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Quantitative Analysis of Phytoconstituents and *in Vitro* Antioxidant Activity of *Pyrostegia venusta* Leaves

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KEYWORDS

Pyrostegia venusta, DPPH, FRSA, $IC_{50,}$ Antioxidant

ABSTRACT:

Pyrostegia venusta (Ker-Gawl) Miers (family, Bignoniaceae) is a neotropic evergreen vine, also known as Orange trumphet. The present study aimed to investigate quantitative analysis of leaves of Pyrostegia venusta in different extracts using standard methods. From the preliminary examination it has been found that extracts gave result for phenolic, flavonoids and protein contents. The quantitative studies for phenol, flavonoids and protein were performed present in different extracts like chloroform, ethyl acetate, methanol and water. The quantitative estimation was carried out using modified folin-ciocalteu method, taking gallic acid as standard, flavonoids content was based on aluminum chloride method taking quercetin as standard, protein content estimated by Lowry's method taking bovine serum albumin as standard. Also, the in vitro antioxidant activity of methanolic extract was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Hydrogen Peroxide method calculating the IC50 values. In DPPH method, ascorbic acid was taken as standard having IC50 value 14.55 µg/ml while methanolic extract gave IC50 value 33.38 µg/ml and in hydrogen peroxide method the IC50 value calculated for ascorbic acid is 7.58 µg/ml and that of methanolic extract was 62.70 µg/ml. So, a comparison of antioxidant potential was being made using the two methods.

1. Introduction

Medicinal plants or Traditional medicines are gaining importance in the field of medicine as they produce many phytoconstituents. A single plant is holding a wide variety of phytochemicals in different parts of it like roots, stem, leaves, flowers, fruits, etc.[1] The phytochemicals are secondary metabolites of plant and these secondary metabolites have anti-inflammatory, antioxidant, anticancer, antimicrobial, antihyperglycemic and many more activities.[2] One such plant is *Pyrostegia venusta* (Ker-Gawl) *Miers* family, Bignoniaceae is an ornamental plant known as orange trumpet vine, flame creeper, golden shower vine has been used as a traditional medicine for the treatment of dysentery, vitiligo, cough & flu, diarrhea, antioxidant, anti-inflammatory, antibacterial, anthelmintic activity.[3,4,5,6] The antioxidant potential of the plant is of great importance in the pharmaceutical industry as the main phytoconstituents like phenols and flavonoids possesses antioxidant activity occurring naturally to fight against the oxidative stress and free radicals that are generated as the lifestyle diseases are emerging nowadays. This research targets the quantitative estimation of different plant extracts and antioxidant activity of methanolic extract.[7]

2. Materials and Methods

Plant Material

The leaves of Pyrostegia venusta were collected from local area of Bhopal in the month of June, 2022 and authenticated by Dr. Suman Mishra at Vindhya Herbals, Bhopal, Madhya Pradesh (India) and shade dried which was further powdered and were defatted with petroleum ether using maceration method. The defatted plant material was extracted in four solvents of different polarity viz water, methanol, ethyl acetate and chloroform by maceration method. All the solvents like methanol, ethyl acetate and chloroform used in the experiments were of analytical grade. The other chemicals and reagents like DPPH, Hydrogen peroxide, and Folin-Ciocalteu reagent, were obtained from Merck Life sciences Pvt. Ltd. India . The remaining chemicals used were obtained from the local vendors.

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Qualitative Phytochemical Analysis

The Preliminary Phytochemical analysis was carried out for different plant extracts for the presence of alkaloids, glycosides, flavonoids, diterpenes, phenol, proteins, carbohydrates, saponins and tannins as described before.[8,9,10]

Quantitative Phytochemical Analysis

After Phytochemical screening of the extracts, estimation of flavonoids, phenol and protein were carried out which showed positive result.

1. 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 filtered. 2 ml (1mg/ml) of this extract was taken 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 15sec. and allowed to stand for 10 minutes for color development. The absorbance was measured at 765 nm using a spectrophotometer.[11]

2. Total flavonoids content estimation

Determination of total flavonoids content was based on Aluminum 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. 3 ml (1mg/ml) of this extract was used for the estimation of flavonoids. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15 minutes at room temperature, absorbance was measured at 420 nm.[11]

3.Total protein content in estimation

The amount of protein was estimated by Lowry's method. Following reagents were used: 2% Na2CO3 in 0.1 N NaOH[A], 1% Sodium potassium tartrate in H2O [B], 0.5% $CuSO_4$.5 H_2O in H_2O [C].

