

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF AERIAL PARTS OF *BAUHINIA VARIEGATA*

Abstract

The goals of this research are to screen phytochemicals, perform thin layer chromatography, assess the quantity of phenolic and flavonoid compounds, and measure the antioxidant potential of *Bauhinia variegata*. The well-known test methodology available in the literature was used to determine the qualitative analysis of various phytochemical elements as well as the quantitative analysis of total phenol and flavonoids. The hydro alcoholic extract of *Bauhinia variegata* aerial parts was investigated for qualitative and quantitative analyses, as well as antioxidant activity using an in vitro model, namely the 1,1-diphenyl, 2-picrylhydrazyl (DPPH) assay. The presence of phenols and flavonoids was discovered by phytochemical research. The total phenolic and flavonoid content of *Bauhinia variegata* hydroalcoholic extract was 1.454 and 1.112mg/100mg, respectively. Ascorbic acid, which was employed as a control, was also assessed for comparison. In the investigated models, the extract demonstrated dose-dependent free radical scavenging activity. *Bauhinia variegata* aerial parts For the DPPH technique, hydroalcoholic extract had an IC_{50} value of 60.22g/ml, which was close to that of ascorbic acid (IC_{50} =17.68g/ml). The IC_{50} value for the hydrogen peroxide technique was determined to be 76.97g/ml, which compares favourably to ascorbic acid (IC_{50} =18.69g/ml). The current study discusses the phytochemical profile, antioxidant activity, and TLC of *Bauhinia variegata*, which will be employed for medical purposes in the future.

Keywords: *Bauhinia variegata*, Qualitative, Quantitative phytochemical, TLC, Antioxidant activity.

INTRODUCTION

Exogenous substances or endogenous metabolic activities in the human body produce free radicals or highly reactive oxygen species. These are capable of oxidising biomolecules such as nucleic acids, proteins, lipids, and DNA and can cause a variety of degenerative illnesses such as neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, and arthritis [1, 2]. Antioxidants are substances that stop the onslaught of free radicals, lowering the risk of certain illnesses [3]. Almost all organisms are protected against free radical damage to some extent by enzymes such as superoxide dismutase and catalase, as well as antioxidant substances such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione. According to Prior and Cao [4], antioxidant supplements or dietary antioxidants

protect against the harmful effects of free radicals. At the moment, great emphasis is being placed on the utilisation of natural antioxidants to protect the human body, particularly brain tissues, from the oxidative damage produced by free radicals. Several medicinal plants have demonstrated similar efficacy in the last two decades using established methods of psychoneuropharmacology [5]. The bark of *Bauhinia variegata* Linn. (Leguminosae) has long been used as a tonic and in the treatment of ulcers. It is also beneficial in the treatment of skin problems. The roots are used as a snake venom antidote [6]. This herb is also utilised in traditional medicine to treat a variety of ailments, including inflammatory problems [7]. Keeping this in mind, the current study was carried out to assess the antioxidant activity, quantitative assessment of total phenolic and flavonoid content of *Bauhinia variegata*, which is historically well known.

MATERIAL AND METHODS

Material

In the month of February 2020, aerial portions of *Bauhinia variegata* were harvested from Vindhya Herbals (MFP-PARC) Bhopal (M.P.). Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) supplied all of the chemicals utilised in this work (Mumbai, India). This study's chemicals and solvents were all of analytical grade.

Methods

Procedure for extraction

The following method was used to prepare extract from shade dried and powdered aerial components [8-9].

Maceration extraction technique [10]

The shade dried material was coarsely pulverised before maceration extraction with petroleum ether. The extraction was maintained until the material had been defatted. 77 gramme of dried aerial portions of *Bauhinia variegata* were maceration extracted using a hydroalcoholic solvent (methanol: water: 70: 30). The extracts were evaporated above their boiling temperatures and kept in an airtight container free of contamination until they were utilised. Finally, the dried extracts' % yields were computed.

