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Research Article

The pharmacognostic study, phytochemical screening, and TLC of *Allamanda Cathartica* L. Plant

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ABSTRACT

Objective:
This study aimed to investigate the phytochemical properties of Allamanda cathartica L. (Golden Trumpet) and explore its potential therapeutic applications.

Methods:
Various parts of the plant, including leaves, stems, and flowers, were collected and subjected to phytochemical screening and thin-layer chromatography. The extracts were analyzed for alkaloids, tannins, flavonoids, phytosterols, saponins, fixed oils, and carbohydrates. Total flavonoid content was estimated using the aluminum-chloride spectroscopic assay. Microscopical analysis and physicochemical tests were also performed.

Results:
Phytochemical screening revealed the presence of flavonoids and phytosterols in all extracts, while alkaloids were absent. Tannins were present in the petroleum ether extracts of the flower and stem, but absent in the hydroalcoholic extracts. The total flavonoid content was higher in the hydroalcoholic extracts compared to the petroleum ether extracts. Microscopical analysis showed characteristic features of Allamanda cathartica, including whorled leaves, large yellow flowers, and a twinning woody stem. Physicochemical analysis indicated the presence of ash, fiber, and moisture content within acceptable limits.

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Conclusion:
Allamanda cathartica possesses significant phytochemical constituents with potential therapeutic benefits. Further studies are warranted to explore its pharmacological properties and clinical applications.

INTRODUCTION

Under the family of Apocynaceae, Allamanda cathartica L., often known as Golden Trumpet, is a 6 m tall woody climbing green shrub. It is cultivated in India for its eye-catching Karnataka flowers and is close to Brazil and Central America. Using phytochemical research, a class of chemicals found in plant extracts can be identified as plants for therapy. The identification of the active ingredients found in plants is the primary goal of phytochemical research. This plant reproduces vegetatively through cuttings and sexually through seeds. Around the world, A. cathartica is commonly grown as an ornamental in tropical and subtropical climates1. Antimicrobial substances can still be found in large quantities in medicinal plants. Globally, there has been a rise in interest in the medicinal properties of natural goods. It is said that the world's flora, nature's medicine, has the solution for any crippling human ailment. Human health has been significantly maintained by phytochemicals. Allamanda Cathartica has been chosen for the current investigation. This plant is said to have several therapeutic uses, including being an effective purgative and an antidote for poisoning, inflammation, constipation, and ascites2. It has been utilized to treat malaria-related jaundice and enlarged spleen. It is effective on human nasopharyngeal cancer both in vitro and in vivo in mice. Both the watery extract and the alcohol have high blood pressure. It has antimicrobial and perhaps anticancer properties, and it is cathartic (milky sap). Plant alkaloids are known to have significant biological activity. The leaf of A. cathartic contains alkaloids, sterols, and flavonoids. Strong anti-oxidative cell damage and robust anti-cancer activity are exhibited by flavonoids, which are water-soluble antioxidants and free radical scavengers. Flavonoids have been shown to reduce blood pressure and enhance blood circulation. The flowers of Allamanda cathartica were extracted using acetone, petroleum ether, chloroform, ethanol, and water. Various phytoconstituents, including alkaloids, phenolic substances, saponins, flavonoids, and glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins, and carbohydrates, were found in these extracts. Allamanda cathartica extracts in petroleum ether and chloroform showed encouraging antifungal activity3.

Description4:

Table no. 1: Detailed description of the plant. (Allamanda Cathartica L.)

Sr. No.	Parts of plant	Description
1.	Leaves	They are whorled, obviate to oblong-lanceolate, 8-12*2.5-4.0cm, wavy, and may be either opposite or whorls of three or four.
2.	Flowers	Flowers are very large, usually yellow, in the few-flowered terminal and auxiliary cymes. Corolla with short tubular base, then suddenly campanulate, lobes rounded, contorted to the left, throat with a ring of ciliate scales. Ovary one-celled with two parietal placentae and many ovules.
3.	Stems	Woody and twinning stem. It contains a milky sap.

Fig 1: Allamanda cathartica L.

(Golden Trumpet)

PLANT PROFILE5:

Table no. 2: Taxonomical classification of plant.

