

# Pharmacognostical, Physicochemical Analysis and Phytochemical Screening of the Leaves of *Allium fistulosum* Linn.

Jesvy Joby\*, G.N Pramodini

Department of Pharmacognosy, Nehru College of Pharmacy, Pampady, Thrissur, Kerala, INDIA.

## ABSTRACT

**Introduction:** Natural products have long served as an essential foundation for drug discovery owing to their, unique structural diversity and therapeutic properties. *Allium fistulosum* L. (Welsh onion), widely used in traditional Chinese medicine, exhibits broad therapeutic activities including Inflammation-inhibiting, Tumor-suppressive, Infection-controlling, and Glucose-lowering Properties. The present study aims to provide a comprehensive Pharmacognostical, physicochemical, and phytochemical evaluation of the leaves of *Allium fistulosum* to support its authentication and standardization. **Methodology:** Fresh leaves of *Allium fistulosum* were collected and taxonomically authenticated. Macroscopic and microscopic examinations, including transverse section and powder analysis, were performed to assess morphological features. Physicochemical parameters such as extractive values, ash levels, moisture content, and foaming index were determined using standard pharmacopoeial methods. Preliminary phytochemical screening was conducted on the ethanolic extract to identify the presence of secondary metabolites. **Results:** Macroscopic evaluation revealed hollow cylindrical leaves with a mild onion-like odour. Microscopic analysis showed a fistular lamina with closed collateral vascular bundles. Physicochemical analysis recorded a moisture content of 16%, total ash value of 8.23%, and foreign matter content below 1%. Among the solvents tested, ethanol and chloroform yielded the highest extractive values (12.5% and 10.3%, respectively), indicating their efficiency in isolating bioactive compounds. Preliminary phytochemical evaluation indicated that the ethanolic extract contains glycosides, flavonoids, tannins, phenols, proteins, terpenoids, and amino acids, but lacks alkaloids, carbohydrates, and saponins. **Conclusion:** This study provides essential baseline data for the quality control and authentication of *Allium fistulosum* leaves. The results affirm its potential as a source of bioactive phytoconstituents and justify further pharmacological investigations to explore its therapeutic applications.

**Keywords:** Ash content, Extractive value Physicochemical, Phytochemical, Soxhlet extraction.

## Correspondence:

Jesvy Joby

M Pharm Student, HOD, Department of Pharmacognosy, Nehru College of Pharmacy, Pampady, Thrissur-680588, Kerala, INDIA.

Email: jjesvy@gmail.com

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## INTRODUCTION

For thousands of years, medicinal chemicals have been found in nature, and a staggering number of modern medicines have been found to have natural roots. Folk herbal remedies have long been made from plants to cure a variety of illnesses, and the wide range of natural compounds they generate has inspired the development of new drugs. With the emergence of novel protein based molecular targets, there is an increasing demand for fresh chemical diversity in screening processes. Continued exploration of the planet's largely undiscovered biodiversity will play a vital role in fulfilling this need, with natural products

remaining a key resource in future drug discovery efforts. Asthma, influenza, cancer, TB, diabetes mellitus, coronary artery disease, diarrhea, and other life-threatening conditions can be treated with innovative biotechnology applied to plants to create medications made from natural components.<sup>1</sup> In addition to complementing synthetic molecules, natural products have drug-relevant qualities that no synthetic chemical can match. The vast structural and chemical variety of natural goods is one of their main characteristics. In actuality, modern medicinal chemistry lacks over 40% of the molecular scaffolds present in natural compounds, making them complimentary to molecules that are synthesized. Their past success in drug development is probably due in part to the fact that natural chemicals or their derivatives make up 45% of the best-selling drugs on the market today.<sup>2</sup>

Worldwide, *Allium* species will be utilized as spices, vegetables, and medicinal plants due to their economic necessity.<sup>3</sup> *Allium*



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**Figure 1:** *Allium fistulosum* Linn.

*fistulosum* L. is a perennial monocot that is widely grown, particularly in China, Japan, and Korea, from tropical Asia to Siberia. Welsh onion is the name given to this species, which comes from the German word "welshche," which means "foreign." In Chinese folk medicine, it is used to cure colds, headaches, stomach-aches, diarrhea, eye conditions, and recurrent abortions. Sulphur molecules are abundant in the essential oil found in the bulb. It is a promising source of bioactive components including flavonoids and quercetin, which show a lot of pharmacological activities, including inflammation reducing, cancer fighting, anti-microbial, anti-platelet, anti-diabetic, anti-asthmatic, anti-thrombotic, hypolipidemic, and anti-hypertensive properties.<sup>4</sup>

