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RESEARCH ARTICLE

Phytochemical Analysis and Evaluation of *In Vitro* Antimitotic Activity of *Allamanda cathartica* Methanolic Extract

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Abstract: *Allamanda cathartica*, a perennial shrub of the Apocynaceae family, has garnered significant attention in pharmacological research due to its diverse bioactive compounds and potential therapeutic applications. This study aimed to investigate the phytochemical composition and evaluate the *in vitro* antimitotic activity of *A. cathartica* methanolic extract. Comprehensive phytochemical screening revealed the presence of carbohydrates, alkaloids, saponin glycosides, proteins, tannins, flavonoids, and amino acids in the extract. The antimitotic potential was assessed using a seed germination assay with Vigna radiata L. (green gram) seeds. The methanolic extract exhibited dose-dependent growth inhibition, with 100 µg/mL concentration demonstrating complete inhibition comparable to the standard drug methotrexate. At lower concentrations of 25 µg/mL and 50 µg/mL, the extract showed 50% and 65.5% inhibition, respectively. These findings suggest that *A. cathartica* possesses significant antimitotic properties, potentially attributable to its rich phytochemical profile. The study also highlights the plant's other pharmacological activities, including antioxidant, anti-inflammatory, wound healing, and antimicrobial properties. Given the growing interest in natural products for cancer therapeutics, *A. cathartica* presents a promising candidate for further investigation.

Keywords: *Allamanda cathartica*; Phytochemistry; Antimitotic activity; Traditional medicine; Seed germination assay.

1. Introduction

In recent years, there has been a resurgence of interest in natural products as sources of novel therapeutic agents. Among the vast array of medicinal plants, *Allamanda cathartica* Linn., commonly known as Golden Trumpet Flower, has emerged as a subject of significant scientific inquiry due to its diverse pharmacological properties and traditional uses [1]. This perennial shrub, belonging to the Apocynaceae family, is widely distributed across tropical and subtropical regions of the world, including parts of South America, Asia, and Africa [2]. *A. cathartica* has been used in various traditional medicine systems for centuries. In Trinidad, it has been employed to treat malaria and jaundice, while in Southeast Asia, its latex is used as a purgative [3]. The plant's versatility in traditional medicine has prompted researchers to investigate its phytochemical composition and potential therapeutic applications.

The phytochemical profile of *A. cathartica* is remarkably diverse, encompassing a wide range of secondary metabolites. These include flavonoids, polyphenols, iridoids, tannins, alkaloids, hydrocarbons, alcohols, esters, ethers, aldehydes, ketones, fatty acids, phospholipids, volatile compounds, phenol compounds, steroids, terpenes, lactones, and carbohydrates [4]. This rich array of bioactive compounds contributes to the plant's pharmacological activities and potential medicinal value.

Recent pharmacological studies have revealed a spectrum of bioactivities associated with *A. cathartica* extracts. Notable among these are its antioxidant, anti-inflammatory, antimicrobial, and wound healing properties [5]. The plant has also demonstrated potential anti-mitotic or antiproliferative effects, suggesting possible applications in cancer research [6]. Furthermore, investigations have uncovered membrane-stabilizing, thrombolytic, and hepatoprotective activities, broadening the scope of its potential therapeutic uses [7,8].

The antioxidant capacity of *A. cathartica* is particularly noteworthy. Studies have shown that extracts from various parts of the plant, especially the roots, exhibit significant free radical scavenging activity [9]. This property is attributed to the presence of enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), as well as high concentrations of polyphenols [10]. These antioxidant properties may contribute to the plant's potential in preventing oxidative stress-related

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disorders. The anti-inflammatory effects of *A. cathartica* have been demonstrated through various in vitro and in vivo studies. Ethyl acetate extracts of the flowers have shown particular promise in this regard, with mechanisms potentially involving membrane stabilization and inhibition of inflammatory mediators [11]. These findings suggest possible applications in the management of inflammatory conditions. *A. cathartica* has shown efficacy against a range of pathogenic microorganisms. Studies have reported activity against both gram-positive and gram-negative bacteria, as well as certain fungal species [12]. This broad-spectrum antimicrobial action underscores the plant's potential in developing new antimicrobial agents, particularly in an era of increasing antibiotic resistance.

