ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bougainvillea spectabilis, a good source of antioxidant phytochemicals

Ravi Narayan Venkatachalam, Kanchanlata Singh, Thankamani Marar*

Dept of Biotechnology & Bioinformatics, Padmashree Dr. DY Patil University, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, India.

INTRODUCTION

Uncontrolled generation of free radicals together with reduced level of antioxidative vitamins and enzymes is considered to be the main contributor to oxidative stress. Free radicals attack membrane lipids, protein and DNA, which is believed to be involved in many health disorders. Growing knowledge about the health promoting impact of antioxidants in foods, combined with the knowledge that a number of common synthetic preservatives may have hazardous effects has led to multiple investigations in the field of natural antioxidants. Determination of phytochemical content and radical scavenging assays for evaluation of *in vitro* antioxidant activity of methanolic and aqueous extracts of *Bougainvillea spectabilis* leaves were carried out. It was found that the methanolic extracts showed greater amount of phytochemicals and higher antioxidant activity than aqueous extracts. This observation was supported by the amount of phytochemical constituents and antioxidant activity that exhibited a significant linear relationship. On the basis of our results, bougainvillea appears to have potential for treatment of oxidative stress related diseases. It should, however, be explored as a functional medicinal plant for isolating the active ingredients along with animal studies *in vivo*.

Keywords: DPPH, Bougainvillea, Oxidative stress, Phytochemicals, Tannins, Phenols

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Oxidative stress arises from production of free radicals and subsequent ensuing cascade of reactions [1]. Reactive oxygen species (ROS) are major sources of primary catalysts that initiate oxidation in vivo and in vitro and create oxidative stress which results in numerous diseases and disorders [2].The extreme toxicity of oxygen is related to its high capability of generating free radicals and in turn destroying many major biological molecules. They can attack on lipids and proteins and destroy membranes. ROS can damage DNA and lead to mutation and chromosomal damage. Oxidized cellular thiols abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids [3].

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Antioxidant-based drugs and formulations from plants for the prevention and treatment of oxidative stress related diseases have attracted a great deal of research interest in natural antioxidants [4]. Numerous studies have been carried out on the leaves of plants because they are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, phenols and flavonoids [5]. Flavonoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. [6].

Bougainvillea spectabilis (Common name: Bougainvillea) is a popular woody scandent shrub that grows in tropical and sub tropical forests in India. It contains a number of phytochemicals such as quinones, saponins, triterpenoids, flavonoids, phenols, sterols, glycosides, tannins, furanoids and small amounts of sugars. The alcoholic extract of the leaf has been reported to possess hypoglycemic effect and has been used for the management of Diabetes Mellitus. The hypoglycemic principle of the leaf extract has been isolated and named pinitol [7]. *Bougainvillea* leaf extracts were shown to inhibit tomato spotted wilt tospovirus on capsicum annum and ground water in laboratory tests. Anti-inflammatory activities were also observed in the extracts [8].

Although antioxidant activity has been demonstrated in extracts from leaves of bougainvillea, only a limited number of tests have been performed for its determination. Information pertaining to phytochemicals in leaves of bougainvillea is also scarce. Hence, the present research was designed to determine the phytochemical constituents and in vitro antioxidant activity of methanolic and aqueous extracts from leaves of bougainvillea through a number of testing methods.

MATERIALS AND METHODS

Preparation of Extracts

Fresh, young leaves of *bougainvillea* were collected, authenticated and soaked in water for 3 hours. The leaves were dried at room temperature and ground to a fine powder.

The dry powder was weighed and was used for extract preparation. Extracts for the plant leaves were prepared using both methanol (methanol extract) and distilled water (aqueous extract) as solvents. Using 150 ml of the respective solvent, 30 g of the dry powder was ground to a paste in pestle and mortar and was filtered twice through Whatman filter paper. The resulting filtrate was collected in a beaker and was subjected to evaporation in a Rotary Evaporator for 10 min at 100^{o} C (for aqueous extraction) and 60^{o} C (for methanol extraction). The extracts were diluted appropriately before use.

Estimation of Phytochemical constituents

1. Estimation of total phenol content (TPC)

The total phenol content was determined by Folin- Ciocalteu method [26]. 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added and after 30 min absorbance measured at 760 nm[12]. The total phenol content was expressed in terms of gallic acid equivalent (mg/g).

2. Estimation of total flavonoids (TF)

The total flavonoid content was determined by aluminum chloride method [27]. The reaction mixture comprising of extract, aluminum chloride (1.2%) and potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercitin equivalent (mg/g).

3. Estimation of sugars

Estimation of Sugars in the extract was done by DNSA method [28]. 1 ml of the extract was added to 1 ml DNSA. The contents were mixed and allowed to boil for 5 min. 2 ml of distilled water was added to the mixture and absorbance was measured at 525 nm. Sugar content was expressed in terms of maltose equivalent (mg/g).

4. Estimation of tannins

The tannin content was determined by Folin-Ciocalteu reagent method. 0.5 ml of extract and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. The tannin content was expressed in terms of tannic acid equivalent (mg/g) [12].