Kingdom	Plantae
Order	Gentianales
Family	Apocynaceae
Subfamily	Rauvolfioideae
Genus	Allamanda L.
Species	*A.Cathartica*
Flower	Oval, Smooth Edges, and hairy
Inflorescence	Compound cyme
Odor	Fresh Floral and Jasmin-like Scent

MATERIAL AND METHODS4:

Collection of plant material:

From the surrounding Wardha areas, we gathered the leaves, stems, and flowers of a cathartic plant. The leaves were dried after being cleaned of pollutants using tap water. We gathered fresh stems and let them dry in the sun. The maceration method involved the use of dried flowers. Afterward, the dried stems were ground into a fine powder and, depending on the polarity of the solvents, were extracted using a Soxhlet with petroleum ether and hydroalcoholic (ethanol).

Instruments and Glassware used:

Measuring cylinder, beakers, desiccator, water bath, Petri dishes for weighing balance, test tubes, pipette, evaporating dish, silica crucible, muffle furnace, and funnel for the Soxhlet apparatus.

Reagents and solvents Use:

The concentrated hydrochloric acid solution, sodium hydroxide, dilute hydrochloric acid, chloroform, ethanol, ethyl acetate, methanol, petroleum ether phloroglucinol, safranin, glacial acetic acid, concentrated sulphuric acid, anhydrous acetic anhydride, molisch reagent, Fehling's solution, flavonoid test, tannins test, Hagar'sreagent, dragendroff's reagent and Mayer's reagent, sulphuric acid.

Extraction6,7,8:

The Allamanda Cathartic flower is taken in a maceration bottle and filled with petroleum ether for a 7-day maceration process. Later we dry the flower After this, process we take another solvent, Hydroalcoholic (80:20 ethanol: distilled water). For the extraction of the Allamanda cathartica L. stem, we used petroleum ether solvent in soxhlet assembly for 3 days (16 cycles). Afterward, we dry the crude drug (stem) and change the solvent, Hydroalcoholic for 4 days (18 cycles).

Fig 2: Soxhlet assembly

Fig 3: Maceration

Microscopical analysis9:

1. Transverse section of leaves: A cut-through leaves showing their internal structure.
2. Transverse section of stem: Cut through a stem showing a single-layered epidermis with trichomes.
3. Longitudinal section of stem: A cut along the length of a stem.
4. Transverse section of the ovary: Cut through an ovary showing its structure.
5. Powder Characteristic: Evaluation of medicinal plants using staining reagents.
6. Palisade ratio: Average number of palisade cells under one epidermal cell in leaves.
7. Stomatal number & Stomatal Index: Number of stomata per sq mm and ratio of stomata to epidermal cells.
8. I = S / E + S, where I is a stomatal index.
9. Vein islet & vein termination number: Number of vein islets and veinlet terminations per sq mm of leaf surface.

These techniques are used to study and identify plant structures for various purposes, such as research, quality control, and taxonomy.

Fig 4: T. S. of leaves

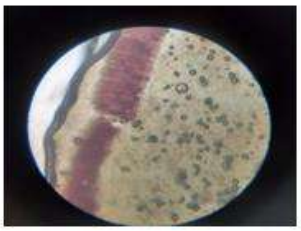


Fig 5: T. S. of stem

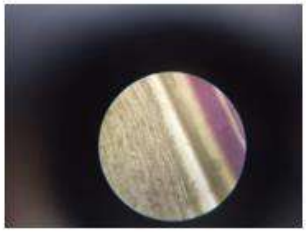


Fig 6: L. S. of stem

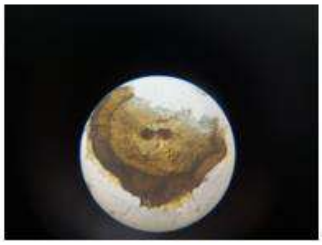


Fig 7: T. S. of ovary

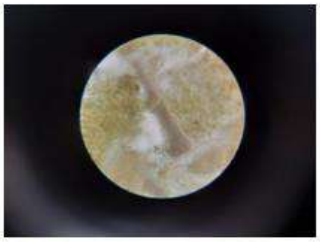


Fig 8: Powder slide

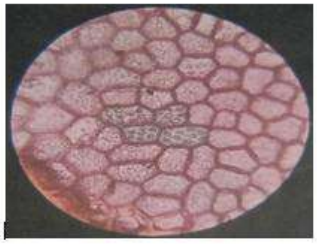


Fig 9: Palisade cells

Fig 10: Stomata

Fig 11: Vein islet and termination number

Physicochemical analysis3:

