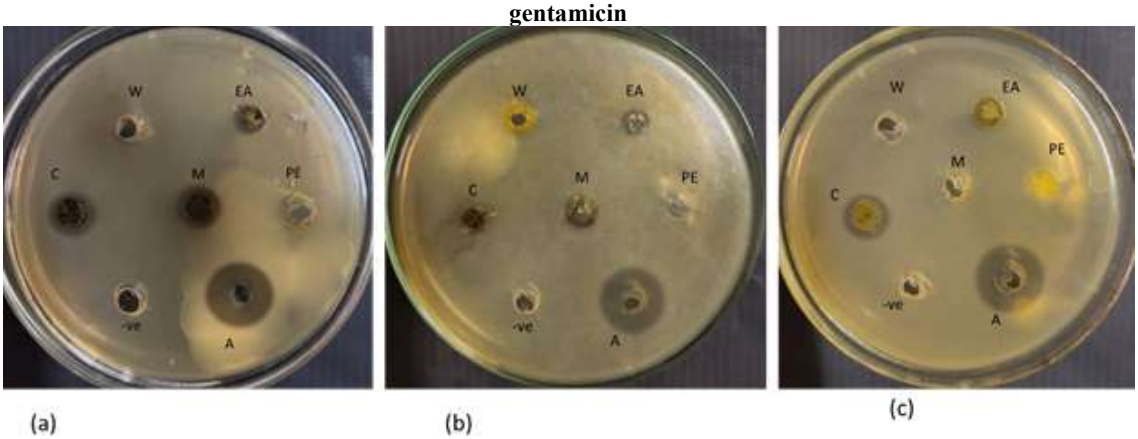


Fig. 5: Antibacterial activity of *S. lurida* against *S. typhimurium*. (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

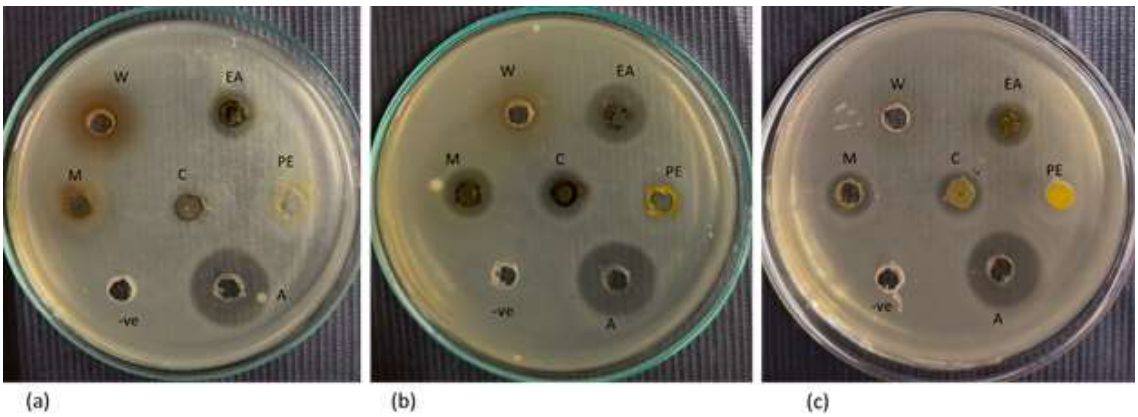


Fig. 6: Antibacterial activity of *S. lurida* against *K. pneumoniae.* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A- Antibiotic gentamicin

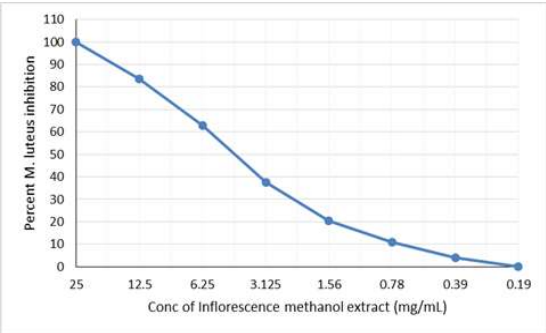
Based on the results of the well diffusion method, only active extracts, viz., methanol, chloroform, and ethyl acetate extracts of three different plant samples (Leaf, inflorescence, and stem) were subjected to the Determination of minimum inhibitory concentrations (MIC), Minimum bactericidal/fungal concentration (MBC/MFC) and IC_{50} concentrations.

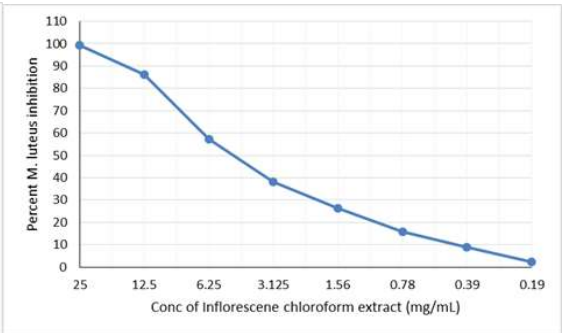
Determination of minimum inhibitory concentrations (MIC), Minimum bactericidal concentration (MBC), and IC_{50} concentration

