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PREMILARY PHYTOCHEMICAL SCREENING AND INVITRO ANTIOXIDANT ACTIVITY OF BOUGAINVILLEA SPECTABILIS WILLS

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ABSTRACT

Bougainvillea spectabilis (Nyctingaceae) is a thorny woody evergreen shrub in India,leaves are used for the treatment of diabetics and fungal infection and as laxative. The literature survey revealed the presence of tannins, phenolic compounds, flavanoids and alkaloids. The previous study of this plant revealed the preliminary phytochemical studies for the leaves was not so far. An attempt was proceeded to investigate the preliminary phytochemical screening and in vitro antioxidant studies. Leaves were collected, authenticated, shade dried, coarsely powdered was extracted with 70% hydroalocohol, the resulting extract was (HAEBS) hydroalcoholic extract(70%) of *Bougainvillea spectabilis* was subjected to preliminary phytochemical studies and In vitro-antioxidant activity. The inhibitory concentration (IC_{50}) of *Bougainvillea spectabilis* leaf against hydrogen peroxide scavenging effect and nitric oxide assay was found to be (79 µg/ml& 88 µg/ml) in comparison with ascorbic acid (76 µg/ml & 75 µg/ml) respectively. The inhibitory concentration (IC_{50}) of reducing power assay and total antioxidant capacity was found to be (74 µg/ml &75 µg/ml) in comparison with ascorbic acid (68 µg/ml &77 µg/ml) respectively. The plant showed moderate antioxidant effect when compared with ascorbic acid.

INTRODUCTION

Bougainvillea spectabilis Willd is a climbing shrub with spikes grow as an evergreen ornamental woody plant in gardens and inhabiting in warmer climate. It is commonly known as Bougainvillea belongs to family Nyctaginaceae [1,]. The ethno medicinal information reveals that leaves are used to treat diabetes by tribals of Chittoor and Nagaland, India. Leaf, flower, and stem preparation are used by the tribals of Mandasaur, India to treat stomach acidity and leuchorrea. Fresh leaves are used by tribals of Sriharikata, Andra Pradesh to treat wound. In folklore practice of Assam and Maharashtra, leaves are used to treat fungal infection, jaundice and dysentery [2-7] . The Phytochemical review of leaf, flower and root revealed the presence of alkaloids, terpenoids, tannins, saponins, flavonoids and glycosides. Pinitol, a mehyl ester of chiroinsitol was isolated [8-10].The plant exhibited antihyperglycemic activity, anti-ulcer, antibacterial, thromobolytic and antioxidant effect[11-16]. The previous study of this plant revealed the preliminary phytochemical studies for the leaves was not so far. An investigation was proceeded to study the pharmacognostical for the fresh leaves and phytochemical analysis of hydroalcholic extract of HAEBS were determined.

MATERIALS AND METHODS

Collection &Authentication of plant material: Fresh leaves of *Bougainvillea spectabilis* were collected from the village Poovanthi Sivagangai Dist, Tamilnadu in the month August 2015. The plant was identified and authenticated by Dr.Stephen, M.Sc. Ph.D., Dept of Botany, The American College, Madurai. A herbarium was preserved in the department for future reference.

Preparation of Hydroalcoholic Extract of *Bougainvillea spectabilis* (HAEBS)

The leaves were collected, shade dried and coarsely powdered, passed through sieve no 40, was extracted with 60% hydroalcohol by maceration technique, was concentrated to drynesss and stored in a closed container for further use. Hydroalcoholic extract of *Bougainvillea spectabilis* (leaf) **(HAEBS)** was subjected to and quantitative and qualitative chemical analysis..

Qualitative chemical analysis

Phytochemical studies

Hydroalcoholic extract of *Bougainvillea spectabilis* (leaf) **(HAEBS)** was subjected to qualitative chemical analysis .The various chemical tests were performed on hydro alcoholic extract for the identification of secondary metabolites determined as per (Harbone; 1998 [18]and the results are displayed in table1.