1. **Total Ash Value:**

Total ash is a measure of the mineral oxide content of activated carbon on a weight basis. It is determined by converting the mineral constituents to the respective oxides at 800°C. The ash primarily consists of silica and aluminum, and its amount depends on the base raw material used for production.

2. **Acid Insoluble Ash:**

Acid insoluble ash is determined by dissolving ash in dilute hydrochloric acid (10% m/m), filtering the liquid through ashless filter paper, and then washing it with hot water. The filter paper is ignited, cooled, and weighed.

3. **Water-soluble Ash:**

This is the part of the total ash that dissolves in water under specified conditions.

4. **Sulphated Ash10:**

Sulphated ash, like crude ash, can indicate the level of known metal-containing additives or impurities in organic material. The procedure involves heating the ash with sulfuric acid, which can be hazardous and corrosive.

5. **Loss on Drying (LOD):**

This test determines the moisture content of a sample by measuring the loss of volatile matter. It does not usually refer to molecularly bound water or water of crystallization.

6. **Foaming Index:**

The foaming index is a measure of the ability of a substance to produce foam. It is calculated based on the volume of plant material decoction in a test tube that produces a 1 cm foam height. The procedure involves boiling the material, filtering, and then shaking the filtrate in test tubes to measure foam height.

Foaming index = 1000/a.

a =volume in ml of the decoction in the test tube showing 1 cm height.

7. **Fibre Content:**

Fibres in pharmacognosy are defined as elongated thick-walled cells with cellulose walls, which may or may not contain lignin. In the context of surgical dressings, fibre includes both natural and artificial fibres.

Fig 12: Total ash

Fig 13: Muffle furnace

Fig 14: Acid insoluble ash

Fig 15: Sulphated ash

Fig 16: Loss on drying

Fig 17: Fiber content

Phytochemical Screening11:

Table no. 3: All phytochemical tests for screening of the phytoconstituents in the plant.

Test	Procedure	Observation
Alkaloids	a. **Dragendorff test:** 1ml of leaf extract + 2ml of 1% HCl, boil, add 2-3 drops of Dragendorff's reagent. b. **Wagner's test:** 1ml of leaf extract + 1ml of 1% H2SO4, add Wagner's reagent. c. **Mayer's test:** 1ml of leaf extract + 2ml of 1% HCl, add Mayer's reagent dropwise.	Formation of reddish-brown precipitate (Dragendorff); formation of precipitate (Wagner's and Mayer's).
Flavonoid	**a.** 2.5 ml of leaf extract + 1 ml of 10% NaOH, add drops of conc. HCl. **b. Shinoda test:** Dissolve 0.5 portion in ethanol, warm, filter, add magnesium chips, then conc. HCl.	Yellow to colorless (NaOH + conc. HCl); pink, orange, or red to purple coloration (Shinoda).
Anthraquinone	1ml of leaf extract + 2ml of 5% KOH.	Pink coloration.
Saponin	1ml of leaf extract + 2ml of NaHCO3, shake to form foam.	Formation of foam.
Terpenoids	1ml of extract + 400µl of chloroform, add 4-5 drops of conc. H2SO4 from the walls of the test tube.	Formation of reddish-brown ring.
Cardiac glycoside	2.5ml of extract + 2ml of glacial acetic acid, few drops of FeCl3 and conc. H2SO4 from the sides of the test tube.	Reddish-brown ring.
Tannin	**Method A:** Boil 1ml of extract, add few drops of FeCl3. **Method B:** 1ml of extract + 500µl of lead acetate.	Blue, black, or green coloration (Method A); yellow color (Method B).
Starch	1ml of extract + 500µl of iodine.	Blue coloration.
Phlobatannin	1ml of extract + 1% HCl, boil.	Formation of precipitate.
Gums and mucilage	**Ruthenium red test:** Mount test extract on a slide, add ruthenium red solution, observe under the microscope.	Red-pink color seen.

