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In-Vitro Antioxidant Activity of *Bauhina variegata*

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Abstract

Bauhinia variegate, commonly known as Kachnar, belongs to family Leguminosae. The leaves of this plant used in the treatment of diabetes, ulcers, inflammation etc.Diabetes mellitus (DM) is denotation to a mixed group of a syndrome characterized by an elevated level of blood glucose due to imbalanced oxidation and utilization of glucose, which is related to the malfunction of insulin-producing pancreatic β-cells.The aim of present research is to study *in-vitro*antioxidant activity of *Bauhinia variegata.*The petroleum ether and methanol extracts prepared from the powdered leaves of *Bauhinia variegata* were subjected to qualitative phytochemical screening in order to check the presence of particular class of components present in them. The antioxidant activity of methanol extract of *Bauhinia variegata* against superoxide dismutase was found to be 58.64 and 95.8±0.4.The EC_{50} of methanol extract *of Bauhinia variegata* was 232.375 µg/ml respectively, and for ascorbic acid it was found to be 0.708 µg/ml. The IC_{50} of ascorbic acid was found to be 3.178 µg/ml whereas IC_{50} of methanol extract of *Bauhinia variegata* was calculated as 5.2 µg/ml. The radical scavenging ability of the compound is measured by stable radical DPPH (2, 2-diphenol-2-picryl hydrazyl). The DPPH scavenging activity of methanol extract at 5µg/ml was found to be 65.69 ± 0.10. The present study clearly indicated that *B. variegata* extract have appreciable *in-vitro* antioxidant effect.

Keywords: *Bauhinia variegate*, Antioxidant, DPPH, Scavenging Activity

Introduction

Bauhinia variegate, commonly known as Kachnar, belongs to family leguminosae. All the parts of the plant (leaves, flower bud, root, seed, bark, stem, and flower) were used as traditional medicine. It was traditionally used in the treatment of bronchitis, leprosy, tumors. The leaves of this plant used in the treatment of diabetes, ulcers, inflammation etc. [1]

Bauhinia variegata is a medium sized deciduous tree. The genus includes tree, vines, shrubs that are frequently planted for their use of pharmacological and pharmaceutical properties. The leaves are 10-15cm long and broad and deeply cordate. It is mainly found in garden, park and roadsides as ornamental plant in many warm temperate and subtropical regions. It was native to Southeast Asia and grows in tropical and subtropical climate. Infusion of leaves was used as an anti-diabetic which is increasing day by day in normal population [2].

Diabetes Mellitus

Diabetes mellitus (DM) is denotation to a mixed group of a syndrome characterized by an elevated level of blood glucose due to imbalanced oxidation and utilization of glucose, which is related to the malfunction of insulin-producing pancreatic β-cells. [3]. DM is primarily caused by dysfunction in insulin secretion (inadequate production of insulin), reduction in peripheral response to insulin (insufficient sensitivity of cells to insulin) or both. The cytokines, lipotoxicity and glucotoxicity are three major stimuli for β-cell apoptosis.

World Health Organization (WHO) classified DM into two broad categories Type I or also called insulin-dependent DM (IDDM) and Type II or non-insulin-dependent DM (NIDDM) [4-6]. The level of hyperglycaemia associated DM increases the risk of microvascular damage (nephropathy, neuropathy and retinopathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischemic heart disease, stroke and peripheral vascular disease), and diminished quality of life [7,8].

Materials and Methods

Materials

The leaves of *Bauhinia Variegata are* collected and dried in shade and coarsely powdered in the blender. All the chemicals used in the present investigation were of analytical grade. All the glasswares and equipments were sterilized before use.

Methods

Preparation of Alcoholic Extract

Alcoholic extract was prepared by taking different concentration of dry *Bauhinia variegata* leaf powder (25,75mg). Each concentration was dissolved in ratio of alcohol (60:40) in soxhlet apparatus and heat at a particular temperature 50-100 $^{\circ}$C, until the stiffen tube was cleared and extract was collected.

Preparation of the Extracts

The *Bauhinia variegata* leaves were dried under shade for 15 days and then powdered. The powdered plant material (1 kg) was sequentially macerated (15 hour at room temperature) with ethanol followed by hydro ethanol (water and ethanol, 60:

40v/v) by using soxhlet apparatus.The obtained extracts were filtered with Whatman's filter (paper no. 4) and dried under vacuum. The percentage yield of ethanolic and hydroethanolic extracts was 15.5% and 12.3% w/w with respect to air dried plant material, respectively [9].

Qualitative Phytochemical Analysis

The following procedures were adopted to test for the presence of various chemical constituents in the extracts.

4.4 Preliminary Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical investigation for the detection of the different metabolites. [10,11].

1. Test for Alkaloids

5 ml of methanolic extract was evaporated to dryness. The ethanolic residues were taken in 5 ml of 2% hydrochloric acid, saturated with sodium chloride and filtered. The filtrate was tested with alkaloidal reagents:

A. **Mayer's Reagent** (KI + Hg_2Cl_2 solutions) produced cream coloured precipitate, indicate presence of alkaloids.
B. **Dragondorff's Reagent** (excess of KI + Bismuth Subnitrate Solutions) produced reddish brown coloured precipitates, indicating presence of alkaloids.
C. **Wagner's Reagent** (I2 + KI solutions) produced reddish brown coloured precipitate indicating presence of alkaloids.
D. **Hager's Reagent** (Picric acid) produced yellow coloured precipitate, indicating presence of alkaloids.

2. Test for Carbohydrates

i. **Molisch Test:** To 2 ml of BVHE and BVHAE, 3 drops of α-naphthol (20% in ethanol) was added. Then 1ml of concentrated sulphuric acid was added along the side of the test tube. Reddish-violet ring at the junction of the two layers indicating the presence of carbohydrates.
ii. **Reduction of Fehling's Solution:** 1 ml of Fehling's Solution (Copper Sulfate in alkaline condition) was added to the concentrated extract and heated on a steam bath. Brick red precipitate indicating the presence of carbohydrates.

3. Test for Glycosides

i. **Keller–Killiani Test:** 1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of concentrated sulphuric acid were added to the extract carefully. A reddish brown colour formed at the junction of the 2 layers and the upper layer turned bluish green indicating the presence of glycosides.
ii. **Borntrager's Test:** 1 ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A reddish pink colour indicating presence of glycosides.
iii. **Legal's Test:** Concentrated ethanolic extract was made alkaline with drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside solution was added. The presence of blue coloration indicating the presence of glycoside.

