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# *In-vitro* Antioxidant and Anti-lipid Peroxidation Activity of Ethanolic Extracts of *Bougainvillea shubhra, Bougainvillea peruviana* and *Bougainvillea bhuttiana Golden Glow*: A Comparative Study

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## Abstract

The aim of our present study was to evaluate the antioxidant and anti-lipid peroxidation activity of ethanolic extracts of *Bougainvillea species viz B. shubhra, B. peruviana* and *B. bhuttiana golden glow*. Phytochemical constituents *viz.* total phenolics, flavonoids and tannins were assayed using standard protocol. The antioxidant activity was studied by DPPH assay, FRAP assay, reducing power assay and in-vitro inhibition of lipid peroxidation. Of the three species, *B. bhuttiana golden glow* showed highest amount of phenols (6.78±0.001), flavonoids (27.7±0.012) and tannin (11.08±0.008) contents. It also showed highest antioxidant activity of 15.15± 0.008 and 8.08± 0.018 as determined by FRAP assay and reducing power assay respectively. The percent DPPH radical scavenging activity was 82.72% and in-vitro inhibition of lipid peroxidation in mitochondrial membrane was also found to be highest in *B. bhuttiana golden glow.*

**Keywords:** Antioxidant activity, *Bougainvillea*, DPPH, flavonoids, lipid peroxidation

## 1. Introduction

An endogenous antioxidant system of the body takes care of reactive oxygen species (ROS) that are generated under normal physiological conditions. However overproduction of ROS and/or inadequate presence of antioxidants have been related to pathogenesis of disease conditions like diabetes, cancer, atherosclerosis etc [1, 2]. Our body's self antioxidant system comprises of enzymes like catalase, superoxide dismutase and glutathione which react with reactive species and neutralizes them thereby protecting the body from damaging effect of free radical species and preventing oxidative stress [3].

Plants have ability to synthesize various aromatic substances viz phenols or their oxygen substituted derivatives which play an important role in plants defense mechanisms against predation by microorganisms, insects and herbivores [4]. Phytochemical screening of these secondary metabolites synthesized by plants have shown the presence of many active components which have properties like antimicrobial, antifungal, antidiabetic, anticancer, antioxidant etc.

*Bougainvillea,* a common ornamental plant of *Nyctaginaceae* family is widely grown all over the world in tropical or subtropical gardens. Three horticulturally

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important species are *B. spectabilis*, *B. glabra* and *B. peruviana*. Different hybrid species and cultivars of this genus have also been produced. Many phytochemical studies have been done on *B. spectabilis and B. glabra*and have shown that the leaf extracts are rich source of antioxidants and have various biological activities like anticancer, antidiabetic, anti-inflammatory, antimicrobial [4–7]. *B. spectabilis* leaves extract was shown to inhibit tomato spotted wilt tospovirus on *capsicum annum* and ground water in laboratory tests [8].

The present study aimed to determine in-vitro antioxidant and anti-lipid peroxidation activity of ethanolic leaves extracts of three *Bougainvillea species* viz. *B. shubhra, B. peruviana* and *B. bhuttiana golden glow.*

## 2. Materials and Methods

### 2.1 Collection of Plant material

Fresh *Bougainvillea* plant samples were collected from plant nursery at Nerul, Navi Mumbai and authenticated at Bhabha Atomic Research Centre, Trombay, Mumbai. Taxonomically they were identified as *B. shubhra, B. peruviana* and *B. bhuttiana golden glow.*

### 2.2 Preparation of Extracts

Leaves were washed, oven dried, ground to fine powder and stored in airtight containers until further use. 20gm of dried powder was defatted with petroleum ether using soxhlet apparatus followed by successive solvent extraction using acetone, ethanol and distilled water [9]. For better extraction, 300 ml volume of each solvent and 15 cycles of extraction were followed. The resulting fractions were concentrated using rotary evaporator, final residue was reconstituted in DMSO and stored at -20°C. This crude extract was suitably diluted and assayed for phytochemical and antioxidant activity.

### 2.3 Estimation of Phytochemical Constituents

#### 2.3.1 Estimation of Total Phenols

The total phenol content in ethanolic leaf extract was determined spectrophotometrically as described by Singleton and Rossi [10] with little modification.0.5 ml of extract (1:200 dilution) was mixed with 5.0 ml of 0.5 N Folin-Ciocalteu reagent and 4.0 ml of saturated sodium carbonate (1M) and incubated at 45ºC in water bath for 15 min. The absorbance was measured at 765 nm. Gallic acid prepared in methanol:water (50:50 $^{v}/_{v}$) was used as standard and total phenol content in leaf extracts was expressed as gallic acid equivalent (mg/g).