Welsh onions are said to have numerous medicinal benefits, particularly in Chinese medicine. It prolongs life and improves the function of internal organs and metabolism. Additionally, it is said to help with digestion and sweating, improve vision, and speed up the healing process from wounds, festering sores, headaches, and common colds. Children can be sedated by a tea made from the roots. Internal parasites are hindered when the bulb is incorporated into the diet. The bulb can be used externally as a poultice to remove pus from boils, abscesses, and wounds. The plant's juice is used to ward off moths.<sup>5</sup> Amino acids, phenols, coumaric, ferulic, glycosides,  $\beta$  sitosterol, campesterol, stigma sterol, flavonoids, and D-limonene are all found in *A. fistulosum*. In addition,  $\alpha$ -pinene, 1-Buten-3-yne, 1-chloro-, (Z), TMS derivative, and thymol dichloroacetic acid were identified as minor bioactive components.<sup>6</sup> Among plants, luteolin, myricetin, apigenin, kaempferol, and quercetin are the five most common flavonoids.<sup>5</sup>

Pharmacognostical, phytochemical and physicochemical studies are essential for the standardization and quality evaluation of medicinal plants. Pharmacognostical analysis uses microscopic and morphological characteristics to guarantee accurate identification. Phytochemical studies reveal the presence of Active constituents such as flavonoids, phenolic acids, sulfur compounds,

and sterols, which contribute to the plant's therapeutic effects. Physical-chemical constants, including moisture content, ash values, and extractive values, help assess the purity, stability, and overall quality of the plant material. Some Pharmacognostical, physicochemical and phytochemical research were found in the current literature. This study's primary goal is to offer useful information about the Characterization and validation of *Allium fistulosum* L. leaf, which may be useful in regards to authenticity, purity, and quality features.

## METHODOLOGY

### Collection of plant material

*Allium fistulosum* was purchased from the Thrissur local market. The plant was submitted to the botany department, at NSS College Ottapalam. The botanist Dr. Ranjusha A.P. taxonomically identified and verified the plant.

### Macroscopy

Fresh leaves of *Allium fistulosum* were analyzed for their morphological features, including organoleptic properties such as color, odor, taste, shape, and texture, following the standard procedures outlined in the WHO guidelines.

### Microscopy

Histochemical and microscopic studies of the fresh and powdered samples were performed in accordance with the methods described by Kokate and Khandelwal.

### Preparations of plant powder

The entire *Allium fistulosum* plant was washed and allowed to dry in the shade for a period of 20 to 25 days. After being ground in a mixer to a coarse powder, the dried plant was sealed in an airtight container. In the phytochemical research, extraction is the first step. It involves applying certain solvents and following established protocols to separate the parts of plants that have therapeutic value.

## Physicochemical evaluations

### Identification of foreign matters

About 100 g of *Allium fistulosum* powder was evenly spread in a thin layer, and any foreign materials were identified through visual examination, removed, and subsequently weighed. The formula was utilized to ascertain the proportion of foreign material.<sup>7</sup>

$$\text{Foreign Matter (\%)} = \left( \frac{\text{Weight of foreign matter}}{\text{Total sample weight}} \right) \times 100$$

### Identification of moisture content

Tiny quantity of dust is taken and put in a covered crucible and kept in a hot air oven set at  $100 \pm 10^\circ\text{C}$  for the entire night in order to remove the moisture content. Once at room temperature, the dried specimens were measured using a crucible covered with a lid. We used the following formula to determine the moisture content.<sup>7</sup>

$$\text{Moisture Content (\%)} = \left( \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \right) \times 100$$

### Determination of total ash contents

Ash is a representation of the sample's inorganic matter composition, as established by the AOAC technique. A dried sample weighing about one gram was placed in a crucible, burned in a small fire, and then maintained for two to 3 hr at  $550$  to  $600^\circ\text{C}$  in a muffle furnace in order to verify that the ashing was finished, it was weighed after cooling in a desiccator. After another half-hour of heating in the furnace, it was cooled and weighed. Until the weight remained constant, the process was repeated in turn. The following calculation was used to get the total ash content.<sup>7</sup>

$$\text{Total Ash (\% w/w)} = \left( \frac{\text{Weight of ash (g)}}{\text{Weight of air-dried sample (g)}} \right) \times 100$$

### Identification of water-soluble ash

A total of 100 mg of ash was boiled with 10 mL of distilled water for five minutes. The insoluble residue was collected on ash-free filter paper or in a silica crucible, rinsed thoroughly with hot water, and ignited at a low temperature until a constant weight was achieved. The weight of the insoluble residue was subtracted from the total ash weight, and the values obtained were used to determine the water-soluble ash content and its percentage.<sup>8</sup>

$$\text{Water-Insoluble Ash (\% w/w)} = \left( \frac{\text{Weight of water-insoluble residue (g)}}{\text{Weight of air-dried sample (g)}} \right) \times 100$$