4. Test for Phenolic Compounds

i. **Ferric Chloride Test:** 3 ml of BVEE and BVHAE were evaporated to dryness, extracted with 5 ml of distilled water; ferric chloride solution (5%) was then added in the extract, blue green colour indicating the presence of phenolic compounds.

ii. **Lead Acetate Test**: Yellow precipitates were obtained by the addition of 3 drops of lead acetate solution (5%) indicating phenolic compounds.

iii. **Gelatin Test**: 3 ml of 0.1% of gelatin solution was added to 5ml of ethanolic extract. Precipitation indicating the presence of phenolic compounds.

5. Test for Flavonoids

i. **Ammonia Test:** Filter Paper strips were dipped in the alcoholic solution of the extract and ammoniated. The filter strips turned yellow indicating the presence of flavonoids.

ii. **Shinoda / Pew Test:** Two drops of hydrochloric acid were mixed to 1 ml of extract along with an addition of metallic piece of magnesium. The deep red colour indicating presence of flavonoids in the extract.

6. Test for Proteins and Free Amino Acids

i. **Millon's Test:** Few ml of BVEE and BVHAE, 5 ml distilled water was added and filtered. To 2 ml of filtrate, 5 drops of Millon's Reagent (solution of mercury nitrate and nitrous acid) were added. Red precipitates were not formed, indicating absence of proteins and amino acids.

ii. **Xanthoprotein Test:** 2 ml of extract, 3 drops of nitric acid were added by the side of the test tube. Absence of yellow coloration indicating the absence of proteins and free amino acids.

iii. **Biuret Test:** Ammoniated alkaline filtrate of the extract, 2 drops of 0.02% copper sulphate solution was added. Absence of red or violet coloration indicating absence of proteins and free amino acids.

7. Test for Saponins

Both Extracts of leaves of BV were tested for the presence of saponins.

8. Test for Sterols

The BVEE and BVHAE were evaporated to dryness and the residue was extracted with petroleum ether. The insoluble residue was tested for sterols:

a. **Salkowski Reaction:** To the extract, 2 ml of concentrated sulphuric acid was added. The presence of a yellow ring at the junction which finally turns red after one minute indicated the presence of sterols.

b. **Hersche's Son's Reaction:** To the residue, 2 ml of trichloroacetic acid was added. Presence of red to violet colour on heating, indicating the presence of sterols.

9. Test for Acidic Compounds

a. To 2 ml of BVEE and BVHAE, 1ml sodium bicarbonate solution was added. The effervescence produced indicating the presence of acidic compounds.

b. 2 ml of BVEE & BVHAE was taken in warm water and filtered. The filtrate was then tested with litmus paper and methyl orange. The appearance of blue colour indicating the presence of acidic compounds.

10. Test for Steroids

i. **Hesse's Test**: Extract (50 mg) was dissolved in chloroform (4 ml) and then slowly added conc. sulphuric acid along the sides of test tube. Pink color indicating the presence of sterols.

ii. To 1 ml BVEE and BVHAE taken in a test tube, few drops of Liebermann Burchard Reagent were added. Development of greenish color indicating the presence of steroids. [12,13].

11. Test for Fixed Oils and Fats

Few amounts of extracts was pressed and applied in the layers of 2- filter papers. Presence of fixed oils was indicating by the oily stain on filter paper.

12. Test for Terpenoids

Salkowski Test: Small amount of extracts was mixed with chloroform (2 ml) and conc. sulphuric acid (3 ml) was added from the sides of the test tube. Reddish- brown ring formed on the junction of 2 layers indicating the presence of terpenoids.

Standardization of Extracts

TLC Analysis

TLC plates were coated using Silica-gel G and were activated in hot air oven at 120°C for 10-15 minutes. BVEE and BVHAE were dissolved in respective solvents and spotted on activated TLC plates employing capillary tubes. A solvent system of chloroform, ethanol and acetic acid in ratio of 6: 3.5: 0.5 was prepared in TLC jar. After saturation of TLC jar, TLC plate spotted with samples was placed in the jar and solvent was allowed to run. After 5 minutes, plate was taken out and allowed to dry. For identification of the separated compounds, dried plate was kept in iodine chamber for five minutes. Spots were marked and R_f values were calculated.

***In- Vitro* Antioxidant Assay**

Estimation of Total Phenolic Compounds

Estimation was done with Folin-Ciocalteau reagent using gallic acid as a standard phenolic compound [14]. According to the method, 1 mg/mL of extract solution was taken in a volumetric flask. The final reaction mixture was prepared by mixing 0.5 mL of plant extract solution with 2.5 mL of 10% Folin Ciocalteu reagent dissolved in water and 2.5 mL of 7.5% $NaHCO_3$ aqueous solution. The samples were placed for 45 minutes at 45°C. The absorbance of the blue color was observed at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract [15]. All determinations were made in triplicate. The total content of phenolic compounds in plant extract was determined as mg of gallic acid equivalents (GAE).

Total Antioxidant Capacity (TAOC)

Total antioxidant capacity (TAOC) was carried out as per the method suggested [16, 17]. Different concentrations (50–450mg/ml) of extracts were prepared in water; 0.3 ml of the extract was mixed with 3 ml of reagent (a mixture of sulfuric acid [0.6 M], ammonium molybdate [4 mM], and sodium phosphate [28 mM]). It was then incubated for 90 minutes at 95°C, and absorbance was recorded at 695 nm against the blank. EC_{50} values of the extracts as well as the ascorbic acid were calculated from the concentration-absorbance graph.

Determination of 2, 2-Diphenyl-1 Picrylhydrazyl Radical Scavenging Activity

Scavenging activity of the extracts was evaluated by the hydrogen donating or radical scavenging ability using the stable radical DPPH. In this, add 1.0 mL of 0.1 mM solution of DPPH in methanol to 3.0 ml of extract solution in water at different concentrations (10–320mg/ml). After 30 minutes, absorbance was determined at 517 nm. The reference compound used in the test was ascorbic acid.

$$\% \text{ Inhibition} = (A_0 - A_t) / A_0 \times 100$$

Here, A_0represents the absorbance of the control i.e., blank (without extract), and A_t represents the absorbance in the presence of the extract. This procedure was repeated three times and the mean values ± standard deviations (SD) were calculated. The inhibitory concentration 50 (IC_{50}) value was calculated from the concentration-absorbance graph.

