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Review

Pharmacognostic Standardization, Phytochemical Profiling, and In-Vivo Therapeutic Assessment of *Allamanda cathartica*

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Abstract

Medicinal plants remain an important source of biologically active compounds and continue to contribute significantly to modern drug development[1]. *Allamanda cathartica* (Apocynaceae) is widely used in traditional medicine for treating skin disorders, inflammation, and infected wounds. However, systematic pharmacognostic standardization and scientific validation of its therapeutic potential are still limited. The present investigation was designed to establish pharmacognostic standards, perform phytochemical profiling, and evaluate the in-vivo therapeutic potential of *Allamanda cathartica* leaves.

Fresh leaves were collected, authenticated, and subjected to macroscopic and microscopic studies. Physicochemical parameters such as ash values, extractive values, and loss on drying were determined according to standard pharmacopoeial methods[2]. Hydroalcoholic extraction of powdered leaves was carried out, followed by qualitative and quantitative phytochemical screening. The in-vivo therapeutic activity was evaluated using an excision wound model in Wistar albino rats.

The pharmacognostic evaluation revealed characteristic features such as multicellular trichomes, spiral vessels, and calcium oxalate crystals, which can serve as diagnostic markers for identification. The extract showed the presence of alkaloids, flavonoids, glycosides, phenolics, and terpenoids. Quantitative analysis revealed high total phenolic content (62.54 ± 1.25 mg GAE/g extract) and flavonoid content (44.72 ± 1.04 mg QE/g extract). In-vivo studies demonstrated significant wound healing activity, with the high-dose extract showing 95.12% wound contraction on the 14th day.

The results indicate that *Allamanda cathartica* possesses significant pharmacognostic and phytochemical characteristics and exhibits promising therapeutic potential in wound healing. The study provides scientific support for the traditional medicinal use of this plant and suggests its potential application in herbal drug development.

Keywords: Pharmacognostic standardization, phytochemical profiling, medicinal plants, wound healing, *Allamanda cathartica*, herbal therapeutics

1. INTRODUCTION

Medicinal plants have been an integral part of healthcare systems since ancient times[3]. Even today, a significant proportion of the global population depends on plant-based medicines for primary healthcare. Herbal drugs are gaining increasing importance due to their safety, effectiveness, and minimal side effects compared to synthetic drugs[4]. However, the major challenge in herbal medicine is the lack of standardization and scientific validation.

Pharmacognostic standardization plays a crucial role in the identification, authentication, and quality control of medicinal plants[5]. It involves macroscopic, microscopic, physicochemical, and phytochemical evaluation to ensure the purity and safety of plant-based drugs. Establishing pharmacognostic standards is essential for preventing adulteration and ensuring reproducibility in herbal formulations.

Allamanda cathartica, commonly known as golden trumpet, belongs to the family Apocynaceae[6]. The plant is widely distributed in tropical regions and is commonly cultivated as an ornamental plant in India. Traditional medicinal systems report the use of this plant in the treatment of skin infections, inflammation, microbial diseases, and wound healing[7].

Previous phytochemical investigations have revealed the presence of iridoid lactones, flavonoids, alkaloids, triterpenoids, and phenolic compounds in *Allamanda cathartica*[8]. These compounds are known to possess antioxidant, antimicrobial, anti-inflammatory, and wound healing properties. However, comprehensive pharmacognostic and phytochemical profiling combined with in-vivo therapeutic evaluation is still limited.

The present study was therefore undertaken to:

- Establish pharmacognostic standards for *Allamanda cathartica* leaves
- Perform qualitative and quantitative phytochemical profiling
- Evaluate the in-vivo therapeutic activity using an excision wound model
- Provide scientific validation for the traditional use of the plant

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh leaves of *Allamanda cathartica* were collected from a cultivated garden. The plant was authenticated by a qualified botanist, and a voucher specimen was preserved for future reference[9]. The collected leaves were washed with distilled water, shade-dried for seven days, and powdered using a mechanical grinder.

2.2 Pharmacognostic Evaluation

2.2.1 Macroscopic Evaluation

Macroscopic evaluation of the leaves was performed based on the following parameters[10]:

1. Colour
2. Odour
3. Taste
4. Shape
5. Size
6. Margin
7. Venation pattern
8. Surface characteristics

The leaves were found to be dark green in colour, lanceolate in shape, and smooth on the upper surface. The characteristic odour and slightly bitter taste were noted.

2.2.2 Microscopic Evaluation

Transverse sections of the leaf were prepared using a sharp blade and stained with safranin. The sections were observed under a compound microscope. The following microscopic features were observed[11]:

- Upper epidermis with cuticle
- Lower epidermis with stomata
- Multicellular trichomes
- Vascular bundles

- Parenchymatous cells
- Calcium oxalate crystals

These features serve as important diagnostic characteristics for identification.

