Research Article

# Phytochemical Profile, *In Vitro* Antioxidant Property and HPTLC Analysis of Methanol Extract of *Bougainvillea glabra* (Nyctaginaceae)

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**ABSTRACT**

The current study was aimed to evaluate phytochemical composition and antioxidant property of red, white and yellow flower yielding varieties of *Bougainvillea glabra* leaves by various *in vitro* methods. Antioxidant activity of all the three varieties was analyzed by DPPH radical scavenging assay. Red variety showed highest DPPH radical scavenging activity and hence was further analyzed by $H_2O_2$ radical scavenging and ferric ion reduction activities. Total phenolic and flavonoid content estimation was performed that resulted in maximum content of phenolics and flavonoids in the red variety of *B. glabra* leaves. Thus, HPTLC fingerprint was developed for polyphenols identification that detected five polyphenols within the extract. Since, red variety of *B. glabra* leaves showed good antioxidant property for the tests performed and high polyphenolic content within them, the bioactive compound in the red variety of *B. glabra* leaves could be further isolated, purified, characterized and used as a safe antioxidant compound.

**Keywords:** *Bougainvillea glabra*, DPPH activity, $H_2O_2$ radical scavenging activity, HPTLC analysis

## INTRODUCTION

The degree of oxidation of an atom in a chemical compound is called oxidation state. It is defined as the addition of oxygen to a compound with loss of electrons. At cellular level, it leads to the generation of free radicals. These free radicals are collectively known as Reactive Oxygen Species (ROS). They are mainly produced inside cellular organelles, such as mitochondria[1]. Examples of free radicals includes oxygen ions, peroxides ($H_2O_2$), superoxides, nitric oxide radicals (NO) and the lipid peroxyl radical (LOO)[2]. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis[3]. The antioxidant defense system protects the cell against the free radicals. The antioxidant defense mechanism is affected by age, diet and health condition of an individual[4]. Mal-functioning of anti-oxidant defense system leads to the damage in nucleic acids, mitochondria, proteins and enzymes[5]. An antioxidant supplement could be used to strengthen the anti-oxidant defense system. Thus a natural antioxidant from plant, algae and microbial source could be a successful alternative to synthetic ones. There is search for effective and nontoxic, natural compound with antioxidant activity in recent years[6]. For the development and discovery of antioxidant compounds, scientists are looking forward to the alternative sources and in last few decades, medicinal plants have been extensively studied for their bioactive compounds to develop new molecules for pharmaceutical use[7]. *Bougainvillea glabra*, also called as paper flower is a climbing, evergreen, woody and ornamental shrub which inhabited to warmer climates is a native to Brazil, also seen in areas like Middle East, Indian Sub-continent, and North America. *B. glabra* from the family of Nyctaginaceae belongs to the genus Bougainvillea and this genus has 18 species of plants of which three of them *B. spectabilis, B. glabra* and *B.peruviana* have gained a lot of importance in the horticulture field. Traditional practitioners in Mandsaur use the leaves for a variety of disorders, such as diarrhea, and to reduce stomach acidity[8]. In Panama, an infusion of *B. glabra* is used as treatment for low blood pressure. *B. glabra* is reported to have a wide range of medicinal properties like anti-inflammatory, anti-pyretic, analgesic[9], antibacterial[10], anti-diarrheal[11] and antioxidant activities[12]. In scientific medicinal system, *B. glabra* leaf extracts is known to have improved sensitivity of insulin receptor, improves insulin utilization, stimulates the transport of glucose into muscle and prevents diabetic complications (such as cataract, hyperlipidemia). Although, there are recent scientific investigations on the antioxidant activity of *B. glabra*[12], a comparative study on the methanol extract of *B. glabra* was not yet studied. Hence this study was aimed to perform a comparative study on antioxidant activity of three different varieties of *B. glabra* leaves.

## MATERIALS AND METHODS

### Chemicals

DPPH was purchased from Sigma Eldrich, USA. Methanol, sodium phosphate dibasic, sodium phosphate monobasic, ferric chloride, trichloroacetic acid, potassium ferricyanide, sodium bicarbonate, aluminum chloride, gallic acid, Ascorbic acid and Folin-Ciocalteau were purchased from SRL Pvt Ltd, Mumbai, India. All other chemicals used were of analytical grade.

