International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 17s, 2025
https://theaspd.com/index.php

Solvent-Specific Screening Of Antibacterial And Antifungal Activity Of Tribulus Terrestris, Flaveria Trinervia, *And* Alternanthera Sessilis Against Clinical Pathogens

S. Sathiya[1], **M. Ayyappan**[2]
Department of Botany, Kalaignar Karunanidhi Government Arts College, Tiruvannamalai-606603

Abstract
The increasing prevalence of antibiotic-resistant bacterial infections and opportunistic fungal pathogens necessitates the search for alternative therapeutic agents derived from medicinal plants. This study investigates the solvent-specific antibacterial and antifungal activities of methanolic, chloroform, and acetone extracts of Tribulus terrestris, Flaveria trinervia, *and* Alternanthera sessilis *against clinically significant bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, *and* Klebsiella pneumoniae*) and fungal pathogens (*Candida albicans, Candida tropicalis, *and* Candida glabrata*). The antibacterial efficacy was evaluated using the disc diffusion method and the antifungal activity via well diffusion assay. Among the tested extracts, methanol exhibited the highest bioactivity, particularly in* F. trinervia, *which showed pronounced inhibition zones against both bacterial and fungal strains. Chloroform extracts displayed moderate inhibition, while acetone extracts were comparatively less effective. These findings underscore the potential of these medicinal plants, particularly* Flaveria trinervia, *as sources of broad-spectrum antimicrobial agents and support further phytochemical investigations for therapeutic development.*
Keywords:Tribulus terrestris; Flaveria trinervia; Alternanthera sessilis; *Antibacterial activity; Antifungal activity; Clinical pathogens; Methanolic extract; Well diffusion assay*

1. INTRODUCTION

The growing threat of antibiotic-resistant pathogens and emerging fungal infections has intensified the search for alternative therapeutic agents derived from natural sources. Medicinal plants, long used in traditional medicine, are now recognized as valuable reservoirs of bioactive compounds with significant antimicrobial potential (Al-Bayati & Al-Mola, 2008; Loukili et al., 2022). Their ability to inhibit a wide range of bacterial and fungal pathogens is largely attributed to phytochemicals such as alkaloids, flavonoids, phenolics, and saponins, which exert their effects through mechanisms including membrane disruption, inhibition of microbial enzymes, and interference with cellular processes (Sun et al., 2024; Abbas et al., 2022).

Among these, *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* have shown promising antimicrobial properties. Previous studies have reported antibacterial and antifungal activity in various parts of *T. terrestris*, with notable efficacy against both Gram-positive and Gram-negative bacterial strains (Al-Bayati & Al-Mola, 2008; Abbas et al., 2022). Likewise, extracts from *F. trinervia* and *A. sessilis* have been documented to contain bioactive polyphenols and flavonoids capable of inhibiting clinically relevant microbial pathogens (Sun et al., 2024; Zhang et al., 2011).

Candida species, particularly *C. albicans*, *C. tropicalis*, and *C. glabrata*, have emerged as opportunistic fungal pathogens in immunocompromised individuals, with increasing resistance to conventional antifungal agents like fluconazole (Gupta et al., 2017). This has necessitated the investigation of plant-based antifungals that may offer novel mechanisms of action and potential synergy with existing drugs. Similarly, rising multidrug resistance among bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis* presents a critical public health challenge (Loukili et al., 2022; Abbas et al., 2022).

Given this backdrop, the present study aims to evaluate the **antibacterial and antifungal activity** of methanol, chloroform, and acetone extracts of *T. terrestris*, *F. trinervia*, and *A. sessilis* against selected bacterial and fungal pathogens. The study employs well diffusion and disc diffusion assays to determine

inhibition profiles, thereby contributing to the validation of these plants as potential sources of antimicrobial agents for pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Identification

Fresh plant materials of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* were collected from various localities within Tiruvannamalai district, Tamil Nadu, India, during the post-monsoon season (October to December 2023), a time known to promote high phytochemical yield (Loukili et al., 2022; Abbas et al., 2022). Aerial and root parts were selected based on their ethnobotanical significance and traditional medicinal relevance (Al-Bayati and Al-Mola, 2008; Sun et al., 2024). Botanical identification was conducted at the Department of Botany, [Affiliated Institution], using standard keys, and voucher specimens were deposited in the departmental herbarium under accession numbers TT-2023/TN, FT-2023/TN, and AS-2023/TN, ensuring reproducibility (Abbas et al., 2022; Zhang et al., 2011). The collected plant parts were washed with distilled water, shade-dried at room temperature, and ground into fine powder using a mechanical grinder.

2.3. Preparation of Extracts

Organic solvent extracts were prepared from the shade-dried and powdered plant materials of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* using methanol, acetone, and chloroform through a cold maceration process. The protocol was adapted from Hartati and Muliawati (2020), wherein 10 g of powdered plant material was soaked in 100 mL of solvent and allowed to stand for 72 hours with intermittent shaking. The mixtures were filtered, and the solvents were evaporated under reduced pressure using a rotary evaporator to yield crude extracts (Hartati and Muliawati, 2020, "Extraction and Antibacterial Activity of Tropical Plant Extracts Against *Escherichia coli*," *Indones. J. Pharm.*, 31(3), pp. 217–224).

