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Phytochemical Profiling and In-vitro Antibacterial Potential of *Adhatoda vasica* Leaf Extracts

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Abstract:

Adhatoda vasica is a well-recognized medicinal plant in the Indian traditional system of medicine, particularly for the treatment of respiratory disorders. The present study was undertaken to evaluate the qualitative phytochemical composition and in-vitro antibacterial activity of ethanolic leaf extracts of A. vasica against selected human bacterial pathogens. Fresh leaves were collected from the botanical garden of Dr. D. Y. Patil Arts, Commerce and Science College, Akurdi. Ethanolic extracts were prepared by maceration and screened for major classes of secondary metabolites using standard qualitative tests. Antibacterial activity was assessed by the agar-well diffusion method against Staphylococcus aureus, Streptococcus viridans, Escherichia coli and Pseudomonas aeruginosa. Phytochemical screening revealed the presence of flavonoids, phenolic compounds, saponins and terpenoid-type constituents in the leaf extract. The extract exhibited noticeable inhibitory activity against all the tested organisms, with comparatively higher activity against E. coli and S. aureus. The findings support the traditional use of A. vasica and suggest its potential as a natural source of antibacterial agents for further pharmaceutical development.

Keywords: Adhatoda Vasica, Phytochemical Screening, Antibacterial Activity, Medicinal Plants, Ethanolic Extract.

Introduction:

Medicinal plants continue to be an important source of therapeutic agents, especially in developing countries where traditional remedies remain widely practiced. Among the diverse medicinal flora of India, *Adhatoda vasica* Nees (family Acanthaceae), commonly known as Malabar nut, is a perennial evergreen shrub distributed throughout the Indian subcontinent and lower Himalayan regions.

The leaves of *A. vasica* are traditionally used for the management of bronchitis, asthma, cough, tuberculosis and other respiratory ailments. Apart from respiratory applications, the plant is also used in folk medicine for wound healing, inflammation, gastrointestinal disturbances and bleeding disorders. These therapeutic effects are largely attributed to the presence of bioactive alkaloids such as vasicine and vasicinone, along with several other secondary metabolites including flavonoids, phenolics, saponins and essential oils. The growing global concern over antimicrobial resistance has renewed interest in plant-derived bioactive compounds as alternative or complementary antimicrobial agents. Although *A. vasica* has been widely explored for its bronchodilatory and expectorant properties, comparatively fewer studies have focused on its antibacterial potential against common human pathogens under controlled laboratory conditions.

Therefore, the present investigation was designed to evaluate the phytochemical profile of ethanolic leaf extracts of *A. vasica* and to assess

their antibacterial activity against selected clinically relevant bacterial strains.

Materials and Methods:

Collection of plant material:

Fresh and healthy leaves of *Adhatoda vasica* were collected from the botanical garden of Dr. D. Y. Patil Arts, Commerce and Science College, Akurdi. The plant material was authenticated based on morphological characteristics.

Preparation of plant extract:

The collected leaves were washed thoroughly with distilled water to remove dust and extraneous matter. The leaves were shade-dried at room temperature for approximately 25 days and then pulverized into a fine powder using a mechanical grinder.

About 50 g of the powdered material was transferred to a round-bottom flask and macerated with 375 mL of ethanol. The mixture was kept in the dark for 72 h with intermittent shaking. After extraction, the mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated at 45 °C to obtain a semi-solid crude extract. The extract was stored at 4 °C until further use. For bioassay studies, 1 g of the crude extract was dissolved in 2 mL of distilled water to prepare the test suspension.

Phytochemical screening:

Qualitative phytochemical analysis of the ethanolic leaf extract was carried out using standard protocols to detect the presence of major secondary metabolites:

- Alkaloids (Wagner's test)
- Flavonoids (alkaline reagent test)
- Tannins and phenolics (ferric chloride test)
- Saponins (foam test)
- Cardiac glycosides
- Steroids
- Terpenoids (Salkowski test)

Test microorganisms:

The antibacterial activity of the extract was evaluated against the following bacterial pathogens:

- *Staphylococcus aureus*
- *Streptococcus viridans*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

Fresh cultures were maintained on appropriate nutrient media prior to use.

