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Antimicrobial Efficacy And Phytochemical Profiling Of Adina Cordifolia Bark Extract: A Gc-Ms And Mic-Based Study

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Abstract
This study explores the antimicrobial potential and phytochemical profile of Adina cordifolia bark extracts. Using solvents of varying polarity, the extracts were tested against Gram-positive and Gram-negative bacteria, as well as fungal strains. The methanolic and acetone extracts showed significant activity, particularly against E. coli and C. albicans, with MIC values between 2–8 µg/mL. GC-MS analysis of the methanolic extract identified bioactive compounds such as benzaldehyde derivatives and diphenylsulfones, suggesting their role in antimicrobial efficacy. The results support the ethnomedicinal relevance of A. cordifolia and indicate its potential as a natural antimicrobial source.

Keywords:- *Adina cordifolia, Antimicrobial activity, GC-MS analysis, Phytochemicals, Minimum Inhibitory Concentrations (MIC), Methanolic extract, Bioactive compounds, Medicinal plants.*

INTRODUCTION :

Infectious diseases remain a significant global health issue, contributing to 41% of the overall disease burden as measured by Disability-Adjusted Life Years (DALYs) (Noah D et al, 2000). Medicinal plants serve as foundational sources for natural and herbal medicines. Their diverse phytochemical structures make them valuable for discovering new lead compounds in the development of treatments for both acute and chronic diseases. The Indian subcontinent, known for its rich biodiversity, possesses one of the most extensive traditional plant-based medicinal systems in the world. Herbal medicines are widely used in healthcare due to their minimal side effects and greater acceptance. The Indian Himalayan region, particularly the Garhwal Himalayas, is home to approximately 1,748 species of medicinal plants and is recognized for its rich biological and ethnocultural diversity. Many drugs originate from natural sources—including plants, animals, microorganisms, and minerals—which have long been used in treating diseases in both humans and animals (chopra et al.,1986 and Kirtikar KR et al.,1975). Traditionally, fresh bark is ground together with brown sugar and consumed orally to relieve stomachaches. Both the bark and leaves are also used in folk medicine to treat ailments such as cholera, colds, coughs, fevers, headaches, skin scars, and conditions associated with yellowing of the skin and urine. Earlier phytochemical investigations have resulted in the isolation and identification of several compounds, including 10-deoxyadifoline, 10-deoxycordifoline, cordifoline, adifoline, dihydroxytetra-O-methyl flavones, naphthoquinone, and adinine.(Prakash v et al., 2015). UReactive oxygen species (ROS) are a group of chemical compounds generated during oxygen metabolism. These molecules play a significant role in oxidative stress, which can lead to the damage of lipids, proteins, and DNA(Sharma P et al,. 2012). The Rubiaceae family is one of the largest plant groups known for its diverse range of secondary metabolites. Numerous species within this family have shown potential as valuable sources for developing novel bioactive compounds and drug candidates, owing to their chemical diversity and pharmacological activities (Martins D et al., 2015). They produce a diverse array of secondary metabolites such as anthraquinones, alkaloids, coumarins, flavonoids, and terpenes, many of which exhibit notable pharmacological activities (Heitzman ME et al., 2005).

MATERIAL AND METHODS:-

Collection of plants :- The bark of *Adina cordifolia* (Roxb.) Benth. & Hook.f. ex Brandis was collected from the Botanical Garden, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, Maharashtra, India (Pin code: 431001) during the month of [September 2024]. The plant was taxonomically identified and authenticated with the help of regional floras, including Flora of Maharashtra State and Flora of Marathwada, and cross-verified by a qualified taxonomist

Preparation of plant extract:-

The plant leaves were air-dried at room temperature for 14 days after which they were ground into a fine, uniform powder. This powdered material was then used for the preparation of various extracts.