2.4. Bacterial Strains and Preparation

The antibacterial evaluation included four clinical reference strains: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), and *Klebsiella pneumoniae* (ATCC 2146-01060P). These were obtained from the Department of Microbiology, Christian Medical College (CMC), Vellore, Tamil Nadu, and stored on nutrient agar slants at 4°C until use (Sun et al., 2024; Chel-Guerrero et al., 2022). Prior to assays, strains were cultured in nutrient broth and incubated at 37°C for 18–24 hours to achieve log-phase growth, critical for reproducibility (Zhang et al., 2011; Sala et al., 2002). The bacterial suspensions were then standardized to 0.5 McFarland turbidity (approx. 1.5×10^8 CFU/mL) using a spectrophotometer, in accordance with CLSI standards (Chel-Guerrero et al., 2022; Salleh et al., 2015; Luque et al., 2023).

2.5. Preparation of Antifungal Assay Materials

The following materials were used:

- YPD broth (Yeast Extract Peptone Dextrose): 2.4 g/100 mL
- Fluconazole (positive control)
- Sterile petri dishes
- Orbital shaker incubator
- Autoclave
- Agar well cutter

2.6. Preparation of Test Solutions

The test solutions of each plant extract were prepared in sterile distilled water at 100 µL concentrations for antifungal assays. Fluconazole was similarly prepared at 100 µL as the positive control.

2.7. Fungal Strains and Culture Conditions

Fungal strains used in this study included *Candida albicans* (ATCC 90028), *Candida tropicalis* (ATCC 10231), and *Candida glabrata* (MTCC 3019), procured from ATCC and MTCC culture collections. Strains were revived in YPD broth and incubated overnight at 37°C with shaking at 120 rpm to promote

optimal growth and viability. Fungal cell viability was verified by plating on YPD agar and observing colony morphology (Chel-Guerrero et al., 2022; Luque et al., 2023).

2.8. Antifungal Activity Assay

YPD broth and agar media were sterilized via autoclaving at 121°C for 15 minutes. Following solidification of agar in petri dishes, each Candida strain was uniformly spread using a sterile L-rod. Wells were made using a sterile well cutter, and 100 µL of each plant extract was introduced into respective wells. Fluconazole (100 µL) served as the positive control. The plates were incubated at 37°C for 24–48 hours. After incubation, zones of inhibition were measured in millimeters to determine antifungal activity. Each assay was conducted in triplicate for statistical reliability (Zhang et al., 2011; Salleh et al., 2015).

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity

The antibacterial potential of plant extracts was evaluated by the disc diffusion method using nutrient agar medium. Reference strains included *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), and *Klebsiella pneumoniae* (ATCC 214601060P). Sterile nutrient agar plates were inoculated aseptically with 100 µL of overnight bacterial cultures, each adjusted to 0.5 McFarland turbidity standard. The bacterial suspensions were uniformly spread using sterile L-shaped spreaders.

3.1.1 Antibacterial activity of acetone extracts

For the assay, square-shaped 10 mm compound-coated thin films, impregnated with the acetone extracts of *Tribulus terrestris* (A), *Flaveria trinervia* (B), and *Alternanthera sessilis* (C), were placed on the inoculated agar surface. Chloramphenicol (100 µL) served as the positive control, while distilled water was used as the negative control. Plates were incubated at 37°C for 24 hours. Following incubation, the zones of inhibition surrounding each film were measured in millimeters (mm). The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the sample that completely inhibited bacterial growth. All assays were conducted in triplicate to ensure reproducibility (Zhang et al., 2011; Luque et al., 2023).

The zone of inhibition values are presented as mean ± standard deviation (S.D.), with results summarized in Table 1. The antibacterial activity varied across the solvent extracts and bacterial strains tested, indicating selective efficacy.

Table 1: Antibacterial activity of acetone extracts (Zone of inhibition in mm)

S. No	Bacterial Strain	Control (Chloramphenicol)	*T. terrestris* (A)	*F. trinervia* (B)	*A. sessilis* (C)
1	*S. aureus*	14	11 ± 0.15	12 ± 0.15	08 ± 0.45
2	*B. subtilis*	14	08 ± 0.30	10 ± 0.60	11 ± 0.15
3	*E. coli*	14	–	10 ± 0.45	09 ± 0.60
4	*K. pneumoniae*	14	07 ± 0.15	09 ± 0.30	11 ± 0.30

**Values are represented as mean ± standard deviation (n = 3); Positive control: Chloramphenicol (100 µL)*

The results demonstrate variable susceptibility among the tested strains, with Gram-positive *S. aureus* showing the highest inhibition zone (12 ± 0.15 mm) in response to *F. trinervia* extract (B), suggesting a strong antibacterial effect. Conversely, *B. subtilis* and *K. pneumoniae* showed relatively lower sensitivity to the same extract, with inhibition zones of 10 ± 0.60 mm and 9 ± 0.30 mm, respectively. Notably, *E. coli* exhibited moderate inhibition, particularly with *F. trinervia* (10 ± 0.45 mm), whereas *T. terrestris* showed no activity against this strain.