Reducing Power Assay

The reducing power of the extracts was evaluated considering ascorbic acid as a standard compound. Various concentrations, from 10 to 320mg/ml, of extracts as well as ascorbic acid were prepared and one millilitre was taken in different test tubes. The solutions were diluted up to 2 ml using distilled water and were mixed into the mixture of 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated for 20 minutes at 500°C. After incubation, 2.5 ml of 10% trichloroacetic acid was added to the mixture and then centrifuged for 10 min at 3000 rpm. The solution's upper layer (2.5 ml) was diluted with 2.5 ml of distilled water and then 0.5 ml of ferric chloride ($FeCl_3$) was added to mixture. The absorbance of all the reaction mixtures was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased, reducing power. This procedure was done in triplicate, and the mean values ± standard deviations were calculated. Effective concentration 50 (EC_{50}) value was calculated from the concentration absorbance graph using ascorbic acid as a reference standard agent.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide (H_2O_2) scavenging activity of extracts was measured according to the process. The extracts (20–320mg/ml) were mixed in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed 600ml of 43 mM solution of H_2O_2. The absorbance of this reaction mixture was measured at 230 nm. For background subtraction, a separate blank sample was used for each concentration. The percentage (%) of H_2O_2scavenging effect is calculated using the following formula.

$$\%H_2O_2 \text{ Scavenging Effect} = 1-(A_1 - A_2)/A_0 \times 100$$

Here, A_0 represents the absorbance of the control (water instead of the sample), A_1 represents the absorbance of the sample, and A_2represents the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The IC_{50} value represented the concentration that inhibited 50% of H_2O_2.

Results and Discussion

Plant Collection and Identification

The powder of leaves was extracted successively, using soxhlet apparatus with suitable solvents.

Extraction

About 50 gm of powdered leaves of *Bauhinia variegata* were uniformly packed into a thimble and extracted with 250 ml of solvent.

Table 1: Percentage Yield of *Bauhinia variegata*using Pet. Ether and Methanol

S. No.	Extract	%w/w yield of the *Bauhinia variegata*
1.	Petroleum Ether	3.49
2.	Methanol	14.15

Preliminary Phytochemical Screening

The results of phytochemical screening are shown: **(P = Present; A = Absent)**

Table 2: Phytochemical Evaluation Using Petroleum Ether Extract and Methanol Extract

S. No.	Test	Test Performed	Pet. Ether Extract	Methanol Extract
1	Alkaloids	1. Dragendroff Test	P	P
		2. Hager's Test		
2	Saponins	1. Froth Test	P	P
		2. Foam Test		
3	Fixed Oils and Fats	1. Spot Test	A	A
		2. Saponification Test		
4	Carbohydrates	Molisch Test	A	P
5	Reducing Sugar	1. Benedict's Test	A	P
		2. Fehling Test		
6	Flavonoids	1. Ferric Chloride Test	P	P
		2.Alkaline Reagent Test		
7	Glycosides	1. Killer Killani Test	A	A
		2. Bromine Water Test		
8	Tannins	1. Gelatine Test	P	P
		2. Test with Phenazone		
9	Phenols	1. Ferric chloride Test	P	P

		2. Lead acetate Test		

***In-vitro* Antioxidant Activity**

Total Phenol Content

The total phenolic content present in methanol extract of *Bauhinia variegata* was found to be 362.10 and 266.95 mg/gallic acid equivalent (GAE) respectively. Due to their scavenging ability because of hydroxyl groups, they are known to play a vital role in antioxidant activity.

Hydrogen Peroxide Scavenging Activity

Ascorbic acid was used as a reference compound. The IC_{50} of ascorbic acid was found to be 343µg/ml and IC_{50} of methanol extract of *Bauhinia variegata* was found to be 301.85 µg/ml. As hydroxyl radicals are produced by hydrogen peroxide in the body, the antioxidant activity of methanolic extract of *Bauhinia variegata* can be measured by scavenging of these radicals. Thus, antioxidant activity of *Bauhinia variegata* was found to be 76.17 ± 1.523 µg/ml respectively, whereas, that of ascorbic acid was 94.9443 ± 1.146%.

Reducing Power Assay

TheEC_{50} of methanol extract *of Bauhinia Variegata* was 232.375 µg/ml respectively, and for ascorbic acid it was found to be 0.708 µg/ml. This assay is used to measure the antioxidant's reductive ability, which can be evaluated by transformation of (Fe^{+++}) to (Fe^{++}) in the presence of test compounds which in turn reacts with ferric chloride to form ferric-ferrous complex having absorption maximum at 700nm.

Superoxide Scavenging Activity

The IC_{50} was found to be 437.19 µg/ml for ascorbic acid whereas for methanol extract was calculated as 240 µg/ml. The antioxidant activity of methanol extract of *Bauhinia variegata* against superoxide dismutase was found to be 58.64 and 95.8±0.4.

DPPH Scavenging Activity

The IC_{50} of ascorbic acid was found to be 3.178 µg/ml whereas IC_{50} of methanol extract of *Bauhinia variegata* was calculated as 5.2 µg/ml. The radical scavenging ability of the compound is measured by stable radical DPPH (2, 2-diphenol-2-picryl hydrazyl). The DPPH scavenging activity of methanol extract at 5µg/ml was found to be 65.69 ± 0.10.

Table 3:Gallic Acid Standard Curve

Gallic Acid Standard Curve	
Conc. (µg/ml)	**Absorbance**
10	0.113
20	0.14
40	0.195
60	0.271
80	0.34
100	0.3
200	0.72