Preparation of various extracts from air-dried *Adina cordifolia* powder using different **solvents:-**

The leaf extract was prepared using a Soxhlet apparatus. For this process, 10 grams of air-dried leaf powder were placed in 100 ml of the desired solvents (water, methanol, Ethanol, chloroform, Acetone, petroleum ether).The Soxhlet system operates at elevated temperatures and utilizes continuous evaporation and condensation cycles. Each extraction was carried out for 12 hours for every solvent used. After completion, the extracts were filtered through filter paper (De castro et al., 2010).

Concentration of compounds:-

Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried out by taking concentration 100 microrgram per disk.Hi-media antibiotics disk: Streptomycin (10 microgram/disk), moistened with water are used as standard for antimicrobial sensitivity, Fluconazole (25mcg) for *c.albicans* and *Aspergillus niger.*

Test organism :

Microorganism type	Name	Strain
• Gram positive bacteria	*Staphylococcus aureus*	NCIM 2079
	Bacillus subtilis	NCIM 2250
• Gram negative bacteria	*Escherichia coli*	ATCC 25922
	Klebsiella pneumoniae	NCIM 2719
• Fungi	*candida albicans*	
	Aspergillus niger	ATCC 16404

Table 1.

Antimicrobial activity analysis:

Disc diffusion method :-

Sterile discs measuring 6 mm in diameter were loaded with 20 µL of each plant extract, prepared at a concentration of 100 mg/mL, and subsequently air-dried at room temperature. The plant extracts were obtained using a Soxhlet apparatus with a range of solvents, including water, methanol,acetone,petroleum ether.Each disc was estimated to carry around 2 mg of crude extract.(Jorgensen JH et al.;2015, Espinel-Ingroff A et al.;2017,(EUCAST);2024,CLSI;2023)

GC-MS analysis:-

Phytochemical constituents present in the methanolic bark extract of *Adina cordifolia* were identified using a Shimadzu GC-MS instrument equipped with a DB-5MS capillary column. The initial oven temperature was set at 60°C and maintained for 2 minutes, then increased at a rate of 10°C per minute until reaching 300°C, where it was held for a final 10 minutes. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1 µL sample volume was injected in split mode at a ratio of 1:10, with the injector temperature maintained at 250°C. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with the ion source and interface temperatures set at 230°C and 280°C, respectively. Scanning was performed across a mass range of m/z 40–600. Compound identification was accomplished by comparing the obtained mass spectra with entries in the NIST library, and the relative abundance of each compound was assessed based on its corresponding peak area.

The oven temperature was increased from 60°C to 300°C to separate compounds with different boiling points. This helped in better detection of both low and high boiling compounds, improved peak clarity, and accurate identification of phytochemicals in the methanolic bark extract of *Adina cordifolia*

Minimum Inhibitory Concentration (MIC):-

The bark of *Adina cordifolia* was initially shade-dried to preserve its phytoconstituents and then finely powdered using a mechanical grinder. This powdered material was subjected to Soxhlet extraction using methanol and acetone as the solvent to obtain a crude extract rich in bioactive compounds.

For antimicrobial testing, stock solutions of each extract and fraction were prepared at a concentration of 10 mg/mL using 1% Tween 80 in normal saline to ensure adequate solubility and dispersion. These were serially diluted in nutrient broth using a two-fold dilution method to achieve a range of concentrations. Microbial cultures were prepared to match a 0.5 McFarland turbidity standard, ensuring a uniform inoculum density (~1×10^8 CFU/mL). The diluted extracts were then inoculated with the standardized microbial suspensions in sterile test tubes.