The broth dilution method determined the MIC of S. lurida methanol, chloroform, and ethyl acetate extracts of three different plant samples (Leaf, inflorescence, and stem) against selected bacterial cultures. The least concentration of the plant extracts inhibited the growth of microorganisms completely (absence of turbidity) after 24 hrs. of incubation. The MIC was reported by observing the visual turbidity. This was performed in triplicate to confirm its value for the tested organisms. All the extracts of three different plant samples showed the MIC response at 25mg/mL against all the tested bacterial strains used (Table. 1). The minimum bactericidal concentration (MBC) of methanol and chloroform inflorescence extract against M. luteus and methanol, chloroform, and ethyl acetate extracts against S. typhimurium, stem methanol extract for *M. luteus*, stem methanol and chloroform extracts for *K. pneumoniae*, stem chloroform extract for *S. aureus* and methanol, chloroform, and ethyl acetate leaf extracts against *K. pneumoniae* was found to be >25mg/mL whereas, other extracts indicated 25mg/mL against all the tested pathogens (Table. 1). Based on the MIC and MBC values, IC50 values of methanol, chloroform, and ethyl acetate extracts of S. lurida were calculated by treating the pathogens with different concentrations of the extract (0.19 – 25mg). The percentage of bacterial growth gradually decreased with an increase in concentration. The other crude extracts of three plant samples obtained their respective IC50 values against tested organisms (Table 1 & Fig. 7-14). The IC50 value for selected microorganisms was calculated using a linear regression curve in MS Excel 2016. The present work is compared with an earlier study by Sundaram et al. (2021), from the methanol root extract of *S. heyneanus, S. aureus* and *S. typhi* showed no change in colour of resazurin, and *B. subtilis and K. pneumoniae* showed no change in colour of resazurin. This indicated a lower MIC value, which refers to the tested organisms. Venkatachalapathi and Ravi (2013) evaluated the antibacterial activity of the petroleum ether and methanolic extracts of *S. ciliatus*. Both extracts exhibited inhibitory activity against Gram-positive and Gram-negative bacteria. The leaf extract of *S. crispus* exhibited inhibitory activity against *S. aureus* and *Streptococcus pneumoniae*. In contrast, no visible inhibition was observed against *K. pneumoniae* and *P. aeruginosa* as studied by Lim et al. (2015).

Table 1:** Minimum inhibitory concentrations (MIC), Minimum bactericidal concentration (MBC), and IC_{50} values of different extracts of **S. lurida

Sample	Pathogen	MIC (mg/ml)	MBC (mg/ml)	IC_{50} (mg/ml)
Inflorescence-Methanol	*M. luteus*	25	>25	4.68
Inflorescence-Chloroform	*M. luteus*	25	>25	5.47
Inflorescence-ethyl acetate	*M. luteus*	25	25	3.90
Stem-methanol	*M. luteus*	25	>25	4.6
Stem-chloroform	*M. luteus*	25	25	3.90
Stem-ethyl acetate	*M. luteus*	25	25	2.73
Leaf-methanol	*M. luteus*	25	25	2.73
Leaf-chloroform	*M. luteus*	25	25	3.125
Leaf-ethyl acetate	*M. luteus*	25	25	4.68
Inflorescence-methanol	*S. typhi*	25	>25	3.125
Inflorescence-chloroform	*S. typhi*	25	>25	6.25
Inflorescence-ethyl acetate	*S. typhi*	25	>25	7.81
Leaf-methanol	*K. pneumoniae*	25	>25	6.25
Leaf-chloroform	*K. pneumoniae*	25	>25	3.90
Leaf-ethyl acetate	*K. pneumoniae*	25	>25	6.25
Stem-methanol	*K. pneumoniae*	25	>25	5.47
Stem-chloroform	*K. pneumoniae*	25	>25	6.25
Stem-ethyl acetate	*K. pneumoniae*	25	25	2.73
Stem-methanol	*P. aeruginosa*	25	25	2.73
Stem-chloroform	*P. aeruginosa*	25	25	3.90
Stem-ethyl acetate	*P. aeruginosa*	25	25	3.125
Stem-methanol	*S. aureus*	25	25	3.125
Stem-chloroform	*S. aureus*	25	>25	4.68
Stem-ethyl acetate	*S. aureus*	25	25	3.90





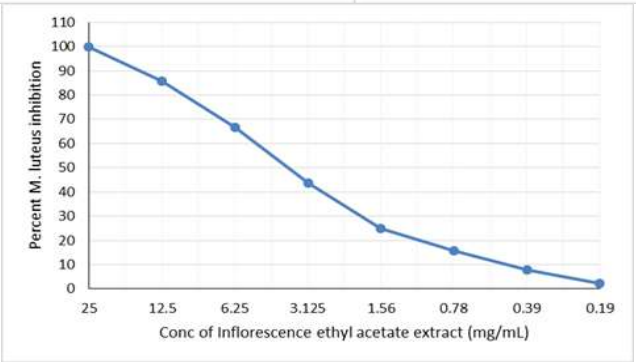


Fig. 7: Percentage inhibition of *M. luteus* with different concentrations of methanol, chloroform, and ethyl acetate inflorescence extracts of *S. lurida*