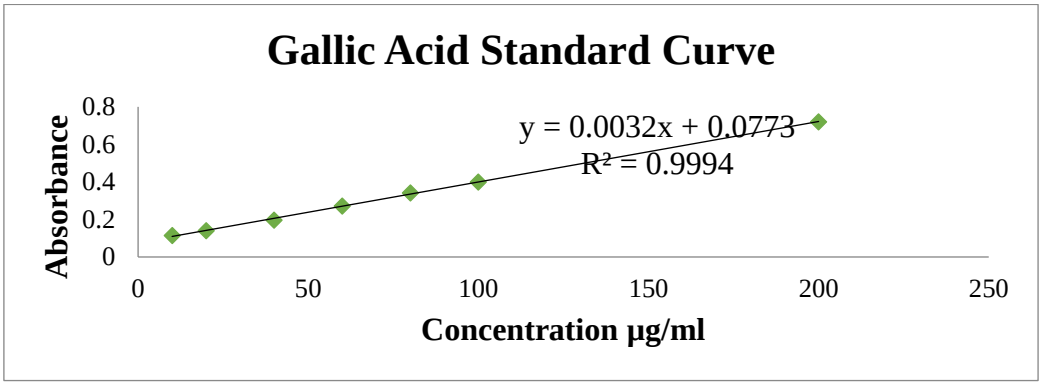

Figure 1: Gallic Acid Standard Curve

Table 4: Standard Curve of Ascorbic Acid

Conc. (µg/ml)	Abs 1a	Abs 1b	Abs 1c	Abs 2a	Abs 2b	Abs 2c
25	0.224	0.221	0.223	0.076	0.079	0.075
50	0.227	0.228	0.226	0.109	0.108	0.106
100	0.244	0.244	0.246	0.145	0.145	0.149
200	0.256	0.253	0.256	0.188	0.185	0.188
400	0.267	0.267	0.267	0.255	0.252	0.258

% age Inhibition			Mean	SD
100.00	100.00	100.00	100.000	0.000
44.36	45.80	43.51	44.558	1.158
55.64	54.20	54.20	54.679	0.832
62.78	62.21	62.98	62.658	0.397
74.44	74.05	74.05	74.176	0.225
95.49	94.27	96.56	95.443	1.146

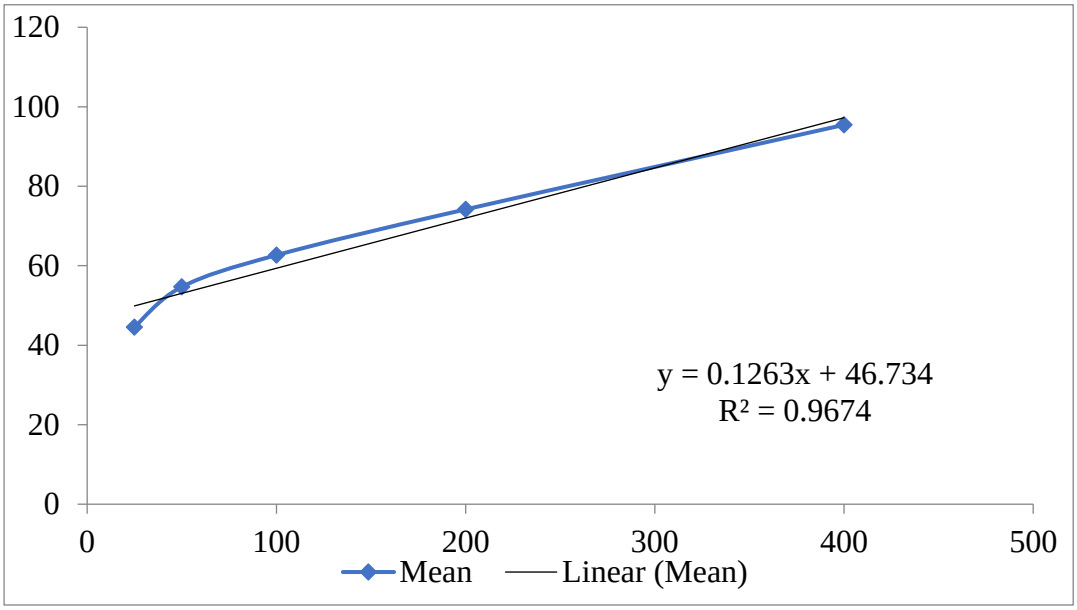

Figure 2: Standard Curve of Ascorbic Acid

Table 5: Hydrogen Peroxide Scavenging Activity of Methanol Extract of*Bauhinia variegata*

Conc. (µg/ml)	Abs 1a	Abs 1b	Abs 1c	Abs 2a	Abs 2b	Abs 2c
25	0.256	0.257	0.255	0.064	0.068	0.064
50	0.28	0.28	0.279	0.102	0.102	0.105
100	0.308	0.31	0.307	0.15	0.153	0.151
200	0.32	0.322	0.324	0.194	0.194	0.191
400	0.355	0.349	0.35	0.294	0.295	0.29

% age Inhibition			Mean	SD
100.00	100.00	100.00	100.000	0.000
27.82	28.95	28.20	28.321	0.574
33.08	33.08	34.59	33.584	0.868
40.60	40.98	41.35	40.977	0.376
52.63	51.88	50.00	51.504	1.355
77.07	79.70	77.44	78.070	1.423

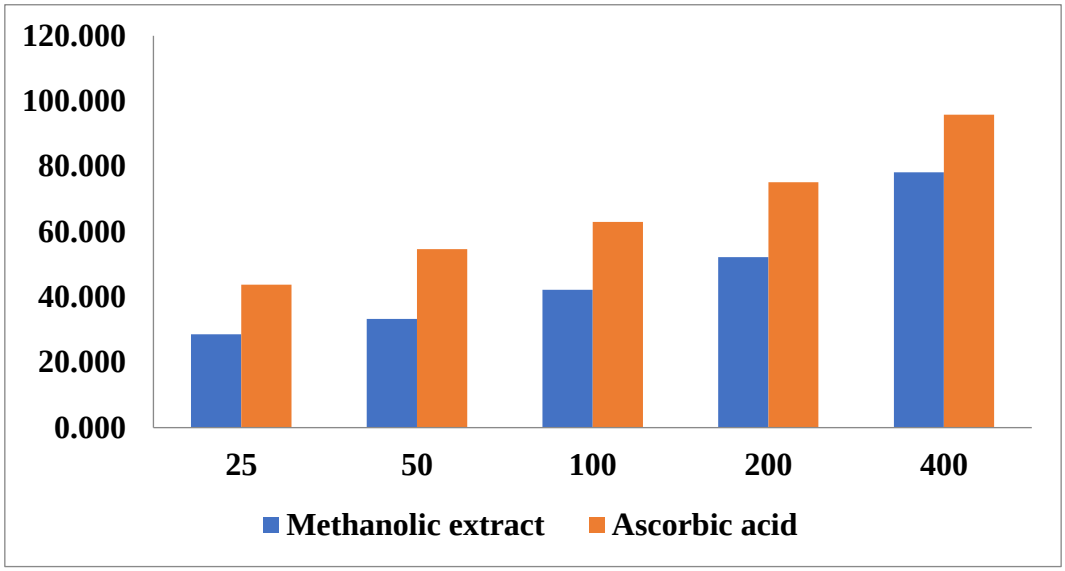

Figure 3: Hydrogen Peroxide Scavenging Activity of Methanol Extract of *Bauhinia variegata*