All samples were incubated at 37 °C for 18–24 hours. The Minimum Inhibitory Concentration (MIC) was determined as the lowest extract concentration at which no visible turbidity was observed, indicating complete inhibition of microbial growth.(W Fabry et al.,1998)

RESULT AND DISCUSSION

Antimicrobial activity :

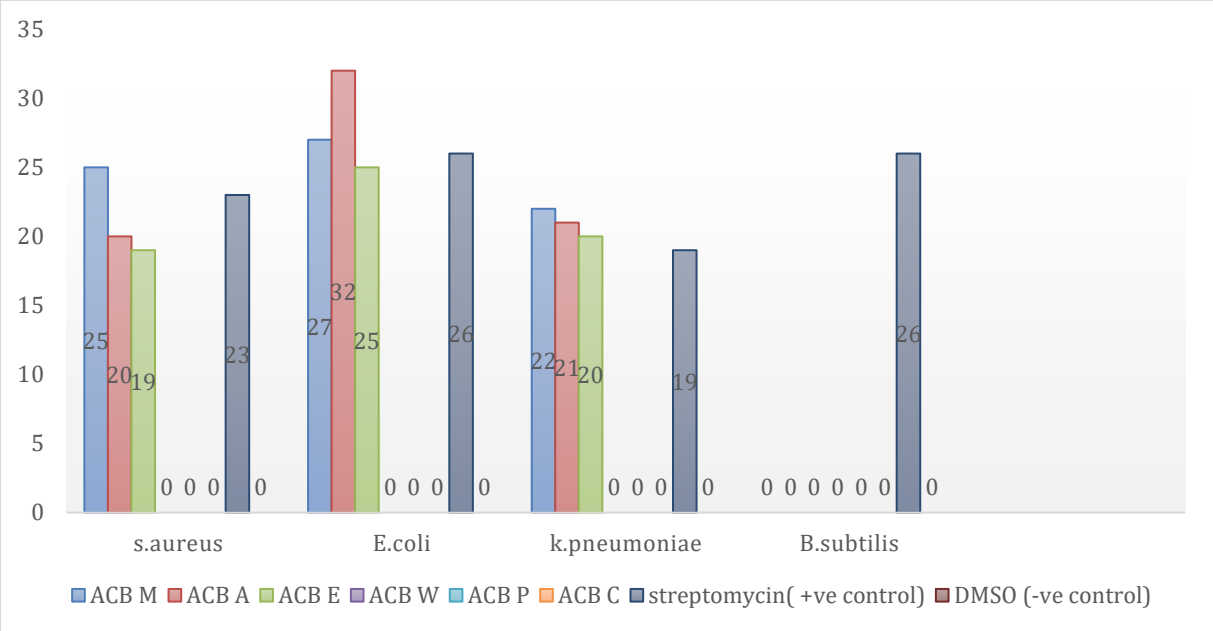
All *Adina cordifolia* extracts (except ACB W against B. subtilis) demonstrated measurable antibacterial activity against *S. aureus, E. coli*, and *K. pneumoniae*, but none showed activity against *B. subtilis*. The leaves and stems of *Adina cordifolia* exhibited minimal zones of inhibition, suggesting a negligible contribution to antimicrobial activity(Mohan SC et al.,2014) . Conversely, the methanolic and acetone extracts of *Adina cordifolia* bark demonstrated significant zones of inhibition, indicating strong antimicrobial potential.

	S.aureus	*B.subtilis*	*E.coli*	*k.pneumoniae*
ACB M	25mm	0mm	27mm	22mm
ACB A	19mm	0mm	32mm	21mm
ACB E	22mm	0mm	25mm	20mm
ACB W	0mm	0mm	0mm	0mm
ACB P	0mm	0mm	0mm	0mm
chloroform	0mm	0mm	0mm	0mm
Streptomycin	23mm	26mm	26mm	19mm
DMSO	0mm	0mm	0mm	0mm

Table 2.

