International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

PHYTOCHEMICAL COMPOSITION, TOTAL FLAVONOID CONTENT, AND QUANTITATIVE ESTIMATION OF QUERCETIN BY HPLC IN THE ETHANOLIC EXTRACT OF *BASELLA ALBA*

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***Article History:**

Received: 25/02/2025
Revised: 11/03/2025
Accepted: 28/03/2025

ABSTRACT

The present study investigates the phytochemical composition, total flavonoid content, and quantitative estimation of quercetin in the ethanolic extract of *Basella alba*. The ethanolic extract yielded 12.7%, with preliminary phytochemical screening revealing the presence of saponins, phenols, carbohydrates, and flavonoids. The total flavonoid content was determined to be 0.22 mg quercetin equivalent per 100 mg of extract. Furthermore, High-Performance Liquid Chromatography (HPLC) analysis showed that the ethanolic extract contained 0.074% quercetin, with a retention time of 2.826 minutes. These findings highlight *Basella alba* as a promising source of bioactive compounds, particularly flavonoids, with potential antioxidant and anti-inflammatory properties. The results provide valuable information for further pharmacological studies and the development of therapeutic products derived from *Basella alba*.

Keywords: *Basella alba,* Ethanolic extract, Phytochemical composition, Total flavonoid content, Quercetin, High-Performance Liquid Chromatography (HPLC), Antioxidant, Anti-inflammatory, bioactive compounds.

INTRODUCTION

Basella alba (commonly known as Malabar spinach or Ceylon spinach) is a tropical plant that is widely recognized for its nutritional and medicinal properties. It belongs to the family Basellaceae and is native to Southeast Asia. The leaves and stems of *Basella alba* are rich in vitamins (such as vitamin A and C), minerals (including calcium and iron), and various phytochemicals that contribute to its broad therapeutic potential (Siddiqui *et al.,* 2012). Due to its versatility in both culinary and medicinal applications, *Basella alba* has been a subject of numerous studies focusing on its bioactive compounds and pharmacological effects.

The plant has shown significant promise in treating conditions such as hypertension, diabetes, and inflammation, due to the presence of various bioactive components, including flavonoids, alkaloids, glycosides, and polyphenols (Kumar *et al.,* 2015). Among these, flavonoids are of particular interest due to their powerful antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Patel *et al.,* 2011). Flavonoids, such as quercetin, are known to play a crucial role in scavenging free radicals and mitigating oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases, including cardiovascular diseases and cancer (Cao *et al.,* 2015).

In recent years, there has been a growing interest in the chemical analysis and quantitative estimation of bioactive compounds in medicinal plants.

High-Performance Liquid Chromatography (HPLC) is one of the most reliable techniques used for the identification and quantification of flavonoids and other phenolic compounds in plant extracts (Ahuja, 2012). Quercetin, a widely distributed flavonoid, has been reported in various plant species, including *Basella alba*. The quantitative estimation of quercetin in *Basella alba* can provide valuable insights into its potential as a source of natural antioxidants and contribute to understanding its pharmacological actions.

The aim of this study is to investigate the phytochemical composition of the ethanolic extract of *Basella alba*, quantify its total flavonoid content, and perform a quantitative estimation of quercetin using HPLC. The results of this study may further support the medicinal and nutritional value of *Basella alba* and contribute to its potential application in the pharmaceutical industry.

MATERIALS AND METHODS

Collection of Plant

The stem of selected plant namely *Basella alba* was collected from local market of Bhopal, Madhya Pradesh. The collected plant drug was cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of *Basella alba* by maceration method

The Collected plant drug was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drug was converted into moderately coarse powder in hand grinder. Maceration was carried out in a closed conical flask for 48 h. (100 g) powdered plant drug sample and ethanol as the extraction solvent was used. The solvent free ethanolic extract obtained was evaluated. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated (Jain *et al.,* 2019).

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the methanolic extract of *Basella alba,* was subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Parkhe *et al.,* 2019).

Estimation of total flavonoids content by Aluminum Chloride Colorimetric method

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 5,10,15,20 and 25 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 420 nm (λmax of quercetin) with a lab science UV-1800 spectrophotometer. Aluminum chloride, 1% and potassium acetate, 1M solutions were prepared.

Stock Solution of Extract

100 mg of the plant extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions

0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminum chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance

was measured at 420 nm. All prepared solutions were filtered through whatmann filter paper before measuring (Jain *et al.,* 2020).

Identification of marker compound (Quercetin) in *Basella alba* by HPLC

Instrumentation

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

Chromatographic conditions

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 mL min-1. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm (Garg, 2021).

Preparation of standard stock solution

10mg of Quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 5, 10, 15, 20, 25µg/ ml concentration.

