



PHYTOCHEMICAL ANALYSIS AND IN-VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF BOUGAINVILLEA GLABRA

Trinichia Rodriguez E.¹, Pruthviraj.R.D², B.V.Somashekaraiiah³

^{1,2}Department of Chemistry, Rajarajeswari College of Engineering, Bangalore-74

³Department of Chemistry, St. Joseph's College, Post Graduate and Research Centre, Bangalore-27

Abstract

The usage of plant based remedies and their derivatives have been an integral part of traditional medicine throughout the world with the discovery of new therapeutic agents and their versatile applications. The majority of medicinal properties shown by plants and its extracts are a result of the phytochemicals they possess, therefore screening these bioactive organic compounds would help in determining various therapeutic activities exhibited by them. It has been observed that the administration of leaf extract of plants decreases the oxidative damage caused by the radical species in in-vivo and in-vitro studies. The present study was carried out to determine the phytochemical analysis and in-vitro antioxidant activity of *Bougainvillea glabra* (Nyctaginaceae) which is a widely grown ornamental plant. The in-vitro antioxidant activity of methanolic extract of *Bougainvillea glabra* leaves were determined by DPPH, reducing power, nitric oxide, superoxide anion, metal ion chelation and hydrogen peroxide radical scavenging assays. The results of this research work showed significant inhibition of radical production in DPPH assay, reducing power assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, metal ion chelation assay and hydrogen peroxide radical scavenging assay. The present investigation revealed that the methanolic extract of leaves of *Bougainvillea glabra* exhibited significant antioxidant activity which is comparable to known standards.

Keywords: Antioxidant activity, *Bougainvillea glabra*, DPPH assay, phytochemicals.

1. INTRODUCTION

Nature by itself is the best physician and a complete abode of remedies which if put to use effectively can prove highly beneficial to human life. Before the era of advanced medical science, modern day medicines, pills and its pharmacopeia of synthetic drugs, there were plants and they have been used extensively in traditional healthcare system since time immemorial [1]. Our ancestors knew how to use them strategically to treat common ailments and even life – threatening diseases. The onus is on us to discover the bioactive constituents responsible for the observed therapeutic effect and establish it with scientific support so that in future it can be successfully incorporated as a pharmaceutical ingredient. Scientific studies are being carried out globally to verify the efficacy and some of the findings have now become part of leading synthetic drugs. On the contrary, many medicinal plants are still obscured and needs scientific evaluation.

Recent researches have shown that these phytochemicals can protect humans against various diseases such as diabetes, Alzheimer's disease, cancer, atherosclerosis, arthritis, neurodegenerative diseases and aging process [2], [3]. Phytochemicals are non-nutritive plant chemicals that have the potential to either be protective or disease preventive [4]. These are plants secondary metabolites i.e. chemicals that are not required for the immediate survival of the plant but which are synthesized to increase the fitness of the plant to sustain itself by allowing it to interact with the environment, including pathogens, herbivorous and symbiotic insects. There are more than 1000 known phytochemicals. To list a few- alkaloids, anthocyanins, carotenoids, flavonoids,

isoflavones, lignans, monophenols, saponins, phenolic acids, phytosterols, organosulphides and many more.

The phytochemical constituents of plants are widely distributed in the food products. However, there is a lack of an accurate comprehensive database and therefore estimation of intake of these remains a challenge. Consumption of phytochemicals should be within the permissible limits due to the theoretical risk of long term effects of consuming them due to which their determination and estimation are of prime importance.

Cellular reaction produce by products called free radicals. Free radicals can cause damage to our cells if they are not neutralized by antioxidants. Oxidative stress is the state of our body when the level of antioxidants in our body are not high enough to counteract the damaging effect of free radicals . Oxidative stress is an important risk factor in the pathogenesis of numerous acute and chronic diseases.

This stress is induced by exogenous sources such as ultraviolet light, ionizing radiations and chemical reactions and endogenous sources like metabolic processes which results in the production of reactive oxygen species (ROS). Supplementation of plant based products as exogenous antioxidants enhances the endogenous antioxidant defense mechanism of the body and is considered to be a better way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage.

Plants have been known to harbour antioxidants(secondary metabolites produced by the plants for their sustenance), which are molecules capable of preventing the oxidation of other molecules and terminate these chain reactions by removing free radical intermediates produced during these reactions. These secondary metabolites are involved in various redox processes, responsible for the observed antioxidant capability of the plants. ROS primarily consist of superoxide radical anion, hydroxyl radical, hydrogen peroxide, singlet oxygen, etc [5]. These radicals are involved in a large number of diseases including cancer, cardiovascular disease, neurodegenerative disorders and aging process.