Among the tested bacterial strains, *Escherichia coli* emerged as the most susceptible, particularly to the acetone (ACB A) and methanolic (ACB M) extracts of *Adina cordifolia* bark, exhibiting inhibition zones of 32 mm and 27 mm respectively—both surpassing the zone produced by the standard antibiotic streptomycin (26 mm). The acetone extract (ACB A) demonstrated the highest overall antibacterial activity, consistent with literature reports indicating that acetone-based plant extracts often exhibit greater efficacy than those prepared with methanol or ethanol (S. Maji et al., 2010). The methanolic extract (ACB M) also showed substantial and broad-spectrum activity against *E. coli (27 mm), S. aureus (25 mm), and K. pneumoniae (22 mm*), aligning with findings that methanol effectively extracts a wide range of antimicrobial phytoconstituents (Borges et al., 2020). The ethanolic extract (ACB E) produced moderate but consistent inhibition zones, whereas the aqueous extract (ACB W) exhibited minimal activity, likely due to poor solubility of bioactive compounds in water—a trend commonly observed in antimicrobial studies (Alfredi A. et al., 2023). Petroleum ether (ACB P) and chloroform (ACB C) extracts displayed negligible to no

activity, further supporting the inference that essential antimicrobial constituents are not effectively extracted by these non-polar solvents. Streptomycin served as a positive control, validating bacterial susceptibility, particularly in *Bacillus subtilis* (26 mm), where none of the plant extracts were effective. Dimethyl sulfoxide (DMSO), used as a negative control, exhibited no inhibitory effect, confirming that the observed antimicrobial activity originated solely from the plant extracts



(Fig.1) comparison of antimicrobial activity of *Adina cordifolia* bark extract and streptomycin against pathogenic bacteria.

Fig 2. Zone of Inhibition in *E. coli*: Streptomycin vs. Active ACB Extracts

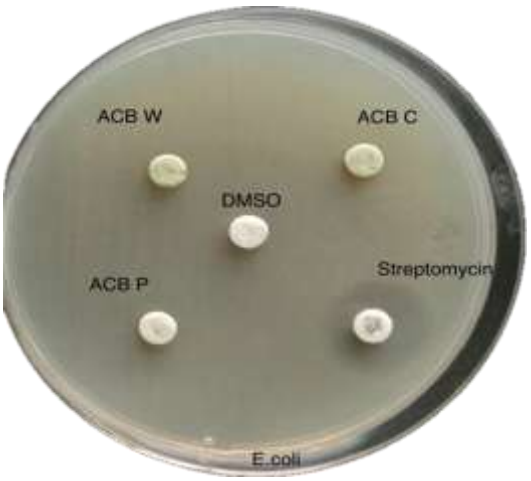


Fig 3. zone of Inhibition in *E.coli* Streptomycin vs. Inactive ACB extracts

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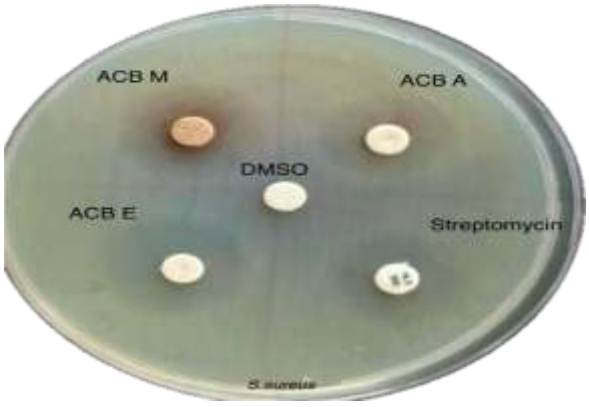

