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In-vitro antioxidant potential of various stem bark extracts of *B. variegata* Linn.

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Abstract
Antioxidant agents are intensifies that can defer or repress the oxidation of lipids or different atoms by hindering the inception or engendering of oxidizing chain responses. The cell reinforcement movement of phenolic mixes is basically because of their redox properties, which can assume a significant function in adsorbing and killing free revolutionaries, extinguishing singlet and trio oxygen, or decaying peroxides. As of late, intrigue has expanded extensively in finding normally happening cell reinforcements for use in nourishments or restorative materials to supplant engineered cancer prevention agents, which are being limited because of their cancer-causing nature. .
In this study various antioxidant activity like DPPH scavenging activity, Nitric oxide scavenging activity, hydrogen peroxide scavenging activity, Superoxide dismutase and reducing power assay performed to evaluate antioxidant potential of Bauhinia Variegata Linn. It indicated greatest % scavenging activty for DPPH extremist rummaging, hydrogen peroxide scavenging and super-oxide dismutase scavenging individually at 400 µg/mL when compared with BHA and ascorbic acid. It additionally demonstrated most extreme absorbance at 400 µg/mL. The concentrate of *B. variegata* stem bark indicated the positive trial of flavonoids. The cell reinforcement action might be because of flavonoid and phenolic content present in the stem bark of this plant. Thusly, further investigations might be performed for detachment and recognizable proof of more cell reinforcement segments of *B. variegata* plant.

Keywords: Antioxidant, Bauhinia Variegata Linn., stem bark extract

Introduction
Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity. Herbs have been used for a large range of purposes including medicine, nutrition, flavorings, beverages, dyeing, repellents, fragrances, cosmetics, charms, smoking, and industrial uses. Since prehistoric times, herbs were the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century. Today, herbs are still found in 40% of prescription drugs(Zheng & Wang, 2001) [7] From the ancient time, herbal plants are widely used for the treatment of various human disorders all over the world because of the presence of ac- tive constituents of therapeutic value. According to World Health Organization (WHO) more than 80% of the world's population still relies on tradi- tional medicine for their primary health care needs.(Kumar *et al*., 2019) [4] among the hundreds of medicinal plant, Bauhinia ariegata Linn. (family: leguminosae), medium sized tree with hairy branches is one of them, and its value in medicine is known since ancient age (Bansal *et al*., 2014). The various parts of the tree like flowers, flowers bud, stem bark, stem, leaves, seeds and roots are popular in various system of medicines like ayurveda, unani and homeopathy in India for the cure of variety of disease (Sahu and Gupta, 2012).

Materials and Methods
Collection of plant samples: Sample of *B. variegata Linn.* (family leguminosae) stem were collected from the plant grown in Botanical Garden, *Department of Botany and Microbiology, Gurukul Kangri University, Haridwar (India)* and were positively identified by the experts of the department (specimen identification No: 291/Bot and Micro/16-01-16). The plant samples were washed with running tap water to remove the adhered dust, dirt and other foreign material and dried in shade at room temperature. The dried samples were powdered which were stored in air tight container at room temperature for further studies.

Preparation of crude extracts: The plant samples were powdered separately in shade and subjected to hot extraction in Soxhlet continuous extraction apparatus with alcohol solvents for 48 - 72 h. The extracts were collected in a beaker, filtered separately and considered as the 100% concentrated stock extract. These extracts were evaporated by vacuum at reduced pressure (Kew *et al*., 2018). The extracts were then dried and weighed, and yield was calculated using the formula: yield % = X/Y ×100, where X = weight of the beaker with dried drug-weight of the beaker, and Y = total amount of the dried drug. (Kumar *et al*., 2019) [4]

Antioxidant Activity

D.P.P.H Free Radical Scavenging Activity

1-diphenyl-2-phenylhydrazine (D.P.P.H) radical model, is broadly used to antioxidants agents. It depends on abatement in D.P.P.H radical (purple) in nonconventional structure D.P.P.H-H (yellow) within sight of hydrogenation antioxidants agent. How discoloration demonstrates immaculateness of antioxidants agents. Absorbance of arrangement is estimated at 517 nm utilizing UV range meter.

Procedure: 1) DPPH free radical scavenging activity Scavenging of DPPH free radicals by plant extracts was determined by the methods of Brand Williams in a UV-Visible spectrophotometer. 4mg of DPPH was dissolved in 10ml of methanol to prepare a standard solution of DPPH (400µg/ml). For the control measurement, 200µl of DPPH solution from this stock solution was made upto 3ml by adding methanol to make a test solution. The absorbance of the tst solution was recorded at 517nm. All investigated samples were also tested for their absorbance, if any, at 517nm.