### Determination of acid insoluble ash

The entire amount of ash was heated in 25 mL of 10% HCl for 5 min. An ash-less filter paper or a silica crucible was used to collect the insoluble ash. Before being burned and weighed, it was cleaned with hot water. The ratio of the total ash weight to that of the insoluble residue was calculated, and the difference in their weights indicated the amount of acid-insoluble ash. Calculations

were made using the quantity of ash collected to determine the proportion of acid-insoluble ash.<sup>8</sup>

$$\text{Acid-Insoluble Ash (\% w/w)} = \left( \frac{\text{Weight of acid-insoluble residue (g)}}{\text{Weight of air-dried sample (g)}} \right) \times 100$$

### Determination of swelling index

In a 25 mL measuring cylinder, 1 g of powder was added. After adding 25 mL of water, it was thoroughly shook every 10 min for 1 hr before being let to stand for 3 hr. Represent a possible source of new therapeutics, and kept it at room temperature for 3 hr. The plant material's volume was measured and contrasted with the dry powder's volume.<sup>9</sup>

$$\text{Swelling Index (mL/g)} = \frac{\text{Final volume of swollen material (in mL)}}{\text{Weight of sample (in g)}}$$

### Determination of foaming index

100 ml of water is taken in a conical flask; one gram of powder was added. It was then heated for 30 min, cooled, and filtered into a 100 mL volumetric flask, with the volume being adjusted with water. In ten test tubes, the decoction was prepared in 1 mL, 2 mL, 3 mL, and 10 mL increments. The foaming index was computed using the following formula, which is recommended in the Quality Control Methods for Medicinal Plants, for 15 sec test tubes were shaken, then kept it for another 15 min.<sup>9</sup>

$$\text{Foaming Index (FI)} = \frac{1000}{\text{Dilution factor (D)}}$$

Where D = is the amount of decoction that was used to make the tube dilution.

### Determination of extractive value

Ethanol, Acetone, Ethyl Acetate, Chloroform, Petroleum Ether, and n-Hexane were used in a maceration method to extract the dry powdered plant material of *Allium fistulosum* L. 2 g of the coarsely powdered plant material were put into a dry 250 mL conical flask after being weighed in a weighing vial. Subsequently, 30 mL of each solvent was added to the flask individually. The flasks were corked and left at room temperature for a full day, shaking regularly. Whatmann No. 1 filter paper was used to filter the combinations before they were transferred into a 50 mL measurement cylinder. The filtrate was then put into weighed petry plates after it was acquired. The resulting extracts were dried out by allowing the filter to completely evaporate the solvent.<sup>10</sup>

$$\text{Extractive Value (\% w/w)} = \left( \frac{\text{Weight of dried extract}}{\text{Weight of crude drug sample}} \right) \times 100$$

### Soxhlet extraction method using ethanol as solvent

Using a soxhlet apparatus, 25 g of the coarsely ground dried plant material of *Allium fistulosum* L. was extracted with a high-polarity solvent (ethanol) at  $60^\circ$  to  $70^\circ$  for 18 hr, or until the solvent became colourless in the siphon tube. Using rotary evaporator extracts were concentrated. The resulting semisolid residue was gathered and kept in desiccators.<sup>11</sup>

## Preliminary phytochemical screening<sup>12</sup>

Preliminary chemical tests for ethanolic *Allium fistulosum* leaf extracts were carried out to determine the presence of different phytoconstituents in the extracts, including carbohydrates, proteins, lipids, flavonoids, tannins, glycosides, alkaloids, essential oils, and other compounds.

### Test for Glycosides

#### Keller-Killani test

An equal volume of water and 0.5 mL of concentrated lead acetate solution were added to the hexane extract of the drug, followed by thorough shaking and subsequent filtration. Extract the filtrate using an equivalent amount of chloroform. The residue from the dry evaporation of the chloroform extract was diluted in 3 mL of glacial acetic acid, and then a few drops of FeCl<sub>3</sub> solution were added. 2 mL of concentrated solution were added to the final solution in a test tube. Because of the presence of digitoxose, the reddish-brown layer of H<sub>2</sub>SO<sub>4</sub> turns bluish green when it stands.

#### Borntrager's test

Two hundred grams of the crude extract were combined with 2 mL of dilute sulfuric acid and 2 mL of 5% aqueous ferric chloride solution, then heated for five minutes. The resulting oxidation to anthraquinones confirmed the presence of glycosides.

### Test for Carbohydrates

#### Molisch's test

A few drops of  $\alpha$ -naphthol reagent were added to 1 mL of the sugar solution in a test tube, and the mixture was thoroughly blended. Keep the test tube angled while you add the acid by running H<sub>2</sub>SO<sub>4</sub> along its side (do not shake the test tube). At the interface between the sugar solution and the concentrated sulphuric acid, a purple ring form.