Reagent I: 48 ml of [A], 1 ml of [B], 1 ml [C]

Reagent II- 1part folin-phenol [2N]: 1part water.

1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serving as blank. Added 4.5 ml of reagent I and incubated for 10 minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph.[12]

In-vitro* antioxidant activity of extract of *Pyrostegia venusta

1. DPPH method

DPPH scavenging activity was measured by the spectrophotometer.[13] 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 with 3 ml methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. 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.

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

2. Hydrogen peroxide method

In-vitro antioxidant activity of extract of *Pyrostegia venusta* using hydrogen peroxide FRSA was performed.[14] Added 2ml hydrogen peroxide (43 mol) and 1.0 ml methanol sample [20-100 µl different extracts (4 mg / ml) methanol] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Three times all measurements were repeated. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

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RESULTS AND DISCUSSION

1. Estimation of total phenolic, flavonoids and protein content

Total Phenolic content estimation (TPC)

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 = 0.012x + 0.007, R2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table No. 1: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	10	0.135
3	20	0.256
4	30	0.374
5	40	0.501
6	50	0.613

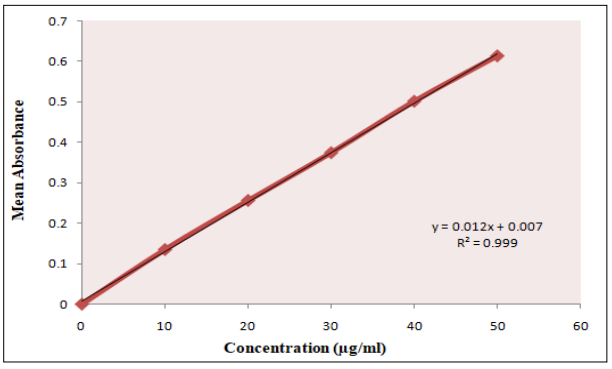

Figure 1: Graph of Calibration curve of Gallic acid

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$y = 0.034x + 0.014$, $R^2=0.998$**, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 2: Preparation of Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	5	0.202
3	10	0.367
4	15	0.524
5	20	0.702
6	25	0.874

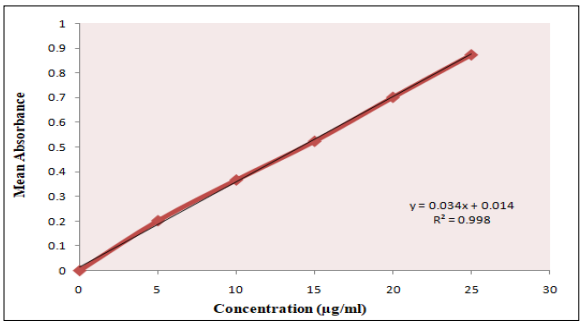

Figure 2: Graph of calibration curve of Quercetin

Estimation of total protein content (TPC)

Total protein content was calculated as Bovine serum albumin equivalent mg/100mg using the equation based on the calibration curve: **$Y = 0.002X + 0.006$, $R^2=0.999$**, where X is the BSA equivalent (BE) and Y is the absorbance.

Calibration curve of BSA (Bovine serum albumin)

Table 3: Calibration curve of BSA (Bovine serum albumin)

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	50	0.127
3	100	0.245
4	150	0.366
5	200	0.474

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6	250	0.593

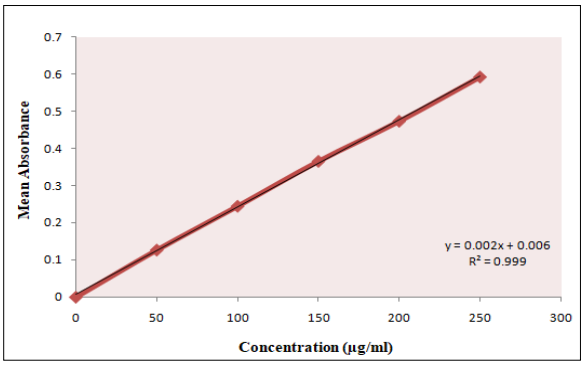

Figure 3: Graph of calibration curve of Bovine serum albumin

Table No. 4: Estimation of total phenolic, flavonoids and protein content of *Pyrostegia venusta* in different extracts