QUANTITATIVE ESTIMATION OF PHYTO-CONSTITUENTS

Quantiative analysis

Hydroalcoholic extract was estimated quantitatively for the content of phytoconstituents such as tannic acid, gallic acid and flavonoid.

DETERMINATION OF TANNIN ACID EQUIVALENTS

Procedure

0.2 mL of (1 mg/mL) hydroethanolic extract of *Bougainvillea spectabilis*, was made up to 1 mL with distilled water. Then add 0.5 mL of Folin Denis reagent and allowed to stand for 15 mins, then 1 mL of sodium carbonate solution was added to the mixture and it was made up to 10 mL with distilled water. The mixture was allowed to stand for 30 mins at room temperature and the tannin content was determined spectrophotometrically at 700 nm. The calibration curve was generated by preparing tannic acid at different concentration (10, 20, 30, 40 and 50 µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract[18]. The results are tabulated in **Table 2** and the calibration graph was presented at **Fig 1.**

DETERMINATION OF GALLIC ACID EQUIVALENTS

Procedure

About 1 mL (1mg/ml and 0.5 mg/mL) of hydroalcoholic extract of *Bougainvillea spectabilis* (HAEBS), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength. The calibration curve was generated by preparing Gallic acid at different concentration (10, 20, 30, 40 and 50 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of HAEBS extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g)[19] .The results are tabulated in **Table 3** and the calibration graph was presented at **Fig 2.**

DETERMINATION OF RUTIN (FLAVONOID) CONTENT

Procedure

1mL of hydroethanolic extract of *Bougainvillea spectabilis*, 0.1 mL of aluminium chloride solution, 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance was measured at 415 nm.A calibration curve was constructed by plotting absorbance reading of rutin at different concentrations (10, 20, 30, 40 and 50 µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was

expressed as milligrams of rutin equivalent per gram of extract[20]. The results are tabulated in **Table 4** and the calibration graph was presented at **Fig 3.**

ANTIOXIDANT EFFECT

Determination of Scavenging Activity against Hydrogen Peroxide

Extract was screened for hydrogen peroxide scavenging as per the Sreejaygan &Ram, 1996 method[21]

Procedure

To 1 ml of test solutions of different concentrations, 3.8 ml of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 ml of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at 230 nm after 10 min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula

% inhibition = [(control - sample) /control]×100

The results are tabulated in Table **5** and the calibration graph was presented at **Fig 4**

Determination of scavenging activity against nitric oxide

Procedure

Hydroalcocholic Extract of Bougainvillea spectabilis was screened effect for nitric oxide scavenging as per Green et al., 1982 [22] .

Procedure: Different concentration of extract solution in phosphate buffer was incubated with sodium nitroprusside for 5 hours at 25°c. Control experiments were performed with equal amount of buffer instead of extract solution. After 5 hours of incubation, 0.5ml of supernatant liquid was removed and 0.5ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization with sulphanilamide and its subsequent coupling was read at 546nm.

The percentage inhibition of hydrogen peroxide was calculated using the formula

% inhibition = [(control - sample) /control]×100

Ascorbic acid was used as standard and its results are presented in Table 6 and figure 5**.**

Determination of reducing power assay

Procedure

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$solution as mentioned by Oyaizu *et al.*, method .[23]

0.1 to 0.5 ml of plant extract solution (1 mg/ml) was mixed with 0.75 ml of phosphate buffer and 0.75 ml of 1 % potassium ferri cyanide [K_3Fe (CN_6)] and incubated at 50°C for 20min. About 0.75 ml of 1 % trichloro acetic acid was added to the mixture and allowed to stand for 10min. The whole mixture was then centrifuged at 3000 rpm for 10min. Finally 1.5 ml of the supernatant was removed and mixed with 1.5 ml of distilled water and 0.1ml of 0.1 %ferric chloride solution and the absorbance was measured at 700 nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer was used as blank solution.