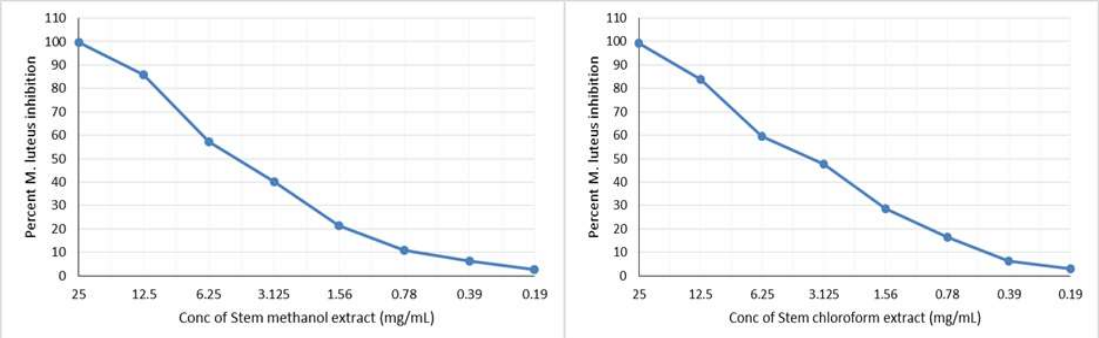


Fig. 8: Percentage inhibition of *M. luteus* with different concentrations of methanol, chloroform, and ethyl acetate stem extracts of *S. lurida*

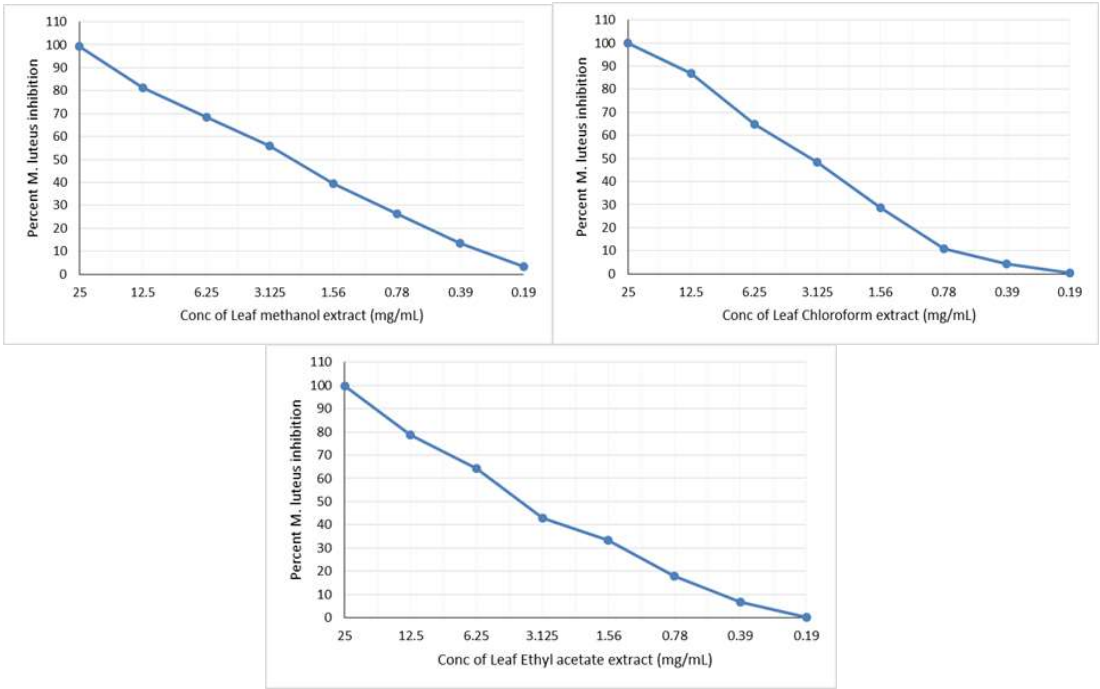


Fig. 9: Percentage inhibition of *M. luteus* with different concentrations of methanol, chloroform, and ethyl acetate leaf extracts of *S. lurida*

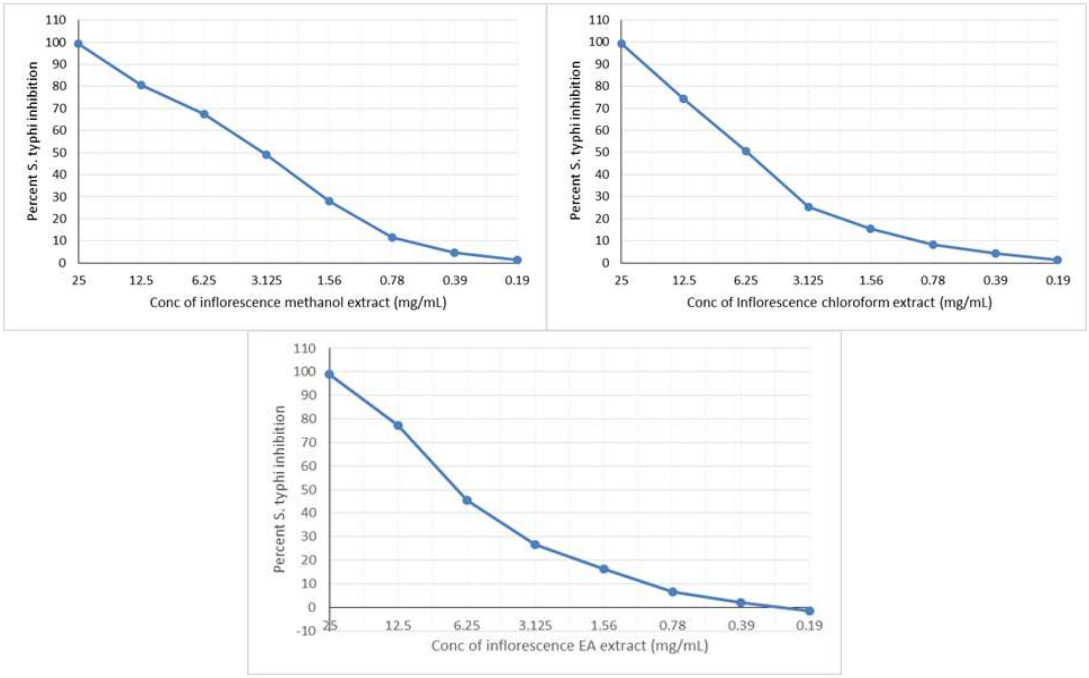


Fig. 10: Percentage inhibition of *S. typhi* with different concentrations of methanol, chloroform, and ethyl acetate inflorescence extracts of *S. lurida*

