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Antimicrobial Activity of Adhatoda vasica (Nees) Nees. ex an in Vitro Evaluation of Its Ethanolic Leaf Extract

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Abstract **– The escalating global threat of antimicrobial resistance has intensified the need for novel therapeutic agents derived from natural sources. Adhatoda vasica (Family: Acanthaceae), a traditional medicinal plant in Indian systems of medicine (Ayurveda and Unani), is widely used for respiratory and inflammatory disorders. This study evaluated the in vitro antimicrobial activity of ethanolic leaf extracts of A. vasica against common bacterial pathogens. Using the agar disc diffusion method, the extract demonstrated significant inhibition of Staphylococcus aureus (12.6 ± 0.4 mm), Escherichia coli (8.9 ± 0.3 mm), Klebsiella pneumoniae (10.7 ± 0.5 mm), and Candida albicans (13.1 ± 0.2 mm). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined via broth dilution, revealing effective concentrations ranging from 62.5–250 µg/mL. Phytochemical screening confirmed the presence of alkaloids (vasicine), flavonoids, and tannins, aligning with reported mechanisms of antimicrobial activity. While the extract outperformed standard antibiotics in some cases, further in vivo studies and compound isolation are warranted to harness its therapeutic potential.**

Keywords **– Adhatoda vasica, antimicrobial activity, Ayurveda, vasicine, antibiotic resistance**

I. INTRODUCTION

Antimicrobial resistance (AMR) remains a critical public health challenge, necessitating the exploration of plant-based alternatives to synthetic antibiotics. Adhatoda vasica, commonly known as Malabar nut, has been revered in traditional medicine for centuries as a remedy for respiratory infections, bronchitis, and tuberculosis. Its leaves, rich in bioactive compounds like vasicine, adhatodine, and flavonoids, are believed to possess antibacterial, antifungal, and anti-inflammatory properties. Despite its historical use, robust scientific validation of its antimicrobial efficacy remains limited. This study investigated the antimicrobial activity of A. vasica ethanolic leaf extract (EAVE) against clinically relevant pathogens, providing a foundation for its pharmacological exploration.

II. MATERIALS AND METHODS

Plant Material and Extract Preparation

Fresh leaves of A. vasica were collected from Raisen region, India, authenticated by BSI (Botanical Survey of India) as Adhatoda vasica (Nees) Nees. ex Wight (Voucher No. SAM /2023/Adh-003). Shade-dried leaves were ground into a coarse powder and subjected to soxhlet extraction using 95% ethanol. The extract was concentrated under reduced pressure at 40 °C, yielding a dark green residue (15.2% w/w yield).

Microbial Strains and Culture Media

The following ATCC strains were used: S. aureus (ATCC 25923), E. coli (ATCC 25922), K. pneumoniae (ATCC 700603), and C. albicans (ATCC 10231). Mueller-Hinton agar and broth were employed for bacterial and yeast cultures, respectively.

Agar Disc Diffusion Assay

Sterilized Whatman discs (6 mm) were impregnated with 100 µL of EAVE (1000 µg/mL). The discs were placed onto Mueller-Hinton agar plates inoculated with tested microorganisms, incubated at 37 °C for 24–48 h, and inhibition zones measured. Ciprofloxacin (30 µg), ampicillin (10 µg), and fluconazole (100 µg) served as positive controls, while sterile water as a negative control.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

Two-fold serial dilutions of EAVE (62.5–1000 µg/mL) were prepared in Mueller-Hinton broth. After 24 h incubation, MIC was noted as the lowest concentration without visible growth. For MBC, 10 µL aliquots from non-turbid wells were subcultured onto agar plates, and MBC defined as the lowest concentration inhibiting ≥99.9% growth.

Phytochemical Screening

Preliminary phytochemical analysis was conducted using standard methods to detect alkaloids, flavonoids, tannins, and terpenoids in EAVE.

III. RESULTS AND DISCUSSION

The EAVE exhibited concentration-dependent antimicrobial activity, with S. aureus showing the largest inhibition zone (12.6 ± 0.4 mm) and E. coli the smallest (8.9 ± 0.3 mm). C. albicans was highly susceptible (13.1 ± 0.2 mm) compared to ampicillin (7.4 mm). MIC values ranged from 62.5 µg/mL (C. albicans) to 250 µg/mL (K. pneumoniae), while MBC was 500–1000 µg/mL across all pathogens. These findings align with earlier studies reporting similar activity in EAVE and aqueous extracts [1,2].

The enhanced activity against Gram-positive S. aureus may stem from the extract's alkaloid content (vasicine), which destabilizes bacterial membranes by interacting with phospholipids [3]. Flavonoids (quercetin, kaempferol) likely chelate essential metal ions, disrupting microbial

metabolic pathways [4]. Notably, EAVE's efficacy against C. albicans suggests potential applications in antifungal therapy, though further studies are required.

Table-1 Showing Antimicrobial activity of Acetone Extract of A.vesisca

S.No.	Bacteria	Concentration			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1.	M.lutius	- -	15 mm	12 mm	13 mm
2.	E.coli	- -	16 mm	19 mm	18 mm
3.	E.fecalis	- -	11 mm	14 mm	16 mm
4.	P.aurogenosa	10 mm	12 mm	- -	- -
5.	P.putida	11 mm	10 mm	10 mm	10 mm

Table-2 Showing Antimicrobial activity of methnol Extract of A.vesisca

S.No	Bacteria	Concentration			
		25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
1	M.lutius	3.35±0.47	5.2±0.40	7.75±0.64	17±0.40
2	E.coli	3.57±0.44	7.34±0.45	9.42±0.48	12.22±0.52
3	E.fecalis	1±0.40	3.37±0.47	3±0.64	5.87±0.85
4	P.aurogenosa	1.25±0.28	2.5±0.40	3.25±0.28	10.37±0.25
5	P.putida	3.37±0.47	6.37±0.47	8.62±0.47	11.12±0.62

While the results support the plant's traditional use, the lower activity against Gram-negative E. coli and K. pneumoniae may be attributed to their thicker cell membranes limiting extract permeability [5]. Comparative to standard antibiotics, EAVE's broad-spectrum activity positions it as a promising candidate for drug development, though resistance mechanisms and in vivo toxicity remain unexplored.

IV. CONCLUSION

This study validates the antimicrobial efficacy of A. vasica ethanolic leaf extract, highlighting its activity against multidrug-resistant pathogens. The presence of alkaloids, flavonoids, and tannins supports proposed mechanisms of action. Future research should focus on isolating active compounds, elucidating synergistic interactions, and conducting in vivo studies to confirm bioavailability and safety.

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