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ANALYSIS OF BIOACTIVE COMPOUND IN HERBAL MEDICINAL PLANT EXTRACT OF *BAMBUSA VULGARIS*

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Abstract

The present study was carried out to investigate the phytochemical profile and quantify the flavonoid marker compound quercetin in the aqueous extract of *Bambusa vulgaris*. The powdered plant material was extracted using water as the extraction solvent, yielding 4.92% w/w dried extract. Preliminary phytochemical screening was performed using standard qualitative tests, which revealed the presence of flavonoids, saponins, phenolic compounds, proteins, and diterpenes, while alkaloids, glycosides, carbohydrates, tannins, and sterols were absent. Thin-layer chromatography (TLC) analysis was conducted using a mobile phase comprising toluene:ethyl acetate:formic acid (5:4:1), which demonstrated a prominent spot corresponding to quercetin with an Rf value of 0.6. The total phenolic and flavonoid contents of the aqueous extract were found to be 0.35 mg/100 mg and 0.80 mg/100 mg, respectively. Quantitative estimation of quercetin by HPLC analysis showed a retention time of 2.290 min and a quercetin content of 0.176% in the extract. The findings indicate that *Bambusa vulgaris* is a rich source of flavonoids and phenolic constituents, particularly quercetin, which may contribute to its pharmacological activities. The study provides a scientific basis for the use of *Bambusa vulgaris* as a potential natural source of bioactive compounds for pharmaceutical and nutraceutical applications.

Keywords

Bambusa vulgaris; Phytochemical Screening; Quercetin; HPLC; Thin Layer Chromatography (TLC); Total Phenolic Content; Total Flavonoid Content; Aqueous Extract; Medicinal Plant; Bioactive Constituents.

Introduction

Medicinal plants have been utilized for centuries as a valuable source of therapeutic agents and continue to play a significant role in traditional and modern healthcare systems. The increasing demand for natural products has stimulated extensive research into plant-derived bioactive compounds due to their diverse pharmacological activities and relatively low toxicity (Jamshidi-Kia et al., 2017). These phytochemicals, including flavonoids, phenolic compounds, alkaloids, glycosides, saponins, terpenoids, and tannins, are responsible for the therapeutic potential of many medicinal plants and have attracted considerable interest for the development of novel pharmaceutical and nutraceutical products (Nwozo et al., 2023).

Bambusa vulgaris Schrad. ex J.C. Wendl., commonly known as common bamboo, belongs to the family Poaceae and is widely distributed throughout tropical and subtropical regions of the world. Traditionally, different parts of the plant have been employed in folk medicine for the treatment of fever, inflammation, wounds, respiratory disorders, gastrointestinal ailments, and various infectious diseases (Ojo and Sadiku, 2023). The medicinal properties of *Bambusa vulgaris* have been attributed to the presence of numerous secondary metabolites that exhibit antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and other biological activities (Fitri et al., 2020).

Among the various phytoconstituents present in medicinal plants, phenolic compounds and flavonoids are of particular importance due to their potent antioxidant properties (Pietta, 2000). These compounds play a crucial role in protecting biological systems against oxidative stress caused by reactive oxygen species (ROS), which are implicated in the pathogenesis of several chronic diseases (Sharifi-Rad et al., 2020). Quercetin, a naturally occurring flavonoid widely distributed in plants, has gained considerable attention because of its strong antioxidant, anti-inflammatory, anticancer, cardioprotective, and neuroprotective activities. Therefore, the identification and quantification of quercetin and other bioactive constituents are essential for the standardization and quality assessment of herbal materials (Vollmannová et al., 2024).

Phytochemical screening serves as a preliminary approach for the identification of various classes of secondary metabolites present in plant extracts, while chromatographic techniques such as thin-layer chromatography (TLC) and high-performance liquid chromatography

(HPLC) provide reliable methods for the separation, identification, and quantification of specific bioactive compounds. HPLC, in particular, is widely recognized as a sensitive and accurate analytical technique for the standardization of herbal extracts and the determination of marker compounds (Ingle et al., 2017).