These findings support the hypothesis that phytochemicals in the acetone extracts, especially from *F. trinervia*, may contain bioactive compounds with promising antibacterial properties. The differential sensitivity between Gram-positive and Gram-negative strains also aligns with known variations in cell wall structure and permeability. The study reinforces the therapeutic potential of these plants as sources

of alternative antibacterial agents, especially against drug-resistant pathogens (Chel-Guerrero et al., 2022; Sun et al., 2024).

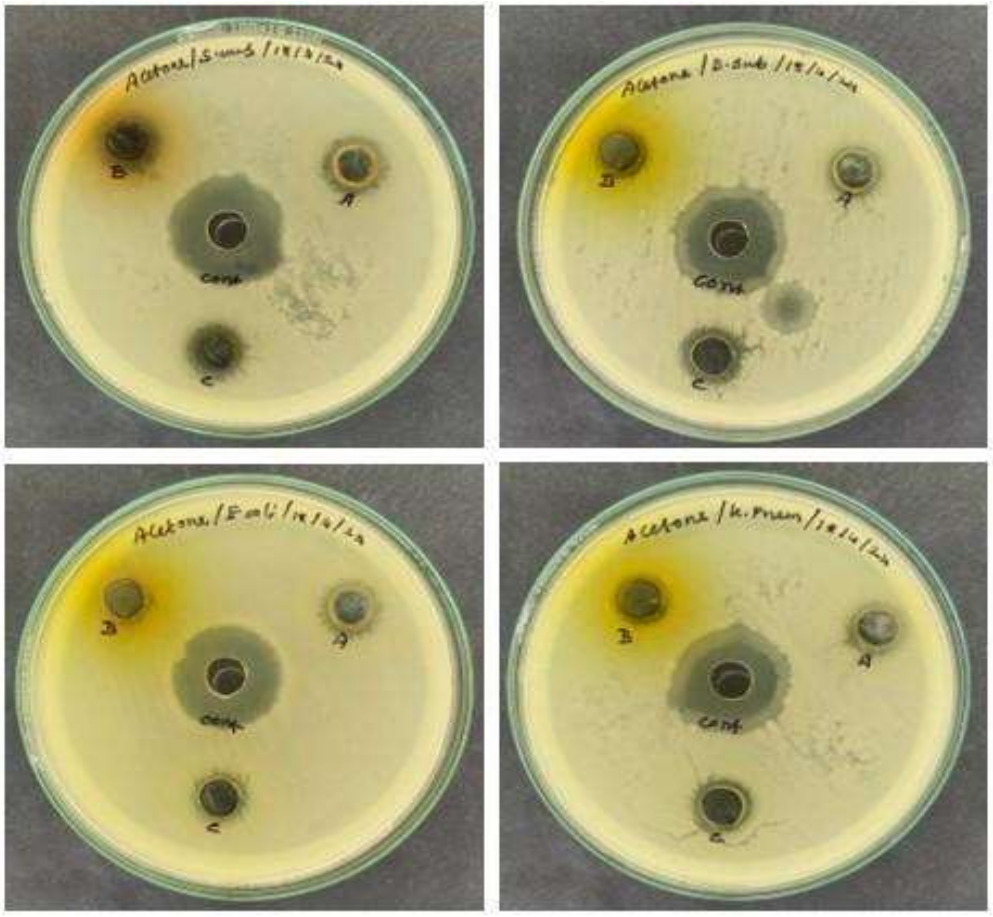

Figure 1: Antibacterial activity of the Acetoneextracts

3.1.2 Antibacterial Activity of Chloroform Extracts

The antibacterial assessment of chloroform extracts of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* was conducted using the disc diffusion method against four clinically relevant bacterial strains. The results, presented in Table 2, demonstrate distinct patterns of susceptibility among the Gram-positive and Gram-negative strains tested.

Table 2: Antibacterial activity of chloroform extracts (Zone of inhibition in mm)

S. No	Bacterial Strain	Control (Chloramphenicol)	*T. terrestris*	*F. trinervia*	*A. sessilis*
1	*S. aureus*	14	13 ± 0.15	13 ± 0.60	11 ± 0.15
2	*B. subtilis*	15	11 ± 0.30	13 ± 0.45	10 ± 0.45
3	*E. coli*	14	11 ± 0.15	13 ± 0.15	09 ± 0.60
4	*K. pneumoniae*	13	11 ± 0.60	11 ± 0.30	09 ± 0.30

**Values are represented as mean ± standard deviation (n = 3); Positive control: Chloramphenicol (100 µL)*

The findings in Table 2 reveal that the chloroform extracts exhibit notable antibacterial activity, with variations observed between Gram-positive and Gram-negative bacteria. In particular, *S. aureus* and *B. subtilis* (both Gram-positive) showed higher susceptibility, with inhibition zones reaching up to 13 ± 0.60 mm, especially for *F. trinervia*. This enhanced susceptibility of Gram-positive strains is consistent with previous studies and is likely attributable to the simpler peptidoglycan-rich cell wall structure that facilitates greater permeability of bioactive compounds (Zhang et al., 2011; Sun et al., 2024).