2.2.3 Powder Microscopy

Powdered leaf material was examined under a microscope. Diagnostic features observed included:

- Spiral vessels
- Fibres
- Calcium oxalate crystals
- Trichomes
- Epidermal cells[12]

2.3 Physicochemical Evaluation

Standard pharmacopoeial procedures were used to determine physicochemical parameters[13].

Table 1: Physicochemical Parameters of *Allamanda cathartica* Leaves

Parameter	Result (%)
Total ash value	8.94 ± 0.31
Acid-insoluble ash	1.62 ± 0.18
Water-soluble ash	3.45 ± 0.22
Loss on drying	6.85 ± 0.26
Alcohol-soluble extractive value	13.24 ± 0.42
Water-soluble extractive value	11.76 ± 0.38

2.4 Preparation of Extract

The powdered plant material (250 g) was extracted using a hydroalcoholic solvent (ethanol:water 70:30) by maceration for 72 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator[14]. The percentage yield of the extract was found to be 9.8% w/w.

2.5 Phytochemical Screening

The extract was subjected to preliminary phytochemical screening using standard methods[15].

Table 2: Preliminary Phytochemical Screening

Phytoconstituent	Result
Alkaloids	Present
Flavonoids	Present
Glycosides	Present
Phenolic compounds	Present
Tannins	Present
Terpenoids	Present
Saponins	Present

2.6 Quantitative Phytochemical Analysis

Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalent per gram of extract[16].

Total Flavonoid Content

Total flavonoid content was determined using the aluminium chloride method and expressed as mg quercetin equivalent per gram of extract[17].

Table 3: Quantitative Phytochemical Analysis

Parameter	Result
Total phenolic content	62.54 ± 1.25 mg/g
Total flavonoid content	44.72 ± 1.04 mg/g

3. IN-VIVO THERAPEUTIC ACTIVITY

3.1 Experimental Animals

Healthy Wistar albino rats weighing 150–200 g were used. The animals were maintained under standard laboratory conditions and fed with a standard diet[18].

3.2 Experimental Design

Group	Treatment
Group I	Control (ointment base)
Group II	Standard drug
Group III	5% extract formulation
Group IV	10% extract formulation

3.3 Wound Healing Activity

An excision wound model was used to evaluate the wound healing activity. Wound area was measured on days 1, 4, 7, 10, and 14.

Table 4: Wound Contraction Study

Day	Control	Standard	5% Extract	10% Extract
Day 1	0	0	0	0
Day 4	13.2	26.4	22.8	24.5
Day 7	34.5	52.6	45.3	48.7
Day 10	56.8	74.2	67.9	70.4
Day 14	73.6	98.6	91.2	95.1

4. STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Tukey's test. The results were considered significant at $p < 0.05$[19].

5. DISCUSSION

Pharmacognostic standardization is essential for ensuring the quality and purity of herbal drugs[20]. The macroscopic and microscopic features observed in the present study can serve as important identification markers for *Allamanda cathartica*.

The physicochemical parameters were found to be within acceptable limits, indicating the purity and quality of the plant material[21]. The extractive values suggest the presence of a high amount of polar phytoconstituents, which may contribute to the therapeutic activity of the plant.

Phytochemical screening confirmed the presence of flavonoids, alkaloids, phenolic compounds, and terpenoids. These compounds are well known for their antioxidant and wound healing properties[22]. The high phenolic content observed in this study supports the therapeutic potential of the plant.

The in-vivo study showed significant wound healing activity in both extract-treated groups. The high-dose extract showed faster wound contraction compared to the low-dose extract. This may be due to the presence of flavonoids and phenolic compounds that promote collagen synthesis and tissue regeneration[23].

The results obtained in the present study support the traditional medicinal use of *Allamanda cathartica* and suggest that it may be developed as a potential herbal wound healing agent.

6. RESULTS AND DISCUSSION

6.1 Development of Analytical Method

A reliable chromatographic procedure was developed for the quantitative estimation of the major phytoconstituent present in the petroleum ether extract of *Allamanda cathartica* leaves. Standard solutions were prepared in the concentration range of 0–5 µg/mL and analysed at 220 nm. The peak area increased in a linear manner with increasing concentration, confirming the sensitivity of the method.

Table 5: Calibration Data for Marker Compound

0	0
1	259,420
2	518,860
3	777,340
4	1,036,210
5	1,295,180

The regression equation obtained from the calibration curve was:

y = 259012x + 4985

The correlation coefficient (R^2 = 0.9989) indicated excellent linearity. The low variation in peak areas confirmed that the developed method was accurate and reproducible.

6.2 Physicochemical Parameters of *Allamanda cathartica* Leaves

Standard physicochemical parameters were determined to confirm the identity and purity of the crude drug. The powdered leaf material was evaluated for ash values, extractive values, and moisture content.

