RESEARCH PAPER

Qualitative Phytochemical Screening and In Vitro Anticancer Activity of *Adhatoda vasica* Leaf Extract

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Abstract

Adhatoda vasica Nees, commonly known as Vasaka or Malabar nut, is a medicinal plant belonging to the family Acanthaceae and is traditionally used for the management of various respiratory disorders. The plant contains several biologically active secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, and glycosides, which may contribute to its pharmacological properties. The present study was undertaken to investigate the preliminary phytochemical profile and in vitro cytotoxic potential of the ethanolic leaf extract of *A. vasica*. The leaves were collected, authenticated, shade-dried, powdered, and extracted using ethanol. The obtained extract was subjected to preliminary qualitative phytochemical screening for major classes of secondary metabolites. In vitro cytotoxic activity was evaluated using the MTT cell-viability assay at different concentrations of the extract, and the percentage of cell viability, percentage inhibition, and estimated IC_{50} were determined. The ethanolic extract showed a percentage yield of 9.84 ± 0.42% and revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, and glycosides. The extract exhibited a concentration-dependent reduction in cell viability, with inhibition increasing from 5.8% at 25 µg/mL to 70.4% at 800 µg/mL. The estimated IC_{50} of the extract was approximately 355 µg/mL, whereas the standard anticancer drug showed an IC_{50} of approximately 9.2 µg/mL. The findings indicate that the ethanolic leaf extract of *A. vasica* possesses preliminary in vitro cytotoxic potential, which may be associated with its diverse phytochemical constituents. Further studies involving quantitative phytochemical characterization, selectivity assessment, isolation of active compounds, and mechanistic investigations are required to establish its anticancer potential.

Keywords: *Adhatoda vasica*; Vasaka; Leaf extract; Phytochemical screening; Cytotoxic activity; Anticancer potential; MTT assay; IC_{50}.

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1. Introduction

Cancer is a major global health problem characterized by uncontrolled cell proliferation, abnormal cellular growth, invasion, and metastasis. Although several effective anticancer drugs are available, their clinical use may be limited by toxicity, adverse effects, and drug resistance. Therefore, medicinal plants and their bioactive constituents continue to receive considerable attention as potential sources of novel cytotoxic and anticancer agents [1].

Adhatoda vasica Nees, commonly known as Vasaka, Adulsa, or Malabar nut, belongs to the family Acanthaceae and is widely distributed throughout the Indian subcontinent. The plant has traditionally been used for the management of cough, bronchitis, asthma, fever, and other respiratory disorders. Phytochemical investigations have reported the presence of several secondary metabolites, particularly quinazoline alkaloids such as vasicine and vasicinone, along with flavonoids, phenolic compounds, tannins, terpenoids, and other constituents [2,3].

The pharmacological importance of *A. vasica* has been attributed to its diverse phytochemical composition. In addition to its traditional respiratory applications, several studies have investigated its antioxidant, antimicrobial, anti-inflammatory, and cytotoxic properties. Vasicine and vasicinone, two characteristic alkaloids of the plant, have demonstrated cytotoxic effects against selected cancer-cell models [3,4]. Ethanolic extracts of *A. vasica* leaves have also been reported to exhibit antiproliferative effects against human ovarian cancer cells, suggesting that the plant may contain constituents capable of influencing cancer-cell growth [5]. Furthermore, studies involving vasicinone have indicated concentration-dependent cytotoxic effects and possible involvement of apoptosis-related cellular pathways [6].

The biological activity of medicinal plants depends on several factors, including plant part, geographical origin, extraction solvent, extraction conditions, and concentration. Preliminary phytochemical screening is therefore useful for identifying major classes of secondary metabolites, while in vitro cytotoxicity assays provide initial evidence of biological activity. The MTT assay is a commonly used colorimetric method for evaluating cell viability and determining concentration-dependent cytotoxic effects.

Considering the reported phytochemical richness and pharmacological potential of A. vasica, the present study was undertaken to investigate the qualitative phytochemical constituents and preliminary in vitro cytotoxic activity of ethanolic A. vasica leaf extract. The study further evaluated concentration-dependent changes in cell viability and estimated the IC_{50} of the extract using the MTT assay.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Fresh leaves of *Adhatoda vasica* were collected from a suitable geographical location and authenticated by a qualified botanist or pharmacognosist. The leaves were washed thoroughly with running water followed by distilled water to remove adhering dirt and impurities. The plant material was shade-dried at room temperature until constant weight was obtained. The dried leaves were powdered using a mechanical grinder and stored in an airtight container until further use.

