



Research Article

Determination and Quantification of Total Flavonoids Content in Malabar Spinach Leaves by Using Colorimeter

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Flavonoids are significant phytoconstituents that are well known for their anti-inflammatory, antioxidant, and medicinal qualities. The goal of the current study was to use a colorimetric approach to identify and measure the total flavonoid content of Malabar spinach leaves (*Basella alba*). To obtain the plant extract, fresh leaves were gathered, shade-dried, ground into a powder, and then macerated with ethanol in a 1:10 ratio. The presence of flavonoids in the extract was verified by preliminary qualitative tests, including the alkaline reagent test, HCl test, ammonia test, and lead acetate test. Thin Layer Chromatography (TLC), which had distinctive yellow-green bands signifying the presence of flavonoids, provided additional proof. Rutin was used as a standard for quantitative flavonoid quantification. A colorimeter was used to measure the flavonoid–aluminium chloride combination generated during the reaction at a maximum absorbance (λ_{max}) of 450 nm. A calibration curve was created with varying rutin concentrations. At 450 nm, the plant extract's absorbance was measured to be 0.45. The calibration curve was used to determine that the total flavonoid content of 5 g of powdered dry Malabar spinach leaf was 0.216 g, or 4.32%. According to the research, *Basella alba* leaves are rich in flavonoids and could be a useful natural source of bioactive substances. For routine phytochemical analysis and herbal material quality monitoring, the colorimetric approach used in this study was simple, inexpensive, and accurate.

Keywords: Flavonoids, *Basella alba*, Colorimetric method, Rutin, Phytochemical analysis.

INTRODUCTION

For millions of years, people have utilised medicinal plants as natural sources of therapeutic chemicals to cure and prevent a wide range of illnesses. Despite the excellent advancements in modern medicine, plant-based treatments continue to be a vital part of healthcare systems worldwide. Many bioactive substances found in plant-based natural products add to their therapeutic efficacy. Plant-derived chemicals continue to draw interest in pharmaceutical and biomedical research due to their therapeutic potential and relatively lower negative effects. The significance of traditional medicine has also been highlighted by the World Health Organization (WHO), which notes that a large section of the world's population receives basic healthcare from plant-based medicines.

Flavonoids are one of the most significant types of polyphenolic compounds among the various phytochemical groups found in plants. They are found in many fruits, vegetables, and therapeutic plants. Antioxidant, anti-inflammatory, antibacterial, and anticancer properties are only a few of the biological actions of flavonoids. By neutralising free radicals, these substances shield biological systems from oxidative stress and may help prevent a number of chronic illnesses. In order to assess the pharmacological and nutritional value of flavonoids, it is crucial to identify and measure them in plant materials. A member of the Basellaceae family, Malabar spinach (*Basella alba*) is a climbing constant plant that grows quickly. Particularly in India and Southeast Asia, it is extensively grown in tropical and subtropical areas. The plant is also referred to as

Ceylon spinach, vine spinach, Indian spinach, and Malabar spinach. Because of their high nutritional content, their thick, heart-shaped, and mucilaginous leaves are frequently eaten as green vegetables. Apart from its nutritional value, *Basella alba* has long been utilised in traditional medicine to treat a variety of ailments. Flavonoids, phenolic compounds, vitamins, and minerals are among the phytochemicals found in the plant that are known to contribute to its therapeutic qualities.¹



Fig. 1. Malabar spinach leaves

Phytochemicals in plant extracts can be quantitatively estimated using colorimetric analysis, simple and popular method. The Beer–Lambert law, which asserts that the absorbance of light by a solution is proportional to the concentration of the absorbing material, is the foundation of this technique. By measuring the amount of light absorbed by a coloured solution at a particular wavelength, a colorimeter makes it possible to estimate the concentration of a component in the sample. In this context, the current work uses a colorimetric approach to determine and measure the flavonoid content of *Basella alba* leaves. The collection and extraction of plant material as well as the initial identification of the phytochemical components found in the extract are also included in the study. The results could support *Basella alba*'s future usage in pharmacological and nutritional applications by highlighting its phytochemical significance.²

MATERIALS AND METHODS:

MATERIALS:

Fresh Malabar spinach (*Basella alba*) leaves, ethanol, rutin powder (standard), sodium nitrite, aluminium chloride, and distilled water were the ingredients

employed in this investigation. These substances were utilised to extract plant material and to employ the colorimetric method to estimate the flavonoid concentration.

Apparatus: A spatula, funnel, tripod stand, measuring cylinder, Whatman filter paper, beakers, volumetric flasks (10 mL and 100 mL), glass rod, pipette, and cuvettes were among the tools utilised in the experiment.

Instruments: A colorimeter was utilised to measure the absorbance of the prepared solutions during flavonoid estimation, and a digital analytical balance was utilised for precise weighing of chemicals and plant material.