The wound healing properties of *A. cathartica* have also garnered attention. Traditional uses of the plant for treating skin conditions have been supported by scientific studies demonstrating enhanced wound closure rates and improved tissue regeneration in experimental models [13]. These findings open up possibilities for developing new topical treatments for wound management. While the therapeutic potential of *A. cathartica* is promising, it is crucial to consider the potential toxicity associated with some parts of the plant. Studies have reported that consumption of leaves and plant juice in large quantities can cause diarrhea, and skin contact may lead to dermatitis in some individuals [14]. The primary aim of this study is to evaluate the therapeutic potential of *Allamanda cathartica*, with a specific focus on its antimitotic properties

2. Plant profile

2.1. Botanical description

Allamanda cathartica is an evergreen climbing shrub that can grow up to 1.5-3 meters high. Its leaves are leathery, yellow-green to dark green, and grow in whorls of two or four. The leaves are lance-shaped or ovoid, approximately 10-20 cm wide, with an acuminated apex and entire leaf margin [4]. The plant is characterized by its large, bright yellow trumpet-shaped flowers, which are terminally borne and measure 5-6.5 cm in diameter. The stems are covered with bristle-like hairs and contain milky sap [5].

Figure 1. Whole plant of *A. cathartica*

2.2. Taxonomical Classification

Domain: Eukaryote

Kingdom: Plantae

Phylum: Spermatophyta

Class: Dicotyledonae

Order: Gentianales

Family: Apocynaceae

Genus: Allamanda

Species: *Allamanda cathartica*

2.3. Phytochemistry

The phytochemical profile of *A. cathartica* is remarkably diverse, encompassing a wide range of secondary metabolites. These include flavonoids, polyphenols, iridoids, tannins, alkaloids, hydrocarbons, alcohols, esters, ethers, aldehydes, ketones, fatty acids, phospholipids, volatile compounds, phenol compounds, steroids, terpenes, lactones, and carbohydrates [6].

Different parts of the plant contain various phytoconstituents:

1. Leaves: β-sitosterol, β-amyrin, ursolic acid, sesquiterpenes, plumericin, and plumieride [7].
2. Stem and Bark: β-sitosterol, β-amyrin, ursolic acid, alkaloids, glucosides, and triterpenoids [8].
3. Flowers: Flavonoid compounds, quercetin, kaempferol, and hesperidin [9].

4. Roots: Iridoids, lactone, allamandin, glucosides, alkaloids, and triterpenoids [10].

2.4. Toxicity Studies

While *A. cathartica* shows promise in therapeutic applications, it is essential to consider its potential toxicity. The plant contains a cardio-toxic glycoside, and any part of the plant can cause dermatitis in susceptible individuals [11]. Studies have reported that consumption of leaves and plant juice in large quantities can cause diarrhea, although the specific compounds responsible for this effect have not been identified [11].

2.5. Traditional and Culinary Uses

In traditional medicine, various parts of *A. cathartica* have been used for different purposes [12-14]:

1. Flowers: Used as a laxative and antibiotic against Staphylococcus species.
2. Whole plant: Used to treat malaria and jaundice in Trinidad.
3. Latex: Used to treat colic and as a purgative in Southeast Asia
4. Leaves: Used to treat coughs and headaches.

3. Materials and methods

3.1. Plant Material Collection and Preparation

3.1.1. Plant Collection

Allamanda cathartica whole plants, including leaves, stems, flowers, and roots, were collected from local gardens in Tadepalligudem. The plant was identified and authenticated by botanist.

1.2 *Plant Material Preparation*

The collected plant material was thoroughly washed with distilled water to remove dirt and contaminants. The plant parts were separated and shade-dried at room temperature (25 ± 2°C) for 7 days. The dried material was ground into a fine powder using an electric grinder and passed through a 40-mesh sieve [15]. The resulting powder was stored in an airtight container at 4°C until further use.

3.2. Preparation of Methanolic Extract

3.2.1. Extraction Process

105 grams of the powdered plant material was macerated in 420 mL of analytical grade methanol (1:4 w/v ratio) in a closed glass container. The mixture was kept at room temperature for 5 days with occasional shaking to ensure thorough extraction. The process was carried out in the dark to prevent potential photodegradation of sensitive compounds [16].

3.2.2. Filtration and Concentration

After the extraction period, the mixture was filtered through Whatman No. 1 filter paper, followed by a second filtration through a white cotton filter to ensure the removal of all plant debris. The filtered extract was then concentrated using a rotary evaporator (Buchi Rotavapor R-300) at 40°C under reduced pressure. The concentrated extract was further dried in a vacuum desiccator to obtain a dry residue [17].