### Collection of Sample

Fresh and healthy leaves of *B. glabra* (red, white and yellow flower varieties) were collected from VIT University campus, Vellore district, TN, India

(12°55′13″N 79°08′00″E) in the month of February, 2011. Plant samples were brought to the Molecular and Microbiology Research Laboratory, VIT University, Vellore. Voucher specimens were maintained in our laboratory for future references.

### Sample Preparation

Fresh and mature leaves of three different varieties of *B. glabra* were washed thoroughly in distilled water and shade dried at room temperature. Dried leaves were powdered uniformly using a mechanical grinder. Pulverized leaf material was extracted with methanol using a Soxhlet extractor. These extracts were concentrated at 40°C under reduced pressure (72 mbar) with a rotary evaporator and dried using lyophilizer. Dried extract was collected in air tight container and stored at 4°C up for further use.

### Phytochemical Screening

Phytochemical screening of three different varieties of *B. glabra* was carried out using standard protocols for the presence of carbohydrates, proteins, phenolics, oils and fats, saponins, flavonoids, alkaloids and tannins[13].

### DPPH Radical Scavenging Activity

The stable DPPH radical was used for the determination of free radical-scavenging activity of the extracts[14,15]. The plant extracts were diluted to obtain different concentrations (10, 20, 40, 60, 80 and 100 µg/ml). Two milliliters of each dilution was mixed with 1 ml of DPPH solution (0.2 mM/ml in methanol) and mixed thoroughly. The mixture was incubated at 20°C for 40 mins. Absorbance was measured at 517 nm using a UV-Visible spectrophotometer with methanol as blank. Experiment was performed in triplicates at each concentration.

The percentage scavenging of DPPH was calculated according to the following formula

$$\%\ \mathbf{DPPH\ Radical\ Scavenging} = \left[\left(\frac{\mathbf{A_c - A_t}}{\mathbf{A_c}}\right)\right] \times \mathbf{100}$$

Where,

Ac is the absorbance of the control

$A_t$ is the absorbance of test.

### $H_2O_2$ Radical Scavenging Activity

A solution of $H_2O_2$ (40 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). Crude extract at 50, 100, 150 and 200 µg/ml concentrations in 3.4 ml phosphate buffer was added to 0.6 ml of $H_2O_2$ solution (0.6 ml, 43 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution contains sodium phosphate buffer without $H_2O_2$[16]. The percentage of hydrogen peroxide scavenging by the extracts and a standard compound was calculated as follows:

$$\%\ \mathbf{Scavenged\ H_2O_2} = \left[\left(\frac{\mathbf{A_o - A_1}}{\mathbf{A_o}}\right)\right] \times \mathbf{100}$$

Where $A_o$ was the absorbance of the control and $A_1$ was the absorbance in the presence of the sample of extract and standard.

### Reducing Power Activity

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. One milliliter of extract at different concentrations (50, 100, 150 and 200 µg/ml) were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6 and 2.5ml of 1% of potassium ferricyanide ($K_3Fe(CN)_6$). The mixture was incubated at 50°C for 20 mints. A volume of 2.5 ml of trichloroacetic acid was added to the mixture and was centrifuged at 3000 rpm for 10 mints in a cooling centrifuge. About 2.5 ml of supernatant was mixed with equal volume of distilled water and 0.5ml $FeCl_3$. Absorbance was measured at 700nm using a UV-Visible spectrophotometer. Ascorbic acid was used as positive control. Higher absorbance of the reaction mixture indicates greater reductive potential. Each experiment was performed in triplicates at each concentration[17].

### Estimation of Total Phenolic Content

Total phenolic content of the methanol extract of three varieties of *B. glabra* was determined using the Folin-Ciocalteau reagent method[18]. The crude methanol extract was diluted to obtain different concentrations (50, 100, 150 and 200µg/ml). A volume of 50µl of each of the three extracts were mixed with 2.5ml of Folin-Ciocateau reagent ($1/10^{th}$ dilution of distilled water) and 7.5ml of $Na_2CO_3$. The mixture was incubated at 45°C for 15mins. The absorbance was measured at 765nm using a UV-Visible spectrophotometer. $Na_2CO_3$ solution (2ml of 7.5% $Na_2CO_3$ in 2.55ml of distilled water) was used as blank. The results were expressed as Gallic acid equivalence in µg. Experiment was performed in triplicates at each concentration.