In present research, ethanopharmacological and nutraceutical investigations of drugs by in vitro antioxidant activity assessment methods are often used to screen for the antioxidant

potential of plant extracts or their phytochemicals. In the case of medicinal plants, these assays are used to evaluate the free radical scavenging potency of plants. These data have great importance in understanding the role of plants in minimizing the oxidative stress linked pathophysiology of diseases. Therefore, multiple assay strategies have frequently been adapted to find antioxidant potential. In these assays, plants are estimated for their function as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators, after which they are classified as primary (chain-breaking) and secondary (preventive) antioxidants. Plants, herbs and spices which are rich in phenolic compounds like flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, antiviral, antiaging and anticarcinogenic activities which can be attributed to their antioxidant properties [6], [7].

II.MATERIALS AND METHODS

Phytochemical analysis [8]

Collection of Plant Materials

The leaves of *Bougainvillea glabra* were collected in the month of June, 2017 from the campus of St. Joseph's college, Post Graduate and Research Centre, Bangalore.

Processing of Plant Materials

The leaves were carefully washed in running water and cut into small bits to facilitate drying. The pieces of leaves were shade dried for about a week. The dried plant material (leaves) was pulverized using an electric blender. The powdered sample was stored in a well closed container free from environmental climatic changes until usage.

Extraction and Phytochemical analysis

100g of powdered sample of the leaves of this plant was subjected to soxhlet extraction using of petroleum ether at 70°C for 8-10 hours, chloroform at 61° C for 8-10 hours and methanol at 64.7°C as a solvent for 8-10 hours and concentrated using rotavapor. Qualitative phytochemical screening was carried out with the petroleum ether, chloroform and methanolic extracts for alkaloids, cardiac glycosides, flavonoids, phenols, saponins, terpenoids, quinones, tannins, sterols, amino acids and proteins and oxalates.

i.Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and

observed for the formation of reddish brown precipitate (or colouration).

ii. Test for Cardiac glycosides (Keller Kelliani's test)

5ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayered with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

iii. Test for Flavonoids (Alkaline reagent test)

2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

iv. Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

v. Test for Amino acids and Proteins (1% ninhydrin solution in acetone).

2ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

vi. Test for Saponins (Foam test)

To 2ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

vii. Test for Sterols (Liebermann-Burchard test)

1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H₂SO₄ and observed for the formation of dark pink or red colour.

viii. Test for Tannins (Braymer's test)

2ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of purple or greenish colour solution.

ix. Test for Terpenoids (Salkowki's test)

1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

x. Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

xi. Test for Oxalates

To 3ml portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates the presence of oxalates.

Estimation of Phytochemicals

i. Quantification of alkaloids

The content of alkaloid in the plant sample was determined by the method described by Harborne (1973) [9]. 5g of the sample was weighed and transferred in a beaker and 200ml of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for 4 hours at ambient temperature. The filtered extract was concentrated on a water bath to 1/4th of the original volume. Concentrated NH₄OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH₄OH and then filtered. The residue obtained was dried and weighed.

ii. Quantification of saponins

The saponin content in the different parts of the plant was determined using the method described by Obadoni and Ochuko (2001) [10]. The leaves were ground and 20g of it were transferred into a conical flask and 100ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20ml diethyl was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added and the combines of n-butanol extracts were washed twice with 10ml of 5% aqueous NaCl. The remaining solution was evaporated on a water bath. After evaporation, the samples were dried in the oven to obtain a constant weight. The saponin content was calculated as percentage.

iii. Quantification of total phenolics

The total phenolic content (TPC) of the methanolic extract of leaves of *Bougainvillea glabra* were determined using the method of Singleton et al. (1999) [11] with slight modifications. To 0.5 ml of test sample, 1.5 ml (1:1 v/v diluted with distilled water) Folin-Ciocalteu reagent was added and allowed to stand for 5 minutes at 22°C. After 5 minutes,

2.0ml of 7.5% of sodium carbonate was added. These mixtures were incubated for 90 minutes in the dark with intermittent shaking. After incubation development of blue colour was observed. Finally absorbance of blue colour in different samples was measured at 725nm by using spectrophotometer (Double Beam UV-VIS-570455). The phenolic content was calculated as gallic acid equivalents GAE/g on the basis of standard curve of gallic acid. The results were expressed as Gallic acid equivalents (GAE)/g of the plant material.