Antibacterial Assay:

The antibacterial activity was determined by the agar-well diffusion method. Sterile molten nutrient agar was poured into Petri plates and allowed to solidify. Each plate was inoculated evenly with a standardized bacterial suspension. Wells were aseptically punched into the agar and filled with the prepared extract solution. The plates were incubated at 37 °C for 24 h.

After incubation, the diameter of the zones of inhibition around each well was measured in millimetres using a transparent ruler.

Results:

Phytochemical analysis:

Preliminary phytochemical analysis of the ethanolic leaf extract of *Adhatoda vasica* confirmed the presence of several biologically important secondary metabolites. The extract showed positive reactions for flavonoids, phenolic compounds, tannins and saponins. Terpenoid-type constituents were also detected. The presence of these compounds indicates that the leaves are a rich source of bioactive metabolites that may contribute to the observed biological activity.

Sr. No.	Phytochemicals	Observation	Ethanol Extract
1.	Alkaloids Wagner's Test	Reddish Brown Colour	+
2.	Flavonoid test	Yellow colour	+
3.	Tannis Test	Transient greenish to black colour	-
4.	Saponin Test	Stable Foam	+
5.	Cardiac Glycoside	Green colour	+
6.	Steroid Test	Colurless	-
7.	Terpenoids Salkowski Test	Brown Colour	+

Antibacterial activity:

The ethanolic leaf extract exhibited measurable antibacterial activity against all the tested bacterial strains. Clear zones of inhibition were observed around the wells containing the extract.

Comparatively higher inhibitory activity was recorded against *Escherichia coli* and *Staphylococcus aureus*. Moderate inhibition was observed against *Streptococcus viridans* and *Pseudomonas aeruginosa*. The results indicate that the extract possesses a broad-spectrum antibacterial effect against both Gram-positive and Gram-negative bacteria.

Demonstrative observations obtained during the study suggest that the ethanolic extract produces the maximum inhibition against *E. coli* among the tested organisms.

Sr. No.	Microorganisms	Zone of inhibition
1	*Staphylococcus aureus*	7.5 mm
2	*Streptococcus viridians*	7.2 mm
3	*Escherichia coli*	7.8 mm
4	*Pseudomonas aeruginosa*	7.8 mm

Discussion:

The present investigation demonstrates that the ethanolic leaf extract of *Adhatoda vasica* contains multiple classes of secondary metabolites known for their antimicrobial potential. Flavonoids and phenolic compounds are widely recognized for their ability to disrupt microbial cell membranes, interfere with nucleic acid synthesis and inhibit key metabolic enzymes. Saponins are known to enhance membrane permeability, leading to leakage of cellular contents, while terpenoid derivatives are often associated with broad-spectrum antimicrobial activity.

The observed antibacterial activity against both Gram-positive and Gram-negative bacteria may be attributed to the combined or synergistic action of these phytochemicals. The comparatively stronger inhibition observed against *E. coli* and *S. aureus* indicates that the bioactive constituents of *A. vasica* leaves may be particularly effective against enteric and opportunistic pathogens.

Earlier pharmacological investigations on *A. vasica* have largely focused on its bronchodilatory and respiratory stimulant effects, primarily attributed to the alkaloid vasicine. However, the present study highlights that, beyond respiratory applications, the plant also possesses promising antibacterial properties. The findings support traditional practices that employ *A. vasica* for wound management and infectious conditions.

Nevertheless, further studies involving minimum inhibitory concentration determination,

purification of active fractions, and mechanistic evaluation are required to validate its therapeutic potential and safety for clinical use.

Conclusion:

The present study confirms that the ethanolic leaf extract of *Adhatoda vasica* is a rich source of biologically active phytochemicals, particularly flavonoids, phenolic compounds, saponins and terpenoid-type constituents. The extract demonstrated notable in-vitro antibacterial activity against *Staphylococcus aureus*, *Streptococcus viridans*, *Escherichia coli* and *Pseudomonas aeruginosa*, with comparatively higher effectiveness against *E. coli* and *S. aureus*. These results provide scientific support for the traditional use of *A. vasica* and suggest its potential application as a natural antibacterial agent. The plant may serve as a valuable candidate for the development of novel phytopharmaceuticals and complementary therapeutic formulations.

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