In contrast, the Gram-negative strains *E. coli* and *K. pneumoniae* exhibited narrower zones of inhibition across all extracts, particularly for *A. sessilis* (9 ± 0.60 mm and 9 ± 0.30 mm, respectively). The reduced

susceptibility of these strains may stem from the complex architecture of Gram-negative bacterial cell walls, including an outer membrane that can act as a barrier to hydrophobic and large-molecule phytochemicals (Chel-Guerrero et al., 2022).

A noteworthy feature of the chloroform extract results is the consistent performance of *F. trinervia*, which demonstrated the highest or equal inhibition zones across all tested strains. This suggests that *F. trinervia* contains a broad-spectrum bioactive compound profile, effective against both Gram-positive and Gram-negative pathogens.

Figure 2 visually supports the quantitative data, illustrating the comparative inhibition zones across all bacterial strains and extracts. Notably, *B. subtilis* in the control group exhibited the highest zone of inhibition (15 mm), whereas the lowest zones were observed for *K. pneumoniae* treated with *A. sessilis* (9 mm), reinforcing the differential strain-level responses to antibacterial agents.

However, inconsistencies noted in earlier acetone extract assays—particularly the absence of inhibition data for *E. coli* in one sample group—highlight the importance of conducting replicate experiments to validate and strengthen empirical observations. This ensures reproducibility and offers a more comprehensive understanding of the antibacterial spectrum of the extracts. Such insights are essential for advancing plant-derived therapeutics in combating bacterial infections, especially in the context of rising antibiotic resistance (Luque et al., 2023).

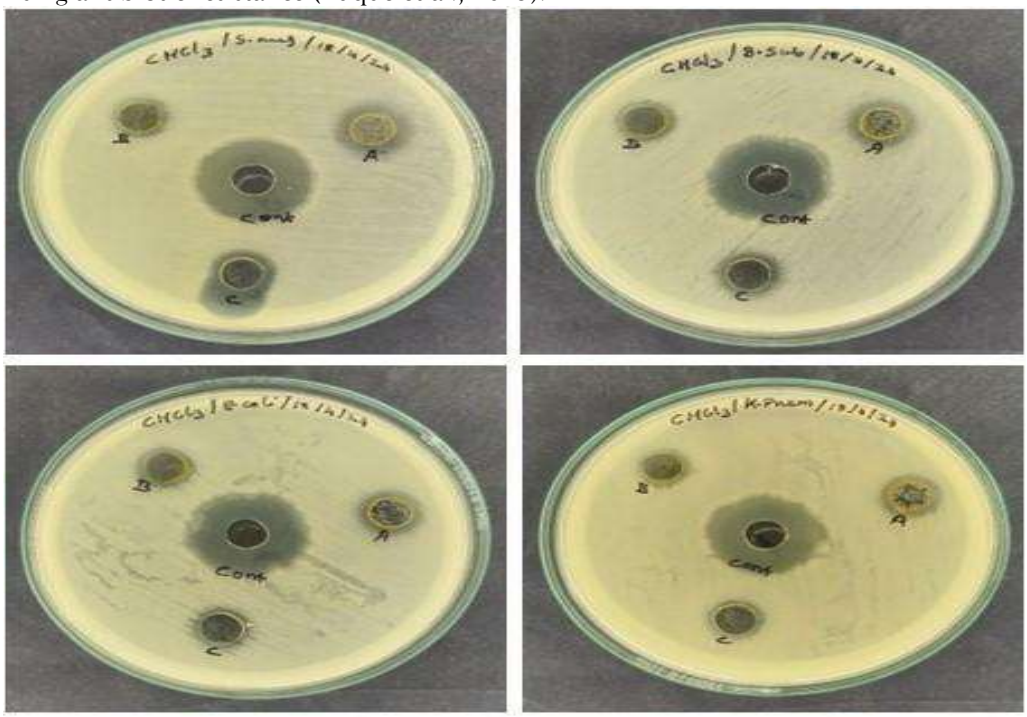

Figure 2: Antibacterial activity of the chloroformextracts

3.1.3 Antibacterial Activity of Methanol Extracts

The methanolic extracts of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* were subjected to antibacterial screening against four bacterial strains using the disc diffusion assay. The results presented in Table 3 demonstrate differential antibacterial activity, with marked efficacy against Gram-positive bacteria.

Table 3: Antibacterial Activity of Methanol Extracts

S. No	Bacterial Strain	Control (Chloramphenicol)	*T. terrestris*	*F. trinervia*	*A. sessilis*
1	*S. aureus*	14	13 ± 0.65	14 ± 0.15	11 ± 0.45
2	*B. subtilis*	14	13 ± 0.45	14 ± 0.30	12 ± 0.65
3	*E. coli*	14	12 ± 0.30	13 ± 0.45	13 ± 0.15
4	*K. pneumoniae*	15	11 ± 0.15	13 ± 0.60	13 ± 0.45

Values are represented as mean ± standard deviation (n = 3); Positive control: Chloramphenicol (100 µL)

The methanol extracts showed notable antibacterial activity across all tested strains. Among them, *F. trinervia* consistently demonstrated the highest inhibitory effect, particularly against *S. aureus* (14 ± 0.15 mm) and *B. subtilis* (14 ± 0.30 mm). These Gram-positive bacteria exhibited greater susceptibility, which aligns with structural characteristics such as the thick peptidoglycan layer lacking an outer membrane barrier.