iv. Quantification of flavonoids

The total flavonoid content (TFC) of methanolic extract of *Bougainvillea glabra* was determined using the aluminium chloride assay through spectrophotometry. An aliquot (0.5 ml) of extracts were taken in test tubes. Then 2ml of distilled water was added followed by the addition of 0.15 ml of sodium nitrite (5% NaNO₂, w/v) and allowed to stand for 6 minutes. Later 0.15 ml of aluminium trichloride (10% AlCl₃) was added and incubated for 6 minutes, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) and the volume was made upto 5ml with distilled water. After 15 minutes of incubation the mixture turns to pink whose absorbance was measured at 425 nm by using spectrophotometer (Double Beam UV-VIS-570455). Distilled water was used as blank. The TFC was expressed in mg of quercetin equivalents (QE) per gram of extract.

In-vitro Antioxidant assays

i. DPPH radical scavenging assay

Free radical scavenging activity of methanolic extract of leaves of *Bougainvillea glabra* was measured by 2, 2-diphenyl-2-picryl-hydrazyl (DPPH). 0.1 mM solution of DPPH in methanol was prepared. This solution (0.2 ml) was added to 3 ml of different extracts in methanol at different concentration (20, 40, 60, 80, 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 20 minutes. Then, absorbance was measured at 517 nm. by using spectrophotometer (Double Beam UV-VIS-570455). Reference standard compound being used was ascorbic acid and experiment was done in duplicates. Lower absorbance of the reaction mixture indicated higher free radical activity.

The percent DPPH scavenging effect was calculated by using the following equation:-

$$\text{Percent inhibition} = [(A_0 - A_1) / A_0] \times 100 \quad (1).$$

where- A₀ was the Absorbance of control reaction and A₁ was the Absorbance in presence of test or standard sample.

ii. Reducing power assay

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml). The absorbance of the coloured solution was measured at 700 nm by using spectrophotometer (Double Beam UV-VIS-570455). A suitable control was prepared. Ascorbic acid at various concentrations was used as standard reference. Increased absorbance of the reaction mixture indicates increase in reducing power.

iii. Nitric oxide radical scavenging assay

In the experiment, sodium nitroprusside (10mM) in phosphate buffered saline (0.025M, pH-7.4) was mixed with different concentrations (100 - 400µg/ml) of methanolic extract of leaves in methanol and incubated at 25°C for 2 and 1/2 hours. The same reaction mixture without the extract but the equivalent amount of methanol served as the control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 5% H₃PO₄ and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 546nm by using spectrophotometer (Double Beam UV-VIS-570455). Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid were calculated relative to the control. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant and calculated using (1).

iv. Superoxide radical scavenging activity

The activity was evaluated using nitro blue tetrazolium (NBT)-NADH

reduction method given by Nishikimi *et al.*, (1972) [2]

The reaction mixture consisted of 1ml of NBT solution (156µM), 1 ml of (468 µM) of NADH and sample solution at different concentration

s (200-100 $\mu\text{g/ml}$). The reaction was started by adding phosphate buffer (pH 7.4) to the reaction mixture followed by incubation at 25°C for 5 minutes and the absorbance at 560nm was measured against blank by using spectrophotometer (Double Beam UV-VIS-570455). Ascorbic acid was used as the reference standard. Inhibition data (percentage inhibition) were linearized against the concentrations of each extract and standard antioxidant and calculated using (1).

v. Hydrogen peroxide radical scavenging activity

The ability of the fraction to scavenge hydrogen peroxide was determined according to the method of Ruch (1989)[13]. A solution of hydrogen peroxide (2 mM) was prepared in phosphate buffer (0.1M, pH 7.4) and concentration was determined spectrophotometrically at 230 nm. Fractions in methanol (20-100 mg/ml) was added to a hydrogen peroxide solution (0.6 ml, 2mM) and the absorbance of hydrogen peroxide at 230 nm

was determined after 10 minutes against a blank solution in phosphate buffer without hydrogen peroxide by using spectrophotometer (Double Beam UV-VIS-570455). The percentage of scavenging of hydrogen peroxide of fraction and standard compounds was calculated by using (1).

vi. Metal ion chelating activity

The ferrous ion chelating potential of the extract was the estimated by 2,2-bipyridyl competition assay by Yamaguchi *et al.*, (2000) [14] with minor modification. 0.25 ml (1Mm) FeSO_4 solution was mixed with equal volume of extracts (50-250 $\mu\text{g/ml}$). 1ml Tris HCl buffer (pH 7.4) and 0.25ml (0.1%) 2,2-bipyridyl solution were added along with 0.4ml hydroxyl amine HCl and 2.5 ml ethanol. The reaction mixture was adjusted to a final volume of 5ml with distilled water and incubated for 10 minutes at room temperature and the absorbance was measured at 522 nm with EDTA as reference chelating compounds by using spectrophotometer (Double Beam UV-VIS-570455). The chelating activity of the extract for Fe^{2+} was determined using (1).