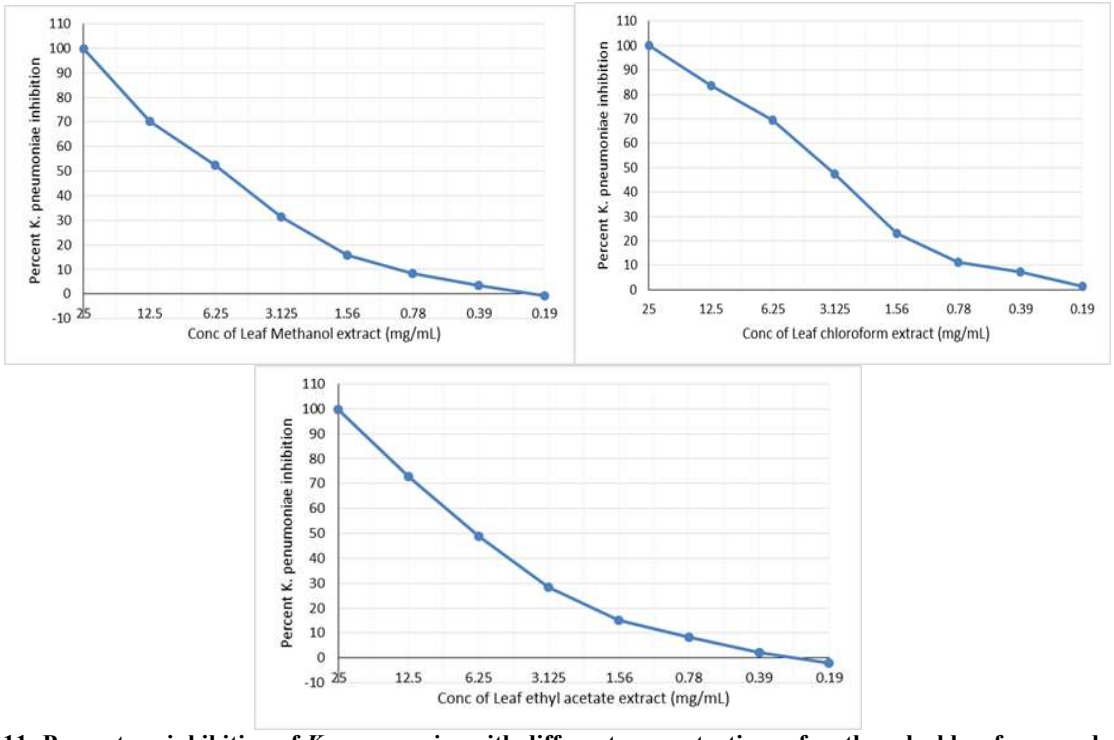


Fig. 11: Percentage inhibition of *K. pneumoniae* with different concentrations of methanol, chloroform, and ethyl acetate leaf extracts of *S. lurida*