Table 6:Standard Curve of Ascorbic Acid

Conc(µg/ml)	Abs1	Abs2	Abs3	Mean	SD
25	0.505	0.506	0.508	0.506	0.002
50	0.615	0.618	0.617	0.617	0.002
100	0.784	0.783	0.782	0.783	0.001
200	1.017	1.016	1.017	1.017	0.001
400	1.429	1.429	1.431	1.430	0.001
800	2.422	2.423	2.423	2.423	0.001

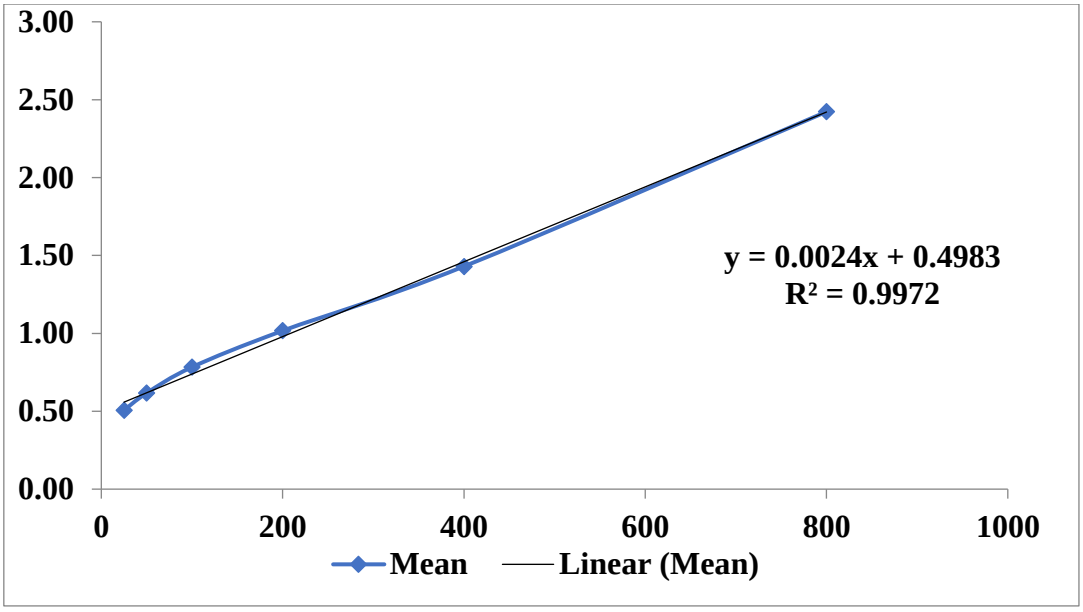

Figure 4: Standard Curve of Ascorbic Acid

Table7: Standard Curve of Methanolic Extract of *Bauhinia variegata*

Conc(µg/ml)	Abs1	Abs2	Abs3	Mean	SD
25	0.299	0.297	0.296	0.297	0.002
50	0.355	0.354	0.357	0.355	0.002
100	0.412	0.412	0.415	0.413	0.002
200	0.505	0.502	0.505	0.504	0.002
400	0.655	0.654	0.657	0.655	0.002
800	0.965	0.967	0.968	0.967	0.002