#### Fehling's test

In a test tube, combine 1 mL of reagents A and B. Heat the tube in a boiling water bath after adding a few drops of the test sample and mixing for 5-10 min. Appearance of orange to brick red Colour.

#### Benedict's test

Divide the test solution by 2 mL and add 2 mL of Benedict's reagent. Bring a tub of water to a boil. A red precipitate forms, which is a sign that carbohydrates are present.

### Test for Alkaloids

#### Mayer's test

2 mL of Mayer's reagent is added to 2 mL of the extract. A creamy precipitate is formed which indicate the presence of alkaloids.

### Dragendorff's test

2 mL of reagent were added to 2 mL of the plant extract filtrate, and the appearance of a reddish-brown precipitate confirmed the presence of alkaloids.

#### Wagner's test

An equal volume (2 mL) of reagent was added to 2 mL of the plant extract filtrate, and the development of a reddish-brown precipitate signified the presence of alkaloids.

### Test for Flavonoids

#### Alkaline reagent test

Two to three drops of Na<sub>2</sub>CO<sub>3</sub> were added to 2 mL of extract. The bright yellow colour is formed when a few drops of diluted HCl were added gradually it turns to colourless, signifying the presence of flavonoids.

#### Zinc hydrochloride solution test

Add concentrated HCl and zinc dust solution to the test solution. It turns crimson after a few minutes.

### Test for Tannins and Phenolic

#### Ferric chloride test

To 2 mL of the aqueous extract, a few drops of 10% ferric chloride solution (light yellow) were added. The appearance of a dark blue coloration confirmed the presence of gallic tannins, whereas a greenish-black hue indicated catechol tannins.

#### Lead acetate test

1 mL of lead acetate is added to two mL of the test solution. large white precipitation is formed.

### Test for Proteins and Amino acids

#### Millon's test

In a test tube, 2 mL of the sample solution is added with 2 mL of Millon's reagent. If a red precipitate does not appear immediately, the test tubes are placed in a water bath for about 2 minutes. Afterwards, they are examined to determine whether a colored precipitate has developed.

#### Biuret test

Fill a test tube with 2 mL of the test solution. Add a 5% sodium hydroxide solution in 2 mL. Mix the solutions. Two drops of a 1% copper sulphate solution should be added.

#### Ninhydrin test

Two drops of ninhydrin solution is added with extract and heated on a water bath. The presents of violet colour were noted.

## Test for saponins

### Foam test

In a graduated cylinder, a 1 mL extract solution was diluted with 20 mL of distilled water and agitated for 15 min. The presence of saponins is indicated by the production of stable foam.

### Lieberman Burchard's test

To drug extract few drops of glacial acetic acid and 2 drops of  $H_2SO_4$  were added colour change from red to green.

## Test for Terpenoids

### Salkowski test

Put 1 mL of oil or fat sample in a test tube, dissolve it in 1 mL of chloroform, and then run an equal volume of strong sulphuric acid along the tube's walls (Do not mix the contents). The presence of steroids was indicated by the red hue that formed at the lower layer, whereas the presence of terpenoids was shown by the production of a yellow layer.

### Liebermann-Burchard test

Fill a test tube with approximately 100 mg of fat or 1 mL of oil, dissolve it in 1 mL of chloroform, and then gradually add equal parts acetic anhydride and concentrated sulphuric acid along the test tube walls. The formation of a deep red colour showed the presence of terpenoids, whereas the upper layer turned green to indicate the presence of steroids.

## Quantitative phytochemical analysis

### Total phenol content [Folin-Ciocalteu Method]

Using a modified Folin-Ciocalteu reagent method, the quantity of phenol in the aqueous extract was ascertained. 1 mL of plant extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 2%  $Na_2CO_3$  solution. For 15 min, the resultant mixture was incubated at room temperature. The absorbance of the sample was measured at 760 nm. Gallic acid (1 mg/mL) was employed as a standard. Triple testing was done on each test. Using the standard curve as a guide, the results were calculated and presented as Gallic acid equivalent (mg/g of extracted molecule).<sup>13</sup>

### Total flavonoid content [Aluminium Chloride Colorimetric Method]

The flavonoid content was estimated using a modified aluminum chloride colorimetric assay. A reaction mixture was prepared by combining 1 mL of the plant extract with 3 mL of methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. The mixture was kept at room temperature for 30 minutes, after which the absorbance was

recorded at 510 nm. Quercetin (1 mg/mL) served as the standard. All analyses were conducted in triplicate, and the flavonoid concentration was calculated from the quercetin calibration curve and expressed as quercetin equivalents (mg/g of extract).<sup>13</sup>

