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

Abstract

The objectives of this study are to screen the phytochemicals, thin layer chromatography, estimate the content of phenolic and flavonoid compounds and determine the antioxidant capacity of the *Bauhinia variegata*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. The hydro alcoholic extract of aerial parts of *Bauhinia variegata* was studied for qualitative and quantitative analysis and antioxidant activity on *in vitro* model namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay, Phytochemical analysis revealed the presence of phenols and flavonoids. The total phenolic and flavonoids content of hydroalcoholic extract of *Bauhinia variegata* was 1.454 and 1.112mg/100mg respectively. Ascorbic acid used as standards was also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. aerial parts of *Bauhinia variegata* Hydroalcoholic extract showed IC_{50} value 60.22µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=17.68µg/ml) . For hydrogen peroxide method, IC_{50} value was found to be 76.97µg/ml, which compares favourable with ascorbic acid (IC_{50}=18.69µg/ml). The present study describes the phytochemical profile and antioxidant activity and TLC of *Bauhinia variegata* which will further used for medicinal applications.

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

INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc [1, 2]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders [3]**.** Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids,

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polyphenols, flavonoids and glutathione. Prior and Cao [4], reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology [5]. *Bauhinia variegata* Linn. (Leguminosae) bark is traditionally used as tonic and in treatment of ulcers. It is also useful in skin diseases. The roots are used as antidote to snake poison [6]. In folklore medicine, this plant is also used for managing several diseases including inflammatory conditions [7]. Keeping this in view, the present study has been conducted to evaluate the antioxidant activity, quantitative study of total phenolic and flavonoid content of *Bauhinia variegata* which are traditionally well known for their various activities.

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MATERIAL AND METHODS

Material

Aerial parts of *Bauhinia variegata* were collected from Vindhya Herbals (MFP-PARC) Bhopal (M.P.) in the month of February, 2020. All the chemicals used in this study were obtained from 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).All the chemicals and solvent used in this study were of analytical grade.

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Methods

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered aerial parts [8-9]

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Extraction by maceration method [10]

The shade dried material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 77 gm of dried aerial parts of *Bauhinia variegata* were exhaustively extracted with hydroalcoholic solvent (methanol: water: 70: 30) using maceration method. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

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Determination of percentage yield

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

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

Phytochemical screening

Phytochemical examinations were carried out extracts as per the following standard methods.

1. Detection of alkaloids: Extracts dissolved individually in dilute Hydrochloric acid and filtered.

a) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

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

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the incidence of saponins.

5. Detection of phenols

a) Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of flavonoids

a) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formations of yellow colour precipitate indicate the occurrence of flavonoids.

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

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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

Thin layer chromatography is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase. Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) for Quercetin and toluene: ethyl acetate: formic acid (7:5:1) for gallic acid solvent system used. 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 (Rf), 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 total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and

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allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

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

DPPH scavenging activity was measured by the spectrophotometer [14]. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. Three test samples were taken and each processed similarly. Finally the mean was taken. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

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

Results and Discussion

The percentage yield of hydroalcoholic extract obtained Aerial parts of *Bauhinia variegata* depicted in the Table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. Phytochemical analysis revealed the presence of flavonoids, diterpenes, phenol, proteins and saponins Table 2. Thin layer Chromatography was performed to confirm the presence of Gallic acid and Quercetin in herbal extract table 3 & 4. Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: 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 calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.032X + 0.018, R^2=0.998, where X is the quercetin equivalent (QE) and Y is the absorbance Table 5. DPPH radical scavenging assay measured hydrogen donating nature of extracts. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *Bauhinia variegata* hydroalcoholic

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extract was found to be 76.97µg/ml as compared to that of ascorbic acid (18.69µg/ml). A dose dependent activity with respect to concentration was observed Table 6.