The percentage inhibition of hydrogen peroxide was calculated using the formula

% inhibition = [(control - sample) /control]×100

The results are tabulated in **Table 7** and the calibration graph was presented in **Fig 6.**

Determination of Total antioxidant activity by Phosphomolybdenum Method

Procedure

Total antioxidant activity of plant extracts was screened by assessing the ability of the test extract to phosphomolybdenum as mentioned by Prieto *et.al*.1999 method [24].

An aliquot of 0.3 ml of different concentrations of sample was treated with 2.7 ml of the reagent (H_2SO_4), sodium phosphate and ammonium molybdate. In case of blank, 0.3 ml of methanol was used in place of sample. The tubes were incubated in a boiling water bath at 95°C for 90 min. The samples were cooled to room temperature; the absorbance of the aqueous solution of each concentration was measured at 695 nm against blank. The standard vitamin C was treated in a similar manner. The antioxidant activity was expressed as inhibition of percentage and results are

tabulated in Table 8 and were presented at Fig 7.

Results and discussion

Phytochemical screening

The phytochemical screening of the hydroalcoholic extract showed the following results and was displayed in table 1. Preliminary phytochemical screening of hydro-alcoholic extract of *Bougainvillea spectabilis* (leaf). Preliminary phytochemical screening of hydroalcoholic extract of *Bougainvillea spectabilis* (leaf) showed the presence of alkaloids, carbohydrate, sterols, tannin, phenolic compound, flavanoids, protein and amino acid, terpenoids, and absence of cardiac glycosides, coumarin glycosides, saponins, volatile oil and fixed oil.

QUANTITATIVE ANALYSIS

Determination of TAE, GAE &RE of *Bougainvillea spectabilis* (leaf)

Quantitative estimation comprises the estimation of tannic acid, gallic acid and rutin (flavanoids) content equivalent content in terms of TAE/g, GAE/g and RE/g of extract. The hydroalcoholic extract of *Bougainvillea spectabilis* (leaf) was found to be 65mgTAE/g, 58mg GAE/g, and 13mgRE/g respectively.

In vitro antioxidant studies

Determination of Hydrogen peroxide scavenging effect.

Procedure

In vitro antioxidant studies include hydrogen peroxide scavenging activity and nitric oxide scavenging effect. The inhibition concentration (IC50) of *Bougainvillea spectabilis* (leaf) against hydrogen peroxide is found to be 79µg/ml in comparison with ascorbic acid 76µg/ml. Table 5 and the calibration graph was presented in Fig 4**.**

Determination of Nitric Oxide Method of HAE *Bougainvillea spectabilis*.

In vitro antioxidant studies include nitric oxide scavenging effect. The inhibition concentration (IC50) of *Bougainvillea spectabilis* (leaf) against nitric oxide method is found to be 75µg/ml in comparison with ascorbic acid 88µg/ml and. **Table 9**and the results are presented in **Fig 7.**

Determination of Reducing Power Assay of HAE *Bougainvillea spectabilis* (leaf)

In vitro antioxidant studies include reducing power assay activity effect. The inhibition concentration (IC50) of *Bougainvillea spectabilis* (leaf) against reducing power assay is found to be 68µg/ml in comparison with ascorbic acid 74µg/ml. **Table 7** and the results are presented in **Fig 6.**

Determination of Total Anti-oxidant Capacity of HAE *Bougainvillea spectabilis* (leaf)

In vitro antioxidant studies include total antioxidant capacity activity effect. The inhibition concentration (IC50) of *Bougainvillea spectabilis* (leaf) against total antioxidant capacity is found to be 75µg/ml in comparison with ascorbic acid 77µg/ml. **Table 8** and the activity was presented in **Fig 7.**

Table: 1 Preliminary phytochemical screening of B*ougainvillea spectabilis (leaf)*

Test for	**Report of Hydro alcoholic extract B*ougainvillea spectabilis (leaf)***
Alkaloids	Positive
Carbohydrate	Positive
Anthraquinone glycoside	Negative
Cardiac glycosides	Negative
Sterol saponins	Positive
tannins and phenolic compounds	Positive
Flavonoids	Positive
Protein and free amino acid	Positive
Mucilage	Positive
Terpenoids	Positive
Fixed oil and gum	Negative
Resin and volatile oil	Negative

***In vitro* antioxidant studies**

Determination of Hydrogen peroxide scavenging effect.