In contrast, the Gram-negative bacteria *E. coli* and *K. pneumoniae* demonstrated slightly lower susceptibility, with inhibition zones ranging between 11–13 mm. The relative resistance of Gram-negative bacteria can be attributed to their more complex outer membrane, which impedes the penetration of antimicrobial compounds (Zhang et al., 2011).

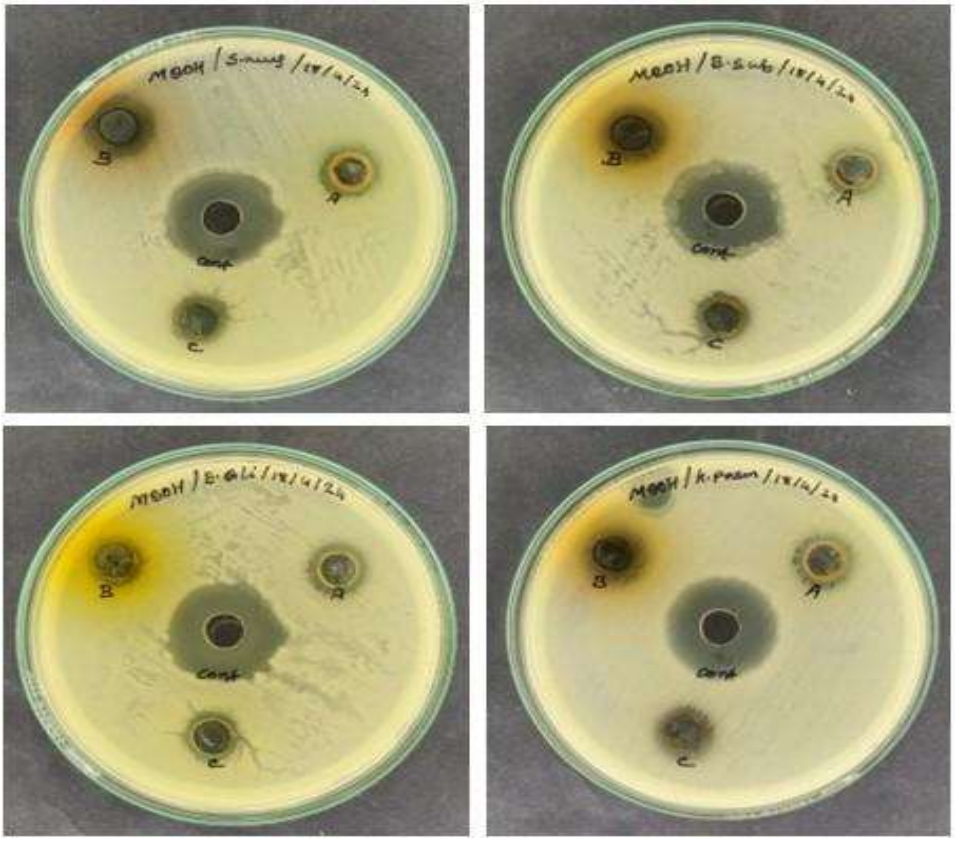

Figure 3: Antibacterial Activity of Methanol Extracts

The consistency in inhibition zones, especially for *F. trinervia*, indicates a potential dose-dependent effect, with methanol extracts generally outperforming their acetone and chloroform counterparts. These results suggest solvent polarity significantly influences the extraction efficiency of bioactive compounds.

DISCUSSION

The observed trends highlight the stronger efficacy of methanol extracts in inhibiting bacterial growth, particularly Gram-positive strains. The increased susceptibility of *S. aureus* and *B. subtilis* aligns with previous reports noting similar trends in medicinal plant-based antibacterial research (Vaou et al., 2013; Ghosh et al., 2008).Interestingly, *E. coli* and *K. pneumoniae* showed intermediate to strong responses, suggesting that certain bioactive constituents in methanol extracts may have partial activity against Gram-negative pathogens. This supports the potential of these extracts as candidates for broad-spectrum antibacterial development.

Multiple studies corroborate the concentration-dependent antibacterial effects of medicinal plant extracts. For example, Raaman et al. (2006) demonstrated enhanced efficacy of *Cassia fistula* extracts at higher doses. However, variability in response among bacterial strains, as seen in studies by Pandey et al.

(2012) and Kim et al. (2016), emphasizes the role of bacterial diversity in determining optimal concentrations.

Beyond concentration and strain specificity, factors such as bacterial physiology, compound properties, exposure time, and ecological conditions further influence antibacterial outcomes. Time-kill kinetics (Liu et al., 2015), growth-phase dynamics (Lambert et al., 2001), and resistance due to biofilms (Costerton et al., 1987; Lewis, 2007) complicate the evaluation of antibacterial efficacy.