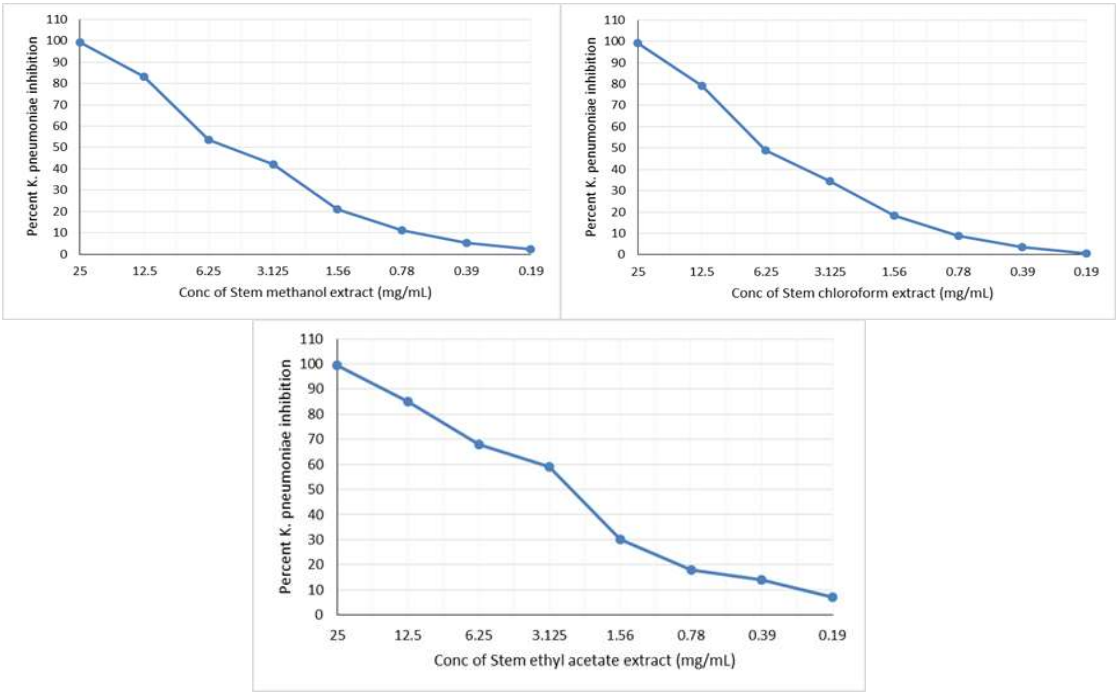


Fig. 12: Percentage inhibition of *K. pneumoniae* with different concentrations of methanol, chloroform, and ethyl acetate stem extracts of *S. lurida*

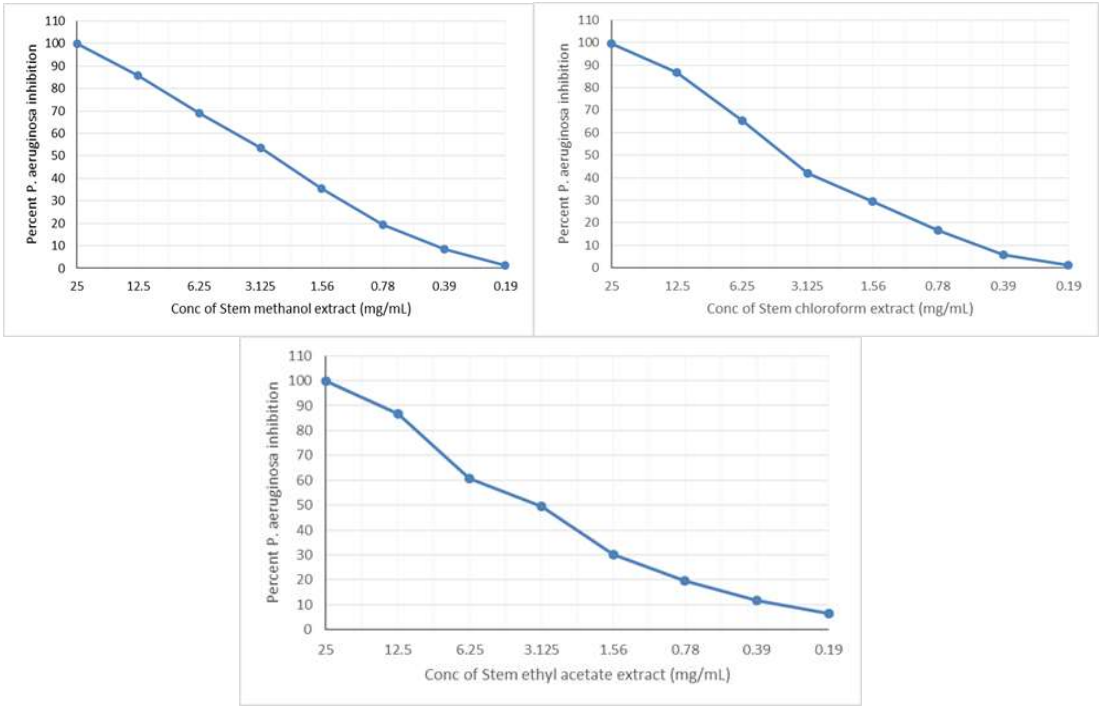


Fig. 13: Percentage inhibition of *P. aeruginosa* with different concentrations of methanol, chloroform, and ethyl acetate stem extracts of *S. lurida*