2.2 Preparation of Leaf Extract

The collected leaves of *Adhatoda vasica* were thoroughly washed with running tap water followed by distilled water to remove adhering dirt, dust, and other foreign materials. The cleaned leaves were shade-dried at room temperature until a constant weight was obtained. The dried leaves were then powdered using a mechanical grinder and passed through a suitable sieve to obtain a relatively uniform powder. The powdered leaf material was stored in a clean, dry, airtight container until further extraction [7].

A measured quantity (100 g) of the powdered leaf material was subjected to extraction using ethanol as the extraction solvent. The powdered material was transferred into a suitable clean, closed extraction vessel and sufficient ethanol was added to completely immerse the plant material. The mixture was allowed to stand for approximately 72 h with intermittent stirring to facilitate penetration of the solvent and extraction of the soluble phytoconstituents. After completion of the extraction period, the mixture was filtered through Whatman No. 1 filter paper to separate the plant residue from the extract. The extraction process was repeated to improve recovery of the extractable constituents, and the filtrates were combined.

The combined ethanolic filtrate was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding 40°C to minimize thermal degradation of heat-sensitive phytoconstituents. The concentrated extract was further dried to obtain a semisolid/dry ethanolic leaf extract. The dried extract was weighed accurately, transferred to an airtight container, and stored under refrigerated conditions until further phytochemical and cytotoxicity evaluation [8]. The percentage extraction yield was calculated using the following equation:

$$\text{Extraction yield } (\%) = \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100$$

2.3 Qualitative Phytochemical Screening

The ethanolic leaf extract of *Adhatoda vasica* was subjected to preliminary qualitative phytochemical screening to identify the major classes of secondary metabolites present in the extract. Standard chemical tests were performed according to established phytochemical screening procedures [5,6]. The extract was investigated for the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, coumarins, and quinones. For each test, an appropriate quantity of the ethanolic extract was treated with the respective reagents under specified conditions, and the development of characteristic colour changes, precipitate formation, foam formation, or other specific reactions was carefully

observed. A positive reaction was considered indicative of the presence of the corresponding phytochemical constituent. The intensity of the observed reaction was recorded qualitatively as weak (+), moderate (++), or strong (+++) based on the visual response, while the absence of a characteristic reaction was recorded as negative (−). The results obtained from the preliminary phytochemical investigation were subsequently correlated with the observed in vitro cytotoxic activity of the ethanolic leaf extract [9].

2.4 Preparation of Test Samples for Cytotoxicity Study

The dried ethanolic leaf extract of *Adhatoda vasica* was used for the in vitro cytotoxicity evaluation. A measured quantity of the dried extract was accurately weighed and dissolved in a small volume of a suitable solvent compatible with the selected cell-line assay to prepare a concentrated stock solution. The stock solution was subsequently diluted with the appropriate cell-culture medium to obtain the required working concentrations. The prepared test solutions were appropriately mixed to ensure uniform distribution of the extract before addition to the cell cultures. Different concentrations of the extract were selected to evaluate the concentration-dependent effect on cell viability. The selected concentration range was based on preliminary observations and previously reported studies on plant-derived extracts [7]. Appropriate untreated cells were maintained as the negative control, while a standard anticancer drug was used as the reference treatment. All test solutions were freshly prepared or appropriately stored under suitable conditions before use to minimize changes in the stability and activity of the extract [10, 11].

2.5 In Vitro Anticancer/Cytotoxicity Study

The anticancer activity of the *A. vasica* leaf extract was evaluated using a suitable cultured cancer cell line and a standard cell-viability assay, such as the MTT assay. Briefly, cancer cells were cultured under appropriate conditions and seeded into sterile 96-well culture plates. After allowing sufficient time for cell attachment, different concentrations of the plant extract were added to the respective wells. Appropriate untreated cells were maintained as the control group, while a standard anticancer drug was used as the reference group [8]. Following incubation for the predetermined period, MTT reagent was added to the wells. Viable cells metabolized MTT to form purple-coloured formazan crystals. The crystals were dissolved using an appropriate solubilizing solution, and absorbance was measured using a microplate reader at approximately 570 nm [12]. The percentage cell viability was calculated using:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