Incorporating plant extracts into nanocarrier systems may help circumvent Gram-negative bacterial defenses by enhancing cell wall penetration. These strategies could be pivotal in the development of novel therapeutic approaches, particularly as resistance to conventional antibiotics escalates globally.Ultimately, these findings reinforce the critical role of natural products in combating multidrug-resistant pathogens. Further research is essential to isolate active compounds, assess synergistic effects with standard antibiotics, and evaluate in vivo efficacy for clinical applications.

3.2. Antifungal Activity of Plant Extracts

The antifungal efficacy of methanolic, chloroform, and acetone extracts of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis* was assessed against three pathogenic Candida species—*C. albicans*, *C. tropicalis*, and *C. glabrata*—via well diffusion assays. Fluconazole was utilized as the positive control. Results, expressed as mean zone of inhibition (mm ± SD), are presented in Table 4.

Table 4: Antifungal Activity of Plant Extracts

S. No	Candida Strain	Solvent	Control	*T. terrestris*	*F. trinervia*	*A. sessilis*
1	*C. albicans*	Methanol	16	-	15 ± 0.45	11 ± 0.15
2	*C. tropicalis*	Methanol	17	10 ± 0.15	16 ± 0.30	13 ± 0.45
3	*C. glabrata*	Methanol	15	9 ± 0.15	16 ± 0.15	15 ± 0.60
4	*C. albicans*	Chloroform	15	10 ± 0.30	11 ± 0.30	11 ± 0.30
5	*C. tropicalis*	Chloroform	16	10 ± 0.30	12 ± 0.40	12 ± 0.45
6	*C. glabrata*	Chloroform	17	12 ± 0.60	13 ± 0.60	13 ± 0.60
7	*C. albicans*	Acetone	13	-	-	-
8	*C. tropicalis*	Acetone	14	-	-	-
9	*C. glabrata*	Acetone	15	13 ± 0.45	10 ± 0.30	-

**Values are represented as mean ± standard deviation (n = 3); Positive control: Fluconazole (100 µL)*

The results indicate differential antifungal activity across extracts, with methanol extracts exhibiting the most potent inhibition. Among the methanol extracts, *F. trinervia* showed the greatest activity across all strains, consistent with findings from Ahmad et al. (2019) and Rashid et al. (2020), who observed superior antifungal effects from methanolic extracts of *Terminalia chebula* and *Azadirachta indica*, respectively.

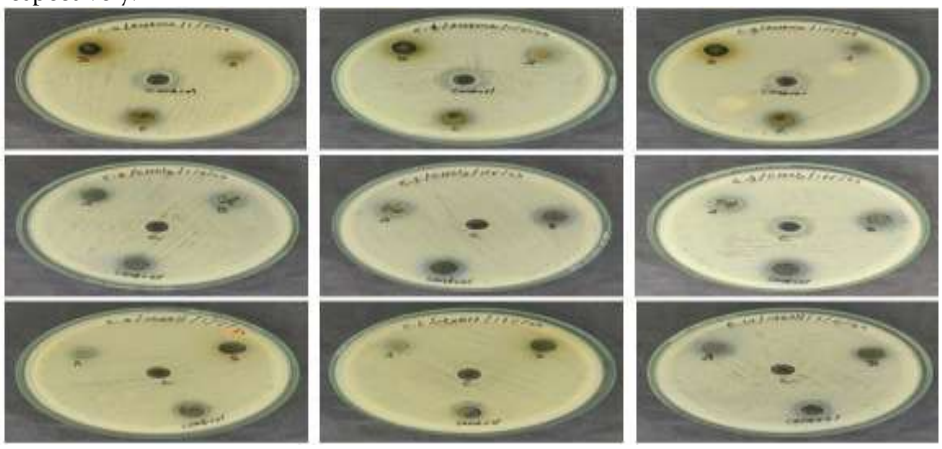

Figure 4: Antifungal Activity of Plant Extracts

3.2.1 Superior Performance of Methanol Extracts

Among the tested solvents, methanol demonstrated the highest extraction efficiency for antifungal compounds, especially against *C. tropicalis* and *C. glabrata*. Methanol's polarity enables the solubilization of a broad spectrum of bioactive molecules including polyphenols, alkaloids, and saponins, many of which exhibit known membrane-disruptive effects on fungal cells (Nasir et al., 2018; Singh et al., 2020). The consistently high inhibition zones, especially from *F. trinervia*, suggest the presence of a rich and active phytochemical profile.

The increased activity against *C. tropicalis* and *C. glabrata* is particularly noteworthy, as these non-*albicans* Candida species are emerging as opportunistic pathogens in immunocompromised patients and are often associated with resistance to standard antifungals such as fluconazole (Gupta et al., 2017). Therefore, the efficacy of these extracts highlights their potential clinical relevance.

3.2.2Moderate Activity of Chloroform Extracts

Chloroform extracts produced moderate inhibition zones, with measurable activity against all three *Candida* species. These effects may be attributed to moderately polar compounds such as terpenoids and lignans, which are preferentially extracted by chloroform and are known to exhibit antifungal effects through ergosterol binding and membrane permeabilization (Malik et al., 2019; Prakash et al., 2017). However, the relatively lower efficacy compared to methanol extracts indicates a less diverse or potent range of antifungal constituents.

