Pharmacognostic and Phytochemical Studies of *Jasminum malabaricum* Wight. Leaves

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Abstract

Jasminum malabaricum Wight. is a climbing shrub belonging to the Oleaceae family, indigenous to the Western Ghats of India, and is known for its significant ethnomedicinal applications. Given its rising importance in traditional medicine, proper identification and evaluation are essential to prevent adulteration and ensure quality. This research article presents a detailed pharmacognostic, physicochemical, and phytochemical evaluation of *Jasminum malabaricum*, focusing on its leaves, to establish reliable identification and standardization parameters. Fresh and powdered leaves were examined for their macroscopic and microscopic characteristics. Physicochemical properties and phytochemical screenings were conducted following the standard procedures. The leaves exhibited a distinct green color, smooth texture, and broadly ovate shape, while microscopic analysis revealed key features such as mesophyll cells, vascular bundles, stone cells, spiral vessels, and glandular trichomes. The physicochemical analysis showed a moisture content of 10.96%, with effective extractive values for ethanol (8.6%) and water (7.6%), along with total ash, water-soluble ash, and acid-insoluble ash values of 3.1%, 1.4%, and 1.2% respectively. Phytochemical screening confirmed the presence of various bioactive constituents, including phenols and flavonoids. The pharmacognostic and phytochemical standards of *Jasminum malabaricum* were established, serving as vital quality control parameters for its purity, identification, and standardization.

Key words: *Jasminum malabaricum*, Pharmacognostic, Physicochemical, Phytochemical.

Abbreviations: JMEs – *Jasminum malabaricum* extracts, JMEE - *Jasminum malabaricum* ethanolic extract,

JMAE - *Jasminum malabaricum* aqueous extract

Introduction

Medicinal plants serve as a vital source of natural products for treating and managing various health conditions. The extraction of bioactive compounds from these plants has laid the groundwork for the development of herbal medicines and phytopharmaceuticals. To gain global acceptance, a healthcare product must undergo rigorous scientific validation to confirm its purity, potency, efficacy, and safety. The World Health Organization has established guidelines for assessing the quality and safety of herbal plants, emphasizing the importance of physicochemical and phytochemical evaluations of crude drugs.

Setting pharmacognostic standards is a critical step in the formulation of monographs for crude drugs, ensuring consistent quality and facilitating their acceptance in conventional medicine. The use of numerical quality standards enhances uniformity, allowing for reliable assessments of herbal products.

Jasminum malabaricum Wight, an indigenous flowering plant native to the Western Ghats of India, belongs to the Oleaceae family. Also known as Malabar jasmine as it is found in Malabar regions of Kerala. This climbing shrub is distinguished by its fragrant white blossoms and holds significant ethnomedical value. Traditionally, it has been utilized for its antibacterial, antioxidant, wound healing, blood-purifying, and antitumor properties. This study aims to fill that gap by conducting a comprehensive evaluation of the physicochemical and pharmacognostic properties of *Jasminum malabaricum*. This plant's leaves have not yet been scientifically assessed for their pharmacognostic standards. Thus, the current research focuses on establishing standardization parameters for *Jasminum malabaricum*, supporting its safe and effective use in herbal medicine.

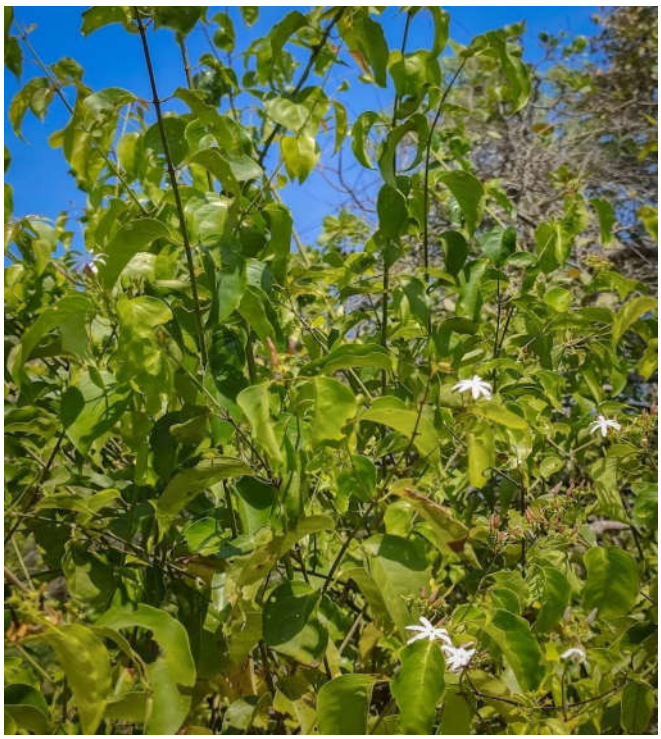


Figure 01: *Jasminum malabaricum* plant

Materials and Methods

Collection, authentication and preparation of plant materials

The leaves of *Jasminum malabaricum* Wt. were collected on February 13, 2024, from Madai Para in the Kannur district of Kerala. The plant specimen had been taxonomically identified and authenticated by Dr. Ranjusha A. P.,