The free-radical scavenging activity of extract was measured by preparing different concentrations (100, 200, 300 & 400µg/ml) of plant extracts and adding 0.1ml from the respective solution of the plant extracts to a test solution of DPPH containing 0.2ml of the stock solution of DPPH and the volume was made upto 3ml by adding methanol. The solution in test tube was shaken and then kept in dark for 30 min. at room temperature. After 30 min, absorbance was measured at 517 nm and compared with standards (5-50 µg/ml). Scavenging activity was expressed as the percentage inhibition calculated using the following formula.

$$DPPH scavenging activity\ \% = \frac{Absorbance of control - Absorbance of sample}{Absorbance of control} \times 100$$

The antioxidant agent movement of fluid concentrate and SNPs was evaluated by spectrophotometry of nearness of D.P.P.H radical, which is regularly used to look at action of watery concentrate and SNPs.

D.P.P.H is steady free extreme which breaks down in methanol and shows trademark ingestion at 517 nm. When antioxidant agent searches free radicals by hydrogen gift, D.P.P.H measure arrangement gets lighter in shading. (Kedare & Singh, 2011) [3]

Nitric Oxide Scavenging Activity Assay

It was assessed by utilization of Griess's response. Sodium nitroprusside in fluid arrangement physiological pH unexpectedly creates nitric oxide that connects with oxygen to deliver nitrite particles that can be evaluated by utilizing Griess' reagent

Nitric oxide forager rivals oxygen nitric oxide creation, which was estimated at 540 nm utilizing spectrophotometer

Procedure: Nitric oxide fouiller movement can be assessed by utilization of response sodium nitroprusside compound is known to break down in watery arrangement at physiological pH (7.2) creating NO. Under vigorous conditions, NO response with oxygen to deliver stable items (nitrate and nitrite). Amounts can be resolved to utilize Griess reagent. Nitric oxide foragers contend with oxygen, lessening creation of nitrite particles. For test, sodium nitroprusside (10 mM) in phosphate cradled saline was blended in with various fixations (100, 200,300 and 400 µg/ml) of methanolic remove from each plant broke down in methanol and brooded at room temperature 30 ° C for 2 hours.

A similar response blend without concentrate yet comparable measure of ethanol filled in as control. After brooding time frame, 0.5 ml (1% sulfanilamide, 2% H3PO4, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) of reagent was included. Chromophore-shaped absorption during nitrite diazotization with sulfonyl amide and resulting coupling with naphthylethylenediamine dihydrochloride was immediately perused at 550 nm.

Inhibition of nitrite arrangement by plant concentrates and standard cell reinforcement ascorbic acid was determined comparative with control. Restraint information (percent hindrance) were linearized comparative with convergences of fluid concentrate, SNPs, and standard (BHT).(Boora *et al*., 2014)

Hydrogen Peroxide Scavenging Activity

The ability of the B. Veriagatalinn. extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100, 200, 300 and 400 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both B. Veriagatalinn. extracts and standard compounds were calculated:

$$S\% = \frac{(A\ control - A\ sample)}{A\ control} \times 100$$

Where control = absorbance of clear control (containing all reagents aside from concentrate arrangement) and example = absorbance of test. (Long *et al*., 1999) [6]

Reducing Power Assay Method

Although reductant isn't cell reinforcement, and antioxidants agent is normally reductant. decreasing limit of compound may fill in as huge pointer of its potential antioxidants agent movement. However, action of antioxidants has been allocated to different instruments, for example, anticipation of chain inception, authoritative of progress metal particle impetuses, deterioration of peroxides, counteraction of proceeded with hydrogen reflection, reductive limit and radical searching.