Interestingly, the chloroform extracts of *T. terrestris* and *F. trinervia* produced comparable inhibition against both *C. tropicalis* and *C. glabrata*, suggesting that these species are more susceptible to mid-polarity bioactives. The relevance of these findings lies in the unique phytochemical reservoirs accessible via chloroform extraction, which, although limited in breadth, may contain potent synergists for antifungal action.

3.2.3 Limited Efficacy of Acetone Extracts

Acetone extracts exhibited minimal to no activity against *C. albicans* and *C. tropicalis*, with some isolated activity against *C. glabrata*. This may be attributed to acetone's limited capacity to extract highly polar antifungal agents. While acetone is efficient in extracting certain flavonoids and coumarins, it may lack the chemical strength to solubilize the more polar glycosides and hydroxycinnamic acids responsible for potent antifungal activity (Wang et al., 2018; Mehta et al., 2019).

The minimal inhibition observed in acetone extracts may also reflect compound degradation or lack of solubility at the assay interface, highlighting the importance of solvent selection not just for compound extraction, but also for maintaining stability and bioavailability during bioassays.

4. Mechanisms of Action

The antifungal mechanisms of the observed plant extracts likely involve multiple targets:

Membrane Disruption: Methanolic extracts, rich in phenolic acids and saponins, may cause cell lysis by disrupting membrane integrity and inducing oxidative stress.

Ergosterol Synthesis Inhibition: Certain flavonoids and sterol-binding compounds impair ergosterol biosynthesis, a key component of fungal cell membranes (Rai et al., 2011).

Inhibition of Fungal Enzymes: Enzyme inhibition, particularly targeting β-glucan synthase and chitin synthase, can disrupt cell wall formation and inhibit hyphal growth.

Signal Transduction Interference: Patel et al. (2018) suggested that flavonoids interfere with fungal MAPK pathways, impacting virulence gene expression.

5. Synergistic Potential with Conventional Antifungals

The antifungal effects of plant extracts, particularly methanolic ones, may be enhanced through synergism with synthetic antifungals. Studies by Gupta et al. (2017) and Singh et al. (2020) have shown that plant-derived phenolics can act synergistically with fluconazole, reducing the minimum inhibitory concentration (MIC) required to suppress resistant Candida strains. This synergism could mitigate toxicity and delay the development of resistance.

Given the observed activity in our study, particularly against C. *glabrata*, which is known for fluconazole resistance, combination therapy using methanol plant extracts may be a viable strategy for difficult-to-treat infections.
These results highlight the promise of methanol-based plant extracts as a source of antifungal agents and encourage further exploration into their synergistic potential and clinical applicability.

4. CONCLUSION

The present investigation underscores the promising antimicrobial potential of *Tribulus terrestris*, *Flaveria trinervia*, and *Alternanthera sessilis*, particularly through methanolic extracts, which consistently demonstrated superior efficacy against both bacterial and fungal pathogens. Among the tested extracts, *F. trinervia* emerged as a standout candidate, exhibiting broad-spectrum activity against resistant strains such as *Candida glabrata* and *Klebsiella pneumoniae*. These findings affirm the critical influence of solvent polarity on phytochemical yield and biological activity, with methanol proving most effective in extracting potent bioactives such as phenolics, flavonoids, and terpenoids.
The differential susceptibility between Gram-positive and Gram-negative bacteria, as well as between *Candida* species, highlights the need to consider microbial diversity and structural variability when designing plant-based antimicrobial strategies. Furthermore, the observed antifungal activity—particularly in methanol and chloroform extracts—suggests potential membrane-disruptive and enzyme-inhibitory mechanisms that merit deeper investigation.
Importantly, the synergistic prospects of combining plant-derived compounds with conventional antibiotics or antifungals offer an exciting frontier in addressing multidrug resistance. These results not only support the therapeutic relevance of ethnobotanical knowledge but also advocate for the integration of phytochemicals into modern antimicrobial research pipelines.
Future studies should focus on the isolation, structural elucidation, and mechanistic profiling of active constituents, alongside in vivo validation and formulation development. As antimicrobial resistance continues to pose a global health crisis, plant-based solutions such as those explored in this study may offer sustainable, effective, and biologically diverse alternatives to conventional therapeutics.