III.RESULTS AND DISCUSSION

Table I. Qualitative Phytochemical Screening of *Bougainvillea glabra*

Phytochemical	Test	Petroleum ether	Chloroform	Methanol
Alkaloid	Wagner	+	+	-
Cardiac glycosides	Keller-Kelliani	+	+	+
Flavonoids	Alkaline	+	+	+
Phenols	Ferric chloride	-	-	-
Saponins	Foam	-	+	+
Terpenoids	Salkowki	+	+	-
Quinones	-	-	-	-
Tannins	Braymer	-	-	+
Sterols	Liebermann-Burchard	-	-	-
Amino acids and proteins	Ninhydrin	-	-	-
Oxalates	-	-	-	-

where '+' indicates positive and '-' indicates negative.

The results of the preliminary phytochemical analysis showed that terpenoids, flavonoids, alkaloids and cardiac glycosides were present in the petroleum ether and chloroform extract. However, the methanolic extract of the leaves contained tannins to the above mentioned

compounds. Phenols, flavonoids, saponins, tannins and glycosides are known for their ability to scavenge free radicals, in normalizing lipid peroxidation and also for possessing hypoglycemic activity (Table I)

Table II. Phytochemical constituents of *Bougainvillea glabra*

Phytochemical	Quantity
Total Phenolics (Methanol extract)	0.44mg of Gallic acid equivalents/g of extract
Total Flavonoids (Methanol extract)	5.5mg of quercetin equivalents/g of extract
Alkaloids (Crude sample)	3g/100g of crude sample
Saponins (Crude sample)	0.8g/100g of crude sample

i. DPPH radical scavenging assay

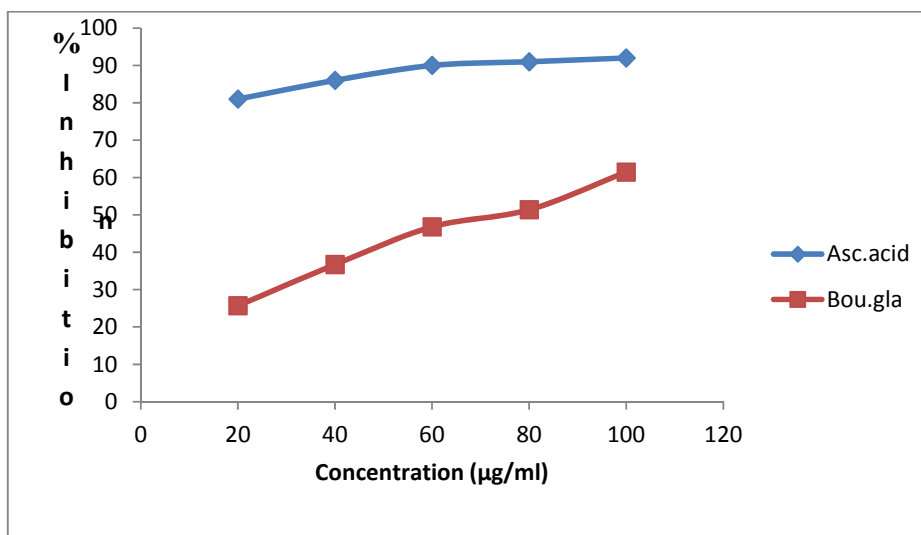


Figure 1. DPPH radical scavenging capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard Ascorbic acid.

The results obtained from the DPPH scavenging assay are shown in Figure 1 for standard (ascorbic acid) and the leaf extract. It was observed that the standard antioxidant (ascorbic acid) showed significantly higher inhibitory effect in comparison to the leaf extract of *Bougainvillea glabra*. However, the percentage inhibition of DPPH radical formation is found to increase in a dose dependent manner. The IC₅₀

values for the methanolic extract was found to be 0.482 mg/ml whereas that of the reference standard was 0.041mg/ml.