Head of the Department of Botany at N. S. S. College, Ottapalam, and the herbarium was deposited at Nehru College of Pharmacy, Thrissur under voucher specimen number NCP/2024/MP/04 for future use. The leaves were separated from the twigs, thoroughly washed and they were shade-dried for 70 days,ground into powder using a mechanical grinder and kept in an airtight storage unit for subsequent studies.

Pharmacognostical Studies

Macroscopic Evaluation

The evaluation of organoleptic properties can be performed using sensory organs. This refers to assessing a crude drug based on its color, smell, size, shape, and flavor, along with distinct features like touch, texture, and leaf characteristics, including margin, apex, base surface, and venation. Macroscopic studyfocuses on the morphological description of plant parts visible to the naked eye or through a magnifying lens.

Microscopic Evaluation

From the entire leaf sample, a small portion of the midrib region was separated to take a section. Thin transverse sections of the leaf were manually taken with sharp razor blades, using a thermocol pith for support. Safranin red was used to stain the sections. A small drop of safranin red was diluted in water, and the thin sections were added to the stain and removed after a few seconds. The stain turned the sections red. The sections were then washed in water to remove excess stain and placed on a clean glassslide, then mounted with glycerin. A clean coverslip was carefully placed over the section without trapping any air bubbles. The slides were then observed under a microscope, first at low power and then at high power. Clear photographs were taken, and details were noted. The slides were observed, and photographs were taken using a Labomed LX-300 Binocular microscope attached to an industrial digital camera.

Powder Microscopy

The powdered sample was kept onto the slides. A few drops of phloroglucinol solution were added (prepared by dissolving a small amount of phloroglucinol in ethanol in a watch glass, covered to prevent evaporation). Then, 1-3 drops of dilute hydrochloric acid and the mixture was stirred with a fine pointed needle to ensure even distribution, if necessary. A cover slip was placed over the sample,and any excess liquid was gently blotted from the edges with filter paper. All lignified tissues were stained pink with phloroglucinol. The powder slides were then observed, and photographs were taken using a Labomed LX-300 Binocular microscope attached to an industrial digital camera.

Determination of Physico-Chemical Constants

Moisture Content

A 2 g sample of powdered leaf was accurately measured and placed in a pre-weighed crucible. The sample was dried in a hot air oven at 100-105°C for several hours until a constant weight was reached. After removing it from the oven, the sample was cooled in a desiccator. Once cooled, the final weight was recorded, and the weight loss was used to calculate the moisture content of the crude drug.

Total Ash Value

A precisely weighed 2 grams of finely ground, air-dried material was transferred to a crucible that had been preheated for 30 minutes. The sample was then evenly divided and incinerated at temperatures exceeding 450°C until all carbon was eliminated. After cooling the crucible in a desiccator, it was weighed again. The total ash content per gram of the air-dried material was subsequently calculated.

Acid Insoluble Ash Value

The total ash was treated with 25 ml of 2 M hydrochloric acid for 5 minutes. Afterward, the insoluble residue was filtered through ashless filter paper (Whatman-41) and washed with hot water. The filter paper with the residue was returned to the original crucible, ignited, and weighed. The percentage of acid-insoluble ash was then calculated based on the weight of the air-dried drug.

Water Soluble Ash Value

To the crucible with the total ash, 25 ml of water was added, and the mixture was boiled for 5 minutes. The insoluble material was collected in sintered glass crucibles, washed with hot water, and ignited in a crucible for a few minutes at a temperature not exceeding 450°C. The weight of the residue in milligrams was subtracted from the total ash weight, and the water-soluble ash content was then calculated per gram of air-dried material.

Sulphated Ash Value

A few drops of H_2SO_4 were added to the crucible containing the total ash, and the mixture was ignited as before. After cooling, the crucible was weighed repeatedly until a constant weight was achieved. The percentage of sulfated ash was subsequently calculated relative to the air-dried drug.