#### 2.3.2 Estimation of Total Flavonoids (TF)

The total flavonoid content in ethanolic leaf extract was determined by aluminum chloride method [11]. The reaction mixture comprising of 1.0 ml of extract (1:200 dilution), 0.5 ml of1.2% aluminum chloride and 0.5 ml of 120 mM potassium acetate was mixed and incubated at room temperature in dark for 30 min. Absorbance was measured at 415 nm. Quercetin was used as standard [12] and flavonoid content in leaf extracts was expressed as quercetin equivalent (mg/g).

#### 2.3.3 Estimation of Tannins

The tannin content was determined by Folin-Ciocalteu reagent method. 0.5 ml of extract (1:200 dilution) and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) was mixed and incubated at room temperature for 15 min. 2.5 ml of saturated sodium carbonate (20%) was added, incubated at room temperature for 30 min and absorbance was measured at 760 nm. Tannin content was expressed as tannic acid equivalent (mg/g).

### 2.4 Evaluation of Antioxidant Activity

#### 2.4.1 α, α-Diphenyl- β-picryl-hydrazyl Radical Scavenging (DPPH)Radical Scavenging Assay

The free radical scavenging activity of ethanolic leaf extracts was measured by using DPPH assay. The reaction mixture comprising of 1.0 ml of DPPH in methanol (0.3 mM), 1.0 ml of extract (1:200 dilution) and 1.0 ml of methanol was incubated in darkfor 30 min. The absorbance was measured at 517 nm [13].Ascorbic acid was used as standard [14]. The percent radical scavenging activity was calculated as:

$$RSA\ (\%) = (A_0 - A_1/A_0) * 100$$

Where, $A_0$ is the absorbance of control and $A_1$ is the absorbance of test sample.

#### 2.4.2 Ferric Reducing Antioxidant Power (FRAP) Assay

0.2 ml of the extract (1:200 dilution) was added to 3.8 ml of FRAP reagent (10 parts of 0.3 M sodium acetate bufferpH 3.6, 1 part of 10 mM TPTZ (2,4,6-Tripyridyl-S-Triazine) solution in 40 mM HCL and 1 part of 20 mM $FeCl_3$ solution) and incubated at 37°C for 30 min and the increase in absorbance was measured at 593 nm [15]. The antioxidant capacity based on the ability of the leaf extract to reduce ferric ions was expressed in terms of ascorbic acid equivalent (mg/g).

#### 2.4.3 Estimation of Reducing Power (RP)

The reducing power of ethanolic extracts was determined by method of Athukorala [16]. 1.0 ml of extract (1:200 dilution) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.5) and 2.5 ml of potassium ferricyanide (1%) and incubated at 50°C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture, centrifuged at 5000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1%$FeCl_3$ and absorbance was measured at 700 nm. Reducing power was expressed in terms of standard ascorbic acid equivalent (mg/g) [17].

### 2.5 Isolation of Rat Liver Mitochondria

Rat liver tissue (20% w/v) was washed thoroughly with buffer, cleaned to remove blood clots and homogenized in mitochondrial extraction buffer (5 mM Tris HCL pH 7.4, 0.15 M sucrose, 1mM EDTA). The homogenate was filtered to remove tissue debri and the filtrate obtained was centrifuged at 1500 rpm for 5 min at 4°C. The pellet-containing cell debri was discarded and the supernatant was again centrifuged at 10,000 rpm for 20 min at 4°C. The pellet obtained was re-suspended in mitochondrial extraction buffer and re-centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was discarded and residue was suspended in 1.15% KCL solution. Isolated mitochondria were characterized by succinate dehydrogenase activity in order to confirm the purity of mitochondria [18]. Fresh mitochondria were used for each experiment. Protein concentration was estimated by Lowry's method using BSA as standard [19]. The protein content of mitochondrial suspension was adjusted to 5mg/ml for assaying lipid peroxidation in mitochondrial membrane.