DPPH radical scavenging assay is relatively a sensitive method for elucidating antioxidant activity by employing stable DPPH radical to find the total antioxidant capacity. This method has advantage over other methods as it is unaffected by side reactions such as enzyme

inhibition and metal ion chelation (Waltasinghe and Shahidi.,1999) [15].The assessed DPPH radical scavenging activity is an indicator of the presence of plant antioxidants with hydrogen

donating ability. Noticeable decolourization of deep purple colour of the DPPH radicals upon addition of plant extract represents the efficient scavenging potential of the plant extract.

ii. Reducing power assay

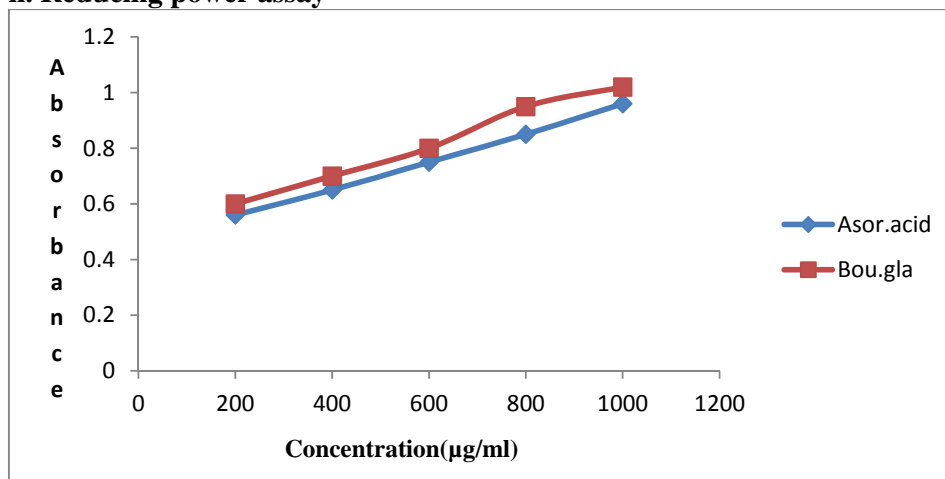


Figure 2. Reductive capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard

Ascorbic acid.

The results of Figure 2 indicate that the methanolic leaf extract of *Bougainvillea glabra* has more reducing power when compared to standard (ascorbic acid). The IC_{50} values for the leaf extract was found to be 0.521 mg/ml and the reference standard was 0.621 mg/ml.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of antioxidant potential of the plant extract. Compounds with reducing power indicate that they are electron donors and can reduce the oxidised intermediates of lipid peroxidation

processes and hence can act as primary and secondary antioxidants.

P.Jayanthi and P.Lalitha [16] have demonstrated the reducing ability of *Eichhornia crassipes* extract in which there was a linear relationship between the concentration and reducing power. It was found that the extract showed greater reducing power than that of standard. The results obtained in the present study also substantiate the above evidence indicating that this plant has the potential of development of useful natural antioxidants.

iii. Nitric oxide scavenging assay

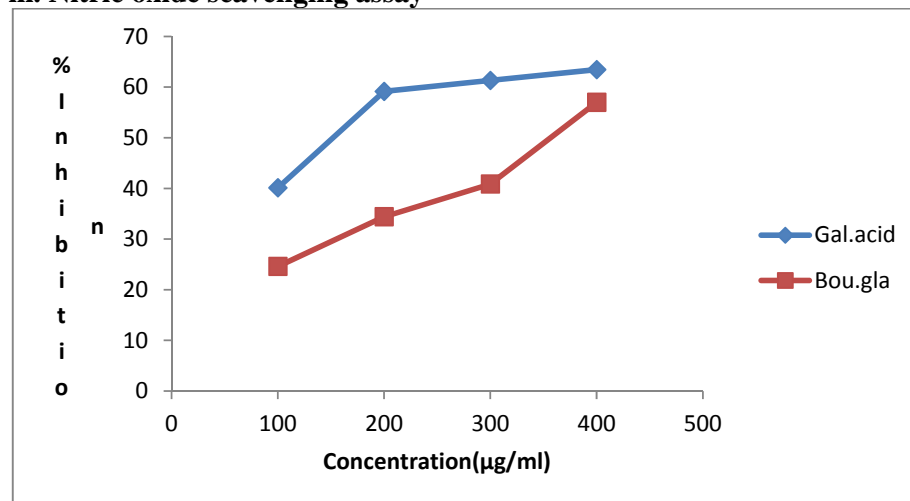


Figure 3 Nitric oxide radical scavenging capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard Gallic acid.