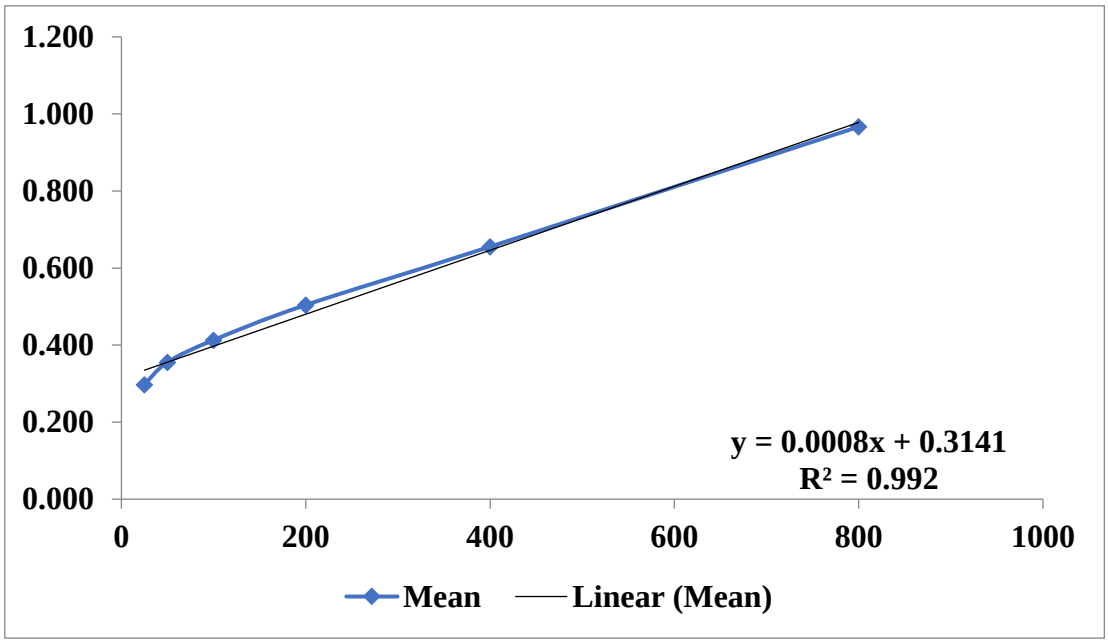

Figure 5: Standard Curve of Ascorbic Acid

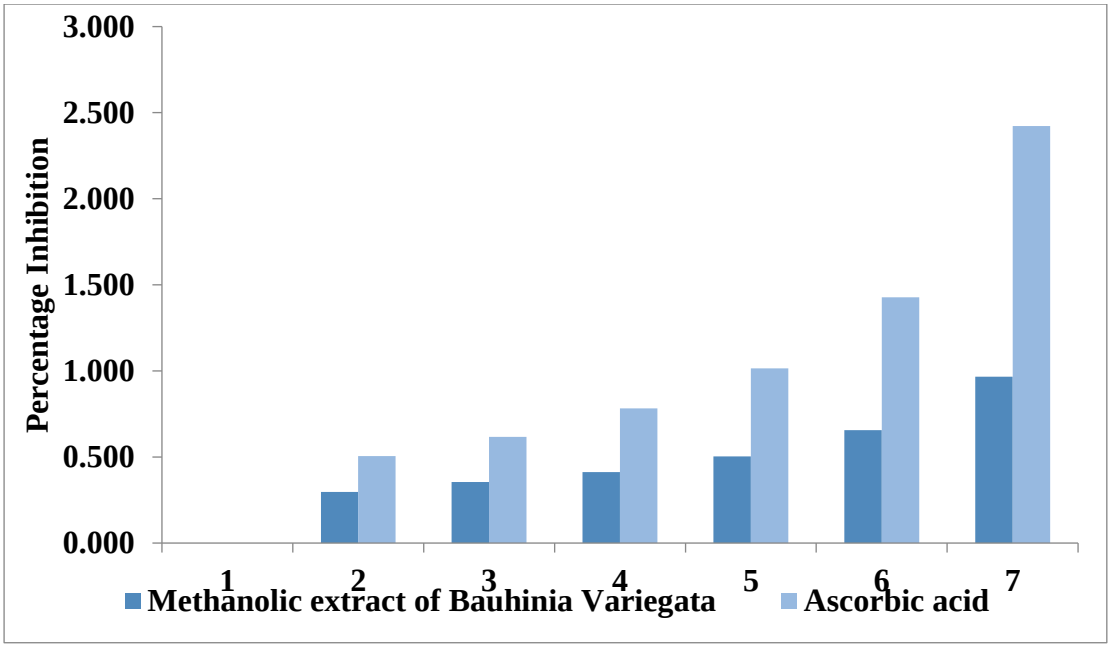

Figure 6: Reducing Power Assay of Methanol Extract of *Bauhinia variegata*

Table 8: Standard Curve of Ascorbic Acid

Conc. (µg/ml)	Abs1	Abs2	Abs3	Mean	SD	% of Inhibition			Mean	SD
1	0.366	0.367	0.368	0.367	0.001	39.20	39.04	38.87	39.04	0.17
2	0.277	0.274	0.276	0.276	0.002	53.99	54.49	54.15	54.21	0.25
3	0.198	0.197	0.196	0.197	0.001	67.11	67.28	67.44	67.28	0.17
4	0.135	0.132	0.134	0.134	0.002	77.57	78.07	77.74	77.80	0.25
5	0.065	0.064	0.063	0.064	0.001	89.20	89.37	89.53	89.37	0.17

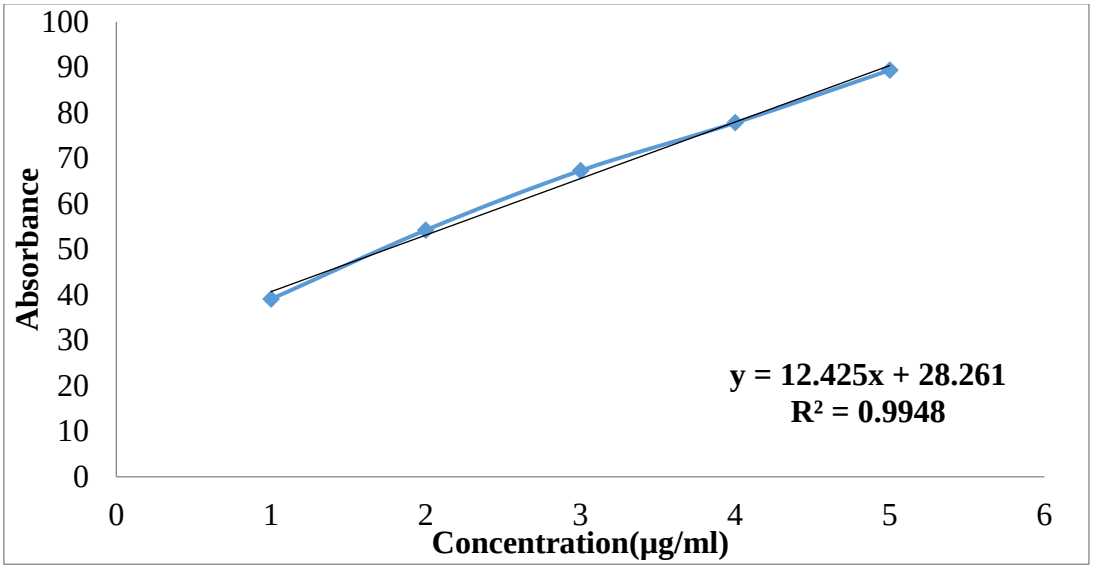

Figure 7: Standard Curve of Absorbance of Ascorbic Acid

Table 9: Standard Curve of Methanolic Extract of *Bauhinia variegata*

Conc. (µg/ml)	Abs 1	Abs 2	Abs 3	Mean	SD
1	0.493	0.491	0.492	0.492	0.001
2	0.433	0.432	0.431	0.432	0.001

3	0.379	0.379	0.38	0.379	0.001
4	0.312	0.312	0.313	0.312	0.001
5	0.262	0.261	0.262	0.262	0.001

Percentage of Inhibition			**Mean**	**SD**
18.11	18.44	18.27	18.27	0.17
28.07	28.24	28.41	28.24	0.17
37.04	37.04	36.88	36.99	0.10
48.17	48.17	48.01	48.12	0.10
56.48	56.64	56.48	56.53	0.10