### 2.6 $FeSO_4$-induced Lipid Peroxidation Inhibiton

The extent of lipid peroxidation in rat liver mitochondria was assayed by estimating thiobarbituric acid-reactive substances (TBARS) by standard assay [20]. Lipid peroxidation was induced in rat liver mitochondria by treating it with 0.5mM of $FeSo_4$ [21], in the presence and absence of different concentrations of the extracts. The reaction mixture consisting of 0.5 ml of mitochondrial sample (5mg/ml), extract (50µg/ml-500µg/ml) and 25µl of $FeSo_4$ (0.5mM) was incubated at 37°C for 30 min. After incubation, 1.5 ml of 20%acetic acid, 0.2 ml of SDS (8.1%) and 1.5 ml of TBA (0.8%) were added, total volume made to 4 ml with distilled water and incubated at 95°C for 1hr. After cooling, 4 ml of n-butanol:pyridine (15:1) was added, mixed well and centrifuged at 10,000 rpm for 5 min. The organic layer was separated and absorbance measured at 532nm. The percent inhibition of lipid peroxidation was calculated as:

$$\% \text{ inhibition} = (\text{control} - \text{test}) \times 100 / \text{control}$$

### 2.7 Statistical Analysis

All assays were performed in triplicate. The results are reported as mean ±standard deviation.

## 3. Results and Discussion

### 3.1 Total Phenol, Flavonoids and Tannin Content

The total phenols, flavonoids and tannin content in crude ethanolic extract of *Bougainvillea species* were estimated by standard protocols. Total phenol levels were expressed as mg gallic acid equivalent/g and were found to be highest in *B. bhuttiana golden glow* (Table 1).

The bitter plant polyphenols, tannins that bind to proteins and other organic compounds e.g alkaloids, form tannin-protein complexes which have antioxidant activity. Tannin content in crude extracts was expressed as mg tannic acid equivalent/g. Highest tannin content of 11.08±0.008 mg tannic acid equivalent/g was found in *B. bhuttiana golden glow* (Table 1). The levels of tannins reported in ethanolic extracts of all the three *Bougainvillea species* was relatively high as compared to

the tannin levels reported in methanolic and aqueous extract of *B. spectabilis,* 1.48 mg/g and 1.33 mg/g respectively [6].

Plant flavonoids have been reported to act by scavenging the free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes responsible for generation of free-radical [22]. Highest flavonoid content of 27.7± 0.012 mg of quercetin equivalent/g was found in *B. bhuttiana golden glow* (Table 1). Thus amongst the three *Bougainvillea species B. bhuttiana golden glow* showed highest levels of phenols, tannins and flavonoids suggesting it to be a potential source of plant phytochemicals.

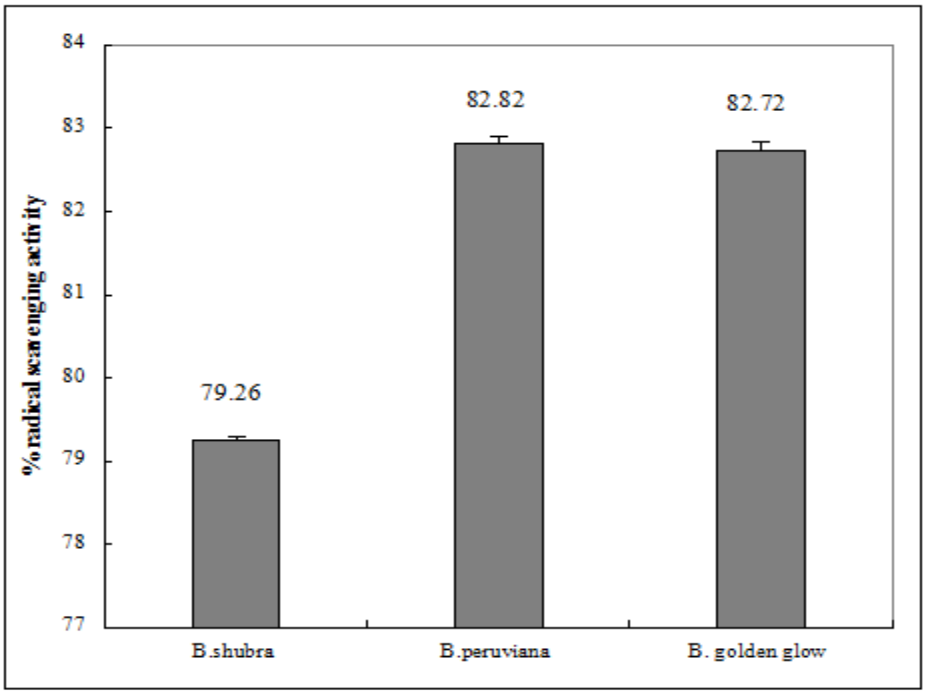
## 3.2 Antioxidant Assay

Different *in-vitro* assays were used to measure the antioxidant activity of crude ethanolic leaf extracts. The DPPH radical scavenging assay has been widely used as a model system to investigate the radical scavenging activity of natural compounds [23]. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidants reduce the stable DPPH radical to a yellow coloured diphenyl-picrylhydrazine with maximum absorption at 517 nm. The percent radical scavenging activity of ethanolic extracts of *Bougainvillea species* under study was found to be about 79 to 83% (Fig. 1). Aqueous leaf