Procedure

In vitro antioxidant studies include hydrogen peroxide scavenging activity and nitric oxide scavenging effect. The inhibition concentration (IC50) of *Bougainvillea spectabilis* (leaf) against hydrogen peroxide is found to be 79µg/ml in comparison with ascorbic acid 76µg/ml. Table 5 and the calibration graph was presented in Fig 4.

Determination of Nitric Oxide Method of HAE *Bougainvillea spectabilis*.

In vitro antioxidant studies include nitric oxide scavenging effect. The inhibition concentration (IC50) of *Bougainvilleaspectabilis* (leaf) against nitric oxide method is found to be 75µg/ml in comparison with ascorbic acid 88µg/ml and. **Table 9**and the results are presented in **Fig 7.**

Determination of Reducing Power Assay of HAE *Bougainvillea spectabilis* (leaf)

In vitro antioxidant studies include reducing power assay activity effect. The inhibition concentration (IC50) of *Bougainvilleaspectabilis* (leaf) against reducing power assay is found to be 68µg/ml in comparisonwith ascorbic acid 74µg/ml. Table 7 and the results are presented in Fig 6.

Table: 2 Determination of Tannic acid equivalent in *Bougainvilleaspectabilis (leaf)*

S.No	Concentration of Tannic acid /Bougainvillea spectabilis(µg/ml)	Absorbance of Tannic acid	Absorbance of Bougainvillea spectabilis(µg/ml)
1	10	0.147 ± 0.058	0.167417 ± 0.002102
2	20	0.536± 0.0033	0.470842 ± 0.001876
3	30	0.746 ± 0.008	0.577955 ± 0.002628
4	40	0.856 ± 0.008	0.669053 ± 0.001581
5	50	0.95 ± 0.006	0.658713 ± 0.002826
	TAE		65mg/g

Table: 3 Determination of Gallic acid equivalent of*Bougainvillea spectabilis*

S.No	Concentration of Gallic acid/Bougainvillea spectabilis (µg/ml)	Absorbance of Gallic acid*	Absorbance Bougainvillea spectabilis*
1	10	0.251 ±0.00203	0.298447 ± 0.002403
2	20	0.339 ±0.0009	0.254425 ± 0.001281
3	30	0.400±0.00186	0.333098 ± 0.009
4	40	0.504±0.00133	0.337057 ± 0.004096
5	50	0.596±0.0032	0.292912 ± 0.001012
GAE			58mg/g

*Mean of three value ± SEM

Table: 4 Determination of Rutin Equivalent of *Bougainvillea spectabilis* (leaf)

S.No	Concentration of Rutin (µg/ml)	Absorbance of Rutin	Concentration of Bougainvillea spectabilic	Absorbance of Bougainvillea spectabilic (µg/ml) *
1	**10**	0.231 ± 0.0667	**100**	1.284897 ± 0.0263
2	**20**	0.299 ± 0.0882	**200**	1.008878 ± 0.0029
3	**30**	0.329 ± 0.0882	**300**	1.199419±0.00493
4	**40**	0.364 ± 0.0577	**400**	1.134624 ±0.0029
5	**50**	0.398 ± 0.0577	**500**	1.351813 ±0.0027
RE				13mg/g

Table: 5 Determination of Hydrogen peroxide scavenging of *Bougainvillea spectabilis*