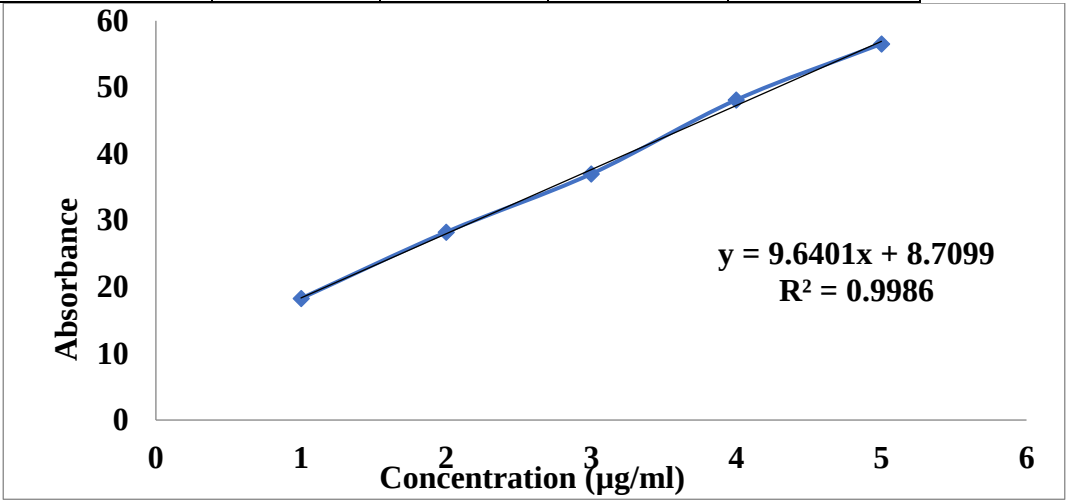

Figure 8: Absorbance of MethanolicExtract of *Bauhinia variegata*

Table 10: DPPH Scavenging Activity of Methanolic Extract of *Bauhinia variegata*

Conc. (µg/ml)	**Methanolic extract of *Bauhinia variegata***	**Ascorbic Acid**
1	18.27	39.04
2	27.30	54.21
3	37.21	67.28
4	48.17	77.80
5	56.64	89.37

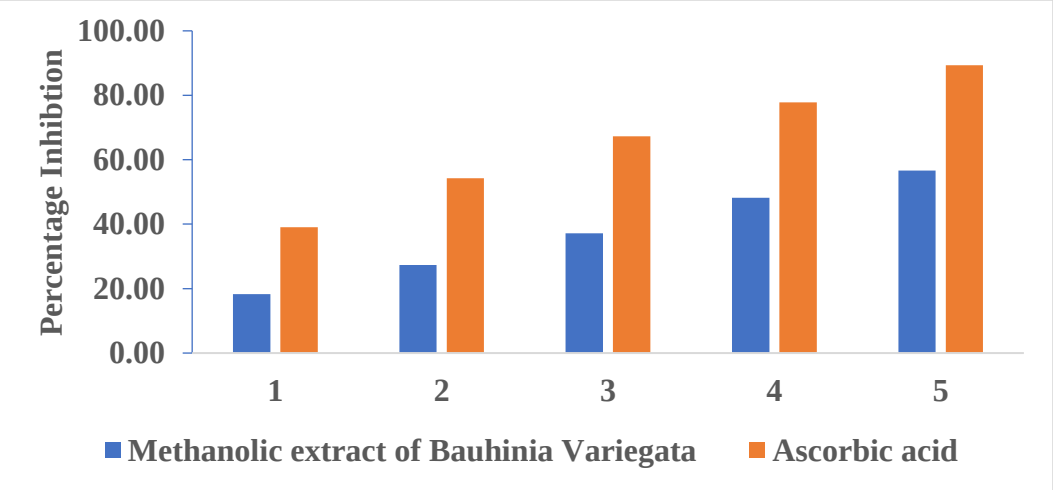

Figure 9: DPPH Scavenging Activity of Methanolic Extract of *Bauhiniavariegata*

Table 11: Absorbance of Ascorbic Acid

Conc. (µg/ml)	Abs 1	Abs 2	Abs 3	% of Inhibition	% of Inhibition	% of Inhibition	Mean	SD
				100.00	**100.00**	**100.00**	**100.000**	**0.000**
25	0.166	0.164	0.164	48.77	49.38	49.38	49.177	0.356
50	0.138	0.136	0.137	57.41	58.02	57.72	57.716	0.309
100	0.105	0.105	0.106	67.59	67.59	67.28	67.490	0.178
200	0.066	0.067	0.066	79.63	79.32	79.63	79.527	0.178
400	0.015	0.013	0.012	95.37	95.99	96.30	95.885	0.471

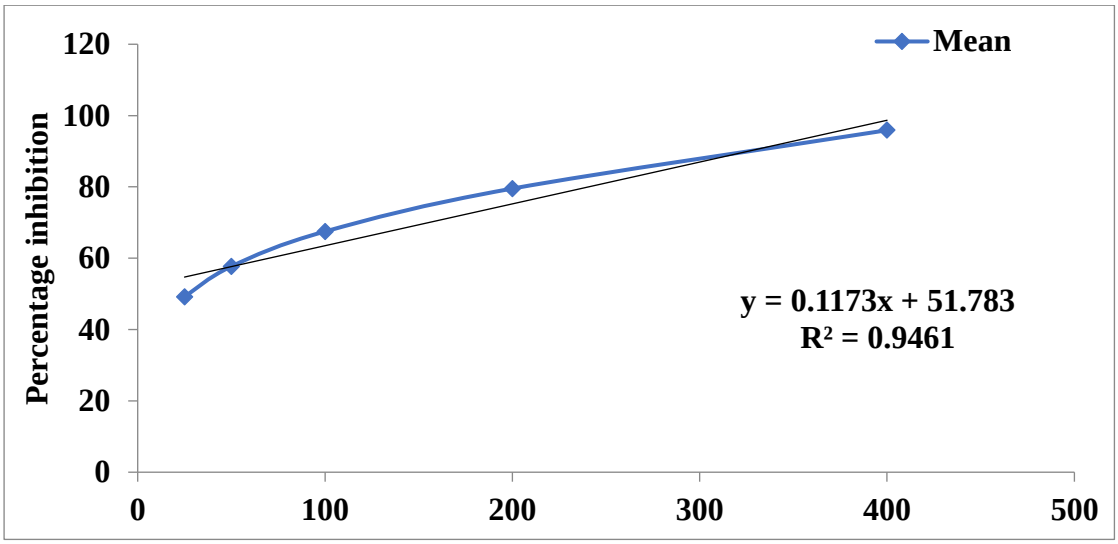

Figure 10: Standard Curve of Ascorbic Acid

Table 12: Absorbance of Methanolic Extract of *Bauhinia variegata*

Conc. (µg/ml)	Abs 1	Abs 2	Abs 3	% of Inhibition	% of Inhibition	% of Inhibition	Mean	SD
				100.00	100.00	100.00	100.000	0.000
25	0.265	0.268	0.27	18.21	17.28	16.67	17.387	0777
50	0.203	0.232	0.234	37.35	28.40	27.78	31.173	5.355
100	0.202	0.203	0.204	37.65	37.35	37.04	37.346	0.309
200	0.182	0.184	0.182	43.83	43.21	43.83	43.621	0.356
400	0.134	0.134	0.134	58.64	58.64	58.64	58.642	0.000