Extractive Values

To determine the extractive values of *Jasminum malabaricum* using various solvents, 5 grams of coarsely powdered, air-dried leaves were accurately weighed for each solvent. The sample was immersed in 100 ml of the chosen solvent (hexane, petroleum ether, benzene, chloroform, acetone, ethyl acetate, 90%

ethanol, or chloroform water) in a sealed container and shaken periodically for the first 6 hours. The mixture was then allowed to stand undisturbed for 18 hours. After 24 hours of total extraction, the solution was rapidly filtered to avoid solvent loss. A 25 ml portion of the filtrate was transferred to a pre-weighed shallow or flat-bottomed dish and evaporated to dryness at 105°C. The dried residue was weighed, and the extractive value was calculated as a percentage of the initial weight of the air-dried drug. This method was repeated for each solvent to determine the respective extractive values.

Foreign Matter

A sample weighing between 100-500 g of the drug was measured and evenly distributed in a thinlayer. Foreign matter was identified through visual observation or using a 6x magnifying lens. The foreign material was then isolated, weighed, and its percentage calculated.

Swelling Index

1 g of powdered drug was taken in a 25 ml stoppered cylinder, and water was added up to the 25 ml marking. The solution was occasionally shaken during 23 hours and then left aside for one hour. The volume before swelling and after swelling was compared.

Foaming Index

1 g of coarse plant powder was taken in a 500 ml conical flask, and 100 ml of boiling water was added while maintaining moderate boiling for 30 minutes. After cooling, the solution was filtered,and the filtrate was collected in a 100 ml volumetric flask, with the volume adjusted to 100 ml by adding sufficient water. The decoction was poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in varying volumes of 1 ml, 2 ml, 3 ml, and so on, up to 10 ml. The volume of liquid in each test tube was adjusted to 10 ml by adding a sufficient quantity of water, and the tubes were stoppered. The test tubes were shaken in a longitudinal motion for 15 seconds, with two shakes per second, and then allowed to sit for 15 minutes. After this period, the foam height was measured.

Phytochemical Studies

Successive Solvent Extraction

The air-dried powdered plant materia55l (50-100 g) was extracted successively with solvents of increasing polarity in a Soxhlet extractor. The solvents used were petroleum ether (60°-80°C), chloroform, ethanol and water. Before extracting with each successive solvent, the powdered material (marc) was dried in air or in an oven at a temperature below 50°C. After the alcoholic extraction. The marc was soaked in chloroform water for 24 hours to obtain the aqueous extract. Each extract was concentrated by evaporating

the solvent via distillation and drying the residue completely in a water bath. The residue collected from each solvent was weighed, and its percentagewas calculated in relation to the air-dried weight of the plant material. (% w/w) to determine the successive solvent extractive values.

Preliminary Phytochemical Screening of Plant Extracts

Preliminary phytochemical screening was performed to identify various constituents present in the extracts, such as carbohydrates, proteins, lipids, flavonoids, tannins, glycosides, alkaloids, essential oils, and others. All the four extracts: petroleum ether, chloroform, ethanol and water were subjected to preliminary phytochemical screening.

a. Chemical Tests for Alkaloids:

- **Mayer's Test:** To test for alkaloids, 2 ml of the extract was treated with 2 ml of Mayer's reagent. A positive result is indicated by the formation of a creamy precipitate.
- **Hager's Test:** In this test, 2 ml of the extract was mixed with 1-2 ml of Hager's reagent. The presence of alkaloids is confirmed by the appearance of a yellow precipitate.
- **Wagner's Test:** Here, 2 ml of the extract was treated with 1-2 ml of Wagner's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

b. Chemical Tests for Carbohydrates:

- **Molisch's Test:** To detect carbohydrates, 1 ml of the test solution was mixed with 2 ml of Molisch's reagent. After shaking, 1 ml of concentrated sulfuric acid (H_2SO_4) was carefully added along the sides of the test tube. A violet ring formed at the junction of the two solutions indicates a positive result for carbohydrates.
- **Fehling's Test:** In this test, 1 ml of the test solution was boiled with 1 ml each of Fehling's solution A and Fehling's solution B in a water bath. The presence of carbohydrates is confirmed by the formation of a red residue at the bottom of the test tube.
- **Benedict's Test:** For this test, 2 ml of Benedict's reagent was mixed with 2 ml of the test solution and then boiled in a water bath. A red precipitate indicates a positive result for carbohydrates.

c. Chemical Tests for Flavonoids:

- **Ferric Chloride Test:** A few drops of ferric chloride solution were added to 2 ml of the test solution. The development of an intense green color indicates the presence of flavonoids.
- **Shinoda's Test:** In this test, 10 drops of dilute hydrochloric acid (HCl) were added to 2 ml of the extract, followed by a small piece of magnesium turnings. The appearance of a pink, reddish, or brown color confirms the presence of flavonoids.
- **Aqueous Sodium Hydroxide (NaOH) Test:** To 2 ml of the extract, a few drops of 10% aqueous NaOH solution were added. A yellow precipitate suggests the presence of flavonoids.

d. Chemical Tests for Glycosides:

- **Baljet's Test:** For glycosides, 2-3 ml of the sample was mixed with 2 ml of sodium picrate solution. A color change to yellow or orange indicates the presence of glycosides.
- **Borntrager's Test:** In this test, 2 ml of the sample solution was added to a mixture of water and chloroform ($CHCl_3$). After separating the layers, the organic layer was collected and shaken with dilute ammonia. The appearance of a deep red color in the lower layer and green or blue-violet in the upper layer indicates glycosides.

e. Chemical Tests for Phenolics and Tannins:

- **Ferric Chloride Test:** Aqueous extract solution was mixed with a few drops of 5% ferric chloride solution. A dark green or bluish-black color confirms the presence of phenolics and tannins.
- **Lead Acetate Test:** In this test, 2 ml of the test solution was mixed with 1 ml of lead acetate solution. The formation of a bulky white precipitate indicates the presence of phenolics and tannins.

f. Chemical Tests for Proteins and Amino Acids:

- **Millon's Test:** To detect proteins, 2 ml of the extract was mixed with 2 ml of Millon's reagent and boiled. The presence of proteins is indicated by a white precipitate that turns red upon warming.
- **Ninhydrin Test:** In this test, 2 ml of the extract was treated with two drops of Ninhydrin solution and heated in a water bath. The appearance of a violet color indicates the presence of proteins or amino acids.

g. Chemical Test for Saponins:

- **Foam or Froth Test:** To test for saponins, 2 ml of the extract was diluted with 20 ml of distilled water in a graduated cylinder. The mixture was shaken vigorously for 15 minutes. The formation of froth confirms the presence of saponins.

h. Chemical Test for Steroids:

- **Liebermann–Burchard's Test:** To test for steroids, 2 ml of the extract was mixed with 1 ml of chloroform and 1 ml of acetic anhydride, followed by the addition of 1 drop of concentrated sulfuric acid (H_2SO_4). The development of a deep red color in the lower portion and green color in the upper portion, eventually changing to blue and violet, indicates the presence of steroids.

i. Chemical Test for Terpenoids:

- **Salkowski's Test:** In this test, 2 ml of the extract was dissolved in chloroform. An equal volume of concentrated sulfuric acid (H_2SO_4) was added. The appearance of a red color in the chloroform layer and greenish-yellow fluorescence in the acid layer indicates the presence of terpenoids.

Quantitative Estimation of Phyto Constituents

Determination of Total Phenolic Content by Folin - Ciocalteu Method

To determine the total phenolic content of *Jasminum malabaricum* extracts, both ethanolic and aqueous extracts were used in the experiment. A 1 ml aliquot of each extract, at a concentration of 1 mg/ml, was combined with 1 ml of Folin–Ciocalteu phenol reagent and permitted to stand for 5 minutes. After this, 10 ml of 7% sodium carbonate solution was added, followed by 13 ml of deionized water. The resulting mixture was incubated in the dark for 90 minutes to ensure proper reaction. Following the incubation, the absorbance of the solution was measured at 760 nm using a spectrophotometer. A calibration curve was established using Gallic acid standard solutions (20–100 µg/ml), and the TPC of the ethanolic and aqueous extracts was determined by extrapolation from the calibration curve. The results were expressed as micrograms of Gallic acid equivalents (GAE) per milligram of extract.

Determination of Total Flavonoid Content by Aluminium Chloride Colorimetric Method

To determine the total flavonoid content of *Jasminum malabaricum* extracts, both ethanolic and aqueous extracts were used in the experiment. 1 ml of each extract was combined in a 10 ml volumetric flask, 4 ml of distilled water was mixed with the sample. To this solution, 0.3 ml of 5% sodium nitrite solutionwas added and allowed to react for 5 minutes. Subsequently, 0.3 ml of 10% aluminum chloride solution was

introduced, and after another 5 minutes, 2 ml of 1 M NaOH was added to the mixture.The total volume was adjusted to 10 ml with distilled water, and the solution was mixedthoroughly. Standard solutions of Kaempferol, ranging from 20 to 100 µg/ml, were prepared using the same method. The absorbance of both the test samples and the Kaempferol standards was measured at 510 nm using a UV-Visible spectrophotometer. The TFC of the ethanolic and aqueous extracts was calculated by extrapolation from the Kaempferol calibration curve, and the results were expressed as micrograms of Kaempferol equivalents (KE) per milligram of extract.