Fig 18: Dragendorff test

Fig 19: Flavonoid test

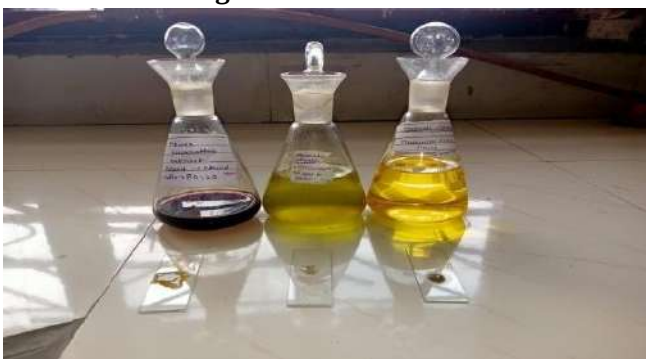


Fig 20: Test for gums and mucilage

Thin-layer chromatography12,13:

TLC is a chromatographic technique that uses a solid fixed phase (silica gel) and a liquid mobile phase (chloroform: methanol = 8:2) to separate compounds in plant extracts based on their polarity. Silica gel plates are activated and used as the stationary phase. Different solvent systems are applied to identify active principles in plant extracts. Visualization is done under UV light, and specific spray reagents are used for enhancement. TLC is rapid, cost-effective, and provides good resolution and sensitivity, making it ideal for phytochemical investigation.

Stationary Phase

Silica gel G, particle size 10 – 40 µm applied as a thin layer on a clean glass plate support and activated (1100C for 30 minutes) just before use.

Mobile Phase

Quantity – 50 ml

The mobile phase was –

1. Chloroform: methanol = 2:8
2. Ethyle acetate: methanol=6:4

Development Method

One dimensional ascending method by using a standard protocol as per IP was followed.

Visualization

After the development of the TLC plate, initially, three spots were visualized in the UV chamber (365 nm).

Flavonoid Estimation14:

Total flavonoid content (TFC) determination. The amount of flavonoids present in the extracts overall was ascertained using the aluminum-chloride spectroscopic assay. In a 10 mL volumetric flask, 1 ml of the extract (1 mg/mL) was combined with 4 mL of methanol. The flask received 0.80 mL of 5% sodium acetate added to it. One milliliter of 10% AlCl3.6H2O solution was added to the mixture after five minutes. Using the same process as for the extracts, a series of standard quercetin solutions (200,180,160,140,120,100, 80, 60, 40, and 20 µg/ml) were made. Using a UV/Visible spectrophotometer, the absorbances of the extracts and standard solutions were measured at 415 nm in relation to the reagent blank. The calibration curve was used to calculate the total flavonoid concentration, which was then represented as milligrams of quercetin equivalent (QE) per gram of extracts. Duplicate measurements of the total flavonoids in the extracts and standards were made.

Fig 21: UV spectrophotometer

Fig 22: Dilutions

Fig 23: Micropipette

RESULTS AND DISCUSSION:

Physicochemical analysis:

This analysis performed for isolation, purification, and identification of active constituents is a chemical method of evaluation.

Table no. 4: Observations of physicochemical analysis.

Test	Calculation	Result
Palisade Ratio	(12 + 8 + 11 + 9) / 4 = 33.25	33.25
Stomatal Index	Stomatal index = (Number of stomata / Number of epidermal cells) x 100	43.2
Vein Islet	4 islets in 0.36 mm², so 11.33 islets in 1 mm²	11.33
Vein Termination	3 terminations in 0.36 mm², so 8.33 terminations in 1 mm²	8.33
Total Ash Value	Total ash = 1.03 gm	1.03 gm

% Total Ash Value	% Total ash value = (1.03 x 100) / 3	34.33%
Acid Insoluble Ash	% Acid insoluble ash value = (0.34 x 100) / 3	11.33%
Moisture Content	Moisture content = ((Initial weight - Final weight) / Initial weight) x 100	11.33% w/w
Foaming Index	Foaming index = 1000 / a (where a is the volume of the decoction in the test tube showing 1 cm height of foam)	Highest foaming index: 2000 cm
Fiber Content	Crude fiber = (Weight of the crucible with fiber - Weight of the empty crucible) / Weight of the sample x 100	Crude fiber: 12%

Phytochemical screening:

Phytochemical screening is used to find out the active constituent of plant extract (crude drug).