The results of Figure 3 depicts Nitric oxide radical scavenging ability of the methanolic extract of leaves of *Bougainvillea glabra*. The present study indicated that the percentage inhibition increases with increase in concentration of the extract. The IC_{50} values for the methanolic extract was found to be 4.281 mg/ml whereas the reference standard was 0.085mg/ml. Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. If the

concentration of Nitric oxide exceeds more than normal, it reacts with oxygen and superoxide anion to generate cytotoxic nitrite and peroxy anions which trigger inflammatory processes [17]. The inhibition of NO radicals by the extract may offer scientific evidence for the use of plant extract to treat inflammatory disorders. The results of the present study indicate the decrease in NO radical production upon addition of plant extract.

iv. Superoxide scavenging activity

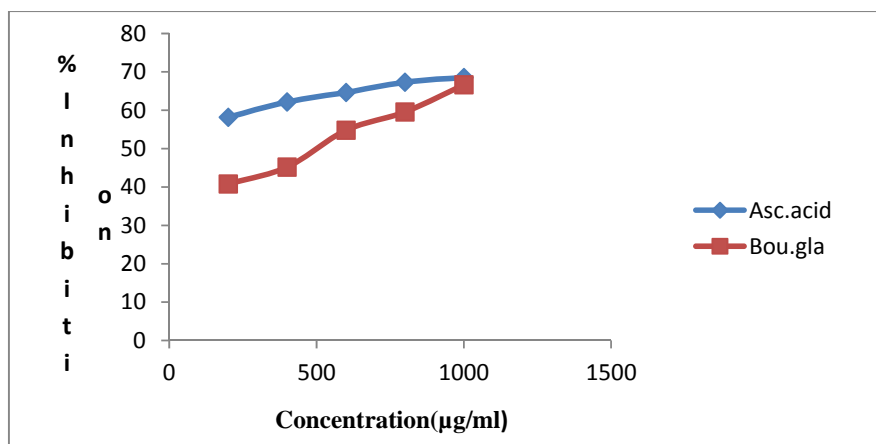


Figure 4 Superoxide radical scavenging capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard Ascorbic acid.

The results showed in the Figure 4 revealed the superoxide scavenging ability of methanolic leaf extract of *Bougainvillea glabra*. The experiments were conducted using ascorbic acid as reference standard. The extract showed significant inhibitory effect on the generation of superoxide anion radicals in in-vitro models. Superoxide radical scavenging activity increases with increase in concentration of extract. However, the percentage of inhibition was less than that of the standard. The IC_{50} values for the

methanolic extract were found to be 0.783mg/ml whereas the reference standard showed 0.705mg/ml.

Superoxide radicals are formed endogenously either by autooxidation or by enzymes and can trigger the formation of dangerous hydroxyl radicals, peroxy nitrile, singlet oxygen and hydrogen peroxides. The plant extracts showed scavenging activity of superoxide radicals which revealed the ability to inhibit the formation of hydroxyl radicals in-vivo.

v. Hydrogen peroxide radical scavenging assay

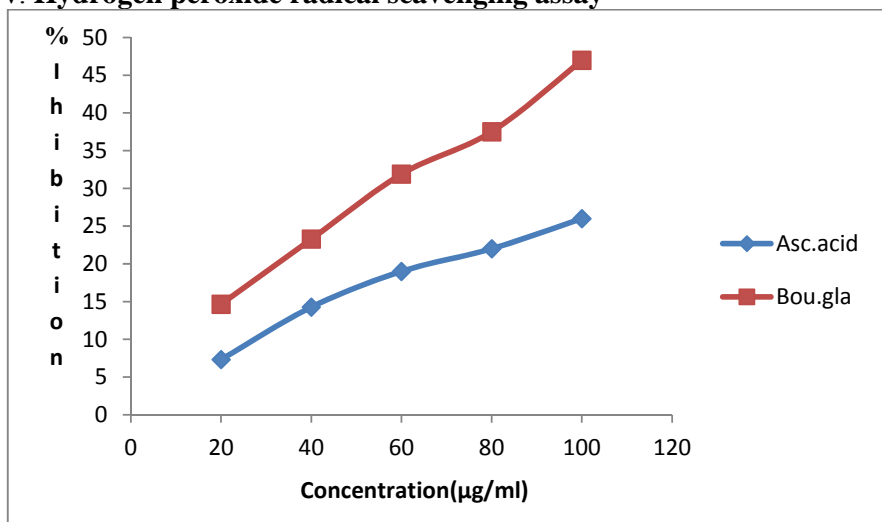


Figure 5 Hydrogen peroxide radical scavenging capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard Ascorbic acid.