## RESULTS AND DISCUSSION

### Macroscopic evaluation

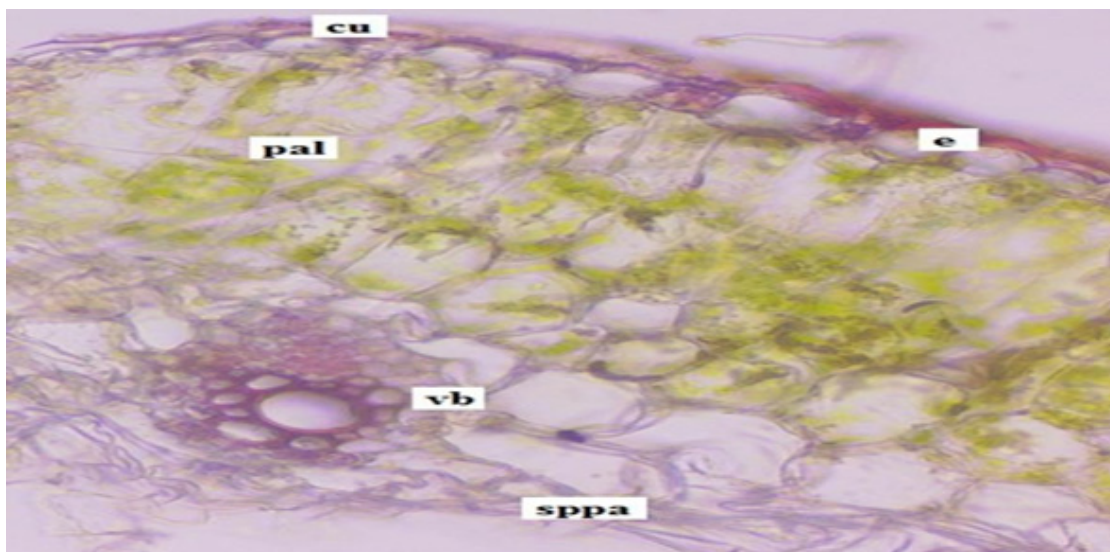
*Allium fistulosum* leaves are long, hollow, cylindrical, and green. They have a silky texture and a subtle onion-like flavour and aroma. It measures 30-60 cm in length and 0.5-2 cm in diameter. It lacks petioles, has an acute to rounded apex, entire margins, a broad cylindrical base forming a false stem, and exhibits parallel venation (Figure 1 and Table 1).

### Microscopic evaluation

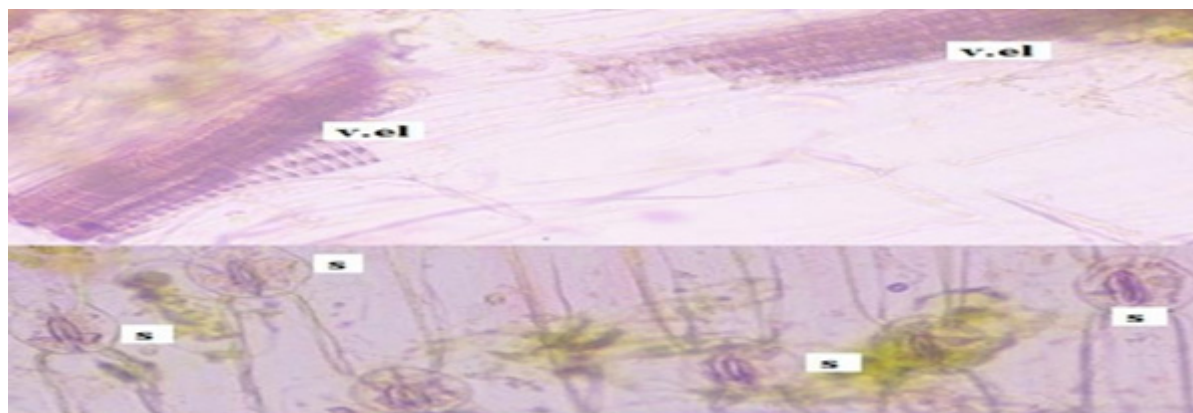
Transverse section of *Allium fistulosum* (Leaf) shows fistular lamina in which the leaf blade develops a central cavity during its growth. During development, leaves transform from solid to hollow. Cells around the cavity break up until the remaining 1-2 layers of cells from the palisade layer show cell wall residues. Epidermis consists of a single layer of large, oval shaped cells with a thin-walled cuticle. Mesophyll includes palisade parenchyma, spongy parenchyma and vascular bundles. Phloem and xylem make up the collateral, closed vascular bundles (Figures 2 and 3).