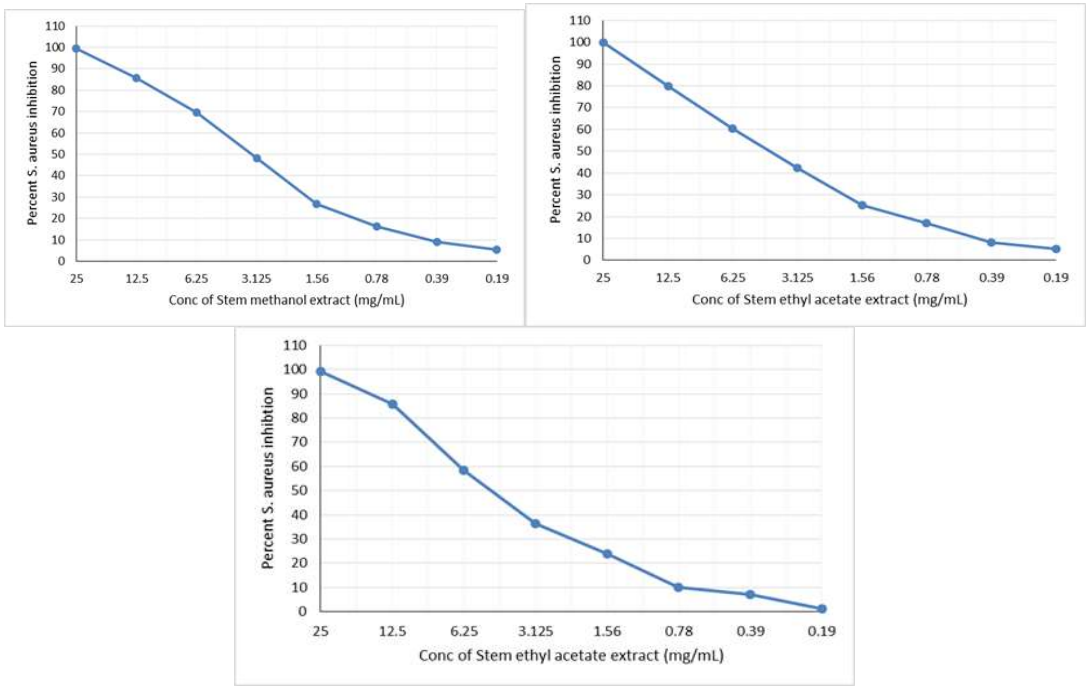


Fig. 14: Percentage inhibition of *S. aureus* with different concentrations of stem methanol, chloroform, and ethyl acetate extracts of the stem of *S. lurida.*

Antifungal Assay by Agar Well Diffusion Method

The agar well diffusion method was tested against antifungal activity of the inflorescence, stem, and leaf extracts of *S. lurida*. *Aspergillus niger* and *Candida albicans* were used for *in vitro* antifungal activity. Six different extracts from three different plant samples (inflorescence, stem, and leaf) showed a lower zone of inhibition for both the tested fungal strains compared to the standard used (Fluconazole - 22mm). Methanol and chloroform extracts from all the plant samples showed a mild zone of inhibition of 10mm against *Aspergillus niger* and *Candida albicans,* while no zone of inhibition was observed for the remaining extracts. **(Fig. 15- a,b,c) & (Fig. 16- a,b,c).** Prashith Kekuda *et al.,* (2018) conducted a well diffusion assay in the methanol leaf extract of *S. heyneana,* which exhibited the reduction of mycelial growth of *A. niger* and *Bipolaris* species by more than 50%. A similar research study was conducted by Raghavendra *et al*. (2017) in the methanol leaf, stem, and inflorescence extracts of *S. sessilis* against *Colletotrichum capsici, Fusarium oxysporum* f.sp. *Zingiberi*, and *Alternaria alternata*. And reported that the extracts were effective in reducing the mycelial growth on the tested fungi. Leaf extract was more effective against the tested fungi, followed by inflorescence and stem extracts.

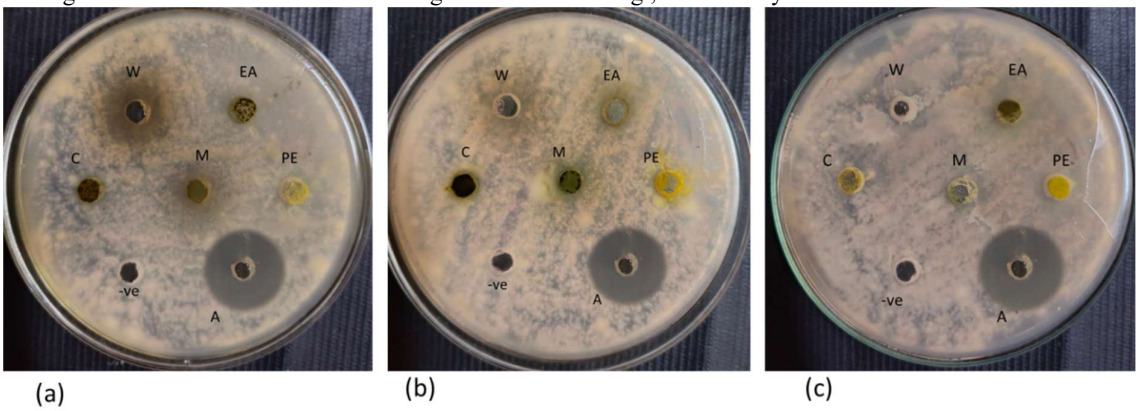


Fig. 15: Antifungal activity of *S. lurida* against *Aspergillus niger* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A: Antibiotic fluconazole

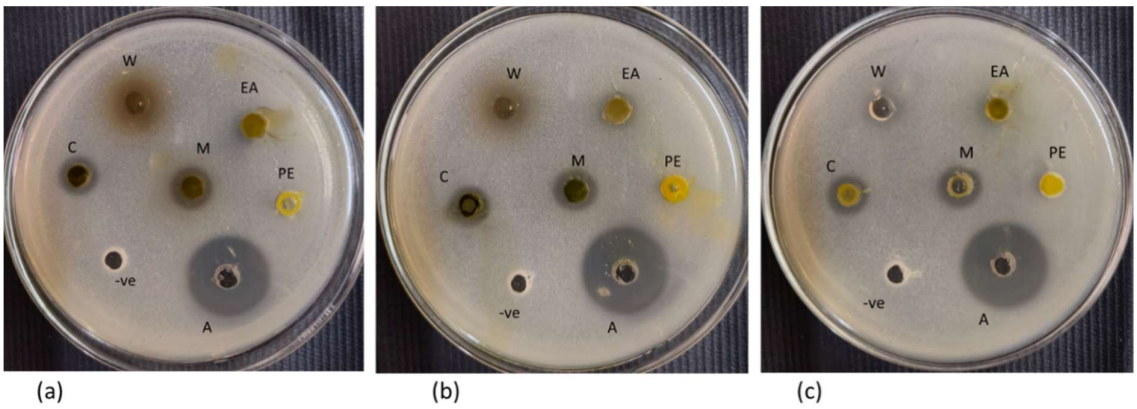


Fig. 16: Antifungal activity of *S. lurida* against *Candida albicans* (a) Inflorescence extract; (b) leaf extract; (c) stem extract. W-water extract; EA-ethyl acetate; M-methanol; C-chloroform; PE-petroleum ether. A: Antibiotic fluconazole