### Determination of Total Flavonoids

The determination of total flavonoids of the methanol extract of *B. glabra* leaves was carried out using the modified procedure[19]. A volume of 1 ml (containing 125, 250, 500 and 1000 µg extract) was mixed with 1 ml of $AlCl_3$ (2% in ethanol). The mixture was incubated at room temperature for 60 mins. $AlCl_3$ solution (1 ml of 2% $AlCl_3$ in 1 ml of water) was used as blank. The absorbance was measured at 420nm using UV-Vis spectrophotometer. Total flavonoid content was expressed as as Quercetin equivalence (QE) in µg. Experiment was performed in triplicates at each concentration.

### HPTLC Analysis

### Sample Application

The given methanol extract of plant sample was centrifuged at 3000rpm for 3 mints and this solution was used as test solution for HPTLC analysis. 2µl of test solution was loaded as 6mm band length in the 2 x 10

Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

### Spot Development

The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Polyphenol) and the plate was developed in the mobile phase [Toluene-Acetone-Formic acid (4.5 : 4.5 : 1)] up to 90mm.

### Photo-Documentation

The developed plate was dried by hot air to evaporate solvents from the plate.

The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV 366nm.

### Derivatization

The developed plate was sprayed with 20% Sodium carbonate reagent followed by Folin-Cio calteu reagent and dried at 100°C in Hot air oven.

The plate was photo-documented in Day light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

### Scanning

After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The Peak table, Peak display and Peak densitogram were noted[20].

### Statistical Analysis

The values of DPPH radical scavenging activity, $H_2O_2$ radical scavenging activity, reducing power activity, total phenolic content and total flavonoid content of *B. glabra* leaves were expressed as mean ± standard deviation of the response of three replicates per sample.

Results were analyzed using Microsoft Excel 2007 and Graphpad Prism 5.

## RESULTS

**Table 1:** Comparative study of phytochemical analysis of *B. glabra* leaves.

| S. No. | Phytochemicals | Variety of *B. Glabra* | | |
|---|---|---|---|---|
| | | White | Red | Yellow |
| 1. | Carbohydrates | +++ | ++ | + |
| 2. | Phenolic compounds | + | ++++ | - |
| 3. | Oil and fats | ++ | +++ | ++ |
| 4. | Saponins | ++ | +++ | ++ |
| 5. | Flavonoids | + | ++ | - |
| 6. | Alkaloids | - | - | - |
| 7. | Tannins | + | +++ | + |
| 8. | Proteins | - | - | - |

**Here,** +: presence; -: absent

### Percentage Yield

Ten grams of dried leaf powders of three varieties of *B. glabra* was extracted in 100 ml of methanol separately. The red variety of *B. glabra* resulted in maximum yield (10%) followed by white variety (7.19%) and yellow variety (4.77%).

### Phytochemical Screening

Phytochemical screening of different variety of *B. glabra* suggests the presence of carbohydrates, phenolic compounds, oils and fats, saponins, flavonoids and tannins as major phytochemical groups in the leaf extracts (Table number 1).

### Antioxidant Activity of *B. glabra*

#### *DPPH Radical Scavenging Activity*

DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers[21]. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accept an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance[22]. All the varieties (methanol extract) of *B. glabra* exhibited dose dependent increase in the DPPH radical scavenging activity. Red variety showed high DPPH radical scavenging activity followed by white and yellow (Figure 1).

Red variety showed highest scavenging activity among all the other variety followed by white and yellow. Therefore red variety was selected for further antioxidant activity or test.

#### *$H_2O_2$ Radical Scavenging Activity*

Scavenging of $H_2O_2$ by extracts be attributed to their phenolics, which can donate electrons to $H_2O_2$, thus neutralizing it to water[23]. The extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. The methanol extract of *B. glabra* exhibited the high $H_2O_2$ radical scavenging activity. Results are reported in Figure 2. The red variety of methanol extract exhibited the dose dependent $H_2O_2$ radical Scavenging activity.

#### *Reducing Power Activity*

In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. Amount of $Fe^{2+}$ complex can be then be monitored by measuring the formation of Perl's prussian blue at 700 nm. Increasing absorbance at 700nm indicates an increase in reductive ability[24]. The results for

ferric reducing power activity of *B. glabra* leaves extract are reported in Figure 3.

Methanol extract of *B. glabra* followed dose dependant increase in the reducing power activity.