3.2.3. Extract Storage

The dried methanolic extract was weighed, and the percentage yield was calculated. The extract was stored in an airtight container at 4°C until further use [18].

3.3. Phytochemical Screening

Qualitative phytochemical analysis of the methanolic extract was performed to identify the presence of various secondary metabolites using standard procedures [19, 20].

3.3.1. Test for Carbohydrates

a) Molisch's test: 2 mL of extract solution was treated with 2 drops of alcoholic α-naphthol solution in a test tube. 1 mL of concentrated sulfuric acid was added carefully along the sides of the test tube. Formation of a violet ring at the junction indicates the presence of carbohydrates.

b) Fehling's test: 1 mL of extract was boiled on a water bath with 1 mL each of Fehling's solutions A and B. A red precipitate indicates the presence of sugar.

3.3.2. Test for Alkaloids

a) Mayer's test: 1 mL of extract was treated with 1 mL of Mayer's reagent. Formation of a creamy white precipitate indicates the presence of alkaloids.

b) Wagner's test: 1 mL of extract was treated with Wagner's reagent. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

3.3.3. Test for Saponin Glycosides

Foam test: 5 mL of extract was shaken vigorously with 5 mL of distilled water in a test tube for 5 minutes. Formation of stable foam indicates the presence of saponins.

3.3.4. Test for Proteins

Biuret test: 2 mL of extract was treated with 2 mL of 10% sodium hydroxide solution and 2 drops of 0.1% copper sulfate solution. Formation of violet/pink color indicates the presence of proteins.

3.3.5. Test for Tannins

Ferric chloride test: 1 mL of extract was treated with 1 mL of 5% ferric chloride solution. Formation of a blue, green, or brown color indicates the presence of tannins.

3.3.6. Test for Flavonoids

Alkaline reagent test: 2 mL of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

3.3.7. Test for Amino Acids

Ninhydrin test: 2 mL of extract was treated with 2 mL of ninhydrin reagent and boiled for few minutes. Formation of purple color indicates the presence of amino acids.

3.3.8. Test for Steroids

Liebermann-Burchard test: 2 mL of extract was treated with 2 mL of acetic anhydride and 2 mL of concentrated sulfuric acid. Formation of blue-green ring indicates the presence of steroids.

3.3.9. Test for Cardiac Glycosides

Keller-Killiani test: 2 mL of extract was treated with 1 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicates the presence of a deoxysugar characteristic of cardenolides.

3.4. ***In vitro*** antimitotic activity assay

3.4.1. Seed Selection and Sterilization

Vigna radiata L. (green gram) seeds of uniform size and weight were selected for the assay. The seeds were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 minutes, followed by rinsing 4-5 times with sterile distilled water [21].

3.4.2. Preparation of Test Solutions

Extract Solution: Stock solution of *A. cathartica* methanolic extract was prepared by dissolving 1 g of extract in 10 mL of distilled water. From this, serial dilutions of 25, 50, and 100 µg/mL were prepared.

Standard Drug Solution: Stock solution of methotrexate (standard anticancer drug) was prepared by dissolving 15 mg (1 mL vial) in 10 mL of sterile water. Serial dilutions of 25, 50, and 100 µg/mL were prepared from this stock.

Seed Germination Assay: Sterilized Petri dishes (90 mm diameter) lined with sterile filter paper were used for the assay. Each Petri dish was moistened with 5 mL of the respective test solution (extract or methotrexate) or distilled water (control). Ten sterilized seeds were placed in each Petri dish, ensuring adequate spacing.

The experimental setup included:

- Control group: Seeds treated with distilled water
- Extract groups: Seeds treated with 25, 50, and 100 µg/mL of *A. cathartica* extract
- Standard drug groups: Seeds treated with 25, 50, and 100 µg/mL of methotrexate
- All Petri dishes were sealed with parafilm and incubated at room temperature (25 ± 2°C) for 72 hours. Each treatment was performed in triplicate.

3.4.3. Measurement and Analysis

After the incubation period, the number of germinated seeds was counted, and the root length of each germinated seed was measured using a ruler. Seeds were considered germinated when the radicle emerged from the seed coat [22].

The percentage of growth inhibition was calculated using the formula:

% Inhibition = (Lc - Lt) / Lc × 100

Where, Lc = Length of root in control

Lt = Length of root in test

3.4.4. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism software (version 8.0). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to determine significant differences between groups. P values < 0.05 were considered statistically significant.