Fig 4. Zone of Inhibition in *S.aureus* Streptomycin vs. Active ACB extracts

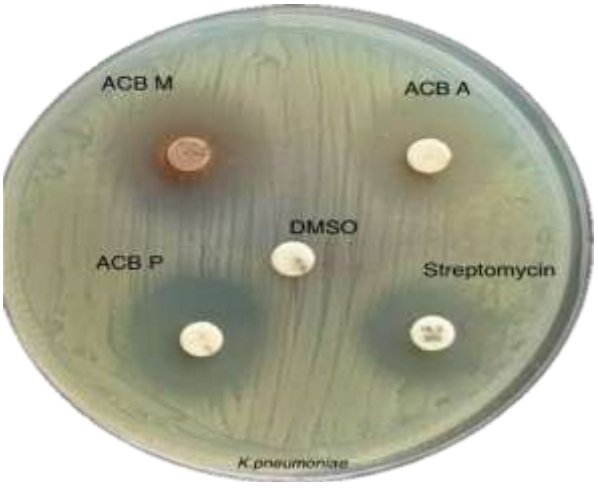

Fig 5.zone of Inhibition *K.pneumoniae* : Streptomycin Vs.Active ACB extracts

Antifungal Activity of *Adina cordifolia* Bark Extracts Against *Candida albicans* and *Aspergillus niger*

The antifungal activity of various *Adina cordifolia* bark (ACB) extracts was evaluated against *Candida albicans* and *Aspergillus niger* using the disc diffusion method. The results revealed that all organic solvent extracts exhibited considerable inhibitory effects against *candida albicans* and no inhibition zone against *Aspergillus niger* , whereas the aqueous extract showed no inhibition zone against *candida albicans.*

Among the extracts tested, the acetone extract (ACB A) demonstrated the highest antifungal activity with a zone of inhibition measuring 25 mm, followed closely by the petroleum ether extract (ACB E) at 24 mm, and the methanolic extract (ACB M) at 21 mm. In contrast, the aqueous extract(ACB W),petroleum ether(ACB P),chloroform extract(ACB C) showed no antifungal activity (0 mm). Fluconazole, used as the positive control, exhibited a clear zone of inhibition measuring 12 mm, whereas no inhibitory activity was observed for the negative control (DMSO), which showed a 0 mm zone. These findings suggest that the antifungal phytoconstituents present in ACB are predominantly soluble in non-polar or semi-polar solvents, as evidenced by the lack of activity in the aqueous extract. The higher inhibition zones produced by ACB extracts compared to Fluconazole indicate the presence of potent antifungal agents, possibly terpenoids, flavonoids, or alkaloids, which are known to be effectively extracted by organic solvents. The considerable efficacy of ACB A and ACB E extracts highlights their potential as sources of bioactive compounds for antifungal drug development.

Extracts	***Candida albicans* (inhibition zone in mm)**	***Aspergillus niger* (inhibition zone in mm)**
ACB M	21	0
ACB A	25	0
ACB E	24	0
ACB P	0	0
ACB C	0	0
Fluconazole(+ve control)	12	6
DMSO (-ve control)	0	0

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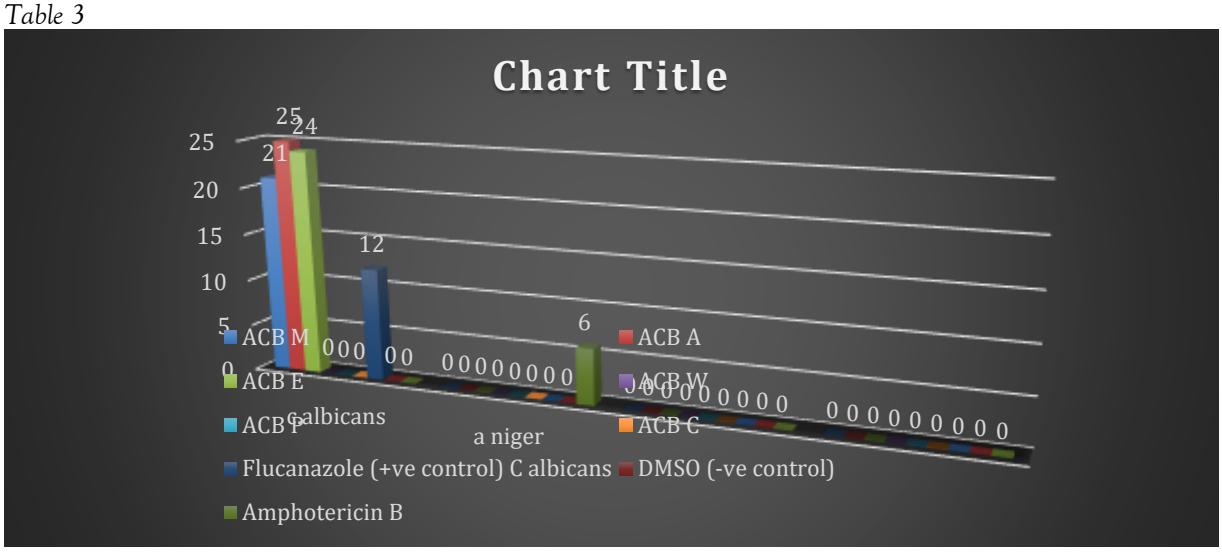
Table 3