**Estimation of Polyphenolic Compounds**

***Total Phenolic Content***

Polyphenols are the major plant compounds with antioxidant activity[25].

Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids[26].

They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals[27,28]. Methanol extract of red variety of *B. glabra* showed dose dependant increase in the phenolic content. The result was expressed as Gallic acid equivalent (GAE) (Figure 4).

***Total Flavonoids***

Flavonoids are phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicines[29].

The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. The result was expressed as Quercitin equivalence (QE) (Figure 5).

**HPTLC Analysis**

The HPTLC analysis is a molecular analysis technique also known as molecular detection. Blue colored zone at Daylight mode was present in the track, it was observed from the chromatogram after derivatization (Figure 6 and Figure 7), which is due to the Presence of Polyphenol 1, 2, 3, 4, 5 in the sample.

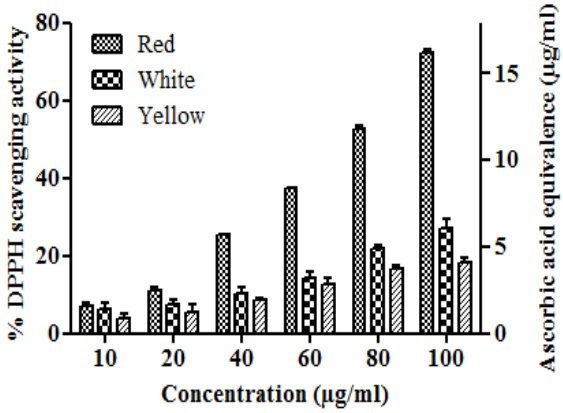


**Figure 1:** DPPH radical scavenging activity of the methanolic extract of *B. glabra* leaves

Results are reported as mean ± standard deviation (n=3).

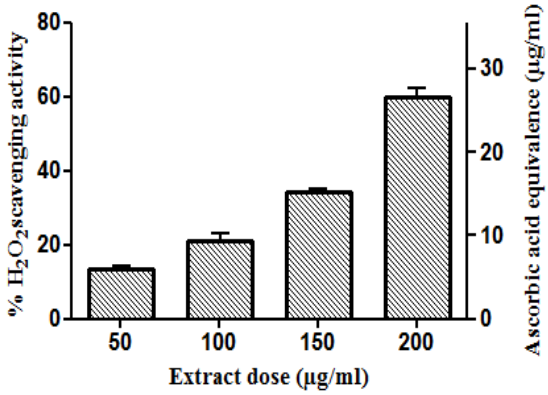


**Figure 2:** $H_2O_2$ Radical Scavenging activity of the methanol extract of *B. glabra* leaves

Results are reported as mean ± standard deviation (n=3).

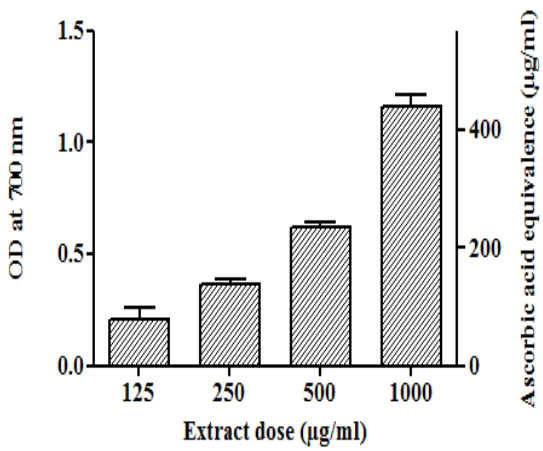


**Figure 3:** Reducing Power activity of the methanol extract of *B. glabra* leaves

Results are reported as mean ± standard deviation

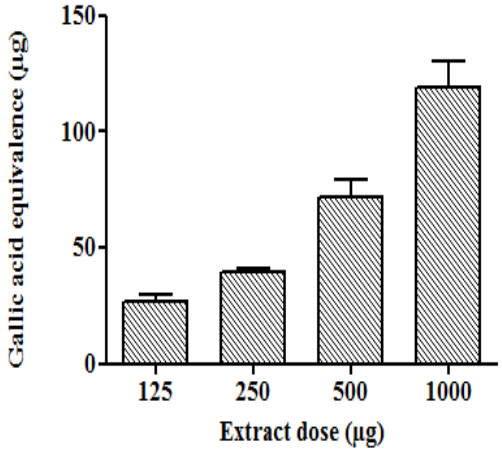


**Figure 4:** Total phenolic content of the methanol extract of *B. glabra* leaves

Results are reported as mean ± standard deviation (n=3).