Table no. 5: Observations of phytochemical screening tests.

Sr. No.	Test	Flower extract (Petroleum ether)	Flower extract (Hydroalcoholic solvents)	Stem extract (Petroleum ether)	Stem extract (Hydroalcoholic solvents)
1.	Alkaloids	-	-	-	-
2.	Tannins	+	-	+	-
3.	Flavonoids	+	+	+	+
4.	Phytosterols	+	+	+	+
5.	Saponins	-	-	-	-
6.	Fixed oils	-	-	-	-
7.	Carbohydrates	-	-	-	-

Thin-layer chromatography:

Observation in different solvent systems.

2:8
Chloroform: methanol
(Fig a)

6:4
Ethyl acetate: methanol
(Fig b)

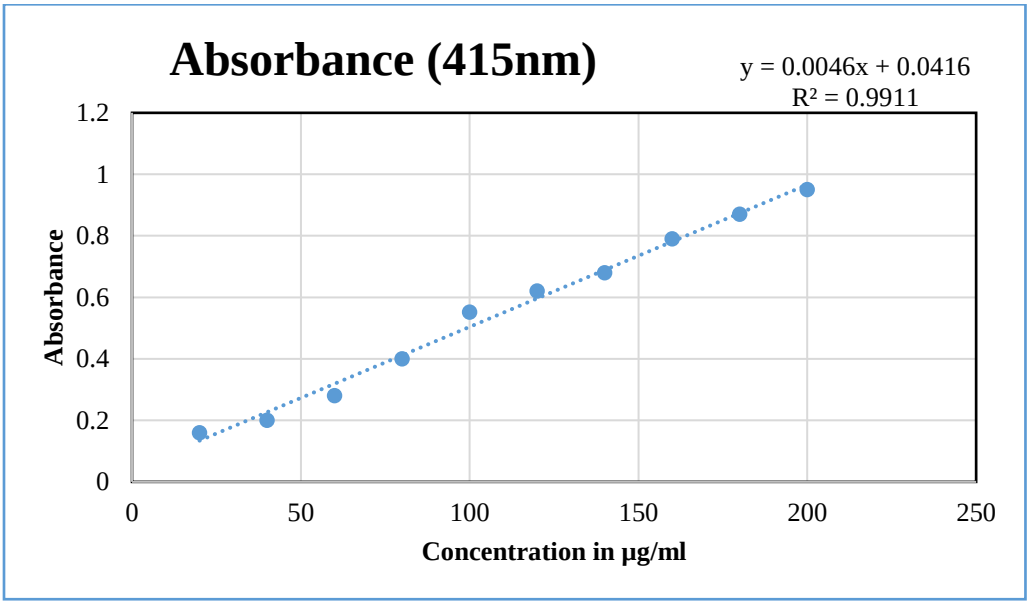
Fig 24: TLC plates

Flavonoid estimation: **Standard: Quercetin**

Table no. 6: Observations of Quercetin in UV-Spectrophotometer

Concentration in µg/ml of Quercetin	Absorbance (415nm)
20	0.16
40	0.2
60	0.28

80	0.4
100	0.552
120	0.62
140	0.68
160	0.79
180	0.87
200	0.95



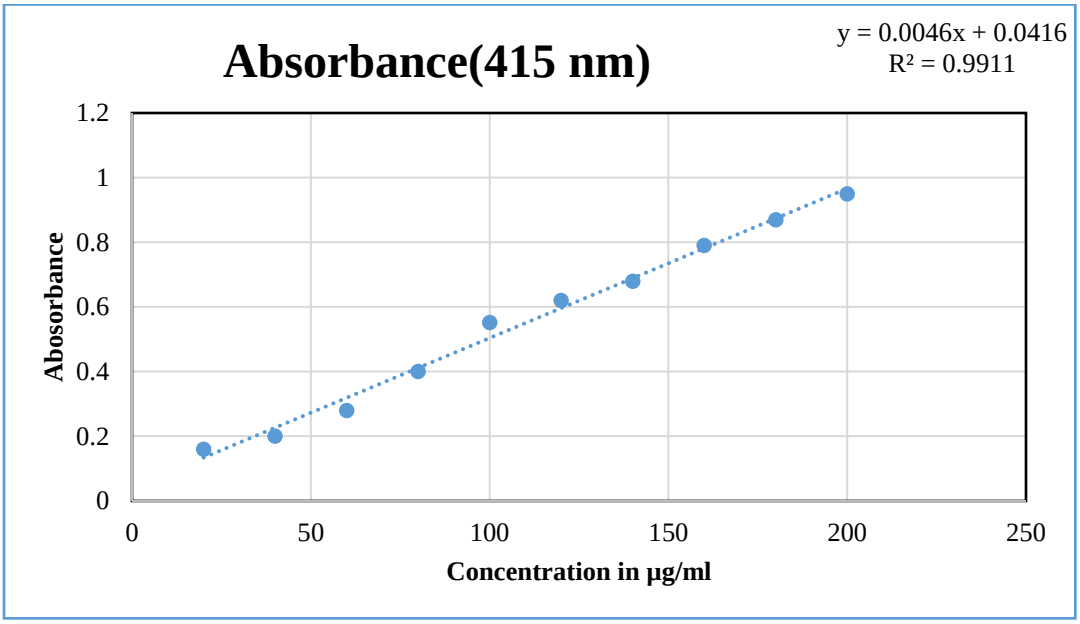


Table no. 7: Observations of different samples in UV-Spectrophotometer.

Sample (1mg/ml)	Absorbance at (415nm)	Concentration in µg/ml	Concentration in mg/g
Petroleum ether extract(stem)	4.000	860.5217391	860.5217391
Hydroalcoholic Extract(stem)	3.725	800.7391304	800.7391304
Petroleum ether extract (Flower)	0.317	59.86956522	59.86956522

Hydroalcoholic Extract (Flower)	0.431	84.65217391	84.65217391

DISCUSSION:

Compounds with antibacterial properties abound in Allamanda cathartica L. Around the world, the therapeutic value has increased. Consequently, the in-depth physicochemical, pharmacognostic, phytochemical, and organoleptic investigations depart from this facility. The presence of flavonoids and phytosterols is evident in all extracts (hydroalcoholic and petroleum ether), while the presence of tannins and saponins is evident in petroleum ether extracts of flowers and stems. Flower and stem extract do not include any