Determination of percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100$$

Phytochemical screening

Phytochemical analyses were performed on extracts using the conventional procedures listed below.

1. Detection of alkaloids: Individual extracts were diluted in dilute Hydrochloric acid and filtered.

a) **Hager's Test:** Filtrates were subjected to the Hager's reagent test (saturated picric acid solution). The presence of alkaloids is established by the production of yellow precipitate.

2. Carbohydrate detection: Extracts were individually diluted in 5 mL distilled water and filtered. The filtrates were examined for the presence of carbohydrates.

a) **Fehling's Test:** Filtrates were hydrolyzed with diluted HCl, neutralised with alkali, and heated in Fehling's A and B solutions. The presence of reducing sugars is shown by the formation of red precipitate.

3. Glycoside detection: Extracts were hydrolyzed with diluted HCl and then tested for glycosides.

a) **Legal's Test:** Extracts were treated in pyridine with sodium nitropruside and sodium hydroxide. The presence of cardiac glycosides is indicated by the presence of pink to blood red colour.

4. Saponin detection

a) **Froth Test:** Extracts were diluted to 20ml in distilled water and shaken in a graduated cylinder for 15 minutes. The presence of saponins is indicated by the formation of a 1 cm layer of foam.

5. Phenol detection

a) **Ferric Chloride Test:** 3-4 drops of ferric chloride solution were applied to the extracts. The presence of phenols is indicated by the formation of a blue black colour.

6. Flavonoids detection

a) **Lead acetate Test:** Extracts were exposed to a few drops of lead acetate solution. The presence of flavonoids is shown by the formation of yellow precipitate.

7. Detection of proteins

a) **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

8. Tannins

a) Gelatin test

To 1 ml of the plant extract was added few drops of 1% Gelatin solution containing 10% Sodium chloride (NaCl). Formation of white precipitate indicates the presence of Tannins.

9. Detection of diterpenes

a) Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [11-13].

Thin layer chromatography

The adsorption phenomenon underpins thin layer chromatography. In this form of chromatography, the dissolved solutes are carried by mobile phase over the surface of the stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) using silica gel 60F254, 7X6 cm (Merck) cut with common household scissors, as per the usual one dimensional ascending procedure. Soft pencil was used to make plate marks. Glass capillaries were used to spot the sample for TLC. A sample volume of one microlitre was applied using a capillary at a distance of one centimetre at five tracks. Toluene: ethyl acetate: formic acid (5:4:1) solvent system was utilised in the twin trough chamber for Quercetin and toluene: ethyl acetate: formic acid (7:5:1) solvent system was used in the gallic acid solvent system. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f), values were calculated for different samples.

Detection and Calculation of R_f Value

Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

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

Quantitative studies of phytoconstituents

Total phenol content estimation

The modified folin-ciocalteu technique was used to determine the total phenol concentration of the extract. 10 mg Gallic acid was diluted in 10 ml methanol, and different aliquots of 10-50g/ml methanol were made. Ten milligrammes of dried extract were dissolved in ten millilitres of methanol and filtered. Two millilitres (1 milligramme per millilitre) of this extract were used to calculate the phenol content. 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml of extract and each standard. The mixture was vortexed for 15 seconds and then left to

stand for 10 minutes to allow the colour to develop. A spectrophotometer was used to measure the absorbance at 765 nm.

Total flavonoids content estimation

The total flavonoids content was determined using the aluminium chloride technique. 10 mg quercetin was dissolved in 10 ml methanol, and different aliquots of 5- 25g/ml methanol were created. Ten milligrammes of dried extract were dissolved in ten millilitres of methanol and filtered. Three millilitres (1 milligramme per millilitre) of this extract were used to estimate flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or standard and allowed to stand for 15 minutes at room temperature before measuring absorbance at 420 nm.