**Table 1: Macroscopic evaluation of *Allium fistulosum* L.**

| Sl. No. | Features [Leaves] | Observations  |
|---------|-------------------|---|
| 1       | Colour            | Green   |
| 2       | Size              | Length: 30 to 60 cm<br>Diameter: 0.5 to 2 cm                                    |
| 3       | Odour             | Characteristic pungent, onion like smell when crushed                           |
| 4       | Texture           | Smooth  |
| 5       | Shape             | Long cylindrical and Hollow (fistular)  |
| 6       | Taste             | Onion like, but generally milder than common bulb onions ( <i>Allium cepa</i> ) |
| 7       | Petioles          | Absent  |
| 8       | Apex              | Acute to rounded  |
| 9       | Margin            | Entire  |
| 10      | Base              | Broad and cylindrical, forming a false stem                                     |
| 11      | venation          | Parallel venation   |



**Figure 2:** Transverse section of leaf. cu.: cuticle; e.: epidermis; pal.: palisade cells; sppa.: spongy parenchyma; vb.: vascular bundle.



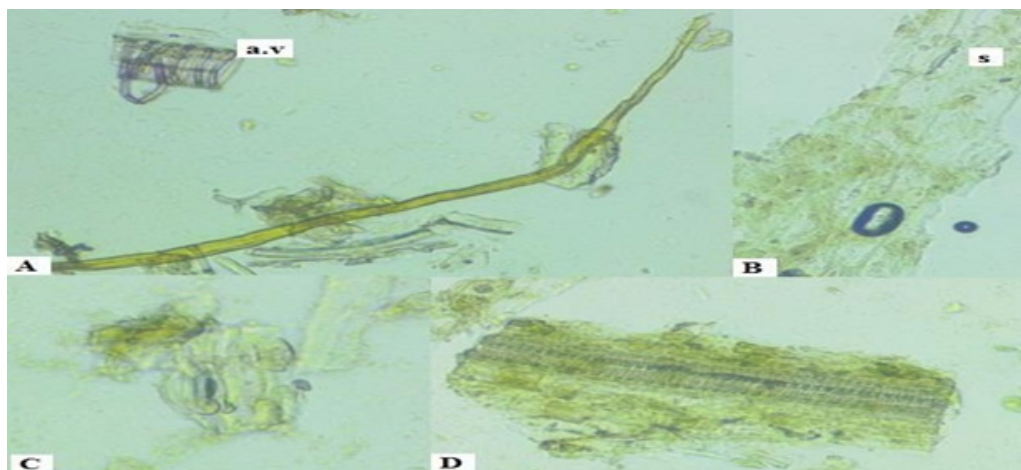
**Figure 3:** Cell inclusions of leaf lamina s.: stomata v.el: vascular elements.

**Table 2: Physicochemical evaluation of *Allium fistulosum* L.**

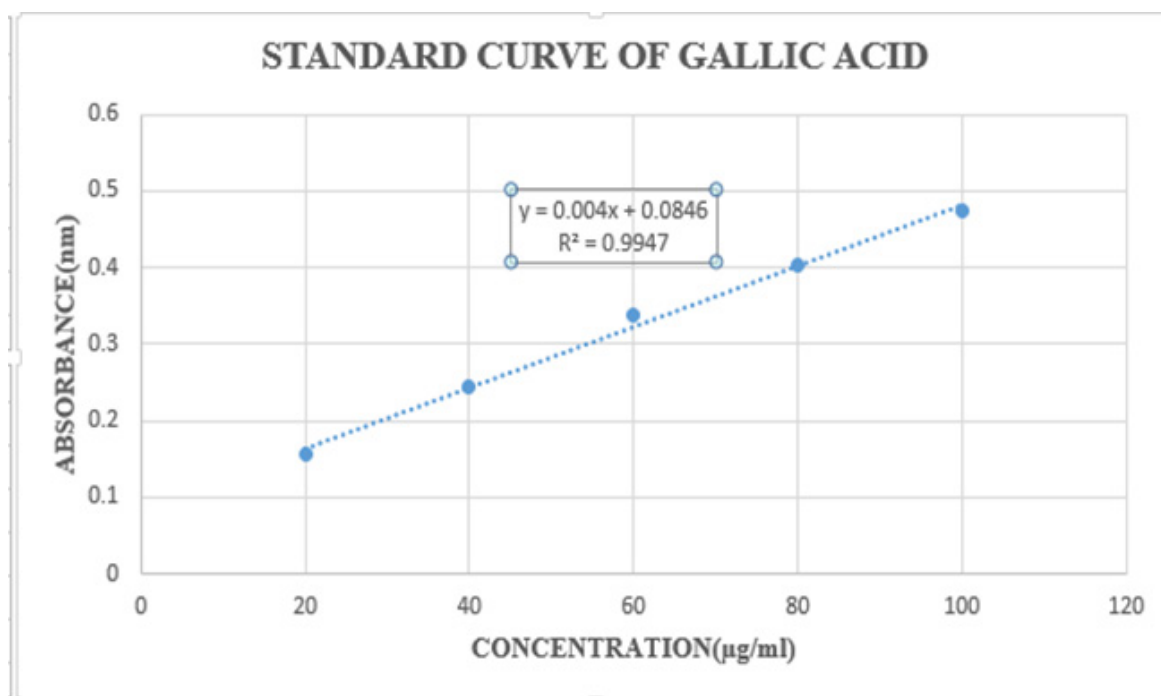
| Sl. No. | Physico-Chemical Constants | Results (%W/W) |
|---------|----------------------------|----------------|
| I       | Moisture Content           | 16             |
| II      | Ash Value                  |                |
|         | Total Ash                  | 8.23           |
|         | Acid Insoluble Ash         | 0.86           |
|         | Water Soluble Ash          | 1.35           |
| III     | Foreign Matter             | <1             |
| IV      | Swelling Index             | Nil            |
| V       | Foaming Index              | <100           |