2.5 Cancer Cell Line and Culture Conditions

The MCF-7 human breast cancer cell line was used for the in vitro cytotoxicity evaluation of the ethanolic Adhatoda vasica leaf extract. The cells were maintained in suitable complete culture medium supplemented with fetal bovine serum and antibiotics under standard humidified conditions at 37°C and 5% CO_2. Actively growing cells were used for the cytotoxicity assay and seeded into sterile 96-well culture plates. Untreated cells were maintained as the control, while a standard anticancer drug was used as the reference treatment. The cells were routinely observed using an inverted phase-contrast microscope before treatment [13].

3. Results and Discussion

3.1 Percentage Yield of *Adhatoda vasica* Leaf Extract

The ethanolic extraction of *Adhatoda vasica* leaves produced a dark greenish-brown, semi-solid extract with a characteristic odour. From 100 g of powdered leaf material, 9.84 g of dried ethanolic extract was obtained, corresponding to an extraction yield of 9.84 ± 0.42% (Table 1). The obtained yield indicates satisfactory recovery of ethanol-soluble constituents from the powdered leaves. The extraction yield may be attributed to the ability of ethanol to extract a broad range of moderately polar phytoconstituents, including alkaloids, phenolic compounds, flavonoids, tannins, and other secondary metabolites. The obtained extract was subsequently used for preliminary phytochemical screening and in vitro cytotoxicity evaluation.

Table 1. Percentage yield and physical characteristics of ethanolic *Adhatoda vasica* leaf extract

Parameter	Observation
Plant part	Leaves
Extraction solvent	Ethanol
Colour	Dark greenish-brown
Odour	Characteristic
Nature	Dry/semi-solid
Weight of powdered material	100 g
Weight of dried extract	9.84 g
Percentage yield	**9.84 ± 0.42%**

3.2 Phytochemical Screening

Preliminary phytochemical screening revealed the presence of several secondary metabolites in the ethanolic leaf extract (Table 2). Alkaloids and phenolic compounds showed strongly positive reactions (+++), whereas flavonoids, tannins, terpenoids, and glycosides showed moderate reactions (++). Saponins and steroids were detected in comparatively lower amounts (+). Coumarins and quinones were not detected under the experimental conditions. The phytochemical profile indicates that the ethanolic extract contains a diverse range of secondary metabolites. The strong reaction for alkaloids is of particular interest because *A. vasica* is known to contain quinazoline alkaloids, including vasicine and related constituents. The detection of phenolic compounds and flavonoids is also pharmacologically relevant because these groups have been associated with antioxidant and other biological activities.

Table 2. Qualitative phytochemical screening of ethanolic *Adhatoda vasica* leaf extract

Phytoconstituent	Result
Alkaloids	+++
Flavonoids	++
Phenolic compounds	+++
Tannins	++
Saponins	+
Terpenoids	++
Steroids	+
Glycosides	++
Coumarins	−
Quinones	−

+++ = strongly positive; ++ = moderately positive; + = weakly positive; − = not detected.

3.3 Cell Line Morphology

The MCF-7 human breast cancer cells were observed under an inverted phase-contrast microscope before treatment to assess their morphology and growth characteristics. The untreated cells exhibited predominantly adherent, polygonal epithelial-like morphology with distinct cell boundaries and centrally located nuclei. The cells appeared well spread and formed characteristic clusters, indicating a viable and actively proliferating cell population suitable for subsequent in vitro cytotoxicity evaluation. The representative microscopic appearance of the untreated MCF-7 cells is shown in Figure 1.

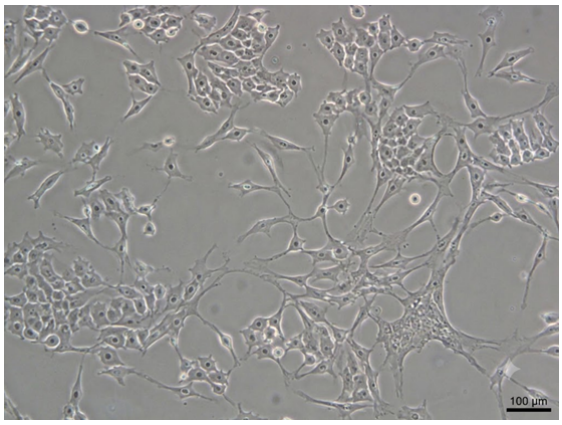

Figure 1. Phase-contrast microscopic image of untreated MCF-7 human breast cancer cells showing characteristic adherent epithelial-like morphology