Results

Pharmacognostical Studies

Macroscopic Evaluation

Figure 02: Macroscopy of leaf

Features	Observations
Color	Green
Size	8-10 cm long and 6-7.2 cm width
Odor	Faint characteristic
Taste	Slightly pungent
Texture	Smooth
Shape	Broadly ovate with a sharp tip. The base of leaf is either rounded or heart shaped.
Petioles	1cm long
Apex	Abruptly shortly acuminate
Margin	Entire
Base	Truncate or subcordate
Venation	Reticulate

Table 01: Macroscopic Characters of leaves of *Jasminum malabaricum*

Microscopic Evaluation

Transverse Section:

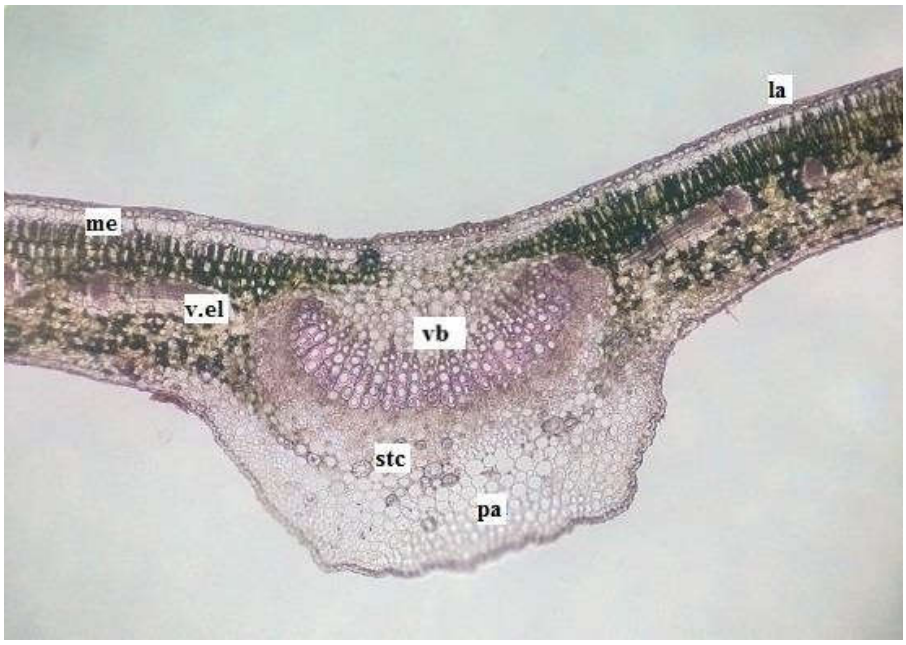


Figure 03: Transverse section of leaf of *Jasminum malabaricum* leaf

la.: lamina; **me.**: mesophyll cells; **v.el.**: vascular elements. **vb.**: vascular bundle; **stc.** Stone cells; **pa.**: Parenchyma cells.