**Table 1:** Phytochemicals and antioxidant activity of *Bougainvillea* species

| Tests | Standard equivalent in ethanolic leaves extract (mg/g) | | |
|---|---|---|---|
| | *Bougainvillea shubhra* | *Bougainvillea peruviana* | *Bougainvillea buttianagolden glow* |
| Total phenol content | 2.34± 0.004 | 2.02± 0.007 | 6.78±0.0015 |
| Total flavonoids | 8.07± 0.008 | 15.05± 0.004 | 27.7± 0.012 |
| Tannin content | 5.23± 0.003 | 3.63± 0.002 | 11.08±0.008 |
| FRAP assay | 3.83± 0.020 | 6.35± 0.002 | 15.15± 0.008 |
| Reducing power assay | 2.01± 0.004 | 3.77± 0.02 | 8.08±0.018 |

Values are expressed as Mean ± S.D. of triplicates




Values expressed as Mean ± S.D. of triplicates

**Fig. 1a.** DPPH radical scavenging activity of *Bougainvillea species*.

extract of *Bougainvillea spectabilis* was reported to have radical scavenging activity of 85.63% [24].

Ferric reducing antioxidant power (FRAP) assay is based on the ability of antioxidants to reduce $Fe^{3+}$ to $Fe^{2+}$ in the presence of TPTZ complex forming an intense blue $Fe^{2+}$-TPTZ complex with an absorption maximum at 593 nm [15]. The ethanolic extracts showed an increase in absorbance indicating an increase in reductive ability. The FRAP activity in the ethanolic extract of *B. bhuttiana golden glow* was almost five times the FRAP activity seen in extracts of *B. shubhra* (Table1). This increased FRAP activity was found to correlate with the higher phenol content in the *B. bhuttiana golden glow.*

Reducing power has been reported to be directly correlated with antioxidant activity of certain plant extracts [25]. Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can act as primary and secondary antioxidants [26]. In reducing power assay, the antioxidant reduces the ferricyanide to ferrous cyanide, resulting in colour change from yellow to greenish blue which can be measured at 700nm. All the ethanolic extracts exhibited reducing power, with highest reducing power ability in extract of *B. bhuttiana golden glow* (Table1). This reducing power ability was relatively high as compared to aqueous and methanolic leaf extracts of *Bougainvillea spectabilis* [6].

### 3.3 Inhibition Of Lipid Peroxidation

The protective effect of ethanolic extracts against$FeSO_4$-induced lipid peroxidation was assessed in rat liver mitochondrial samples. During lipidperoxidation, polyunsaturated fatty acids present in lipid membrane undergo oxidation resulting in formation of malonaldehyde (MDA) which reacts with two molecules of thiobarbituric acid (TBA) to form TBARS, a pinkish red chromagen which is read at 532nm [27]. 0.5 mM $FeSO_4$ effectively induced lipid peroxidation in mitochondrial samples as assayed by TBARS assay. Ethanolic leaf extracts even up to a concentration of 400µg/ml did not induce any lipid peroxidation in mitochondrial membrane. In $FeSO_4$-treated mitochondrial samples, the ethanolic extracts were found to inhibit $FeSO_4$-induced lipid peroxidation in a dose dependent manner. *B. bhuttiana golden glow* extract was more effective in inhibiting $FeSO_4$-induced membrane lipid peroxidation (Table 2).

Thus it can be concluded that *B. shubhra, B. peruviana and B. bhuttiana golden glow,* exhibited antioxidant and anti-lipid peroxidation activities which can be attributed to the presence of plant polyphenols. *B. bhuttiana golden glow* appears to be a promising source of natural antioxidants and a potential candidate which can be evaluated for treatment of oxidative stress related disease, anticancer and antimicrobial activities.

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**Table 2:** Lipid peroxidation inhibition activity of Bougainvillea species

| Concentration of ethanolic extracts µg/ml | % Inhibition of lipid peroxidation | | |
|---|---|---|---|
| | *Bougainvillea shubhra* | *Bougainvillea peruviana* | *Bougainvillea. golden glow* |
| 50 | 5.36% | 6.23% | 24.87% |
| 100 | 12.68% | 10.48% | 29.75% |
| 200 | 32.68% | 28.29% | 37.56% |
| 300 | 45.85% | 45.36% | 45.36% |
| 400 | 56.58% | 64.39% | 51.70% |

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