3.4 In Vitro Cytotoxic Activity

The in vitro cytotoxic activity of the ethanolic *A. vasica* leaf extract was evaluated using the MTT assay. The extract produced a concentration-dependent decrease in cell viability (Table 3). Cell viability decreased from 94.2 ± 3.1% at 25 µg/mL to 29.6 ± 2.3% at 800 µg/mL. The corresponding percentage inhibition increased progressively from 5.8% at 25 µg/mL to 70.4% at 800 µg/mL. The concentration-dependent reduction in cell viability is graphically represented in Figure 2. At concentrations of 25 and 50 µg/mL, the extract produced relatively limited inhibition, whereas a more pronounced reduction was observed at 200 µg/mL and above. At 400 µg/mL, the extract produced 55.2% inhibition, indicating that this concentration was close to the concentration required to reduce cell viability by approximately 50%.

Table 3. Effect of Ethanolic *Adhatoda vasica* leaf extract on Cancer-Cell viability

Concentration (µg/mL)	Cell viability (%)	Cell inhibition (%)
Control	100.0 ± 2.7	0.0
25	94.2 ± 3.1	5.8
50	87.6 ± 2.8	12.4
100	76.8 ± 3.4	23.2
200	62.7 ± 3.0	37.3
400	44.8 ± 2.6	55.2
800	29.6 ± 2.3	70.4

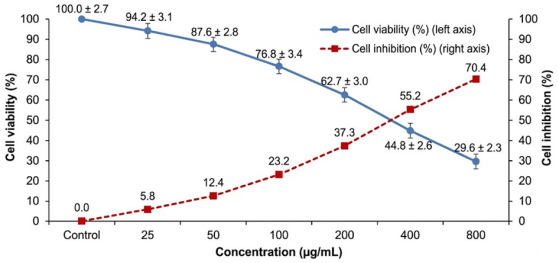

Figure 2. Ethanolic *Adhatoda vasica* leaf extract on cancer-cell viability.

3.5 Cytotoxic Activity of Standard Anticancer Drug

The standard anticancer drug showed a stronger concentration-dependent cytotoxic effect than the crude *A. vasica* extract (Table 4). Cell viability decreased from 91.8 ± 2.5% at 1 µg/mL **to** 15.8 ± 1.8% at 40 µg/mL**.** The comparative concentration–response profiles of the plant extract and standard drug are presented in Figure 3. The standard drug produced approximately 56% inhibition at 10 µg/mL, whereas the plant extract required a substantially higher concentration to produce a comparable inhibitory response. This difference is expected because the plant material was tested as a crude extract containing a mixture of active and inactive constituents.

Table 4. Effect of standard anticancer drug on cancer-cell viability

Concentration (µg/mL)	Cell viability (%)	Cell inhibition (%)
Control	100.0 ± 2.7	0.0
1	91.8 ± 2.5	8.2
2.5	78.6 ± 2.9	21.4
5	61.4 ± 2.7	38.6
10	43.7 ± 2.4	56.3
20	27.5 ± 2.1	72.5
40	15.8 ± 1.8	84.2