Powder Microscopy

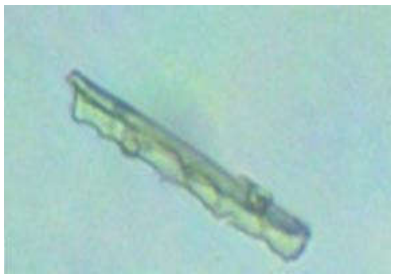


Figure 04: Epidermal cells

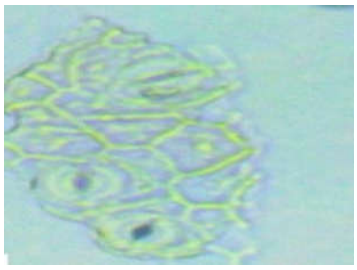


Figure 05: Epidermal cells with stomata

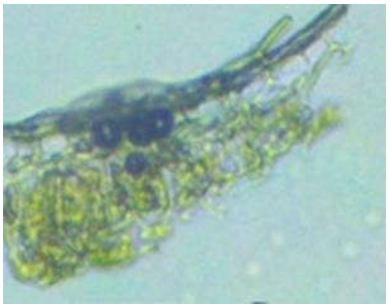


Figure 06: Mesophyll with sectional view

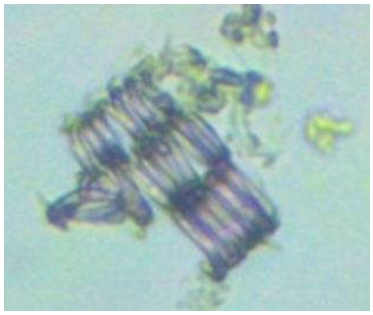
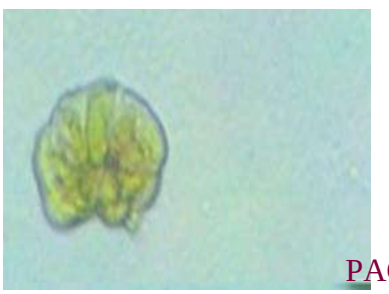


Figure 07: Spiral vessel



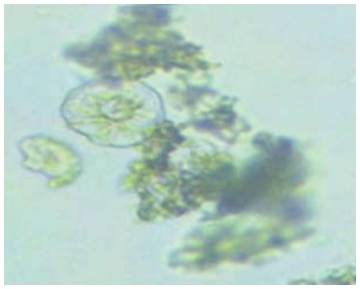


Figure 08: Glandular trichome

Figure 09: Glandular trichome surface view

Physicochemical Studies

Sl. No.	Physico-chemical constants	Result (% w/w)
I	Moisture content	10.96
2	Ash values:	
	a) Total ash value	3.1
	b) Acid insoluble ash value	1.2
	c) Water soluble ash value	1.4
	d) Sulphated ash value	6.0
3	Extractive values:	
	Hexane soluble extractive value	1.3
	Ether soluble extractive value	1.6
	Benzene soluble extractive value	1.8
	Chloroform soluble extractive value	2.8
	Acetone soluble extractive value	6.2
	Ethyl acetate soluble extractive value	7.2
	Ethanol soluble extractive value	8.6
	water soluble extractive value	7.6
4	Foreign organic matter	<1.0
5	Swelling index	Nil

6	Foaming index	<1000

Table 02: Physicochemical analysis of *Jasminum malabaricum* leaves

Phytochemical Studies

Successive Solvent Extraction

SI No	Extract	Percentage yield (%w/w)
1	Petroleum ether	1.2
2	Chloroform	2.0
3	Ethanol	8.2
4	Water	7.2

Table 03: Percentage yield of *Jasminum malabaricum* leaf extracts

Phytochemical Screening of JMEs

Constituents	Petroleum Ether	Chloroform	Ethanol	Water
Carbohydrates	-	-	+	+
Alkaloids	-	++	+	+
Flavanoids	-	++	++	++
Proteins	-	-	-	-
Glycosides	+	++	+	+
Phenols	+	+	++	++
Tannins	+	-	-	+
Terpenoids	-	+	++	++
Steroids	++	-	+	-
Saponins	-	-	-	++

Table 04: Qualitative phytochemical analysis of *Jasminum malabaricum* leaf extracts

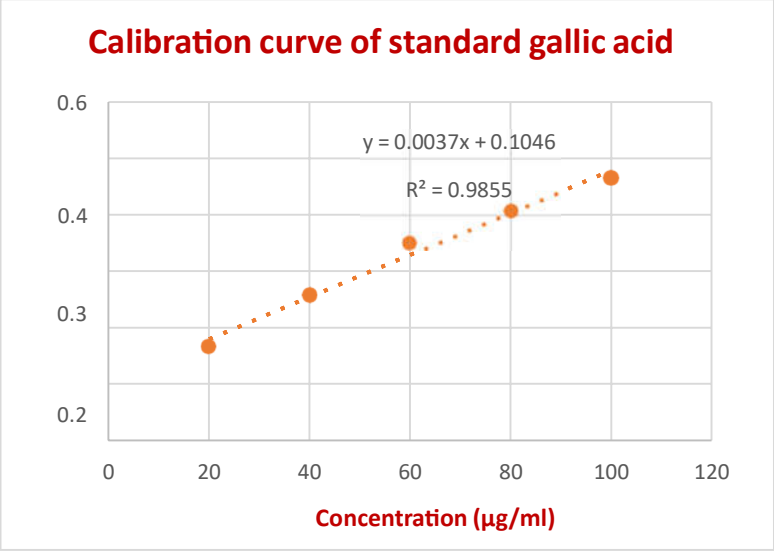
Note: (++) indicate abundance, (+) indicate presence, (-) indicates absence