Plant Collection and Extraction: After gathering fresh Malabar spinach (*Basella alba*) leaves, they were properly cleaned with water to get rid of dust and other contaminants. To preserve the stability of their phytochemical components, the leaves were subsequently shade-dried at room temperature. Following thorough drying, the leaves were ground into a fine powder using an appropriate grinding technique and kept in an airtight container for additional examination. About 5 g of the powdered plant material were combined with 50 mL of ethanol for extraction, keeping the ratio at 1:10 (g/mL). To aid in the effective extraction of constituents, the mixture was macerated for 10 days at room temperature with occasional shaking. Following the extraction phase, the mixture was filtered using filter paper to remove any solid residues. The resulting filtrate was then collected and utilised for additional phytochemical analysis and colorimetric flavonoid content assessment.

Quantitative Estimation of Flavonoids: Using rutin as the standard and the colorimetric method, the total flavonoid content of the *Basella alba* leaf extract was ascertained. After combining distilled water, sodium nitrite solution, and a known volume of plant extract, the mixture was let to stand for a short while. To create a flavonoid–aluminium complex, aluminium chloride solution was then added to the mixture. Sodium hydroxide was added after incubation, and distilled water was used to adjust the final volume. A colorimeter set at 450 nm was used to measure the produced colour intensity in comparison to a blank. A

calibration curve built from various rutin standard concentrations was used to determine the flavonoid content of the sample.

Qualitative tests: Qualitative phytochemical tests, including the alkaline reagent test, HCl test, ammonia test, and lead acetate test, were used to first validate the presence of flavonoids in the ethanoic extract of *Basella alba* leaves. These tests generated distinctive colour changes that indicated the presence of flavonoid components.

Thin layer chromatography:

Using silica gel as the stationary phase and a solvent system comprising ethyl acetate, formic acid, water, and acetic acid, thin-layer chromatography (TLC) was further carried out. Following development, yellow-green bands were seen, indicating the presence of flavonoids, and the conventional formula was used to determine the R_f value.

Standard R_f value for flavonoids = 0.06-0.97

The R_f values were calculated using the formula:

$$R_f = \frac{\text{Distance traveled by pigment}}{\text{Distance traveled by solvent front}}$$

$$R_f = \frac{3.5}{3.9} = 0.89$$

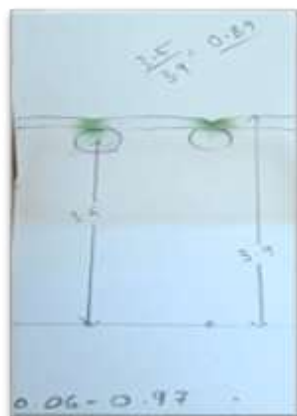


Fig. 2. TLC of Flavonoid.

Determination of total flavonoids content in Malabar spinach leaves:

Preparations:

To estimate flavonoids, standard and reagent solutions were made.

1. A 100 mL volumetric flask was filled with precisely weighed 1 g of rutin to create a standard rutin solution. The rutin was dissolved by adding around 30 mL of ethanol, and the final volume was increased to 100 mL with ethanol.
2. A 5% sodium nitrite solution was made by precisely weighing 5 g of sodium nitrite, dissolving it in distilled water in a 100 mL volumetric flask, and then adding distilled water to bring the volume up to 100 mL.
3. In a similar manner, the necessary quantity of aluminium chloride was weighed, dissolved in distilled water, and the final volume was adjusted to 100 mL in a volumetric flask to create a 10% aluminium chloride solution.

The total flavonoid content was measured colorimetrically using these prepared solutions.

Selection of Wavelength:

Take 3 ml of rutin solution, 0.3 ml of 5% NaNO_2 solution and 0.3 ml of 10% AlCl_3 solution into a 10ml volumetric flask. Makeup the volume to 10ml with distilled water. Take 3 ml of ethanol, 0.3 ml of 5% NaNO_2 solution and 0.3 ml of 10% AlCl_3 solution into a 10ml volumetric flask. Make up the volume 10ml. This serves as the blank solution. Standard and blank solutions to stand for 5mins. Measure the absorbance of the prepared rutin solution at different wavelengths. The wavelengths are 450nm, 470nm, 510nm, 520nm, 540nm, 570nm, 600nm, 670nm. The sample showed an absorbance of 0.55 at 450 nm, which corresponds to its maximum Wavelength (λ_{max}).

Table. 1. Wavelength and absorbance values of Flavonoids

Sl. No	Wavelength	Absorbance
1	450nm	0.55
2	470nm	0.49
3	510nm	0.28
4	520nm	0.15
5	540nm	0.09
6	570nm	0.07
7	600nm	0.05
8	670nm	0.04

Preparation of Calibration curve:

Six different concentrations of rutin solutions were prepared from a standard solution. (They are 1ml, 2ml, 3ml, 4ml, 5ml, and 6ml). Added 0.3 ml of 5% NaNo₂ and 0.3 ml of 10% AlCl₃ solutions for six

concentrations of Solutions. Make up the volume with distilled water up to 10ml. Then solutions stand for 5mins at room temperature. The absorbance measured at 450 nm, and the absorbances were 0.21, 0.36, 0.49, 0.64, 0.81, and 0.91.