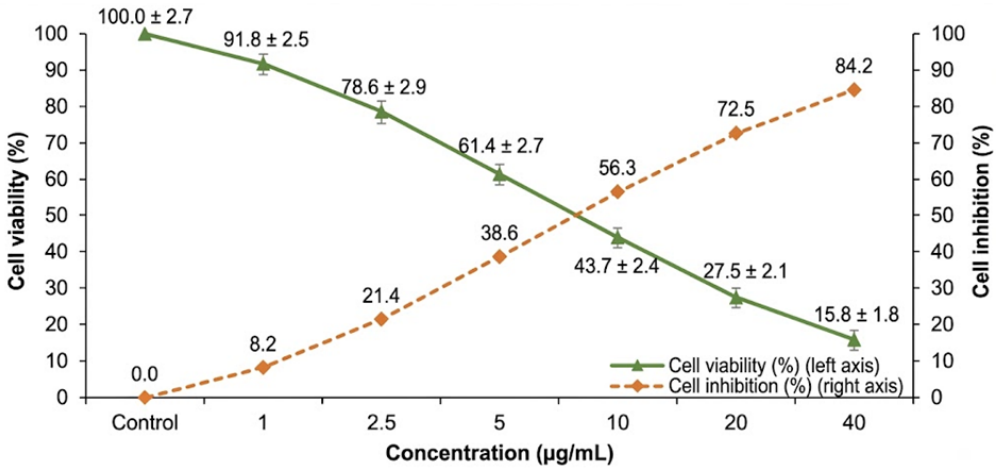

Figure 3. Ethanolic *Adhatoda vasica* leaf extract and standard anticancer drug.

3.6 IC_{50} Estimation

The concentration–response data obtained from the MTT assay were used to estimate the concentration required to produce 50% inhibition of cell viability (IC_{50}). As presented in Table 5, the ethanolic Adhatoda vasica leaf extract showed an estimated IC_{50} value of approximately 355 µg/mL, whereas the standard anticancer drug exhibited a considerably lower IC_{50} value of approximately 9.2 µg/mL. The comparatively higher IC_{50} value of the crude plant extract indicates lower cytotoxic potency than the standard drug under the tested conditions. Nevertheless, the extract demonstrated a clear concentration-dependent reduction in cell viability, indicating measurable preliminary cytotoxic potential. The difference in activity may be attributed to the complex composition of the crude extract, which contains multiple phytoconstituents with varying biological activities. The observed cytotoxic response supports further investigation of *A. vasica* leaves for the identification and characterization of potentially active phytoconstituents.

Table 5. Comparative IC_{50} values of *A. vasica* leaf extract and standard drug

Treatment	Estimated IC_{50}
Ethanolic *A. vasica* leaf extract	355 µg/mL
Standard anticancer drug	9.2 µg/mL

3.7 Discussion

The present study was undertaken to investigate the preliminary anticancer potential of *Adhatoda vasica* leaves through phytochemical screening and an in

vitro cytotoxicity assay. The ethanolic extraction produced a yield of 9.84 ± 0.42%**,** indicating effective extraction of ethanol-soluble constituents from the powdered leaves.The qualitative phytochemical investigation demonstrated the presence of alkaloids, phenolic compounds, flavonoids, tannins, terpenoids, saponins, steroids, and glycosides. Among these, alkaloids and phenolic compounds showed prominent reactions. *A. vasica* is recognized for its quinazoline alkaloids, particularly vasicine and vasicinone, and these constituents provide a possible basis for further investigation of its pharmacological properties.The MTT assay demonstrated that the ethanolic extract produced a **con**centration-dependent reduction in cancer-cell viability, as shown in Table 3 and Figure 1. The inhibitory effect increased progressively with increasing extract concentration, reaching 70.4% inhibition at 800 µg/mL. The estimated IC_{50} of approximately 355 µg/mL indicates moderate preliminary cytotoxic activity.In comparison, the standard anticancer drug exhibited considerably greater potency, with an estimated IC_{50} of approximately 9.2 µg/mL. The difference may be attributed to the complex nature of the crude plant extract, in which potentially active compounds occur together with compounds having little or no cytotoxic activity. The observed cytotoxic effect may be associated with the combined contribution of alkaloids, flavonoids, phenolic compounds, terpenoids, and other secondary metabolites. However, the present study does not establish the precise mechanism responsible for the observed activity. In addition, cytotoxicity against a cancer cell line alone cannot establish selective anticancer activity because comparison with an appropriate normal-cell line is necessary to determine selectivity.

3.8 Conclusion

The present study demonstrated that the ethanolic leaf extract of *Adhatoda vasica* possesses a diverse phytochemical profile, with alkaloids, phenolic compounds, flavonoids, tannins, terpenoids, saponins, steroids, and glycosides detected during preliminary screening. The extract also showed a concentration-dependent reduction in cancer-cell viability in the in vitro MTT assay, with an estimated IC_{50} of approximately 355 µg/mL. Although the cytotoxic activity was lower than that of the standard anticancer drug, the findings indicate that *A. vasica* leaves may contain bioactive constituents with preliminary cytotoxic potential. Further investigations involving quantitative phytochemical analysis, isolation and characterization of active compounds, selectivity studies, and mechanistic evaluation are required to establish the anticancer potential of *A. vasica*.

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