(Fig.6) antifungal activity of *Adina cordifolia* bark extract and fluconazole against *candida albicans.*

Fig 7. Comparative Antifungal Efficacy of ACB Extracts Vs Fluconazole Against C. albicans

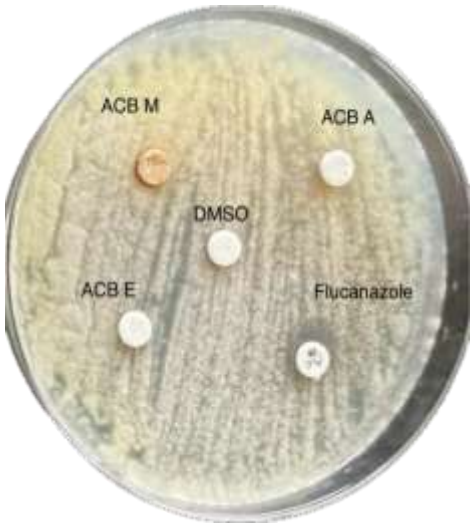


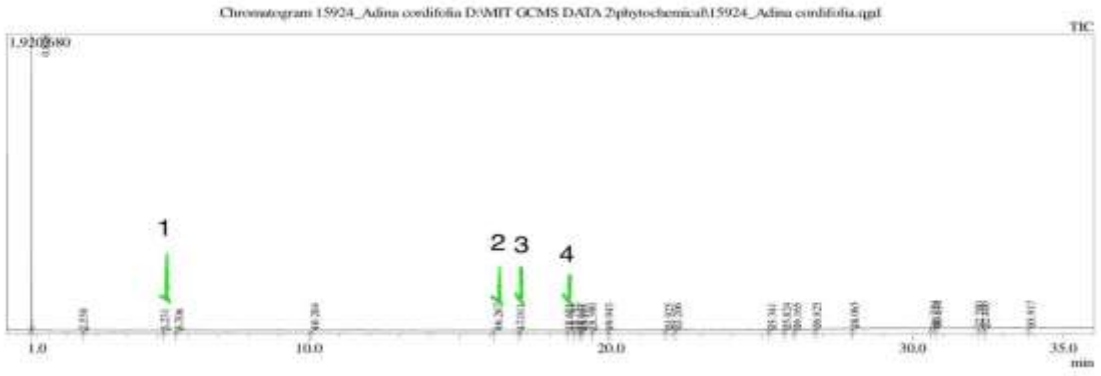
Fig 8. Comparative Antifungal Efficacy of ACB Extracts Vs Fluconazole against A. niger

GCMS analysis :-

The bark extract of *Adina cordifolia* demonstrated antimicrobial activity against certain microbial strains. To explore the potential compounds contributing to this effect, GC-MS analysis was conducted. The results indicated the probable presence of several phytochemicals that may be associated with antimicrobial properties.