Determination of minimum inhibitory concentrations (MIC), Minimum fungicidal concentration (MFC), and IC_{50} concentration

The broth dilution method determined the MIC of *S. lurida* methanol, chloroform, and ethyl acetate extracts of three different plant samples (Leaf, inflorescence, and stem) against *Candida albicans*. The least concentration of the plant extracts was able to inhibit the growth of microorganisms completely (absence of turbidity) after 24 hrs. of incubation. The MIC was reported by observing the visual turbidity. The experiment was performed in triplicate to confirm its value for the tested organisms. All the extracts of three different plant samples showed the MIC response at 25mg/mL against all the tested fungal strains used **(Table. 2).** The minimum fungicidal concentration (MFC) of methanol and ethyl acetate inflorescence extracts, chloroform and ethyl acetate stem extracts, and ethyl acetate leaf extract was shown to be >25mg/mL whereas, other extracts indicated 25mg/mL against all the tested pathogens **(Table. 2)**. Based on the MIC and MFC values, IC50 values of methanol, chloroform, and ethyl acetate extracts of *S. lurida* were calculated by treating the pathogens with different concentrations of the extract (0.19 – 25mg). The percentage of fungal growth gradually increased with an increase in concentration. The other crude extracts of three different plant samples obtained their respective IC50 values against tested organisms **(Table 2 & Fig. 17-19)**. Earlier studies have shown the efficacy of extracts of *Strobilanthes* species against fungi. The inhibitory effect of petroleum ether and methanol extract of *S. ciliatus* was tested against fungi, namely, *Trichophyton rubrum*, *Microsporum gypseum*, and *Monascus purpureus* by Venkatachalapathi and Ravi (2013). The petroleum ether extract was proven to have significant antifungal activity against the tested fungi. The leaf extract of *S. crispus* showed no visible inhibition against *Aspergillus brasiliensis* and *Candida albicans* (Lim *et al*., 2015).

Table 2: Minimum inhibitory concentrations (MIC), Minimum fungicidal concentration (MFC), and IC_{50} values of different extracts of *S. lurida*

Extract	MIC (mg/mL)	MFC (mg/mL)	IC50 (mg/mL)
Inflorescence-methanol	25	>25	5.46
Inflorescence-chloroform	25	25	3.9
Inflorescence-ethyl acetate	25	>25	7.81
Stem-methanol	25	25	3.9
Stem-chloroform	25	>25	6.25
Stem-ethyl acetate	25	>25	6.25
Leaf-methanol	25	25	4.7
Leaf-chloroform	25	25	4.68
Leaf-ethyl acetate	25	>25	5.46

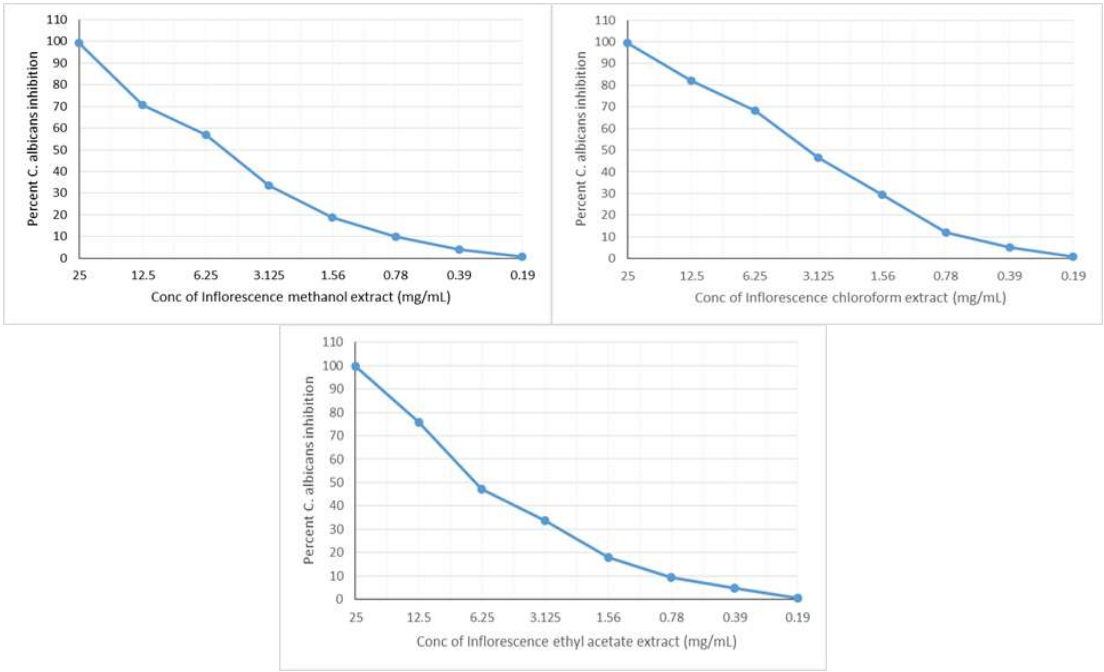
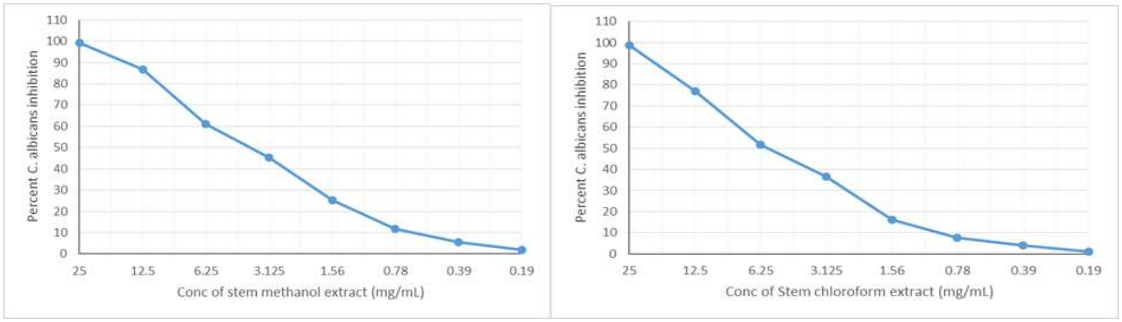


