



Determination of Total Phenols and Flavonoid Content of *Bryonia laciniosa* by Spectrophotometric Method

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ABSTRACT

Bryonia laciniosa Linn. belongs to the family of Cucurbitaceae is one of well known traditional drug. Many pharmacological activities have been reported from this plant including antioxidant activities. As polyphenols are responsible for antioxidant activities, it led to determine the total phenols and flavonoid content in *Bryonia laciniosa*. The content was estimated in methanol extract of leaves and stem by spectrophotometric method. Gallic acid and quercetin were used as standards for the calibration plot of phenols and flavonoid respectively. The total phenolic content was found to be high in the leaves, 9% than the stem 4%. The total flavonoid content was maximum, 6% in leaves and stem contains the minimum, 2%. As per the authors this is the first report of estimation of total phenols and flavonoid in *Bryonia laciniosa*.

Keywords: *Bryonia laciniosa*; Phenols; Flavonoid

INTRODUCTION

Bryonia laciniosa Linn. Syn. *Diplocyclos palmatus* (L). C. Jeffery belongs to the family of Cucurbitaceae, has been used traditionally in the treatment of inflammation, fever, vaginal dysfunction, spasmolytic, infertility [1,2]. According to the literature survey many pharmacological activities have been reported from this herb viz., androgenic activity, anti-diabetic, antitumor, antipyretic, anti-inflammatory and antioxidant activity [3-7]. Phytochemical study showed the presence of carbohydrates, alkaloids, glycosides, saponins, phenols, flavonoids, fixed oils and fats [8]. As antioxidant activity is attributed to the presence of polyphenols, it is important to determine the total phenols and flavonoid content in the herbs [9,10]. But the determination of total phenols and flavonoid content has not been carried out on this herb. Thus the present investigation was carried out to determine total phenols and flavonoid content in the leaf and stem of *B. laciniosa*.

MATERIALS AND METHODS

The plant was collected in the month of August, 2010, from the Vijayapur District, Karnataka, India and authenticated by Dr. Minu Parabha, Botony department, M.S University, Vadodara, India (Ref No. LMCP/MSU/BOT/177/2010/BI (Dp)).

Gallic acid and Quercetin (Hi-media Laboratories Pvt. Ltd., Mumbai, Maharashtra) were used as standards. All the solvents chemicals and reagents used in the present study were purchased from S.D Fine chemicals, Mumbai. Chemicals and reagents used in the present work were Folin Ciocalteu's reagent, Sodium carbonate, Aluminium chloride and Potassium acetate. UV/VIS spectrophotometer (Jasco V630) was used to measure the absorbance of the solutions.

Preparation of the extract

The leaves and stem of the plant were separated, shade dried and powdered individually. Each coarse powder was sieved through 40 # mesh and used for the preparation of the extract.

10 gm of each leaves and stem of *B. laciniosa* were extracted with 100 ml 80% methanol on magnetic stirrer at 40° C for 30 min and filtered through Whatman filter paper. The volume of filtrate was adjusted to 100 ml with 80% methanol. The extract of each were tested for the presence of phenol and flavonoid [11]. Then the Total phenols and flavonoid content were determined.

Chemical test for phenols

Ferric chloride test: Few ml of extract was treated with 5% ferric chloride solution.

Lead acetate test: Few ml of extract was treated with 10% lead acetate solution.

Chemical test for flavonoids

Shinoda test: To the extract, few drops of conc. HCl and 0.5 gm of magnesium turnings were added.

Lead acetate test: To the extract 10% lead acetate solution was added.

Determination of total phenols content

The total phenols content of leaves and stem of *B. laciniosa* were determined using Folin Ciocalteu reagent [12].