The phytochemical composition of the methanolic bark extract of *Adina cordifolia* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS). The resulting chromatogram revealed several distinct peaks, each corresponding to a bioactive compound based on retention time and mass spectral data. For ease of identification and discussion, the major peaks were labeled numerically as Peak 1, Peak 2, Peak 3, and Peak 4, corresponding to Compound Benzaldehyde,(4-amino-5-ethul-1,2,4-triazol-3-yl)hydrazone, Alfaxalone, 2,3,5-Trimethyldiphenylsulfone, and 4-(3,4Dimethoxyphenyl)-3-(methoxycarbonyl)but_3-enoic acid, respectively.

Fig 9. Chromatographic Profile of Bioactive Constituents in Ethanolic Bark Extract of Adina cordifolia



NO.	Co mpound name	Retention time(min)	Area %	Known/Reported Antimicrobial activity
1.	Benzaldehyde,(4-amino-5-ethyl-1,2,4-triazol-3-yl)hydrazone	5.231	1.17	Yes-triazolederivatives known for antimicrobial properties
2.	Alfaxalone	16.110	1.98	Weak evidence – some steroid analogues show antifungal potentials
3.	2,3,5-Trimethyldiphenylsulfone	17.011	1.09	Yes-sulfones are documented to exhibit broad antimicrobial effects
4.	4-(3,4-Dimethoxyphenyl)-3-(methoxycarbonyl)but-3-enoic acid	18.601	1.88	Yes-phenolic ester ,likely antimicrobial due to phenyl ring and methoxy groups

Table 4

The structural framework of Benzaldehyde, (4-amino-5-ethyl-1,2,4-triazol-3-yl)hydrazone closely resembles hydrazone-bearing 1,2,4-triazole derivatives previously reported by (A.N.Yankina et al.,2022)16). These scaffolds are pharmacologically significant due to the presence of a triazole nucleus and hydrazone linkage, both of which are known to contribute to antimicrobial activity. Certain sulfone derivatives can act as

antibiotic enhancers specially combined with amino-glycosides (R Zhang et al.,2020)(17). Although alfaxalone itself lacks antimicrobial activity, multiple studies report that steroidal analogues can possess significant antifungal properties. Notably, steroid saponins (e.g., TTS-12) achieved MIC_{80} values of 4.4 µg/mL against fluconazole-resistant *Candida albicans*, with successful in vivo efficacy (JD Zhang et al.,2005)(18). Structurally related phenolic analogues, particularly those bearing methoxy or carboxyl groups, have demonstrated notable bioactivity, suggesting that similar derivatives may exhibit comparable antimicrobial potential(DS wavhal et al., 2025)(19).

Minimum Inhibitory Concentration(MIC) :

Among the various microbial strains and plant extracts tested, only the methanolic and acetone extracts of *Adina cordifolia* bark exhibited significant antimicrobial activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*, and *Candida albicans*. Therefore, these two extracts were selected for Minimum Inhibitory Concentration (MIC) determination, and the results are presented below.

Microorganism	ACB M MIC (ug/ml)	ACB A MIC (ug/ml)	Streptomycin MIC (ug/ml)	Fluconazole MIC (ug/ml)
E.coli	4	2	30	-
k.pneumoniae	8	32	30	-
s.aureus	8	32	30	-
c.albicans	4	4	-	25