**Table 3: Extractive value of *Allium fistulosum* L.**

| Parameters                       | Value (%WW) |
|----------------------------------|-------------|
| Ethanol Extractive Value         | 12.5        |
| Acetone Extractive Value         | 8.0         |
| Ethyl Acetate Extractive Value   | 5.2         |
| Chloroform Extractive Value      | 10.3        |
| Petroleum Ether Extractive Value | 1.5         |
| Hexane Extractive Value          | 0.8         |



**Figure 4:** Powder microscopy: A.: annular vessels and fibre; B.: surface view of epidermal cells with stomata; C.: stomata; D.: vessel elements.



**Graph 1:** Calibration curve of Standard Gallic acid for EAAF.

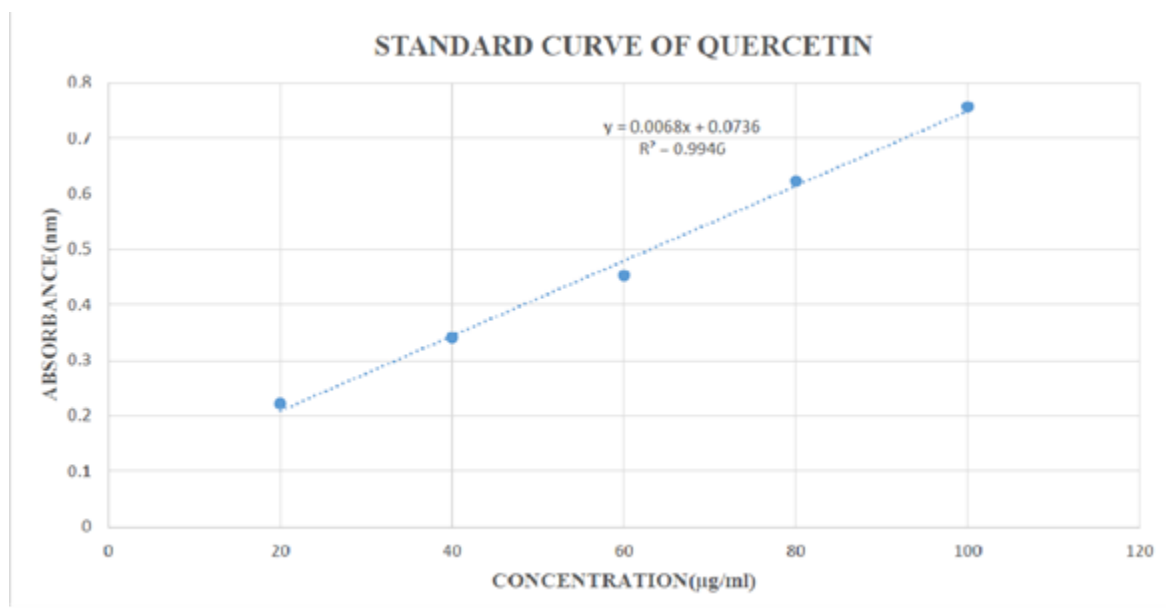
**Table 4:** Phytochemical screening of *Allium fistulosum* L.

| Test For                | Result |
|-------------------------|--------|
| Alkaloids               | -ve    |
| Glycosides              | +ve    |
| Phenolic and Tannins    | +ve    |
| Flavonoids              | +ve    |
| Terpenoids              | +ve    |
| Protein and Amino acids | +ve    |
| Carbohydrate            | -ve    |
| Saponins                | -ve    |

**Table 5:** Absorbance of Standard Gallic acid and EAAF at 760 nm.

| Sl. No. | Concentration (µg/mL)                                    | Absorbance (nm) |
|---------|--|-----------------|
| 1       | 20   | 0.156           |
| 2       | 40   | 0.245           |
| 3       | 60   | 0.388           |
| 4       | 80   | 0.403           |
| 5       | 100  | 0.475           |
| 6       | Ethanollic Extract of <i>Allium fistulosum</i> L. (EAAF) | 0.273           |

Values were expressed as Mean ± SD, n = 3.