carbohydrates. The percentage of total ash of crude drug of the stem (Allamanda Cathartica L.) is considerable as compared to the percentage of acid-insoluble ash of crude drug. that is, 11.33% and 34.33%. Given that the plant's moisture content of 15.33% falls within the drug's suggested range of 14–20%, it can be preserved for an extended period of time with a reduced risk of microbial attack. There were multiple spots in the TLC profile, and the organic extract with the maximum number of components was found in the ethanolic and petroleum ether extract. Spot colour may also be important for component isolation and identification because certain types of compounds, including flavonoids, have been shown to show unique coloration in various ratio stem extracts. According to flavonoid assessment, the stem's petroleum ether and hydroalcoholic extracts have high levels of flavonoids in mg/g, or 860.52 and 800.74 mg/g, respectively.

CONCLUSION:

Based on the phytochemical analysis outcomes, it can be stated that Allamanda cathartica L. yields a large number of secondary metabolites with potential medical use. TLC profiling provided additional evidence for the presence of phenol, tannins, and flavonoids. As a result, this study will be helpful for both the creation of a plant monograph and for the identification and standardization of plant material for quality assurance. In our lab, thorough phytochemical analyses of Allamanda cathartica L. are being conducted to determine whether or not this class of chemicals is present. The results indicate that the stem extract contains a significant percentage of flavonoids, which may be in charge of various biological functions. Thus, several bioactivities of this plant can be investigated.

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