In view of the medicinal importance of *Bambusa vulgaris* and the growing interest in plant-based therapeutics, the present study was undertaken to analyze the bioactive compounds present in the aqueous extract of *Bambusa vulgaris*. The study involved determination of extraction yield, preliminary phytochemical screening, estimation of total phenolic and flavonoid contents, TLC profiling, and quantitative estimation of quercetin using HPLC. The findings of this investigation may contribute to the phytochemical standardization of *Bambusa vulgaris* and provide scientific evidence supporting its potential use as a source of bioactive compounds for pharmaceutical applications.

Material and Methods

Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Mukherjee, 2007). 50 gram of dried powdered leaves of *Bambusa vulgaris* has been extracted with aqueous using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

Phytochemical screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds

Estimation of total phenol content

The total phenolic content of the extract was determined using the modified Folin–Ciocalteu colorimetric method as described by Mishra et al. (2017). Gallic acid was used as the reference standard for the preparation of the calibration curve. Briefly, 10 mg of gallic acid was accurately weighed and dissolved in 10 mL of methanol to obtain a stock solution. From this stock solution, different concentrations ranging from 10–50 µg/mL were prepared by appropriate dilution with methanol.

For the preparation of the test sample, 10 mg of the dried extract was dissolved in 10 mL of methanol and filtered to obtain a clear solution. An aliquot of 2 mL of the extract solution (1 mg/mL) was used for the estimation of total phenolic content. To this, 1 mL of Folin–Ciocalteu reagent, previously diluted with distilled water in a ratio of 1:10 (v/v), was added, followed by the addition of 1 mL sodium carbonate solution (7.5 g/L). The reaction mixture was vortex-mixed for approximately 15 seconds and allowed to stand at room temperature for 10 minutes to facilitate color development.

After incubation, the absorbance of the resulting blue-colored complex was measured at 765 nm using a UV–Visible spectrophotometer against a suitable blank. A calibration curve was constructed using the absorbance values of the gallic acid standard solutions. The total phenolic content of the extract was calculated from the calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract (mg GAE/g extract).

Estimation of total flavonoids content

The total flavonoid content of the extract was determined using the aluminum chloride colorimetric method as described by Mishra et al. (2017). Quercetin was used as the reference standard for the preparation of the calibration curve. Briefly, 10 mg of quercetin was accurately weighed and dissolved in 10 mL of methanol to obtain a stock solution. From this

stock solution, various concentrations ranging from 5–25 µg/mL were prepared by appropriate dilution with methanol.

For the preparation of the test sample, 10 mg of the dried extract was dissolved in 10 mL of methanol and filtered to obtain a clear solution. An aliquot of 3 mL of the extract solution (1 mg/mL) was used for the estimation of total flavonoid content. To this, 1 mL of 2% aluminum chloride ($AlCl_3$) solution was added and mixed thoroughly. The reaction mixture was then allowed to stand at room temperature for 15 minutes to facilitate the formation of a stable flavonoid–aluminum complex.

Following incubation, the absorbance of the resulting yellow-colored complex was measured at 420 nm using a UV–Visible spectrophotometer against a suitable blank. A calibration curve was constructed using the absorbance values obtained for the quercetin standard solutions. The total flavonoid content of the extract was calculated from the calibration curve and expressed as milligrams of quercetin equivalents (QE) per gram of dried extract (mg QE/g extract).

Identification of marker compound (Quercetin) by HPLC

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 (Acharya *et al.,* 2019).

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 of 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 was undertaken to evaluate the phytochemical composition and quantify the bioactive constituents present in the aqueous extract of *Bambusa vulgaris*. The extraction process yielded 4.92% w/w of dried extract, indicating the successful extraction of water-soluble phytoconstituents from the plant material. The moderate percentage yield may be attributed to the presence of polar compounds such as flavonoids, saponins, phenolics, proteins, and other water-soluble secondary metabolites.

Preliminary phytochemical screening revealed the presence of several important bioactive constituents in the aqueous extract. Flavonoids were detected by both alkaline reagent and lead acetate tests, suggesting that *Bambusa vulgaris* is a rich source of flavonoid compounds. Saponins, proteins, diterpenes, and phenolic compounds were also found to be present, whereas alkaloids, glycosides, carbohydrates, tannins, and sterols were absent. The presence of flavonoids and phenolic compounds is particularly significant because these phytochemicals are known to possess antioxidant, anti-inflammatory, antimicrobial, and hair growth-promoting activities. The detection of phenolics by the Folin–Ciocalteu test further supports the occurrence of polyphenolic compounds in the extract.