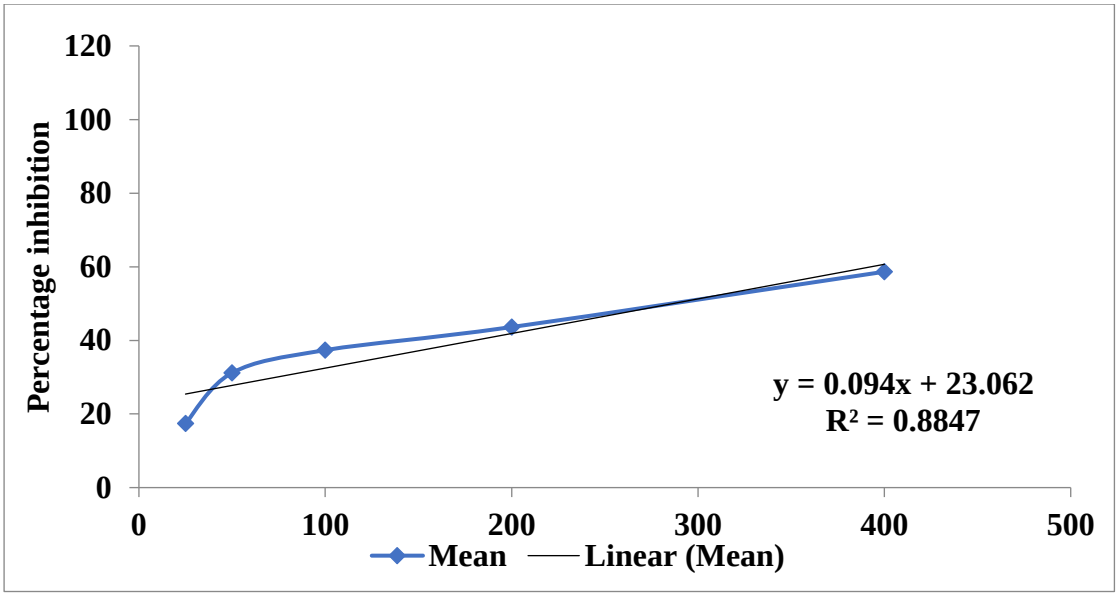

Figure 11: Percentage Inhibition of Methanolic extract of *Bauhinia Variegata*

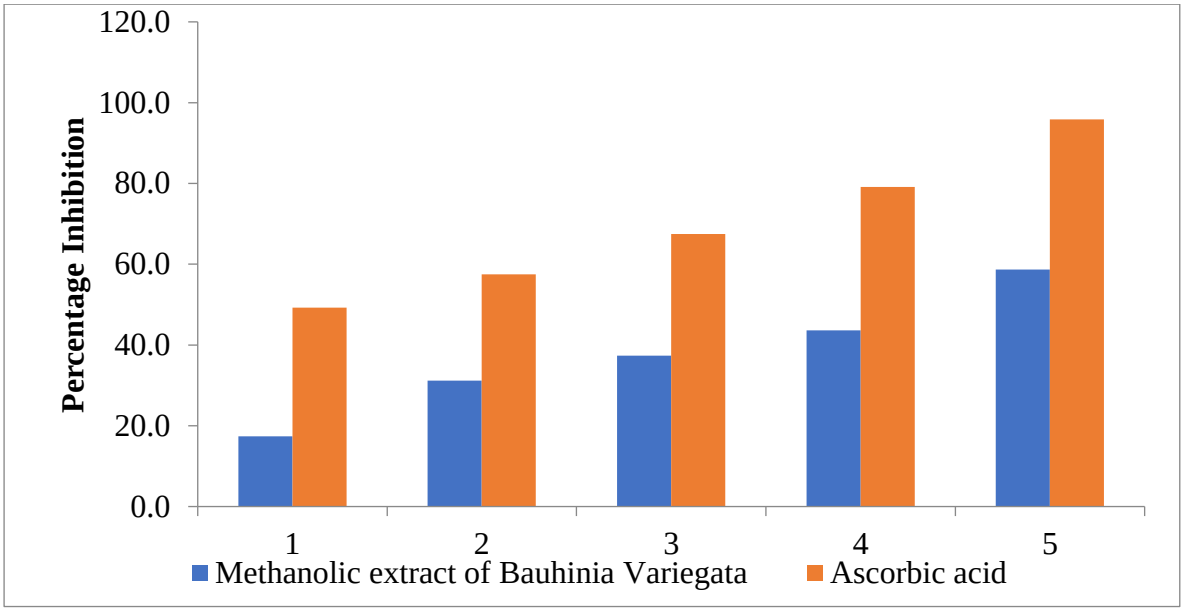

Figure 12: Superoxide Scavenging Activity of Methanolic Extract of *Bauhinia variegata*

Conclusion

Bauhinia variegata leaf extracts exhibits considerable anti-microbial, antidiabetic, antioxidant,anticancer and various other activities. It is traditionally used in the treatment of bronchitis,leprosy and tumors. All parts of the plant like leaves, flower bud, root, seeds, bark, and stemwere used in traditional medicines. The shrub of the plant frequently used for their pharmacological and pharmaceutical properties. Infusion of leaves was used as an antidiabeticand this is increasing day by day in normal population. Powder sample of the leaves of *Bauhinia variegata* was extracted and by alcoholic extract phytochemical analysis is done. This shows the presence of alkaloids, flavanoids, terpenes, saponins and tannins. Alkaloids produce antihyperglycemic action by potentiating pancreatic secretion of insulin from β-cell of islets or by enhancing transport of blood glucose to peripheral tissue. Alcoholic

extract of *Bauhinia variegata* was used for anti-oxidant activities. DPPH is free radical used for the purpose of evaluation of anti-oxidant properties. Anti-oxidants are the moeities which can scavenge free radicals and inhibit anti-oxidant activity. Hydrogen peroxide is also used to evaluate anti-oxidant properties and used in treatment of various human diseases. The present study clearly indicated that *B. variegata* extract have appreciable in-vitro anti-oxidant effect.

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