Quantitative Estimation of Phytoconstituents

Estimation of Total Phenolic Content in JMEE

Sl. No.	Concentration (µg/ml)	Absorbance
1	20	0.166
2	40	0.257
3	60	0.350
4	80	0.407
5	100	0.465
6	Ethanolic extract of *Jasminum malabaricum* Leaf	0.282

Table 05: Absorbance of Standard Gallic acid and JMEE at 760nm



Graph 01: Calibration curve of Standard Gallic acid for JMEE

Sl. No.	Extract (100 µg/ml)	Concentration of phenolic content in µg/ml of sample

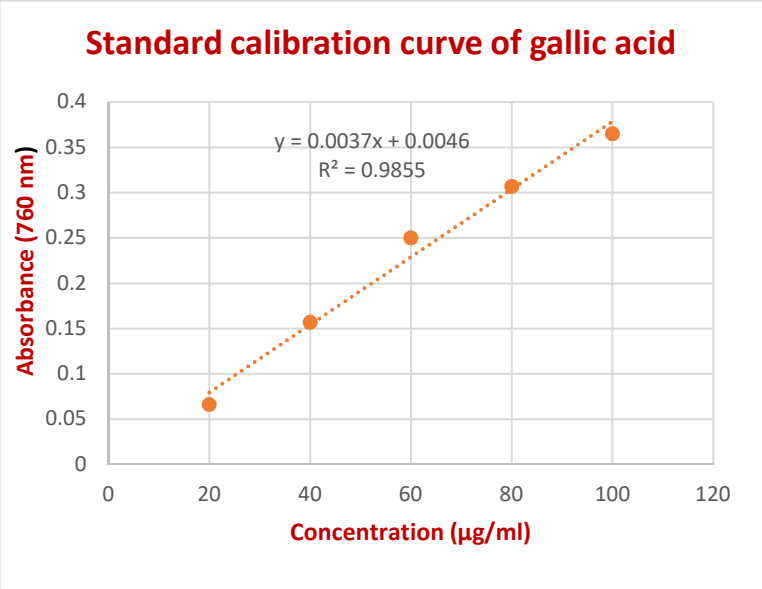
1	Ethanolic extract of *Jasminum malabaricum* leaf	47.9

Table 06: Total Phenolic Content of JMEE

Estimation of Total Phenolic Content in JMAE

Sl. No.	Concentration (µg/ml)	Absorbance
1	20	0.066
2	40	0.157
3	60	0.250
4	80	0.307
5	100	0.365
6	Aqueous extract of *Jasminum malabaricum* leaf	0.201

Table 07: Absorbance of Standard Gallic acid and JMAE at 760nm



Graph 02: Calibration curve of Standard Gallic acid for JMAE

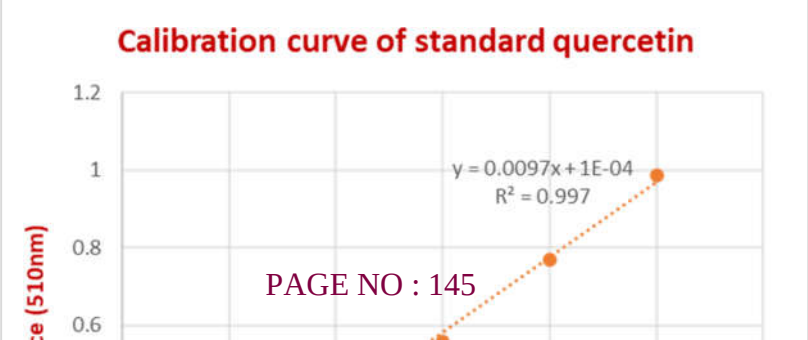
Sl. No.	Extract (100 µg/ml)	Concentration of phenolic content in µg/ml of sample
1	Aqueous extract of *Jasminum malabaricum* leaf	53.0

Table 08: Total Phenolic Content of JMAE

Estimation of total flavonoid content in JMEE

Sl. No.	Concentration (µg/ml)	Absorbance
1	20	0.201
2	40	0.399
3	60	0.557
4	80	0.768
5	100	0.987
6	Ethanolic extract of *Jasminum malabaricum* leaf	0.482