S. No.	Extract	Total phenolic content	Total flavonoids content	Total Protein Content
		(mg/100mg of dried extract)		
1.	Chloroform	0.58	-	-
2.	Ethyl acetate	1.44	-	-
3.	Methanol	1.48	1.49	0.59
4.	Aqueous	-	0.59	0.65

From the above table it is cleared that the total phenolic content of methanolic extract is higher than the ethyl acetate and chloroform extract, the total flavonoids content is also higher in methanolic extract as compared to aqueous extract and the total protein content is slightly higher in aqueous extract than in methanolic extract. The quantitative analysis in different extracts revealed that the methanolic extract can be a basis for further studies as it has more phytoconstituent range.

Results of antioxidant activity of *Pyrostegia venusta*

1. **DPPH method**

Table No. 5: % Inhibition of ascorbic acid and *Pyrostegia venusta* extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Methanolic extract
1	10	47.48	30.22
2	20	52.89	45.59
3	40	62.46	59.19
4	60	70.52	68.13
5	80	78.84	73.80
6	100	90.05	77.83
IC_{50} value		**14.55**	**33.38**

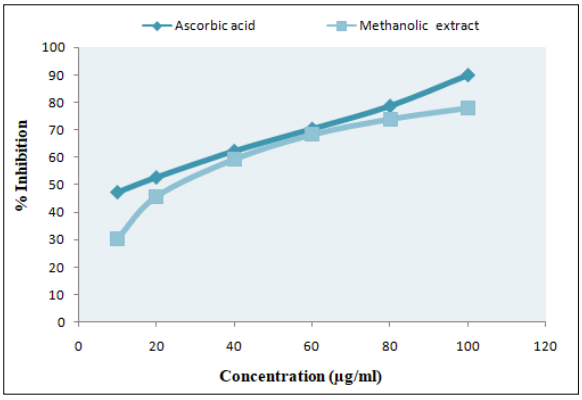

Figure 4: % Inhibition of ascorbic acid and *Pyrostegia venusta* extract using DPPH method

The IC_{50} value is a measure of the concentration of a compound where percent inhibition is equal to 50. The IC_{50} value by DPPH assay of ascorbic acid was found to be 14.55 µg/ml which was taken as a standard and the methanolic extract was found to be 33.38 µg/ml which indicates that the methanolic extract can be a close one for the antioxidant activity.

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2. Hydrogen Peroxide method

Table No. 6: % Inhibition of ascorbic acid and methanolic extract of *Pyrostegia venusta* using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Methanolic extract
1	5	43.02	33.71
2	10	55.77	34.17
3	15	65.25	34.85
4	20	69.94	35.42
5	25	77.02	40.57
IC_{50} value		**7.58**	**62.70**

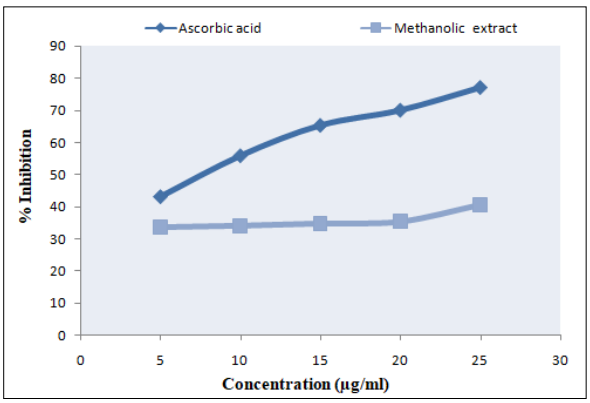

Figure 5: % Inhibition of ascorbic acid and methanolic extract of *Pyrostegia venusta* using hydrogen peroxide method

The IC_{50} value by Hydrogen peroxide assay of ascorbic acid was found to be 7.58 µg/ml which was taken as a standard and the methanolic extract was found to be 62.70 µg/ml which indicates that the methanolic extract require a higher concentration as compared to the standard. Among the two methods applied for the antioxidant potential, the DPPH assay gave a much positive result for the activity as compared to the Hydrogen peroxide free radical scavenging activity.

The research paper highlights the importance of traditional plants for developing many pharmaceuticals which are being used in the formulation like use of natural antioxidants instead of synthetic ones. So, the present work focused on the quantitative estimation of various extracts of *Pyrostegia venusta* leaves and its antioxidant activity as future prospective.

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