**Graph 2:** Calibration curve of Standard Quercetin for EAAF.

**Table 6: Total Phenolic Content of EAAF.**

| Sl. No. | Extract (100 µg/mL)                                      | Concentration of flavonoid content in µg/mL of sample |
|---------|--|---|
| 1       | Ethanollic Extract of <i>Allium fistulosum</i> L. (EAAF) | 52.0  |

**Table 8: Total Flavonoid Content of EAAF.**

| Sl. No. | Extract (100 µg/mL)                                      | Concentration of flavonoid content in µg/mL of sample |
|---------|--|---|
| 1       | Ethanollic extract of <i>Allium fistulosum</i> L. (EAAF) | 48.2  |

**Table 7: Absorbance of Standard Quercetin and EAAF at 510 nm.**

| Sl. No. | Concentration (µg/mL)                                    | Absorbance (nm) |
|---------|--|-----------------|
| 1       | 20   | 0.222           |
| 2       | 40   | 0.341           |
| 3       | 60   | 0.453           |
| 4       | 80   | 0.623           |
| 5       | 100  | 0.757           |
| 6       | Ethanollic Extract of <i>Allium fistulosum</i> L. (EAAF) | 0.353           |

Values were expressed as Mean  $\pm$  SD,  $n = 3$ .

## Powder microscopy

Microscopic characterization of *Allium fistulosum* Linn. powder, displays a fragment of lignified annular vessel and non-lignified fibre. Surface view of epidermal cells with stomata and vessel elements are also present (Figure 4).

## Physicochemical evaluation

The sample contains a moderate amount of water, as indicated by the moisture content of 16%. There are 0.86% acid-insoluble ash and 1.35% water-soluble ash in the 8.23% total ash value. Less than 1% of foreign materials is present, indicating little contamination. The swelling index is reported as nil, indicating that the sample does not exhibit any noticeable swelling. Lastly, the foaming index is less than 100, showing limited foaming ability. These values provide essential insights into the physical and chemical properties of the sample (Table 2).

## Extractive value

The extractive value analysis of the sample showed that ethanol had the highest extractive value at 12.5%, indicating it is the most effective solvent for extracting constituents from the sample. Chloroform also demonstrated a significant extractive value of 10.3%, followed by acetone at 8.0%. Ethyl acetate had a moderate extractive value of 5.2%. On the lower end, petroleum ether showed an extractive value of 1.5%, and hexane had the least at 0.8%. These values suggest that polar solvents like ethanol and chloroform are more efficient in extracting components from this sample compared to non-polar solvents like petroleum ether and hexane (Table 3).

## Phytochemical screening

The leaves of *Allium fistulosum* shows abundance of Glycosides, Phenols, Tannins, Flavonoids, Terpenoids, proteins, and Amino acids. Alkaloids, Saponins and Carbohydrates are not presented (Table 4).

## Quantitative phytochemical analysis

### Estimation of total phenol content

The Total Phenolic Content (TPC) of *Allium fistulosum* L. leaf extract was evaluated using ethanolic (EEAF) extract (Table 5). Based on the gallic acid calibration curve (Graph 1), the phenolic content was found to be 52.0 µg/mL (Table 6).

### Estimation of total flavonoid content

The Total Flavonoid Content (TFC) of *Allium fistulosum* L. leaf extracts was measured using ethanolic (EEAF) extract (Table 7). Based on the Quercetin calibration curve, EEAF showed a flavonoid content of 48.2 µg/mL (Table 8).

## CONCLUSION

The study provides a detailed analysis of *Allium fistulosum* L. leaves, offering essential insights into its morphological, microscopic, physicochemical, and phytochemical characteristics. The results confirm the plant's rich composition of bioactive compounds such as flavonoids, glycosides, phenolics, and proteins, supporting its traditional medicinal use. Ethanol proved to be the most effective solvent for extracting these constituents. The low levels of foreign matter and moderate moisture content indicate good quality of the sample. AFEE is rich in bioactive polyphenolic compounds, with high phenolic and flavonoid content, supporting its traditional use and potential for therapeutic applications. Overall, this research establishes foundational data for the standardization and further pharmacological exploration of *Allium fistulosum*, reinforcing its potential as a valuable natural resource in herbal medicine and drug development.

## ACKNOWLEDGEMENT

The authors are grateful to Nehru College of Pharmacy Pampady, Thrissur for providing the necessary facilities, guidance, and support to carry out this research work. We sincerely thank the faculty and staff of the Department of Pharmacognosy for their valuable assistance during the experimental and analytical phases of the study.

## ABBREVIATIONS

TPC: Total phenol content; TFC: Total flavonoid content; EEAF: Ethanolic extract of *Allium fistulosum*.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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