Table 09: Absorbance of Standard Quercetin and JMEE at 510nm



Graph 03: Calibration curve of Standard Quercetin for JMEE

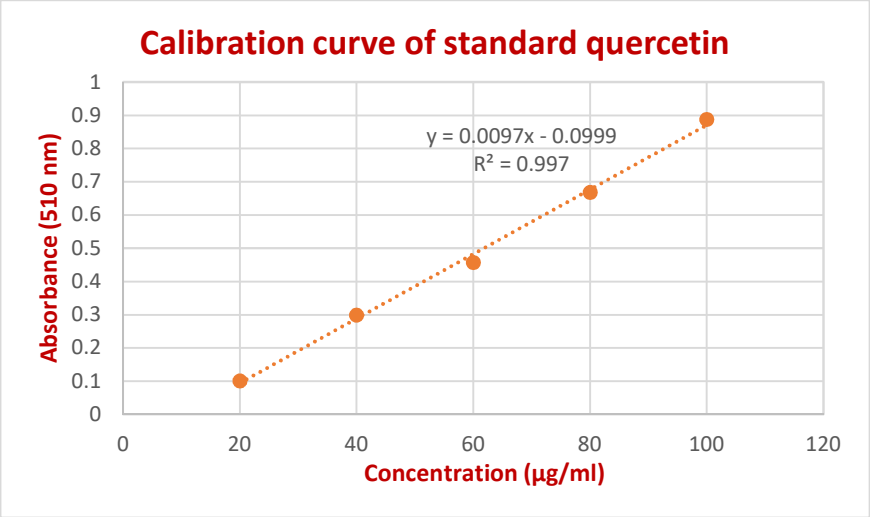
Sl. No.	Extract (100 µg/ml)	Concentration of flavonoid content in µg/ml of sample
1	Ethanolic extract of *Jasminum malabaricum* leaf	49.6

Table 10: Total Flavonoid Content of JMEE

Estimation of total flavonoid content in JMAE

Sl. No.	Concentration (µg/ml)	Absorbance
1	20	0.101
2	40	0.299
3	60	0.457
4	80	0.668
5	100	0.887
6	Aqueous extract of *Jasminum malabaricum* Leaf	0.401

Table 11: Absorbance of Standard Quercetin and JMAE at 510nm



Graph 04: Calibration curve of Standard Quercetin for JMAE

Sl. No.	Extract (100 µg/ml)	Concentration of flavonoid content in µg/ml of sample
1	Aqueous extract of *Jasminum malabaricum* leaf	52.0

Table 12: Total Flavonoid Content of JMAE

Discussion

The study of *Jasminum malabaricum* provides detailed insights into its macroscopic and microscopic characteristics, as well as its physicochemical and phytochemical properties. Macroscopically, the leaves exhibit a distinct green color, smooth texture, and broadly ovate shape, with measurements indicating a size range of 8-10 cm in length and 6-7.2 cm in width. These features facilitate the identification of the species among other *Jasminum* varieties.

Microscopic analysis reveals the internal structure of the leaf, highlighting important elements such as mesophyll cells, vascular bundles, and stone cells. The presence of these structures is indicative of the leaf's functional roles in photosynthesis and mechanical support. Powder microscopy further supplements this analysis by displaying unique features, including spiral vessels and glandular trichomes, which are crucial for the secretion of bioactive compounds and essential oils, thus enhancing the therapeutic potential of the plant.

Physicochemical assessments demonstrate a moisture content of 10.96%, essential for maintaining the stability and quality of the plant material. The extractive values indicate that ethanol (8.6%) and water (7.6%) are effective solvents for the extraction of bioactive compounds, suggesting optimal methods for extracting the plant's medicinal properties.

Phytochemical screening reveals a rich presence of various bioactive constituents across the extracts, notably phenols, flavonoids, alkaloids, and terpenoids. The qualitative analysis indicates that ethanol and aqueous extracts are particularly abundant in these compounds.

Quantitative estimation of total phenolic content shows significant levels in both the ethanolic extract (47.9 µg/ml) and the aqueous extract (53.0 µg/ml), alongside substantial flavonoid concentrations of 49.6 µg/ml and 52.0 µg/ml, respectively. These results indicate that *Jasminum malabaricum* is rich in bioactive compounds, particularly phenolics and flavonoids, which support its traditional use.

Conclusion

Pharmacognostic standardization is essential for the accurate identification, purity, and quality assessment of medicinal plants. This study evaluates the pharmacognostic, physicochemical, and phytochemical properties of *Jasminum malabaricum*. The results obtained provide valuable information that can be utilized for the development of official monographs and the standardization of this plant. Furthermore, the comprehensive characterization supports the potential therapeutic applications of *Jasminum malabaricum*, highlighting the need for further research to establish its efficacy and safety in medicinal use.

Acknowledgement

The authors sincerely thank Nehru College of Pharmacy for their invaluable support and excellent facilities that contributed to the successful completion of this research.

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