4. Results and Discussion

4.1. Phytochemical Screening

The qualitative phytochemical analysis of the methanolic extract of *Allamanda cathartica* revealed the presence of various bioactive compounds, as summarized in Table 1.

Table 1. Phytochemical constituents of *Allamanda cathartica* methanolic extract

Phytochemical Constituents	Result
Carbohydrates	+
Alkaloids	+
Saponin glycosides	+
Proteins	+
Tannins	+
Flavonoids	+
Amino acids	+
Steroids	-
Cardiac Glycosides	-

(+) indicates presence, (-) indicates absence

The presence of diverse phytochemicals in *A. cathartica* extract suggests its potential for various pharmacological activities. Notably, the extract contained alkaloids, flavonoids, and tannins, which are known to possess antioxidant and anticancer properties [23]. The presence of saponin glycosides is particularly interesting, as these compounds have been associated with membrane-permeabilizing and hemolytic activities, which could contribute to the plant's reported antimitotic effects [24].

4.2. *In vitro* antimitotic activity

The antimitotic activity of *A. cathartica* methanolic extract was evaluated using the *Vigna radiata* seed germination assay. The results are presented in Table 2 and Figures 2, 3.

Table 2. Effect of *A. cathartica* extract and methotrexate on seed germination and root length

Treatment	Concentration (μg/mL)	Germination (%)	Root Length (mm)	Inhibition (%)
Control	-	98.33 ± 1.67	25.67 ± 1.25	-
A. cathartica	25	85.00 ± 2.89	12.83 ± 0.62	50.00 ± 2.41
A. cathartica	50	71.67 ± 3.33	8.85 ± 0.43	65.50 ± 1.67
A. cathartica	100	16.67 ± 3.33	1.23 ± 0.15	95.20 ± 0.58
Methotrexate	25	81.67 ± 1.67	11.50 ± 0.50	55.20 ± 1.95
Methotrexate	50	65.00 ± 2.89	7.17 ± 0.44	72.10 ± 1.71
Methotrexate	100	11.67 ± 1.67	0.83 ± 0.17	96.80 ± 0.65

Values are expressed as mean ± SD (n=3)

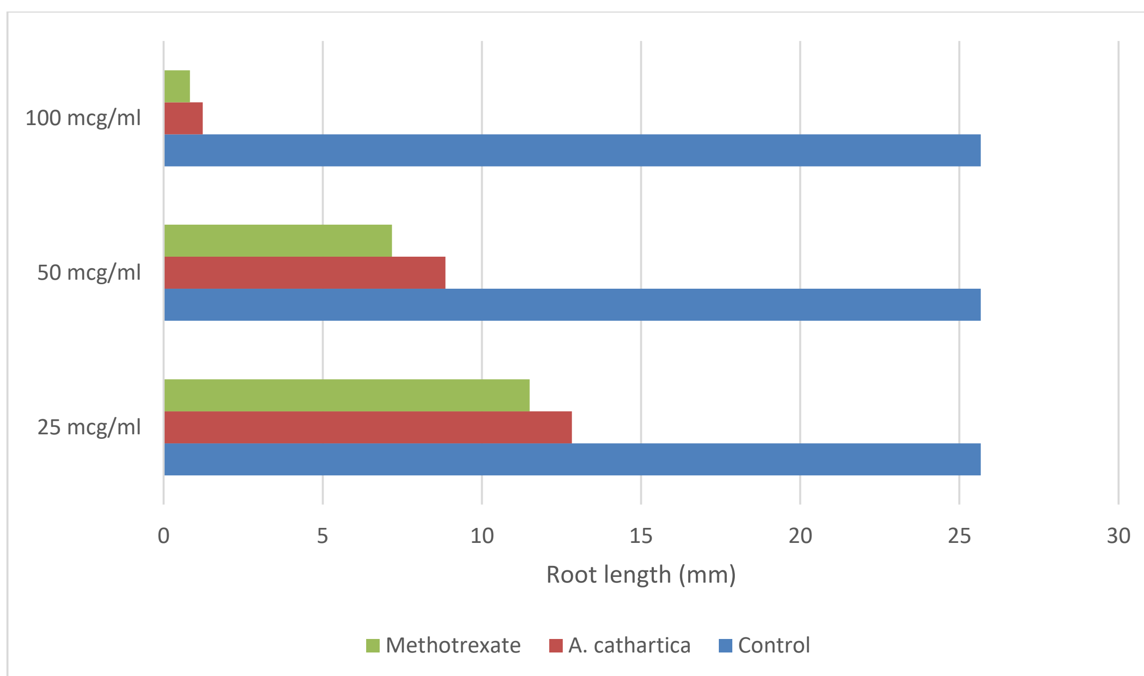

Figure 2. Results of root length by *A. cathartica* extract and methotrexate at different concentrations