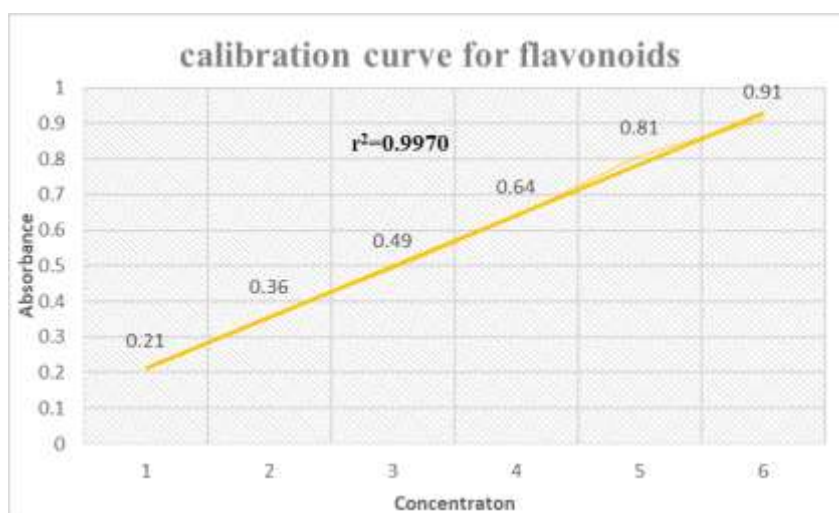
Table. 2. Calibration Results of Flavonoids.

Si. No	Concentrations (Mg/MI)	Absorbance
1	1 mg	0.21
2	2 mg	0.36
3	3 mg	0.49
4	4 mg	0.64
5	5 mg	0.81
6	6 mg	0.91
7	Extract	0.45

Table-2. Determination of extract absorbance:

0.5 ml of Malabar spinach leaves extract were taken into a 10ml of volumetric flask. Then added 0.3 ml of

5% NaNo₂ and 0.3 ml of 10% AlCl₃ solutions. Make up the volume with distilled water up to 10 ml. Then the solution stand for 5mins at room temperature. The absorbance measured at 450nm was 0.45.

**Fig. 3. Calibration curve Flavonoids**

Calculations:

Concentration of flavonoid content in 5 gm of Malabar spinach leaves.

The concentration of Flavonoids in Malabar spinach leaves was found to be 2.7 in 5ml of sample.

Formula:

$$\begin{aligned} \frac{\text{Total flavonoid content}}{\text{Concentration found} \times \text{volume of final extract}} &= \\ \frac{\text{Quantity of extract taken}}{} &= \\ &= \frac{2.7 \times 40}{0.5} \\ &= \frac{108}{0.5} \\ &= 0.216 \text{ gm} \end{aligned}$$

5 gm of Malabar spinach leaves dry powder contains 0.216 gm of flavonoids.

Percentage Of Flavonoids:

$$\begin{aligned} \% \text{ of flavonoids in 5 gm} &= \frac{\text{Concentration in 5 gm}}{\text{quantity of powder}} \times 100 \\ &= \frac{0.216}{5} \times 100 \\ &= 4.32\% \end{aligned}$$

The total flavonoid content in Malabar spinach leaves was found to be 4.32%

SUMMARY:

This study was carried out to determine and quantify the flavonoid content present in Malabar spinach leaves using a colorimetric technique. Flavonoids, which are well known for their therapeutic and antioxidant properties, were extracted from the plant material by the maceration method. Quantitative analysis was based on the formation of a coloured flavonoid–reagent complex, and the absorbance of this complex was measured calorimetrically at 450 nm was 0.45. A calibration curve prepared using standard rutin solutions was employed to calculate the flavonoid concentration in the plant extract. The findings indicated a considerable amount of flavonoids in Malabar spinach leaves, highlighting

their potential pharmaceutical value. Overall, the method proved to be simple, economical, and dependable, making it suitable for routine flavonoid analysis, quality control, and future pharmacological studies.

CONCLUSION:

The colorimetric technique applied in this study effectively enabled the determination and quantification of flavonoid content in Malabar spinach leaves. The extraction and subsequent complex formation proved to be a simple, economical, and dependable method for flavonoid analysis. The results demonstrated a 4.32% of flavonoids, emphasizing the medicinal significance of Malabar spinach. This analytical approach may be effectively utilized for quality control of herbal formulations as well as for supporting pharmacological investigations. Future research may focus on improving the sensitivity of the method and evaluating the biological activities associated with the quantified flavonoids.

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