Table 5

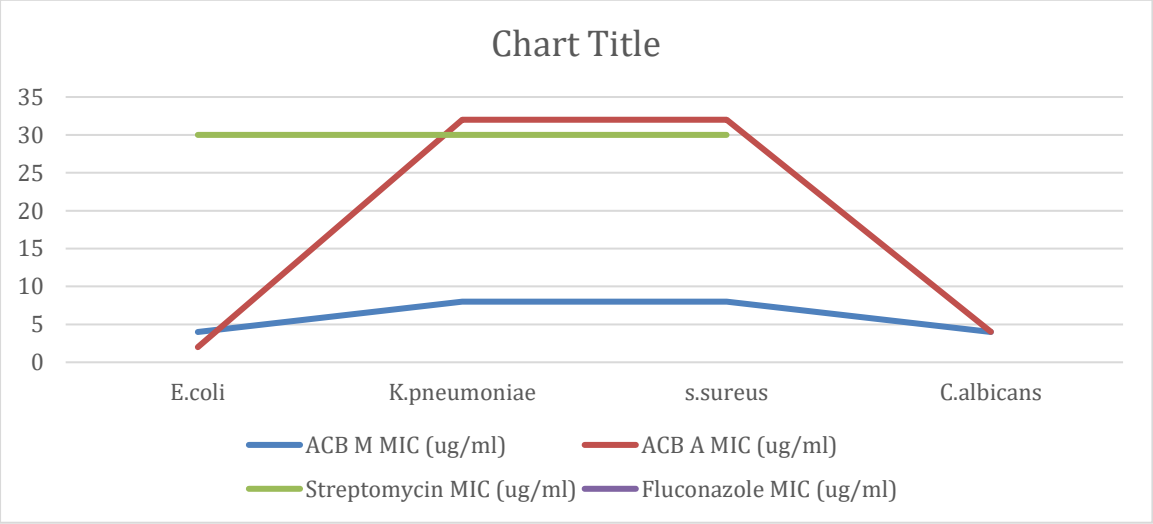


Fig 10. Comparative MIC Values of Methanol and Acetone Extracts of Adina cordifolia Against Clinical Pathogens

The MIC evaluation of *Adina cordifolia* bark extracts revealed promising antimicrobial potential. The methanolic extract (ACB M) demonstrated consistent inhibitory effects against Gram-negative (*E. coli*, *K. pneumoniae*) and Gram-positive (*S. aureus*) bacteria, with MIC values ranging between 4–8 µg/mL. Notably, both ACB M and ACB A were equally effective against *Candida albicans* (MIC = 4 µg/mL), suggesting the presence of phytoconstituents with potential antifungal properties. The superior activity of ACB A against *E. coli* (MIC = 2 µg/mL) may be attributed to the higher solubility of non-polar bioactives in acetone, facilitating better cell membrane penetration in Gram-negative bacteria. However, the reduced activity of ACB A against *K. pneumoniae* and *S. aureus* (MIC = 32 µg/mL) could indicate the selective solubility of certain phytochemicals in methanol that are effective against these pathogens.

When benchmarked against Streptomycin and Fluconazole, the MIC values of ACB extracts—particularly for *E. coli* and *Candida albicans*—approach pharmacological relevance, warranting further phytochemical analysis and compound isolation studies.

CONCLUSION:

Antibacterial activity: The antimicrobial screening highlights that acetone and methanol extracts of *Adina cordifolia* bark possess notable antibacterial activity, especially against *E. coli and S. aureus*. The absence of inhibition against *B. subtilis* across all plant extracts suggests specificity in the extract's phytochemical targeting. This data supports the use of *A. cordifolia* in ethnomedicine and underscores its potential for developing novel antibacterial agents, particularly against Gram-negative pathogens.

Antifungal activity : The present study establishes the antifungal efficacy of *Adina cordifolia* bark extracts against *Candida albicans*, with the acetone and petroleum ether extracts showing superior activity compared to the standard antifungal drug Fluconazole. The absence of activity in the aqueous extract indicates that the active constituents are not water-soluble, further emphasizing the importance of solvent selection in phytochemical extractions. These results support the traditional use of *Adina cordifolia* in ethnomedicine and suggest its potential as a promising candidate for the development of novel antifungal agents. Further studies involving isolation, characterization, and mechanism of action of the bioactive compounds are recommended.GC-MS analysis of the methanolic extract revealed the presence of several compounds with reported antimicrobial potential. These findings validate the traditional use of *A. cordifolia* in ethnomedicine and suggest its suitability as a source of natural antifungal agents. Further studies, including MIC determination, bioassay-guided isolation, and molecular mechanism exploration, are warranted to identify and characterize the active principles responsible for antifungal activity.

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