Figure 3. a. Growth of green gram in tap water b. Effect of *A. cathartica* extract c. Effect of methotrexate

The results demonstrate a dose-dependent inhibition of seed germination and root growth by both *A. cathartica* extract and methotrexate. At the highest concentration (100 μg/mL), *A. cathartica* extract exhibited 95.20% inhibition, which was comparable to the standard drug methotrexate (96.80% inhibition). This strong antimitotic activity of *A. cathartica* extract is likely due to the presence of bioactive compounds that interfere with cell division processes.

The observed antimitotic effect can be attributed to various mechanisms. Alkaloids, for instance, are known to interact with tubulin, disrupting microtubule formation and consequently inhibiting mitosis. Flavonoids have been reported to induce cell cycle arrest and apoptosis in various cancer cell lines [25]. The presence of these compounds in *A. cathartica* extract, as revealed by phytochemical screening, supports its potent antimitotic activity.

Interestingly, even at lower concentrations (25 µg/mL and 50 µg/mL), *A. cathartica* extract showed significant inhibition (50.00% and 65.50%, respectively). This suggests that the extract may contain highly potent antimitotic compounds that are effective even at low doses.

The comparable activity of *A. cathartica* extract to methotrexate, a known anticancer drug, is particularly noteworthy. Methotrexate works by inhibiting dihydrofolate reductase, an enzyme crucial for DNA synthesis [26]. The similar inhibitory effect of *A. cathartica* extract suggests that it may contain compounds that act through similar mechanisms or through alternative pathways that ultimately result in mitotic inhibition.

4.3. Correlation with Other Pharmacological Activities

The observed antimitotic activity of *A. cathartica* aligns well with its other reported pharmacological properties. For instance, the anti-inflammatory activity of *A. cathartica*, particularly attributed to its quercitrin compounds, may contribute to its potential anticancer effects, given the well-established link between inflammation and cancer.

The antioxidant properties of *A. cathartica*, particularly the high levels of enzymatic antioxidants like superoxide dismutase (SOD) and peroxidase (POD) in its root extracts, may also play a role in its antimitotic activity. Antioxidants can protect against oxidative stress-induced DNA damage and mutation, which are often precursors to uncontrolled cell division [27].

Moreover, the reported membrane-stabilizing and thrombolytic activities of *A. cathartica* extracts [10, 11] suggest that the plant possesses compounds that can interact with cellular membranes and proteins. Such interactions could potentially disrupt the cell cycle and contribute to the observed antimitotic effects.

5. Conclusion

This study demonstrated the significant antimitotic potential of *Allamanda cathartica* methanolic extract, comparable to the standard drug methotrexate, using a Vigna radiata seed germination assay. The phytochemical screening revealed a rich profile of bioactive compounds, including alkaloids, flavonoids, and saponins, which likely contribute to its observed pharmacological activities. The dose-dependent inhibition of seed germination and root growth suggests that *A. cathartica* contains potent compounds that interfere with cell division processes. These findings, coupled with previous reports of anti-inflammatory, antioxidant, and other beneficial properties, position *A. cathartica* as a promising source for novel anticancer therapeutics.

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Author's short biography

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Miss Reethu Metilda Manukonda is currently pursuing her second-year master's degree in pharmacy at Sri Vasavi Institute of Pharmaceutical Sciences, Andhra Pradesh, India. Her research interests extend to exploring the therapeutic potential of medicinal plants for various diseases, including cancer, and she aspires to contribute significantly to the field of pharmaceutical sciences through innovative research methodologies.

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Dr. Satapathy Dhabal is a highly respected Associate Professor in the Department of Pharmacology at Sri Vasavi Institute of Pharmaceutical Sciences. His academic trajectory demonstrates a profound interest in pharmacology, as evidenced by his continuous dedication to the field. He has extensive experience in animal handling and experimental pharmacology. His commitment to research and teaching continues to inspire and influence the future of pharmaceutical science, guiding students and contributing to advancements in the field through his research endeavors.