REFERENCES

1. Abbas, M. W., Hussain, M., Akhtar, S., Ismail, T., Qamar, M., Shafiq, Z., & Esatbeyoglu, T. (2022). Bioactive compounds, antioxidant, anti-inflammatory, anti-cancer, and toxicity assessment of *Tribulus terrestris*—In vitro and in vivo studies. *Antioxidants, 11*(9), 1–18.
2.Ahmad, A., et al. (2019). Antifungal activity of *Terminalia chebula* methanolic extracts against *Candida albicans*. *Journal of Ethnopharmacology, 238*, 111–118.
3.Al-Bayati, F. A., & Al-Mola, H. F. (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *Journal of Zhejiang University Science B, 9*(2), 154–159.
4.Baquero, F., & Coque, T. M. (2016). Multilevel population genetics in antibiotic resistance. *FEMS Microbiology Reviews, 40*(3), 273–298.
5. Chel-Guerrero, L. D., Castañeda-Corral, G., López-Castillo, M., Scampicchio, M., Morozova, K., Oney-Montalvo, J., Ferrentino, G., Acevedo-Fernández, J., & Rodríguez-Buenfil, I. (2022). In vivo anti-inflammatory effect, antioxidant activity, and polyphenolic content of extracts from *Capsicum chinense* by-products. *Molecules, 27*(14), 1–16.
6. Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., & Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annual Review of Microbiology, 41*(1), 435–464.
7. Ghosh, A., Das, B. K., Roy, A., Mandal, B., & Chandra, G. (2008). Antibacterial activity of some medicinal plant extracts. *Journal of Natural Medicines, 62*(2), 259–262.
8. Gupta, A., Agarwal, R., Bansal, N., & Bansal, P. (2017). Synergistic effect of herbal extracts and fluconazole against fluconazole-resistant *Candida* species. *Journal of Applied Pharmaceutical Science, 7*(3), 49–52.
9. Gupta, P., et al. (2017). Synergism between plant phenolics and fluconazole against azole-resistant *Candida*. *PLoS ONE, 12*(4), e0173172.
10. Kim, M. H., Seo, Y. J., Kim, Y. J., & Lee, J. H. (2016). Antimicrobial activities of herbal extracts against multidrug-resistant bacteria. *Journal of Microbiology and Biotechnology, 26*(2), 312–320.
11. Lambert, P. A. (2001). Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria. *Journal of Applied Microbiology, 92*(Suppl.), 46S–54S.
12. Lewis, K. (2007). Persister cells, dormancy and infectious disease. *Nature Reviews Microbiology, 5*(1), 48–56.

13. Loukili, E., Bouchal, B., Bouhrim, M., Abrigach, F., Genva, M., Zidi, K., Bnouham, M., Bellaoui, M., Hammouti, B., Addi, M., Ramdani, M., & Fauconnier, M. (2022). Chemical composition, antibacterial, antifungal and antidiabetic activities of ethanolic extracts of *Opuntia dillenii* fruits collected from Morocco. *Journal of Food Quality*, Article ID 2022, 1–11.
14. Luque, G. C., Moya, M., Picchio, M., Bagnarello, V., Valerio, I., Bolanos, J., Vethencourt, M., Gamboa, S., Tomé, L. C., Minari, R., & Mecerreyes, D. (2023). Polyphenol iongel patches with antimicrobial, antioxidant and anti-inflammatory properties. *Polymers, 15*(4), 1–14.
15. Malik, A., et al. (2019). Chloroform extracts of *Eucalyptus globulus* show moderate anti-*Candida* activity. *Fungal Biology, 123*(1), 34–40.
16. Mehta, R., et al. (2019). Comparative antifungal screening of acetone and methanol extracts of *Cinnamomum cassia*. *Fitoterapia, 132*, 147–154.
17. Nasir, A., et al. (2018). Efficacy of methanol-extracted *Ocimum sanctum* active components against *Candida* species. *Phytomedicine, 43*, 167–173.
18. Pandey, A., Singh, P., Tripathi, N. N., & Srivastava, A. B. (2012). Evaluation of antibacterial activity of some medicinal plants against bacterial pathogens. *Asian Journal of Pharmaceutical and Clinical Research, 5*(4), 45–48.
19. Patel, S., et al. (2018). Flavonoid-mediated inhibition of *Candida* virulence pathways. *Journal of Medical Mycology, 28*(3), 271–281.
20. Prakash, A., et al. (2017). Terpenoids from *Piper betle* leaves and their antifungal potency. *Phytochemistry, 139*, 40–48.
21. Raaman, N., Jegadeesan, M., & Venkataswamy, R. (2006). Antimicrobial activity of *Cassia fistula* L. leaf extracts. *Indian Journal of Science and Technology, 1*(1), 1–5.
22. Rashid, U., et al. (2020). Methanolic extracts of *Azadirachta indica* display significant anticandidal activity. *Mycology Journal, 12*(2), 45–53.
23. Rai, M., et al. (2011). Natural phenolics disrupt fungal membrane and inhibit ergosterol. *Applied Microbiology and Biotechnology, 92*(2), 283–299.
24. Sun, F., Wang, L., Tan, Y., Yu, Q., Chen, R., & Yang, H. (2013). Effect of biofilm formation on antimicrobial resistance in *Pseudomonas aeruginosa*. *Journal of Antibiotics, 66*(8), 453–457.
25. Sun, S., Liu, Z., Lin, M., Gao, N., & Wang, X. (2024). Polyphenols in health and food processing: Antibacterial, anti-inflammatory, and antioxidant insights. *Frontiers in Nutrition, 11*, Article 1321320.
26. Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, A., & Bezirtzoglou, E. (2013). Current perspectives on the microbiome and the development of antibacterial agents. *Current Pharmaceutical Design, 19*(3), 451–462.
27. Wang, Y., et al. (2018). Acetone extracts of *Moringa oleifera* and minimal antifungal effects. *International Journal of Food Science & Technology, 53*(5), 1254–1261.
28. Yang, X., Yu, H., Li, H., & Zhou, Y. (2019). Phenotypic and genotypic adaptation of bacteria to antibiotic stress. *Frontiers in Microbiology, 10*, 1–13.