(n=3).

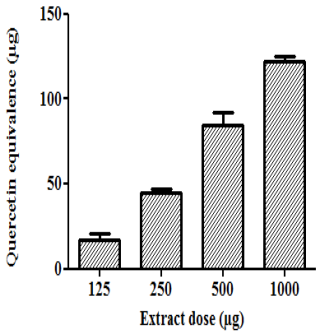


**Figure 5:** Total flavonoids content of the methanol extract of *B. glabra* leaves

Results are reported as mean ± standard deviation (n=3).

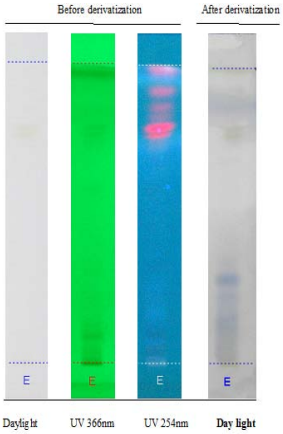


**Figure 6:** HPTLC chromatogram.

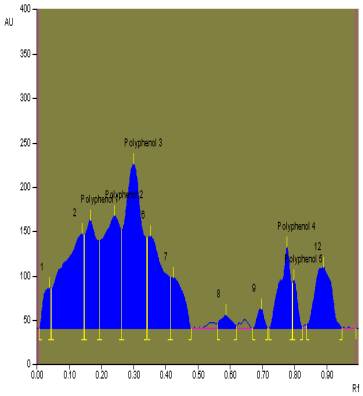


**Figure 7:** Peak densitogram display (Scanned at 500nm).

## DISCUSSION

Plants have been utilized as a natural source of medicines since thousands of years. Traditional Indian literatures such as Rig Veda, Yajur Veda, Atharva Veda, Charak Samhita and Sushrut Samhita reported the use of medicinal plants to cure human diseases in ancient times. There is an increasing interest in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases[30]. Currently, scientists are focusing on the active compound from the natural sources to develop newer drugs. A variety of medicinal plants have been reported to possess different medicinal properties viz, anticancer activity[30], antimicrobial activity[31], antidiabetic activity[32], antioxidant activity[33,34], hepatoprotective activity[35], larvicidal activity[36], hemolytic activity[37], anti-inflammatory activity[38] etc. In this study, we have investigated the phytochemical composition and antioxidant activity of the methanolic extract of *B. glabra* leaves by *in vitro* methods.

Results of phytochemical study of methanol extract of the *B. glabra* leaves are in agreement with the previous studies where *B. glabra* extracts have been reported to possess tannin flavonoids phenolic compound as major phytochemicals[39]. However in our study, it has not exhibited the presence of proteins and alkaloid. But there is presence of some unique compounds like carbohydrates and saponins.

Consumer denunciation towards the synthetic antioxidants provides an opportunity to discover natural antioxidant. Thus in this study antioxidant potential of methanolic extract of *B. glabra* leaves was analyzed against a variety of free radicals by DPPH radical scavenging activity, $H_2O_2$ scavenging activity and reducing power activity. Extract exhibited high antioxidant activity against a variety of free radicals. DNA damage can cause mutagenesis in the cells and initiate the development of cancer which is mediated by free radical[40]. Polyphenolic compound are well accepted antioxidant compound reported from herbal plants[41]. The extract exhibited the presence of three major groups of polyphenolic compounds (phenolic compounds, flavonoids and tannins). Among them, phenolic compounds are the largest group of polyphenols accounted for the antioxidant activity in plants. HPLC analysis of the crude extract showed the presence of four phenolic compounds. These compounds are well known for antioxidant properties and may be the active principle of the extract.

## CONCLUSION

The results obtained in the study represented that the methanolic extracts of *B. glabra* leaves contain a variety of phytochemical compounds, which can effectively protect the body from oxidative damage by free radicals scavenging activity and thus can be used as a potent source of natural antioxidant compounds. In future, further studies could be conducted to establish the antioxidant mechanism of methanolic extract of *B. glabra* leaves. With all these results, we can conclude that *B. glabra* leaves can be used as a source of safe and natural antioxidant compounds.

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