Procedure: The ability of extracts to reduce ferric ions (Fe+3) was assessed by using various concentrations of of extracts (100,200,300 and 400 µg/mL) was mixed with 400 µl phosphate buffer (0.2 M, pH=6.6) and 800 µl of a 1% potassium ferricyanide [K3Fe(CN)6], then the mixture was incubated at 50 °C for 20 min. About 800 µl (10%) of trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min (3000 r/t). Finally, 400 µl of the supernatant solution was mixed with 400 µl of distilled water and 80 µl FeCl3 (0.1%) and the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The results were expressed as µg ascorbic acid equivalent/mg extract. (Ferreira *et al*., 2007) [2]

Estimation of Superoxide Dismutase (Sod) Activity

The superoxide anion radical scavenging activity was determined by a xanthine/xanthenes oxidase generating system coupled with nitro blue tetrazolium (NBT) chloride. In this assay, all solutions were prepared in 0.1 M KH2PO4-KOH buffers (pH 7.6). The reaction mixture contained 1mL of 3mM xanthenes, 0.02mL of 15mM Na2EDTA, 1mL of 0.6mM NBT, 0.5mL of 10mM xanthenes oxidase solution (1u in 10mL buffer) and 0.48mL of 0.1M phosphate buffer. The reaction was initiated by adding of xanthine oxidase at 37 ºC. At pH 7.6, superoxide anion radical reduces NBT to nitro blue formazan (λmax=560 nm). The production of superoxide anion radical (O2•−) was evaluated spectrophotometrically by monitoring the reduction of NBT to nitro blue diformazan at 560 nm.

Then the radical scavenging activity of the plant extracts was studied by preparing different concentrations (100, 200, 300 and 400 µg/mL for plant extracts) and adding 0.1mL from the respective solution of the plant extracts to above reaction mixture and incubated at 37ºC for 30 min and the absorbance maximum at 560nm was measured. The investigated samples were examined before spectroscopic measurements, if these have any absorbance at this region and it was observed that none of the investigated samples has absorbance at this region.

Then, the scavenging of O2•− radical was measured by adding the investigated plant extracts to the solution and the formation of nitro blue di-formazan was decreased and the absorbance at 560 nm reduced. Percentage inhibition of superoxide anion radical was calculated by equation.

$$\% \text{ inhibition} = [(A0-A1)/A0] \times 100$$

Where A0 was the absorbance of the control and A1 was the absorbance of extract or standard compounds.(Li, 2012).

Result

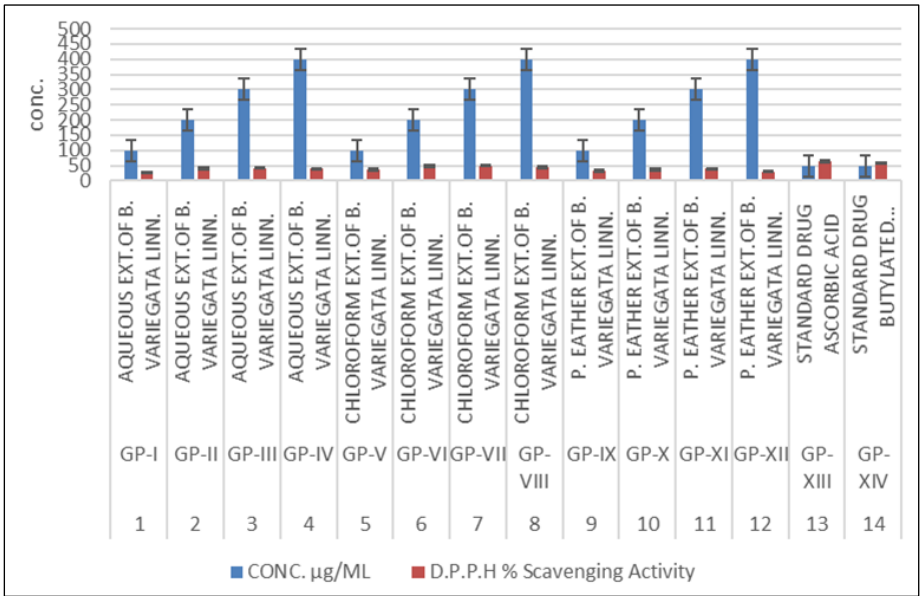

Fig 1: D.P.P.H Free Radical Scavenging Activity

Table 1: D.P.P.H Free Radical Scavenging Activity

S.N	Group	Treated with	CONC. µg/ML	D.P.P.H % Scavenging Activity
1	GP-I	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	100	27.78
2	GP-II	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	200	40.58
3	GP-III	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	300	42.3
4	GP-IV	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	400	39.72
5	GP-V	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	100	36.84
6	GP-VI	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	200	48.66
7	GP-VII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	300	49.77
8	GP-VIII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	400	44.06
9	GP-IX	P. EATHER EXT.OF *B. VARIEGATA LINN.*	100	32.56
10	GP-X	P. EATHER EXT.OF *B. VARIEGATA LINN.*	200	37.11
11	GP-XI	P. EATHER EXT.OF *B. VARIEGATA LINN.*	300	38.92
12	GP-XII	P. EATHER EXT.OF *B. VARIEGATA LINN.*	400	30.89
13	GP-XIII	STANDARD DRUG ASCORBIC ACID	50	64.32
14	GP-XIV	STANDARD DRUG BUTYLATED HYDROXYANISOLE	50	58.92