Figure 5 illustrates the hydrogen peroxide radical scavenging capabilities of methanolic extract of leaves of *B.gla*. The result obtained in this study imply that the percentage of inhibition of H_2O_2 radical increases linearly with increase in concentration which is more in comparison to that of the reference standard (Ascorbic acid). The IC_{50} values for the methanolic extract was found to be 1.260 mg/ml whereas the reference standard showed 0.317mg/ml.

H_2O_2 is formed as a result of superoxide radicals by superoxide dismutase enzyme. In the system H_2O_2 is detoxified by catalase to water and oxygen. In pathological conditions, its concentration increases significantly. Many

medicinal plants have shown to possess H_2O_2 radical scavenging activity.

Priyanka B *et al* [18] have showed that the ethanolic root extract of *Albizia lebbek* have significant antioxidant potential using H_2O_2 radical scavenging assay.

Arulmozhi.S., Papiya Mitra Mazumder, L. Sathiya Narayanan, Prasad A. Thakurdesai [19] screened the fractions of *Alstonia scholaris* for its antioxidant potential and showed that this plant has potential antioxidant capacity using hydrogen peroxide radical scavenging assay.

The results of the present study also stand as evidence to support hydrogen peroxide radical scavenging ability of the leaf extract of *Bougainvillea glabra*.

vi. Metal ion chelating activity

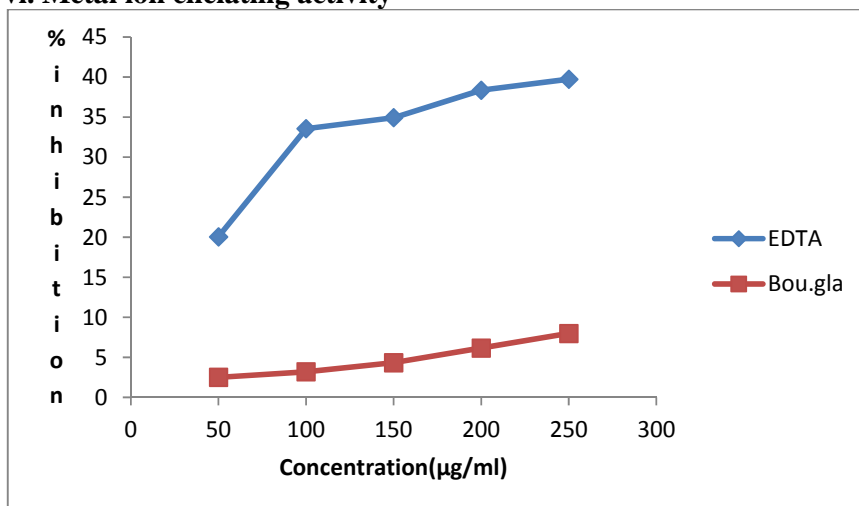


Figure 6 Metal ion chelating capabilities of methanol extract of leaves of *Bougainvillea glabra* and standard EDTA.

Figure 6 depicts the metal ion chelating ability of methanolic extract of leaves of *Bougainvillea glabra*. It was observed that the metal ion chelating ability of the plant extract was found to be much lesser in comparison with that of the reference standard (EDTA) though the percentage of chelation was found to increase with increase in concentration of extract. The IC₅₀ values for the methanolic extract were found to be 0.298 mg/ml whereas the reference standard showed 0.018mg/ml.

The antioxidants present in the plant extract forms a coordinate complex with the metal ions (chelating activity) and inhibits the transfer of electrons. The oxidation reaction is arrested and hence no free radicals are produced.

Silvia Robu *et al* [20] have evaluated the antioxidant potential of some medicinal plants and found it to contain significant antioxidant potential

IV. Conclusion

Over the last few decades, the role of medicinal plants as primary tools in the preservation of health as well as management of diseases is realized with greater concern owing to a large number of people seeking remedies and health approaches free from side effects caused by synthetic chemical drugs consequently adding pressure to look for safe and green alternatives. Even in developed countries like United States, which mostly depends on modern medicines, more than half of the prescribed drugs by doctors every year contain components of natural origin. Thus the study of medicinal plants and drugs mentioned in the traditional systems of medicine in proper scientific perspective is of paramount importance today.

The present study provides scientific evidence for antioxidative and free radical scavenging activity of the leaves of *Bougainvillea glabra* in in-vitro models that are due to various bioactive compounds. Further studies are required to isolate and identify the compounds that are responsible for the various therapeutic activities with proper scientific evaluation and support so that it can be efficiently incorporated into drugs.