S.no	Concentration of ascorbic acid/*B.spectabilis* (µg/ml)	Percentage inhibition of ascorbic acid	Percentage inhibition of B.spectabilis *
1	10	88.16±0.2712	12.42±0.3415
2	20	90.53±0.1974	23.07±0.1972
3	30	110.79±0.1972	24.85±0.1993
4	40	114.79±0.1975	27.85±0.1972
5	50	140.60±0.3150	31.95±0.1972
	IC-50	79(µg/ml)	76 (µg/ml)

*Mean of three value ± SEM

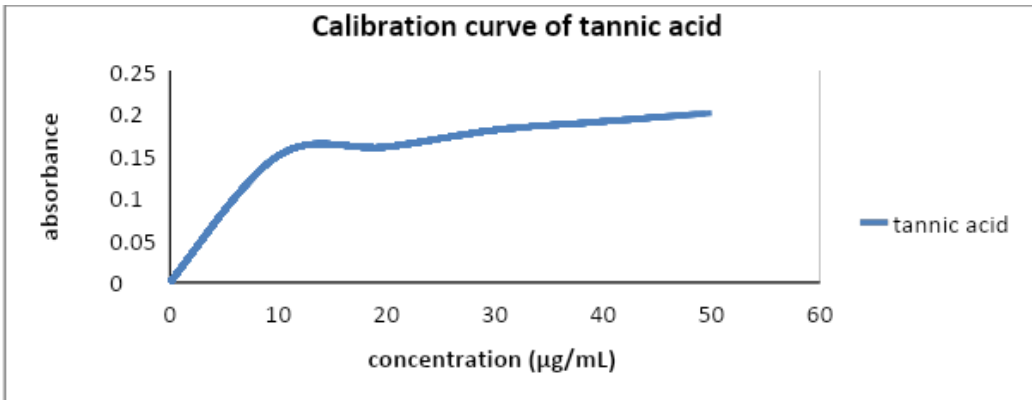

Figure: 1 Calibration curve of Tannic acid