Preparation of the standard solution

Gallic acid was used to make a standard calibration curve. Ten mg of gallic acid was dissolved in 100 ml of 50% methanol (100µg/ml) and diluted to get the different concentration of 10, 20, 30, 40 and 50 µg/ml. One ml solution of each dilution was pipette out into 10 ml of volumetric flask and diluted to 3 ml with distilled water. Then 0.5 ml of 1:1 diluted Folin Ciocalteu's reagent was added. The each solutions were made alkaline with 2 ml of Na₂CO₃ (2.0% w/v) and vortexed. Final volume was adjusted to 10 ml with distilled water. Each volumetric flask was warmed and absorbance was measured at 765 nm against blank, containing distilled water.

Preparation of the sample solution

5 ml of 80% methanol extract of each leaves and stem was concentrated and dissolved in 50ml of 50% methanol and filtered. One ml each filtrate of the sample was taken in a volumetric flask and diluted to 3 ml with distilled water. Then 0.5 ml Folin Ciocalteu's reagent and 2 ml of Na₂CO₃ (2.0% w/w) were added in each volumetric flask and mixed thoroughly. The final volume was adjusted to 10 ml with distilled water. Each volumetric flask was warmed and absorbance was measured at 765 nm.

Estimation of total phenols was done on the basis of calibration curve of gallic acid. The calibration curve of gallic acid was plotted against different known concentration of gallic acid (Figure 1), and the results were expressed as percentage w/w. Percentage (%) content was calculated by using the following formula,

Total phenols content (% w/w) = $GAE \times V \times D \times 10^{-6} \times 100/W$.

GAE - Gallic Acid Equivalent ($\mu\text{g/ml}$), V - Total volume of sample (ml), D - Dilution factor, W - Sample weight (g).

Determination of total flavonoid content

The total flavonoid content of leaves and stem of *B.laciniosa* were determined by aluminium chloride method [13].

Preparation of the standard solution

Quercetin was used to make a standard calibration curve. Ten milligram of quercetin was dissolved in 100 ml of 80% methanol (100 $\mu\text{g/ml}$) and then diluted to get the concentration 25, 50, 100, 125 and 150 $\mu\text{g/ml}$. 0.5 ml of each diluted standard solutions were pipette out in 10 ml test tube and mixed with 1.5 ml of methanol (95%), 0.1 ml of aluminium chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and incubated at room temperature for 30 min. After incubation, the absorbance of the each dilution was measured at 415 nm.

Preparation of sample solution

1 ml of each 80% methanol extract of sample was pipette out in 10 ml of test tube and treated with reagents as mentioned in the of standard solution. The absorbance of each solution was recorded at 415 against the blank without aluminium chloride. The amount of aluminium chloride (10%) was replaced with the same amount of distilled water in blank.

A standard calibration curve was constructed against known concentrations of quercetin. The concentration of flavonoid in the samples was estimated from the standard calibration curve (Figure 2). The results were expressed as percentage w/w. Total Flavonoids content (% w/w) was determined by following formula,

$$\text{Flavonoid content (\% w/w)} = \text{QE} \times \text{V} \times \text{D} \times 10^{-6} \times 100 / \text{W}.$$

QE - Quercetin Equivalent ($\mu\text{g/ml}$), V - Total volume of sample (ml), D - Dilution factor, W-Sample weight (g).

STATISTICAL ANALYSIS

The experiments were conducted in triplicate and data were analysed as mean \pm S.D. The graphs were plotted using M.S Excel 2007.

RESULTS AND DISCUSSION

Phenols and Flavonoids are naturally occurring polyphenolic compounds with wide array of biological activities including potent radical scavenging activity because of their ability to neutralize the free radicals [9,10]. The presence of these active constituent were confirmed by qualitative chemical tests. The extract of each leaves and stem gave deep blue colour and white precipitate with ferric chloride and lead acetate test respectively for the phenols. The Shinoda test and lead acetate test for flavonoid were found to be positive. The Shinoda test showed the formation of pink colour and the lead acetate gave yellow ppt [11].