VI. References

[1] J.Mariajancyrani¹, G.Chandramohan¹, M.Fathima Beevi, A.Elayaraja, "Preliminary Phytochemical Investigation and Antioxidant Activity of *Bougainvillea glabra* choicy

Leaves", 2013 Scholars Academic Journal of Biosciences (SAJB); 72-76.

[2] N. A. Khalaf, A. K. Shakya, A. Al-Othman, Z. El-Agbar, and H. Farah, "Antioxidant activity of some common plants", 2008 Turkish Journal of Biology; Vol. 32, no. 1, pp. 51–55.

[3] V. R. Patel, P. R. Patel, and S. S. Kajal, "Antioxidant activity of some selected medicinal plants in western region India", 2010 Advances in Biological Research; Vol. 4, pp. 23–26.

[4] A.Kumar, "Phytochemicals investigation on a Tropical Plant, *Syzygium cumini* from Kattuppalayam, 2009 Erode District, Tamil Nadu", Pakistan Journal of Nutrition; 8(1), 83-85.

[4] Vasanthi P, Ganapathy M, Evanjelene V.K., Ayyavuv N., Angamuthu J., "Phytochemical screening and antioxidant activity of the extracts of the leaf and bark of *Albizia lebeck* (Benth)", 2014 Academia Journal of Medicinal Plants; 26-31.

[5] S.Chandra Mohan, V.Balamurugan, S.Thiripura Salini, R.Rekha, "Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*", 2012, Journal of Chemical and Pharmaceutical Research; 197-202.

[6] M. Percival, "Antioxidants, *Clinical Nutrition Insight*", 1998 Advanced Nutrition.

[7] F. Aqil, I. Ahmad, and Z.Mehmood, "Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants," 2006 *Turkish Journal of Biology*; 177–183.

[8] Solomon Charles Ugochukwu, Arukwe Uche I. and Onuoha Ifeanyi, "Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker", 2013 Asian Journal of Plant Science and Research; 10-13.

[9] Harborne, J.B., "Phytochemical Methods London, Chapman and Hall Ltd.; 49-188.

[10] Obadoni BO, Ochuko PO, "Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and

- Delta states of Nigeria”, 2001 Global Journal of Pure and Applied Science; 203-208.
- [11] Singleton VL., Orthofer R., Lamuela-Raventos RM., “Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent”, 1999 Methods Enzymol; 152-178.
- [12] Nishikimi M., Rao N.A., Yagi K., “The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen”, 1972 Biochemical Biophysical Research Communications; 849-854.
- [13] Ruch R.J., Cheng S.J., Klaunig J.E., “Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea”, 1989 Carcinogenesis; 1003-1008.
- [14] Yamaguchi F., Ariga T., Yoshimura Y., Nakazawa K., “Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit rind”, 2000 Journal of Agriculture and Food Chemistry; 180-185.
- [15] Waltasinghe M., Shahidi F., “Antioxidant and free radical scavenging properties of ethanolic extracts of defatted borage (*Berapa officinalis* L.) seeds”, 1999 Food Chemistry; 399-414.
- [16] P. Jayanthi and P. Lalitha, ‘Reducing Power of the Solvent Extracts of *Eichhornia crassipes* (mart.) Solms, 2011 “International Journal of Pharmacy and Pharmaceutical Sciences”; 126-128.
- [17] N. A. Khalaf, A. K. Shakya, A. Al-Othman, Z. El-Agbar, and H. Farah, “Antioxidant activity of some common plants”, 2008 Turkish Journal of Biology; 51–55.
- [18] Priyanka B, Anitha K, Shirisha K, Janipasha Sk, Dipankar B, Rajesh K, “Evaluation Of Anti Oxidant Activity Of Ethanolic Root Extract Of *Albizia Lebbeck* (L.) Benth.”, 2013 International Research Journal Of Pharmaceutical And Applied Sciences (IRJPAS); 93-102.
- [19] Arulmozhi.S., Papiya Mitra Mazumder, L. Sathiya Narayanan, Prasad A. Thakurdesai, “In Vitro Antioxidant And Free Radical Scavenging Activity Of Fractions From *Alstonia Scholaris* Linn. R.Br.” 2010; 18-25.
- [20] Silvia Robu, Ana Clara Aprotosoia, Anca Miron, Oana Cioancă, Ursula Stănescu, Monica Hăncianu , “*In Vitro* Antioxidant Activity Of Ethanolic Extracts From Some *Lavandula* Species Cultivated In Romania” 2012, Farmacia, 394.