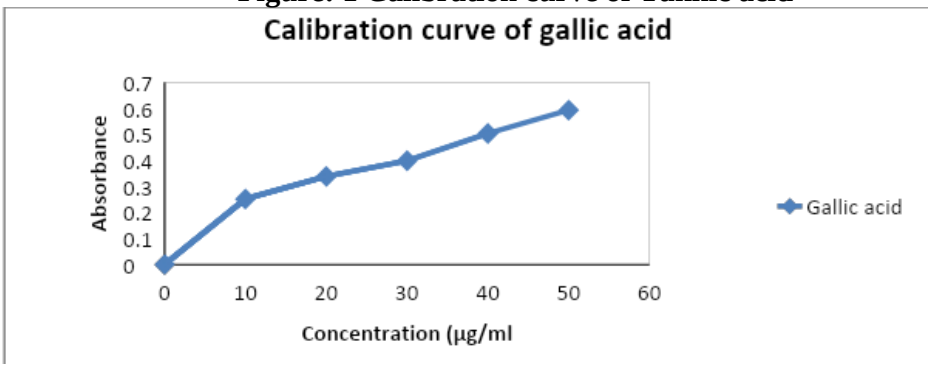

Figure:2 Calibration curve of Gallic acid

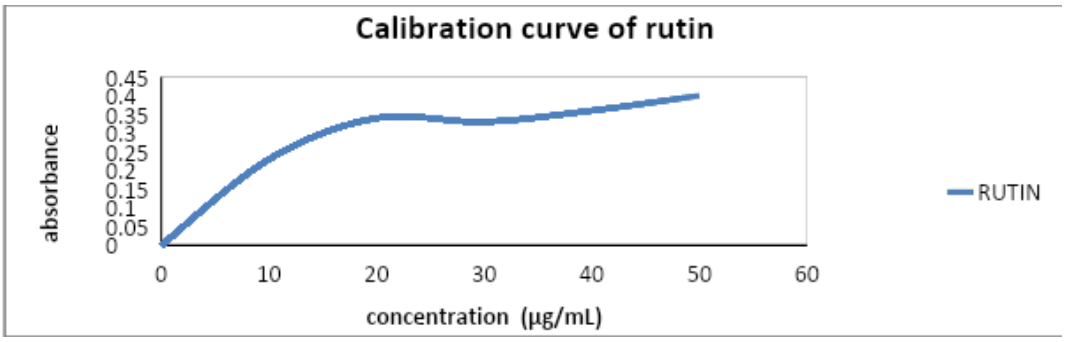

Figure: 3 Calibration curve of Rutin

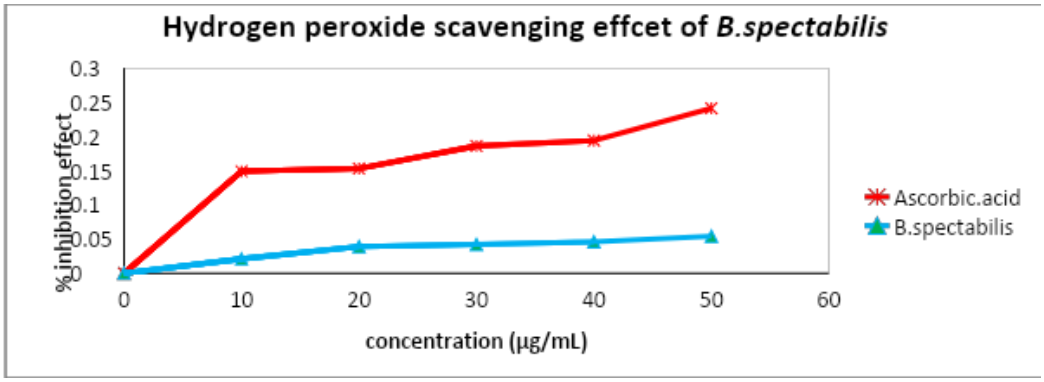

Fig : 4 Determination Of Hydrogen peroxide Method of *Bougainvillea spectabilis*

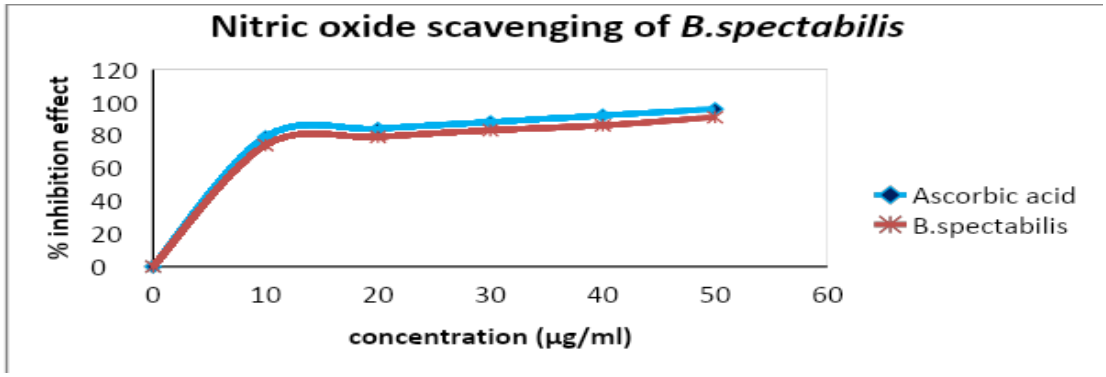

Fig: 5 Determination of Nitric Oxide scavenging method of *Bougainvillea spectabilis*.