Thin-layer chromatographic (TLC) analysis was performed using a mobile phase consisting of toluene:ethyl acetate:formic acid (5:4:1) to identify flavonoid constituents. The standard quercetin exhibited an Rf value of 0.6, while the aqueous extract also showed a prominent spot at Rf 0.6 under long UV light, indicating the probable presence of quercetin or a structurally related flavonoid. Additional spots observed under short UV light (Rf values 0.3, 0.4, 0.54, and 0.64) suggest the presence of multiple flavonoid derivatives and other phenolic constituents in the extract. The similarity of the Rf value between the extract and quercetin standard provides preliminary evidence for the occurrence of quercetin in *Bambusa vulgaris*.

Quantitative estimation of total phenolic and flavonoid contents further confirmed the phytochemical richness of the extract. The aqueous extract contained 0.35 mg/100 mg of total phenolic content and 0.80 mg/100 mg of total flavonoid content. The higher flavonoid content compared to total phenolic content indicates that flavonoids constitute a major fraction of the phenolic compounds present in the extract. Since flavonoids are well-known free radical scavengers and possess various pharmacological activities, their abundance may contribute significantly to the biological potential of *Bambusa vulgaris*.

HPLC analysis was carried out for the quantitative estimation of quercetin in the aqueous extract. The chromatogram showed a peak at a retention time of 2.290 minutes, corresponding to quercetin. The percentage assay revealed that the extract contained 0.176% quercetin, confirming the presence of this important flavonoid marker compound. Quercetin is widely recognized for its antioxidant, anti-inflammatory, anti-aging, and hair growth-promoting properties. The identification and quantification of quercetin in the extract support the traditional medicinal importance of *Bambusa vulgaris* and provide a scientific basis for its potential therapeutic applications.

Table 1: % Yield of *Bambusa vulgaris*

S. No.	Extract	% Yield (w/w)
1.	Aqueous	4.92%

Table 2: Result of phytochemical screening of aqueous extract of *Bambusa vulgaris*

S. No.	Constituents	Aqueous extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 -Ve -Ve
2.	**Glycosides** Conc. H_2SO_4 Test	 -Ve
3.	**Flavonoids** Alkaline Reagent Test: Lead acetate Test:	 +Ve +Ve
4.	**Saponins**	

	Froth Test:	+Ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test	 -Ve +Ve
6.	**Proteins** Xanthoproteic Test:	 +Ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test:	 -Ve -Ve
8.	**Diterpenes** Copper acetate Test:	 +Ve
9.	**Tanins** Gelatin Test	 -Ve
10.	**Sterols** Salkowski Test	 -Ve

[+Ve= Present; -Ve= Absent]

Table 3: TLC of *Bambusa vulgaris* (Flavonoids)

Aqueous extract of *Bambusa vulgaris*		
S. No.	**Mobile phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	***Rf* value**
1.	**(Quercetin)** Dis. travel by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV = 1 No. of spot at normal light= 1	 Long- 0.6 Short- 0.6 Normal- 0.6
2.	**(Aqueous extract)** Dis. Travel by mobile phase=5cm No. of spot at long UV = 1 No. of spot at short UV = 4	 Long- 0.6 Short- 0.3, 0.4, 0.54, 0.64

	No. of spot at normal light= 0	Normal- 0
	Spot Sequence	
	Quercetin	1st
	aqueous extract	2nd

Table 4: Total phenol and flavonoid content of *Bambusa vulgaris*

S. No.	Extract	Total phenol content	Total flavonoid content
		mg/ 100mg	
1.	Aqueous extract	0.35	0.80

Table 5: Quantitative estimation of Quercetin in extract

S. No.	Extract	RT	Area	% Assay
1.	Aqueous extract	2.290	125.43	0.176

Conclusion

The present study demonstrated that the aqueous extract of *Bambusa vulgaris* contains important bioactive constituents, particularly flavonoids and phenolic compounds. TLC and HPLC analyses confirmed the presence of quercetin (0.176%), while quantitative estimations showed appreciable total flavonoid and phenolic contents. These findings indicate that Bambusa vulgaris is a promising natural source of antioxidant bioactive compounds and may be useful for pharmaceutical and nutraceutical applications.

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