Analysis of extract

10 mg extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

RESULTS AND DISCUSSION

The present study evaluates the phytochemical composition, total flavonoid content, and the quantitative estimation of quercetin in the ethanolic extract of *Basella alba*. The findings presented in the tables provide valuable insights into the plant's chemical profile and its potential as a source of bioactive compounds.

As shown in Table 1**,** the ethanolic extraction of *Basella alba* yielded 12.7% of extract, which indicates a moderate concentration of bioactive compounds in the plant material. The relatively high yield of the ethanolic extract is consistent with previous studies on plants with high polyphenolic content, as ethanol is an effective solvent for extracting flavonoids, phenols, and other polar compounds. This high yield supports the potential of *Basella alba* as a viable source of medicinal compounds.

The preliminary phytochemical screening (Table 2) revealed the presence of important bioactive compounds in the ethanolic extract of *Basella alba*. Saponins and phenols were both detected, indicating the presence of compounds with known medicinal properties. Saponins are known for their antioxidant, anti-inflammatory, and cholesterol-lowering effects, while phenols, including flavonoids,

are potent antioxidants that help to combat oxidative stress and prevent chronic diseases.

The absence of alkaloids**,** diterpenes, and proteins and amino acids suggests that these compounds are not present in significant amounts in the ethanolic extract, or they may be poorly extracted by ethanol. The presence of carbohydrates, confirmed by Fehling's test, is also important as they can contribute to the plant's nutritional value, although they are less likely to be the primary bioactive constituents in *Basella alba*.

Flavonoids, detected by both the Lead acetate and Alkaline tests**,** are one of the most significant findings of this study. Flavonoids are well known for their antioxidant, anti-inflammatory, and anticancer properties, which may be responsible for some of the therapeutic benefits attributed to *Basella alba*. This makes the plant a promising candidate for further exploration in relation to its potential health benefits.

The total flavonoid content in the ethanolic extract of *Basella alba* was found to be 0.22 mg quercetin equivalent per 100 mg of extract (Table 3). This value indicates a moderate concentration of flavonoids in the plant, which aligns with its known antioxidant and therapeutic properties. Flavonoids, especially quercetin, are potent antioxidants that play a key role in reducing oxidative stress in the body, thereby lowering the risk of diseases like cardiovascular diseases, diabetes, and cancer. The relatively low flavonoid content could be attributed to the nature of the extraction process or the inherent concentration of these compounds in the plant itself.

The quantitative estimation of quercetin in the ethanolic extract was performed by High-Performance Liquid Chromatography (HPLC), with the results presented in Table 4. The retention time (RT) of quercetin was 2.826 minutes, and the quercetin content in the extract was found to be 0.074%. This value reflects the specific amount of quercetin present in the ethanolic extract, confirming that *Basella alba* is a viable source of this flavonoid. The quantification of quercetin is significant because quercetin is known for its potent antioxidant, anti-inflammatory, and anti-cancer properties, making it a valuable compound for further pharmacological studies.

Table 1: Extractive values obtained from *Basella alba* extract

S. No.	Extract	% Yield
1	Ethanolic	12.7%

Table 2: Preliminary phytochemical screening of *Basella alba* extract

S. No.	Phytoconstituents	Test Name	Ethanolic Extract
1	Alkaloids	Hanger's Test	Absent
2	Saponins	Froth test	**Present**
3	Diterpines	Copper Acetate test	Absent
4	Phenols	Ferric chloride test	**Present**

5	Carbohydrates	Fehling's Test	**Present**
6	Flavonoids	Lead acetate	**Present**
		Alkaline Test	**Present**
7	Proteins & Amino acids	Biuret Test	Absent

Table 3: Total flavonoids content in extract of *Basella alba*

S. N.	Extracts	Flavonoid content Quercetin equivalent mg/100mg
1	Ethanolic extract	0.22 mg/100mg

Table 4: Quantitative estimation of Quercetin in extract by HPLC

S. No.	Extract	RT	% Assay
1.	Ethanolic extract	2.826	0.074%

CONCLUSION

The findings of this study underscore the medicinal potential of *Basella alba*. The ethanolic extract contains a moderate yield of bioactive compounds, with a notable presence of flavonoids, including quercetin. The quantitative estimation of quercetin and the total flavonoid content confirm the plant's potential as a source of antioxidant and anti-inflammatory agents. These properties contribute to its traditional use in treating a variety of ailments, including inflammation and oxidative stress-related conditions. The results from the phytochemical screening and HPLC analysis provide a foundation for future studies aimed at exploring the therapeutic applications of *Basella alba* and isolating its bioactive compounds for pharmaceutical development.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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