Table: 6 Determination of Nitric Oxide scavenging method of *Bougainvillea spectabilis*

S.No	Concentration in ascorbic acid/ *B.spectabilis* (µg/ml)	Percentage inhibition of ascorbic Acid *	Percentage inhibition of *B. spectabilis* *
1	10	79.21±o.1260	74.31±0.3111
2	20	84.60±0.0333	79.06±0.1494
3	30	88.04±0.4082	83.30±0.1494
4	40	92.32±0.2221	86.48±0.3588
5	50	96.03±0.3323	91.80±0.1507
	Ic50	88 µg/ml	75 µg/ml

*Mean of three value ±

SEM

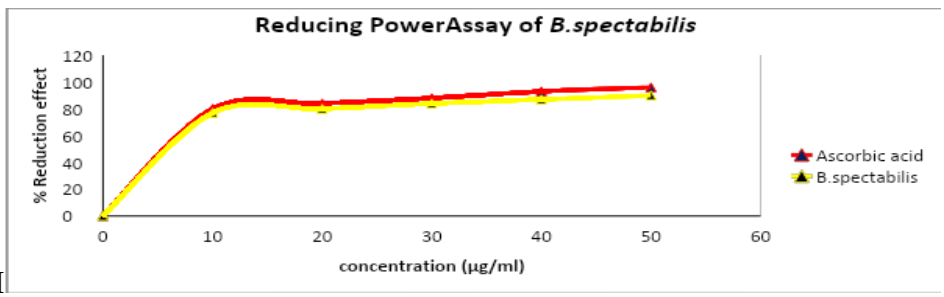

Fig: 6- Determination of Reducing Power Assay of *Bougainvillea spectabilis*

Table: 7 Determination of Reducing Power Assay of *Bougainvillea spectabilis*.

S.no	Conc in ascorbic acid / *B. spectabilis* (µg/ml)	Percentage reduction of absorbance ascorbic acid *	Percentage reduction of absorbance HAEBS *
1	10	80.08±0.1733	77.66±0.0466
2	20	84.79±0.1733	80.12±0.0400
3	30	88.11±0.1766	84.21±0.1834
4	40	93.09±0.3100	87.6±0.3500
5	50	96.13±0.5233	95.06±0.3566
	IC_{50}	74µg/ml	68µg/ml

*Mean of three value ± SEM

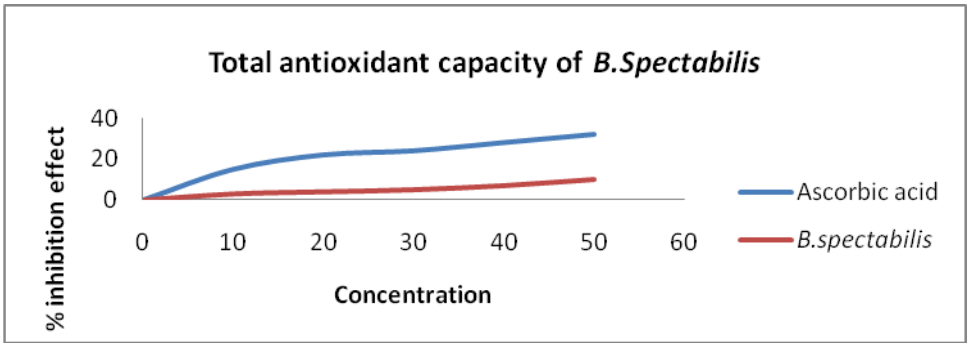

Fig: 7 Determination of Total Anti-oxidant Capacity of HAE *Bougainvillea spectabilis* (leaf)

Table: 8 Determination Of Total Antioxidant Capacity of *Bougainvillea spectabilis*

S.no	Concentration/ *B. spectabilis* (µg/ml)	% inhibition of ascorbic acid *	% inhibition of *B. spectabilis* *
1	10	15.2±0.088194	3.5±0.17321
2	20	22.92±0.36935	4.28±0.122206
3	30	24.9±0.120189	4.95±0.052389
4	40	27.9±0.152757	7.32±0.118653
5	50	32±0.104086	9.64±0.063598
	IC_{50}	77 µg/Ml	75 µg/Ml

*Mean of three value ± SEM

CONCLUSION

The present research studies the phytochemical analysis of the *Bougainvillea spectabilis* which adds additional scientific information to the existing research. It is concluded that this plant preparation may be formulated further such preparations may be added as adjuvant therapy in the management of diseases.

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