Determination of total phenols content (TPC)

Total phenols content of extracts was determined by Folin Ciocalteu reagent method. The principle of this method is the formation of blue coloured complex due to the reaction between the phenols and Folin Ciocalteu reagent [12], which can be measured at a maximum wavelength. Quantification of total phenols was done on the basis of standard calibration plot of gallic acid. It was constructed in the concentration range of 10-80 $\mu\text{g/ml}$ and the coefficient of determination (R^2) was found to be 0.995 (Figure 1). Based on standard plot of gallic acid ($y=$

0.005x+0.021), the leaves found to contain the maximum phenols content of 9.2% and stem contains the minimum of 6.49% (Table 1).

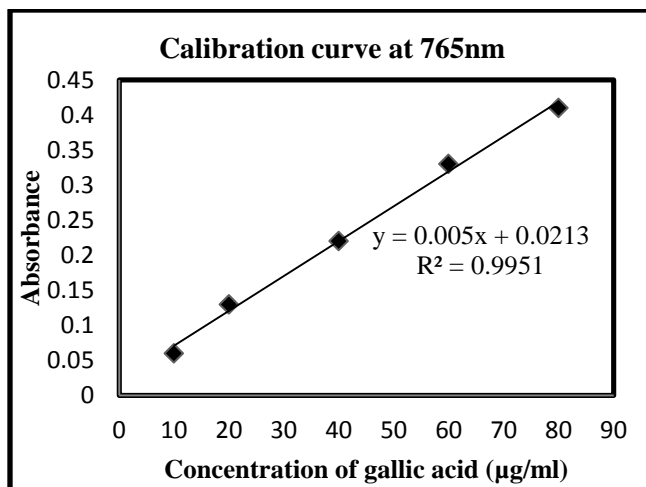


Figure 1. Calibration Curve of Gallic Acid for Phenols Determination

Determination of total flavonoid content (TFC)

Flavonoids are naturally occurring polyphenol compounds with benzo-pyrone structures. Quantification of total flavonoid was done on the basis of standard calibration plot of quercetin. It was constructed in the concentration range of 25-125 µg/ml and the coefficient of determination (R^2) was found to be 0.994 (Figure 2). Based on the standard plot of quercetin ($y=0.006x+0.02$), the leaves found to contain maximum content of flavonoid, 4.85% and stem contains minimum, 2.2% (Table 1).

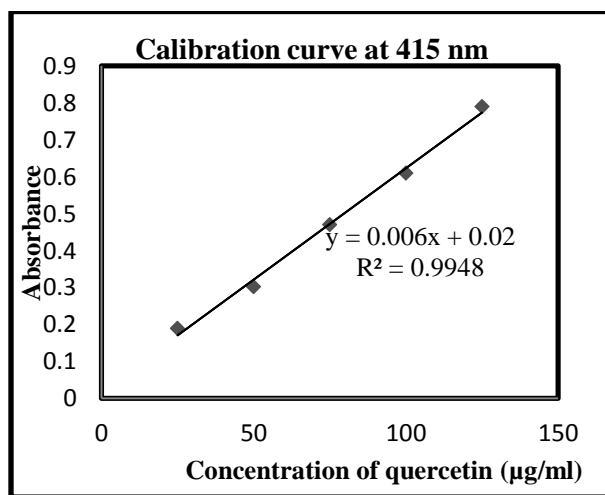


Figure 2. Calibration Curve of Quercetin for Flavonoid Determination

Table 1: Total phenols and Flavonoid Content of *Bryonia laciniosa L.*

Parts of the plant	% Content of Polyphenols (W/W) Mean \pm SD (n=3)		Regression equation	Regression coefficient
	Phenols	Flavonoid		
Leaves	9.2 \pm 0.032	4.85 \pm 0.023	y=0.005x+0.021	0.995
Stem	6.49 \pm 0.012	2.2 \pm 0.039	y=0.006x+0.02	0.994

CONCLUSION

The study confirmed the presence of phenols and flavonoid and revealed that significant amount of these constituents are present in the leaves and the stem of *B. laciniosa*. Therefore the study would further help to correlate the presence of these contents in other parts of this plant and to isolate the flavonoid.

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