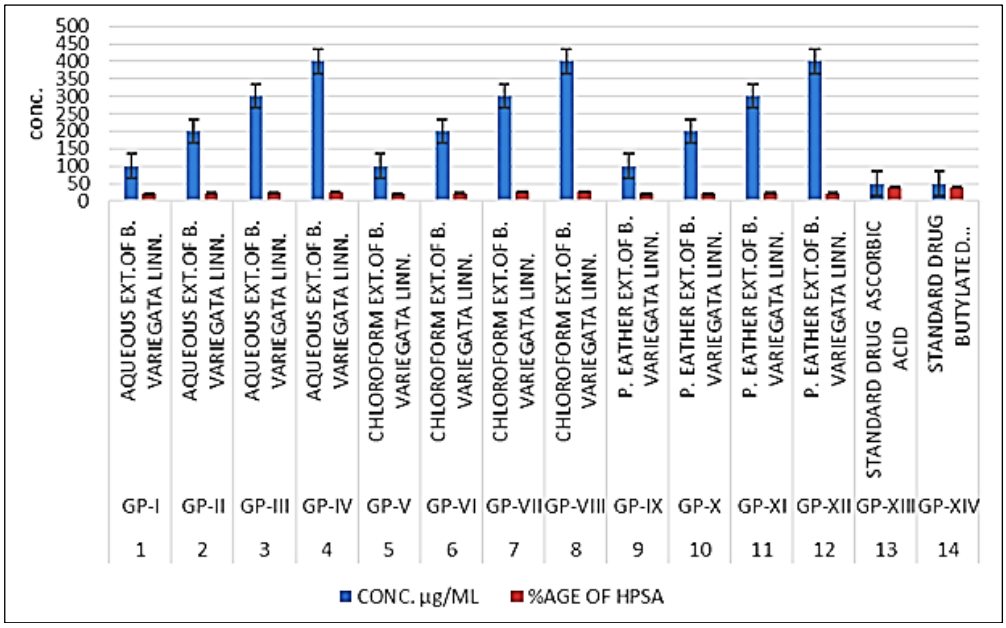

Fig 2: Hydrogen Peroxide Scavenging Activity

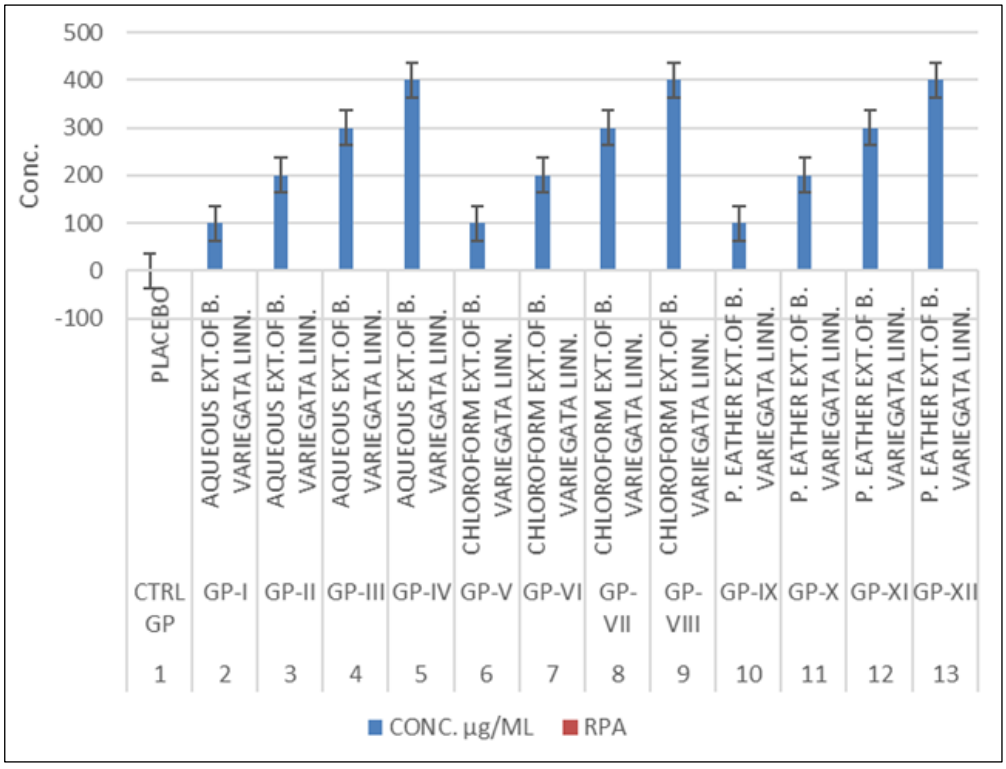

Fig 2: Reducing Power Assay

Table 2: Hydrogen Peroxide Scavenging Activity

S.N	Group	Treated with	Conc. µg/ML	% Age of HPSA
1	GP-I	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	100	20.07
2	GP-II	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	200	22.04
3	GP-III	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	300	24.01
4	GP-IV	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	400	25.98
5	GP-V	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	100	21.04
6	GP-VI	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	200	23.07
7	GP-VII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	300	25.1
8	GP-VIII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	400	27.13
9	GP-IX	P. EATHER EXT.OF *B. VARIEGATA LINN.*	100	20.11
10	GP-X	P. EATHER EXT.OF *B. VARIEGATA LINN.*	200	21.04
11	GP-XI	P. EATHER EXT.OF *B. VARIEGATA LINN.*	300	21.97
12	GP-XII	P. EATHER EXT.OF *B. VARIEGATA LINN.*	400	22.9
13	GP-XIII	STANDARD DRUG ASCORBIC ACID	50	40.05
14	GP-XIV	Standard drug butylated hydroxyanisole	50	39.07

Table 3: Reducing Power assay

S.N	Group	Treated with	CONC. µg/ML	RPA
1	CTRL GP	PLACEBO	-	0.09
2	GP-I	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	100	0.12
3	GP-II	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	200	0.25
4	GP-III	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	300	0.36
5	GP-IV	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	400	0.49
6	GP-V	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	100	0.13
7	GP-VI	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	200	0.25
8	GP-VII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	300	0.38
9	GP-VIII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	400	0.42
10	GP-IX	P. EATHER EXT.OF *B. VARIEGATA LINN.*	100	0.16
11	GP-X	P. EATHER EXT.OF *B. VARIEGATA LINN.*	200	0.28
12	GP-XI	P. EATHER EXT.OF *B. VARIEGATA LINN.*	300	0.39
13	GP-XII	P. EATHER EXT.OF *B. VARIEGATA LINN.*	400	0.44