In-vitro antioxidant activity of extract of *Bauhinia variegata* using DPPH method

The spectrophotometer was used to quantify the DPPH scavenging activity [14]. The stock solution (6 mg in 100ml methanol) was created in such a way that 1.5 ml of it in 1.5 ml of methanol produced an initial absorbance. After 15 minutes, there was a decrease in absorbance in the presence of sample extract at various concentrations (10-100 g/ml). 1.5 ml of DPPH solution was obtained, and the volume was increased to 3 ml with methanol. The absorbance was measured immediately at 517 nm for the control reading. Three test samples were collected and processed in the same manner. Finally, the mean was selected. After 15 minutes, at 517 nm, there was a final decline in absorbance of DPPH with the sample at varied concentrations.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

Results and Discussion

The hydroalcoholic extract yield is a % Table 1 depicts aerial components of *Bauhinia variegata*. The extract was subjected to preliminary phytochemical investigations using known standard procedures. Flavonoids, diterpenes, phenol, proteins, and saponins were discovered by phytochemical investigation. Table No. 2 To validate the presence of Gallic acid and Quercetin in herbal extract tables 3 and 4, thin layer chromatography was used. TPC was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the calibration curve equation: $Y = 0.011X + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was determined as quercetin equivalent (mg/100mg) using the calibration curve equation: $Y = 0.032X + 0.018$, $R^2 = 0.998$, where X represents the quercetin equivalent (QE) and Y represents the absorbance. 5th table The hydrogen donating nature of extracts was determined using the DPPH radical scavenging test. The inhibitory concentration 50 percent (IC₅₀) value of

Bauhinia variegata hydroalcoholic extract was reported to be 76.97g/ml when DPPH radical scavenging activity was compared to that of ascorbic acid (18.69g/ml). Table 6 shows that the action was dose dependent with regard to concentration.

Table No. 1: % Yield of hydroalcoholic extract of *Bauhinia variegata*

| S. No. | Hydroalcoholic extract | % Yield (W/W) |
|--------|---|---------------|
| 1. | Aerial parts of <i>Bauhinia variegata</i> | 3.91 |

Table No. 2: Result of phytochemical screening of hydroalcoholic extract of *Bauhinia variegata*

| S. No. | Constituents | Aerial parts extract |
|--------|---|----------------------|
| 1. | Alkaloids Wagner's Test: | -ve |
| 2. | Glycosides Legal's Test: | -ve |
| 3. | Flavonoids Alkaline Reagent Test: Lead acetate Test: | +ve + ve |
| 4. | Diterpenes Copper acetate Test: | +ve |
| 5. | Phenol Ferric Chloride Test: | + ve |
| 6. | Proteins Xanthoproteic Test: | +ve |
| 7. | Carbohydrate Fehling's Test: | -ve |
| 8. | Saponins Froth Test: | +ve |
| 9. | Tannins Gelatin test: | -ve |

Table No. 3: Calculation of R_f Value of hydroalcoholic extract of *Bauhinia variegata* for

Quercetin

| <i>Bauhinia variegata</i> extract | | |
|--|--|--|
| S. No. | Mobile phase Toluene: Ethyl acetate Formic acid (5:4:1) | R_f 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- 0.58 Short- 0.58 Normal- 0.58 |
| 2. | (Hydroalcoholic extract) Dis. travel by mobile phase= 5cm No. of spot at long UV = 6 No. of spot at short UV = 4 No. of spot at normal light= 4 | Long- 0.58, 0.68, 0.72, 0.8, 0.92, 0.96 Short- 0.58, 0.64, 0.72, 0.8 Normal- 0.58, 0.64, 0.72, 0.8 |

Table No. 4: Calculation of R_f Value of hydroalcoholic extract of *Bauhinia variegata* for