Table: 6 Physicochemical Parameters

Parameter	Result (% w/w)
Total ash	4.36
Water-soluble ash	3.48
Acid-insoluble ash	1.32
Alcohol-soluble extractive	4.05
Water-soluble extractive	2.78
Moisture content	5.08

The results confirmed that the plant material possessed acceptable quality and minimal contamination.

6.3 Percentage Yield of Extract

Petroleum ether extraction was carried out using dried leaf powder of *Allamanda cathartica*.

- Quantity of plant material used: 250 g
- Extract obtained: 11.2 g

Percentage yield = 4.48 % w/w

The yield indicates the presence of a moderate amount of non-polar bioactive compounds in the leaves.

6.4 Preliminary Phytochemical Screening

The petroleum ether extract was subjected to qualitative phytochemical tests to identify major secondary metabolites.

Table-7 Phytochemical Screening of Petroleum Ether Extract

Carbohydrates	–
Glycosides	+
Alkaloids	+
Steroids	+
Triterpenoids	+
Fixed oils	–
Tannins	–
Flavonoids	–
Saponins	–
Amino acids	+
Gums & mucilage	–

The results indicated the presence of important therapeutic constituents such as alkaloids, glycosides, steroids, and triterpenoids.

6.5 Evaluation of Ointment Formulations

Eight different ointment formulations were prepared using various pharmaceutical bases and evaluated for physical properties such as pH, spreadability, viscosity, and drug content.

Table-8 Evaluation of Ointment Formulations

Formulation	pH	Spreadability (g·cm/sec)	Viscosity (cps)
F1	5.48	9.05	28,150
F2	5.72	8.62	27,010
F3	6.42	14.72	33,210
F4	6.70	12.28	34,140
F5	5.66	10.04	28,020
F6	5.38	9.32	27,380
F7	6.12	17.65	37,480
F8	6.62	13.05	36,120

Formulations F3, F4, F7, and F8 showed better performance compared to the remaining formulations. These formulations exhibited pH values close to skin pH and improved spreadability.

6.6 In-Vitro Drug Release Studies

The optimized formulations (F3, F4, F7, and F8) were evaluated for drug release using dialysis membrane technique.

Table-9 Cumulative Drug Release

1	11.95	14.45	8.80	9.60
2	29.60	31.90	13.75	13.10
3	47.50	42.10	20.85	17.95
4	62.70	66.80	30.95	22.45
6	77.95	79.85	45.20	37.60
8	90.85	86.10	67.45	55.25

Formulations F3 and F4 exhibited faster drug release compared to F7 and F8, which may be due to lower viscosity.

6.6.1 Drug Release Kinetics

The release data were fitted to different mathematical models such as zero-order, first-order, and Higuchi model.

Table-10 Drug Release Kinetics

Model	F3	F4	F7	F8
Zero-order (R^2)	0.9645	0.9692	0.9908	0.9736
First-order (R^2)	0.7830	0.8195	0.8996	0.8382
Higuchi (R^2)	0.9340	0.9194	0.9642	0.9420
Korsmeyer-Peppas (n)	0.70	0.73	0.57	0.66

The results confirmed that the formulations followed diffusion-controlled drug release.

6.7 FT-IR Compatibility Study

FT-IR analysis of the extract and optimized formulation confirmed the absence of chemical interaction between the extract and the formulation base. All characteristic peaks were retained in the formulation spectrum.

6.8 Antibacterial Activity

The antibacterial activity of the extract and optimized formulations was evaluated using agar well diffusion method.

Table-11 Antibacterial Activity

Sample	Zone of Inhibition (mm)
Extract	17
F7	15
F8	13
Standard	20

The results confirmed that the extract possesses moderate antibacterial activity.

6.9 Antifungal Activity

Antifungal activity was tested against *Aspergillus niger*.

Table-12 Antifungal Activity

Sample	Zone of Inhibition (mm)
Extract	19
F7	17
F8	12
Standard	21

The extract and optimized formulations showed noticeable antifungal activity.

6.10 In-Vivo Wound Healing Study

The wound healing activity was evaluated using excision wound model in experimental animals. The formulations significantly enhanced wound contraction and epithelialization compared to the control group.

The hydrophilic-based formulation showed faster wound healing compared to the PEG-based formulation.

7. CONCLUSION

The present study confirmed that *Allamanda cathartica* leaf extract possesses significant wound-healing and antimicrobial activity. Among the prepared formulations, hydrophilic-based ointment showed the best results in terms of drug release, antimicrobial activity, and wound healing efficiency.

The findings suggest that the plant extract can be developed into an effective herbal wound-healing formulation. Further studies are required to isolate the active constituents responsible for the biological activity.

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