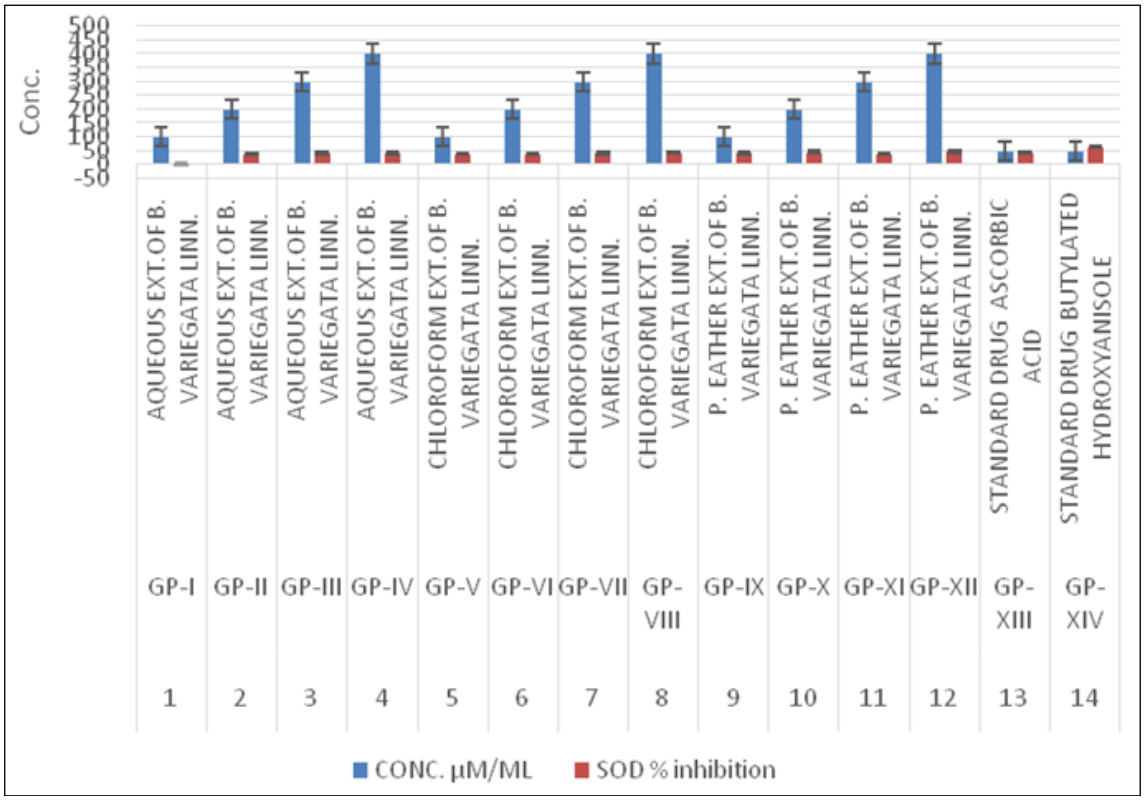

Fig 3: Superoxide Dismutase (Sod) Activity

Table 4: Superoxide Dismutase (Sod) Activity

S.N	Group	Treated with	CONC. µM/ML	SOD % inhibition
1	GP-I	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	100	0.75
2	GP-II	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	200	38.54
3	GP-III	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	300	41.95
4	GP-IV	AQUEOUS EXT.OF *B. VARIEGATA LINN.*	400	42.01
5	GP-V	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	100	40.07
6	GP-VI	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	200	38.77
7	GP-VII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	300	42.18
8	GP-VIII	CHLOROFORM EXT.OF *B. VARIEGATA LINN.*	400	44.77
9	GP-IX	P. EATHER EXT.OF *B. VARIEGATA LINN.*	100	41.83
10	GP-X	P. EATHER EXT.OF *B. VARIEGATA LINN.*	200	45.59
11	GP-XI	P. EATHER EXT.OF *B. VARIEGATA LINN.*	300	39.01
12	GP-XII	P. EATHER EXT.OF *B. VARIEGATA LINN.*	400	47.25
13	GP-XIII	STANDARD DRUG ASCORBIC ACID	50	44.94
14	GP-XIV	STANDARD DRUG BUTYLATED HYDROXYANISOLE	50	65.65

Conclusion

The present study concluded that the aqueous, petroleum ether and chloroform stem bark extract of B. variegata Linn. had significant *in vitro* antioxidant potentials. It showed maximum % scavenging activity for DPPH radical scavenging, hydrogen peroxide scavenging and super-oxide anion scavenging respectively at 400 µg/mL when compared with BHA and ascorbic acid. It also showed maximum

absorbance at 400 µg/mL when compared with placebo group.

The extract of B. variegata stem bark showed the positive test of flavonoids. The antioxidant activity may be due to flavonoid and phenolic content present in the stem bark of this plant. Therefore, further studies may be performed for isolation and identification of more antioxidant components of B. variegata plant.

Acknowledgements

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Reference

1. Boora F, Chirisa E, Mukanganyama S. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents. Journal of Food Processing, *2014*, 1-7. https://doi.org/10.1155/2014/918018
2. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. Food Chemistry. 2007; 100(4):1511-1516. https://doi.org/10.1016/j.foodchem.2005.11.043
3. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. Journal of Food Science and Technology. 2011; 48(4):412-422. https://doi.org/10.1007/s13197-011-0251-1
4. Kumar A, Anand V, Dubey RC, Goel KK. Evaluation of antioxidant potential of alcoholic stem bark extracts of Bauhinia variegata Linn. Journal of Applied and Natural Science. 2019; 11(1):235-239. https://doi.org/10.31018/jans.v11i1.2015
5. Li X. Improved pyrogallol autoxidation method: A reliable and cheap superoxide-scavenging assay suitable for all antioxidants. *Journal of Agricultural and Food Chemistry*; 2012; 60(25):6418-6424. https://doi.org/10.1021/jf204970r
6. Long LH, Evans PJ, Halliwell B. Hydrogen peroxide in human urine: Implications for antioxidant defense and redox regulation. Biochemical and Biophysical Research Communications. 1999; 262(3):605-609. https://doi.org/10.1006/bbrc.1999.1263
7. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry. 2001; 49(11):5165-5170. https://doi.org/10.1021/jf010697n.