



**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF  
LEAVES ETHANOLIC EXTRACT OF *ADHATODA VASICA* NEES.**

**SAGAR VIJAYRAO KATHALE\***

*Vikramshila Polytechnic, Darapur, Dist, Amravati.*

**ABSTRACT**

The development of new antimicrobial agents against resistant pathogens is of increasing interest. Therefore, the ethanolic extract from medicinal plant has been used traditionally as folk medicine over a period of time. The antimicrobial activity of *Adhatoda vasica* was assessed against some pathogen viz. *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* by Agar disc diffusion method the antimicrobial activity was measured at different concentration of 100 µg, 200 µg, 300 µg, 400 µg, and 500 µg. The plant material was extracted by using Soxhlet extractor with ethanol solvent and ethanolic extract was screened phytochemically for the presence of secondary metabolites like alkaloids, phenolics, glycosides, tannins, flavonoids, saponins etc.

**KEYWORDS:** *Adhatoda vasica*, *Ethanolic extract*, *Secondary metabolites*, *Antimicrobial activity*.



**SAGAR VIJAYRAO KATHALE**  
Vikramshila Polytechnic, Darapur, Dist, Amravati.

## INTRODUCTION

The medicinal flora act as a spring for synthetic drugs of traditional systems of medicine, herbal drugs, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and new chemical entities. Herbal medicine involves the use of plants for medicinal purposes. The term "Herb" includes roots, rhizomes, stems, bark, leaves, flowers, fruits, and seeds<sup>1</sup>. *Adhatoda vasica* Nees commonly known as 'Vasaka' or 'Arusha' in Sanskrit<sup>1-2</sup>. The Vasaka plant perennial, evergreen and highly branched with unpleasant smell and bitter taste, the plant lives for multiple seasons and retains its leaves throughout the year. It is a shrub 1.0 m to 2.5 m in height, with opposite ascending branches. The stem, leaf, flower, fruit and seeds were used traditionally as a folk medicine<sup>3</sup>. Vasaka is a bitter quinazoline alkaloid. The major alkaloids are vasicine and vasicinone which are present in all parts of the plant. The leaves contain several alkaloids includes vasicine<sup>4</sup>, vasicinone, vasicinol, adhatodine, adhatonine, adhvasinone, anisotine and peganine and other metabolites includes betaine, steroids carbohydrate and alkanes<sup>5</sup>. According to WHO (1993), 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study of plants to determine their antimicrobial active compounds is a comparatively new field<sup>6</sup>. Exhaustive literature survey reveals that *Adhatoda vasica* has antioxidant activities which may be due to phenolic contain and has the wide range of

biological activities such as antidiabetic, anti-jaundice<sup>7</sup>, antipyretic, antiinflammatory<sup>8</sup>, anti bleeding, antimicrobial<sup>9-12</sup>, bronchodilator, and expectorant activities<sup>13</sup>. Hence, becomes driving force behind this study.

## MATERIALS AND METHODS

### *Plant Collection And Identification*

Fresh leaves of *Adhatoda vasica* Nees free from disease were collected from medicinal garden of 'Vidya Bharati College of Pharmacy, Amravati'. The taxonomical identification of the plant was determined. The plant material was washed with 70% alcohol and then rinsed with sterilized distilled water, shade-dried and coarsely powdered in mixture grinder<sup>14</sup>.

### *Preparation Of Ethanolic Extract*

The ethanolic extract was removed from shade-dried coarsely powdered plant material with the help of Soxhlet extractor for 36 hours at temperature not exceeding to boiling point of solvent. Out of which 10 gm of sample was taken in beaker containing 10 times volume of 80% ethanol and boiled. It was centrifuged at 10,000 rpm for 20 min. supernatant was saved and re-extracted 5 times, with 80% ethanol. Then supernatant was evaporated to dryness, weight was measured.

### *Phytochemical Screening*

Ethanolic extract was subjected to preliminary Phytochemical screening for the evaluation of secondary metabolites. The details of the analysis given in table-1.

**Table 1**  
***Phytochemical analysis of ethanolic leaves extract of Adhatoda vasica***

S. No.	Metabolites	Test (s)	Observation	Inferences
1.	Phenolics	Extract+FeCl <sub>3</sub>	Intense colour	+
2.	Alkaloids	Dragondroffs reagent	Orange colour	+
3.	Glycosides	Anthrone + H <sub>2</sub> SO <sub>4</sub> +Heat	Purple or green	-
4.	Carbohydrates	Molish's reagent+ conc. H <sub>2</sub> SO <sub>4</sub>	Purple colour	+
5.	Flavonoids	Shinoda's test	Red colour	-
6.	Tannins	Extract+FeCl <sub>3</sub>	Intense colour	+
7.	Saponins	Xanthoprotein test	Formation of honey comb like froth.	-
8.	Fixed oils & Fats	Zn-HCl acid	Stains appear after drying	-

**Where: - = Absent, + = Present**

**ANTIMICROBIAL ACTIVITY**

The media and the test bacterial cultures were poured into Petri dishes (Muller Hinton agar media). The test strain (0.2ml) was inoculums size (10<sup>8</sup> cells/ml) care was taken to ensure proper homogenization. The plant extracts were tested for antimicrobial activity in the agar disk diffusion assay.

**Bacterial strain used for assay**

*Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*.

**Agar disk diffusion method:**

The inoculation of microbes was prepared from bacterial culture. About 15- 20ml of Muller- Hinton agar medium was poured in the sterilized Petri dish and allows solidifying. One drop of bacterial strain was spread over the

medium with the help of rod. Sterile filter paper disk of 6mm diameter were impregnated with the different concentration like 100µg, 200µg, 300µg, 400µg and 500µg. of extract of *Adhatoda vasica*. The paper discs were allowed to evaporate and then after placed on the surface of the inoculated agar plates. Then the plates were incubated over night at 37°C for 24 hrs. At the end diameter of zone of inhibition was measured for the evaluation of antibacterial activity.

**RESULTS AND DISCUSSION**

The ethanol extract of *A.vasica* showed maximum activity against *staphylococcus aureus*, than *Proteus vulgaris* and *Pseudomonas aeruginosa* and the least activity was shown against *E. coli*. (Table: 2).

**Table 2**  
**Ethanolic extract of *Adhatoda vasica* against clinical pathogens (µg/ml)**

Pathogens	Zone of inhibition in (mm)				
	100µg	200µg	300µg	400µg	500µg
<i>Proteus vulgaris</i>	0.7	0.6	1	1	2
<i>Pseudomonas aeruginosa</i>	1	1	1	2	1
<i>Escherichia coli</i>	1.8	2	0.5	1	2
<i>Staphylococcus aureus</i>	2	1	2	2	3

The crude extracts obtained from the leaf of *Adhatoda vasica* exhibited the activity against *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The finding of the current study demonstrates that ethanolic extract of *Adhatoda vasica* showed the better activity against Gram-positive and Gram-negative organisms.

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