Fig. 17: Percentage inhibition of *C. albicans* with different concentrations of methanol, chloroform, and ethyl acetate inflorescence extracts of *S. lurida*



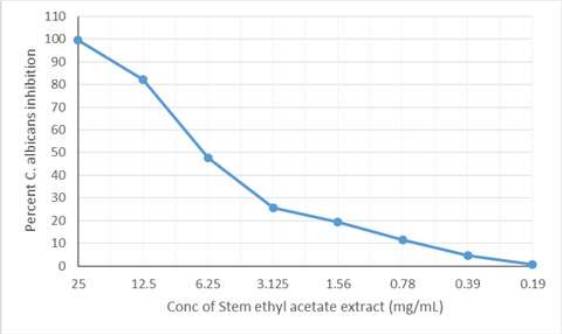


Fig. 18: Percentage inhibition of *C. albicans* with different concentrations of methanol, chloroform, and ethyl acetate stem extracts of *S. lurida*

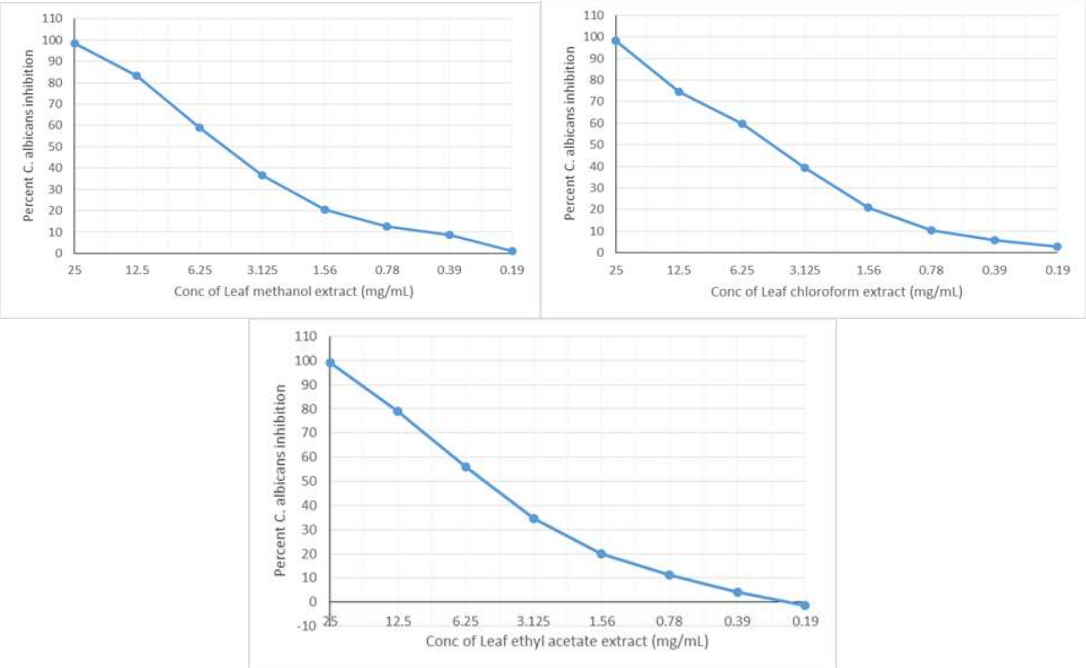


Fig. 19: Percentage inhibition of *C. albicans* with different concentrations of methanol, chloroform, and ethyl acetate leaf extracts of *S. lurida*

Conclusion

The present study clearly concludes that all extracts from the three different plant samples (inflorescence, stem, and leaf) of S. lurida exhibited moderate inhibition against all tested bacterial and fungal strains, as determined by the agar well diffusion method, minimum inhibitory concentrations (MIC), minimum bactericidal/fungicidal concentrations (MBC/MFC), and IC50 concentrations regarding antimicrobial activity. *Strobilanthes lurida* extracts exhibited moderate antibacterial and limited antifungal activity, with ethyl acetate stem extract showing the strongest antibacterial response. These findings support further bioassay-guided isolation and characterisation of active constituents. There is no recorded history of antimicrobial activity on the present plant before this study, and future research would be focused on the structural elucidation of bioactive compounds, followed by their complete characterisation and assessment of their biological effects.

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Conflict of Interest: The authors declare no conflict of interest.

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Data availability: All data generated or analyzed during the study are included in this article.

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