Gallic acid

| <i>Bauhinia variegata</i> extract | | |
|--|---|---|
| S. No. | Mobile phase Toluene: Ethyl acetate Formic acid (7:5:1) | R_f value |
| 1. | (Gallic acid) 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= 0 | Long- 0.34 Short- 0.34 Normal- 0.34 |
| 2. | (Hydroalcoholic extract) Dis. travel by mobile phase= 5cm No. of spot at long UV = 6 No. of spot at short UV = 4 | Long- 0.58, 0.64, 0.74, 0.86, 0.9, 0.98 Short- 0.58, 0.64, 0.74, 0.86, 0.9, 0.98 |

| | | |
|--|--------------------------------|---|
| | No. of spot at normal light= 4 | Short- 0.58, 0.64, 0.74, 0.86 Normal- 0.58, 0.64, 0.74, 0.86 |
|--|--------------------------------|---|

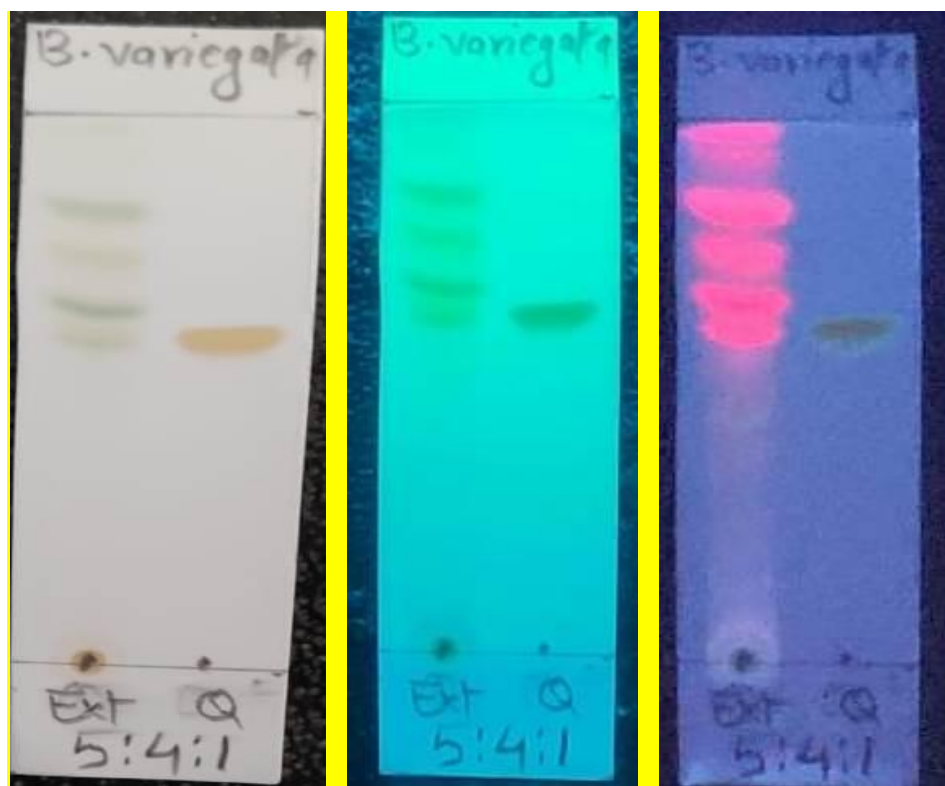


Figure 1: Normal Light Short U.V Long U.V

Spot-1= Quercetin, Spot-2= Aerial parts extract of *Bauhinia variegata*

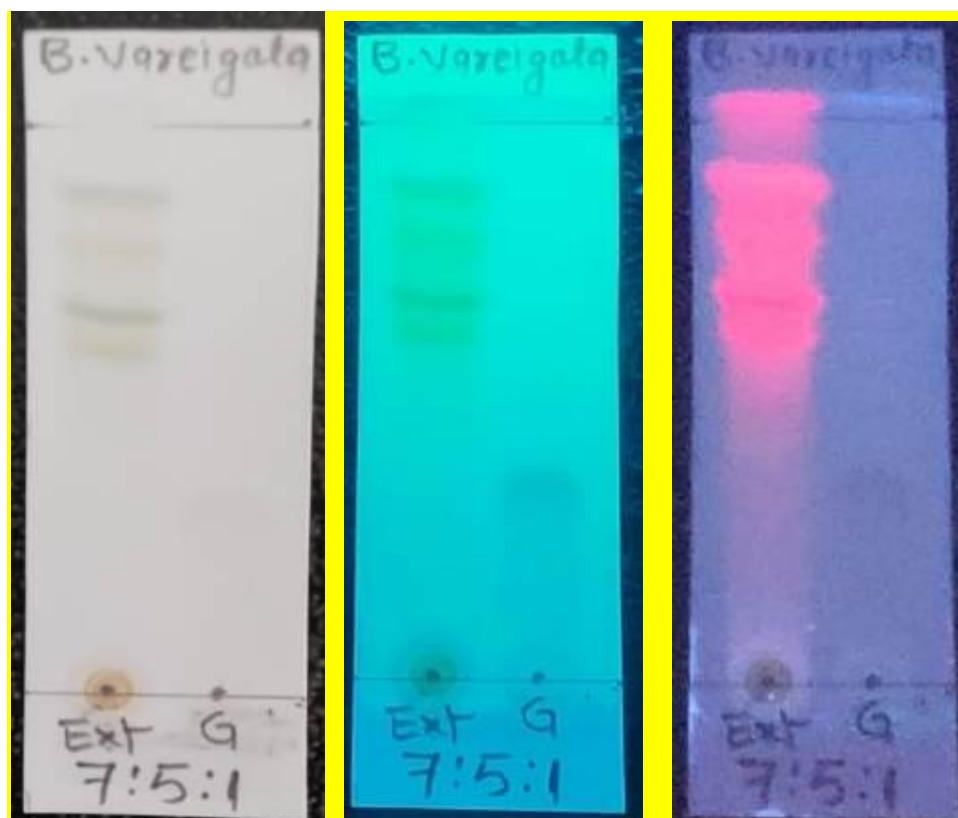


Figure 2: Normal Light Short U.V Long U.V

Spot-1= Gallic acid, Spot-2= Aerial parts extract of *Bauhinia variegata*

Table No. 5: Estimation of total phenolic and flavonoids content of aerial parts of extract *Bauhinia variegata*

| S. No. | Extract | Total phenolic content (mg/100mg of dried extract) | Total flavonoids content (mg/ 100 mg of dried extract) |
|--------|---------------------------|--|--|
| 1. | <i>Bauhinia variegata</i> | 1.454 | 1.112 |

Table 6: % Inhibition of ascorbic acid and hydroalcoholic extract of *Bauhinia variegata* using DPPH method

| S. No. | Concentration (µg/ml) | % Inhibition | |
|--------|--------------------------|---------------|--------------------------------------|
| | | Ascorbic acid | <i>Bauhinia variegata</i> extract |
| 1 | 10 | 30.42 | 18.26 |
| 2 | 20 | 59.11 | 19.13 |
| 3 | 40 | 67.48 | 32.17 |

| | | | |
|------------------|-----|-------|-------|
| 4 | 60 | 75.25 | 46.95 |
| 5 | 80 | 77.58 | 52.17 |
| 6 | 100 | 79.63 | 58.26 |
| IC ₅₀ | | 18.69 | 76.97 |

CONCLUSION

The current study's findings clearly show that the extract may efficiently scavenge numerous reactive oxygen species/free radicals under in vitro settings. This might be due to the large number of stable oxidised products that it can produce upon oxidation or radical scavenging. The extracts' broad spectrum of action shows that antioxidant activity is mediated by numerous pathways. The various antioxidant activity of the extract exhibited in this study clearly demonstrates the potential use of both plants. However, before either plant can be used as an antioxidant component in animal feeds or human health foods, its in vivo safety must be carefully examined in experimental rodent models. The above results showed that aerial parts of *Bauhinia variegata* extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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