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

S. No.	Hydroalcoholic extract	% Yield (W/W)
1.	Aerial parts of *Bauhinia variegata*	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 variegate* for Quercetin

***Bauhinia variegata* extract**		
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- 0.58 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 variegate* for Gallic acid

***Bauhinia variegata* extract**		
S. No.	**Mobile phase** Toluene: Ethyl acetate Formic acid (7:5:1)	***Rf* 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

	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

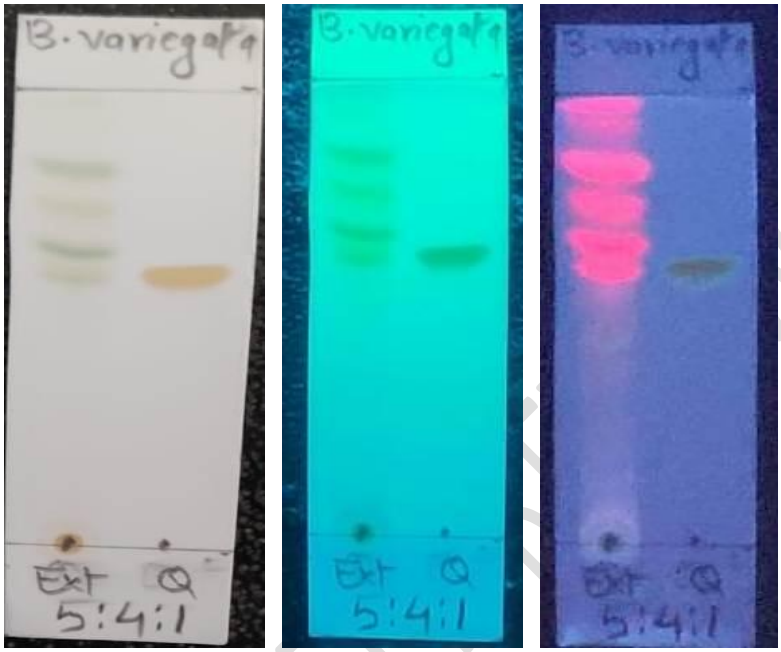

Figure 1: Normal Light Short U.V Long U.V

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

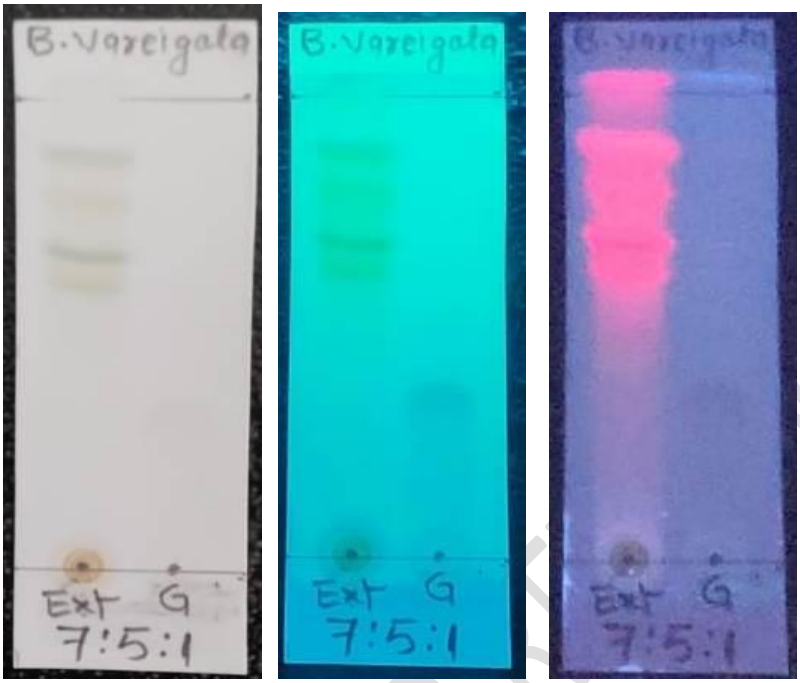

Figure 2: Normal Light Short U.V Long U.V

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.	*Bauhinia variegata*	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	*Bauhinia variegata* 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 results obtained in the present study clearly demonstrate that the extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the both plants. However, the in vivo safety of both plants needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. 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.

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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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