5. Estimation of chlorophyll and carotene

1g of leaf sample was weighed and was ground in pestle-mortar with 5 ml distilled water to a paste. The contents were transferred to a centrifuge tube and the total volume

was made up to 10ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5ml of 80% acetone. The contents were centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at the following wavelengths- 645,663,490,638 nm [29] and the content of chlorophyll was calculated.

Evaluation of antioxidant activity

1. α, α-diphenyl- β-picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picryl-hydrazyl by the method of McCune and Johns [30]. The reaction mixture consisted of DPPH in methanol (0.3 mM) and extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm [12]. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).

2. Nitric oxide (NO) radical scavenging assay

3.0 ml of sodium nitroprusside in phosphate buffer (10 mM) was added to extract. The resulting solution was then incubated at 25°C for 60 min. To 5.0 ml of the incubated sample, 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g) [12, 31].

3. Ferric reducing antioxidant power (FRAP) assay

0.2 ml of the extract was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 30 min and the increase in absorbance at 593 nm was measured [12].The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g).

4. Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala [32]. 1.0 ml extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution was diluted with distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. RP was expressed in terms of standard equivalent (mg/g) [12].

5. Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski[33]. The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer, containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH solution (0.936 mM), 1.0 ml extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8). The reaction was started by adding 0.5 ml PMS solution to the mixture, incubated at 25°C for 5 min and then the absorbance was measured at 560 nm. SO anion scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g) [12].

6. Hydrogen peroxide (H_2O_2) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch[34]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution was added to 1 ml extract. The absorbance at 230 nm was determined after 10 mins. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g) [12].

7. Total antioxidant activity

The antioxidant activity was determined by the conjugated diene method [35].Extract was mixed with 2.0 ml of linoleic acid emulsion (10 mM) in sodium phosphate buffer (pH 6.6) and kept in dark at 37°C to accelerate oxidation. After incubation for 15 hr, 0.1 ml from each tube was mixed with 7.0 ml methanol in deionized water (80%) and the absorbance of the mixture was measured at 234 nm against a blank in a spectrophotometer. Total antioxidant activity was expressed in terms of ascorbic acid equivalent (mg/g) [12].

RESULTS AND DISCUSSION

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [9,10]. Synthetic antioxidants like butylated hydroxy anisole (BHA) butylated hydroxy toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [11].

Antioxidative properties of phytochemicals arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions by termination of the Fenton reaction.

In the present investigation, antioxidant activities of methanolic and aqueous extracts of bougainvillea were assessed. *Bougainvillea* contains a number of phytochemicals such as

quinones, saponins, triterpenoids, flavonoids, phenols, sterols, glycosides, tannins, furanoids and small amounts of sugars. The results of preliminary phytochemical screening of aqueous and alcoholic extracts of bouganvillae revealed the availability of multiple polar and non-polar chemical constituents (Table 1).

Table 1: Phytochemical constituents in *Bougainvillea spectabilis* leaf extracts

TESTS	Standard equivalent in methanolic extract (mg/g)	Standard equivalent in aqueous extract (mg/g)
Total Phenol Content	3.17 ± .06	2.85 ± 0.05
Total Flavonoids	5.40 ± 0.56	4.03 ± 0.05
Sugar Content	2.26 ± 0.03	1.81 ± 0.07
Tannin Content	1.48 ± 0..029	1.33 ± 0.03

(The results obtained were expressed as Mean ± S.D. of triplicates).

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS [12].

Tannin is actually an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids**.** This tannin-protein complex can provide persistent antioxidant activity. Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl [13]. Carotenes have the ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. In terms of its antioxidant properties carotenoids can protect the photosystems in one of four ways–by reacting with lipid peroxidation products to terminate chain reactions or by scavenging singlet oxygen and dissipating the energy as heat or by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen or by the dissipation of excess excitation energy through the xanthophyll cycle [14].

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. These methods are popular due to their high speed and sensitivity.

Table 2: Total chlorophyll and carotene content in *Bougainvillea spectabilis* leaves

PLANT	Total Chlorophyll (g/l)	Chlorophyll a (g/l)	Chlorophyll b (g/l)	Carotene (g/l)
Bougainvillea spectabilis	0.017±0.002	0.006±0.001	0.011±0.001	0.419±0.012

(The results obtained were expressed as Mean ± S.D. of triplicates).

Table 2 shows the reducing power and radical scavenging activity of bougainvillea leaves which are directly correlated to antioxidant activity.

Both aqueous and alcoholic extracts produced DPPH anion scavenging power but the methanolic extract showed a far higher scavenging property. DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids [15-17].

The NO scavenging activity in bougainvillea leaves was estimated to be 77.16 ±0.57 mg ascorbic acid equivalent in methanolic extract and 58.66 ±0.52 mg ascorbic acid equivalent in aqueous extract indicating a significant difference between both extracts of bougainvillea leaves. Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds like flavonoids [18].

The FRAP in bougainvillea leaves was estimated to be 27.83 ±1.52 mg ascorbic acid equivalent in methanolic extract and 43.5 ±0.25 mg ascorbic acid equivalent in aqueous extract. This shows a significant difference between both extracts indicating a high FRAP of bougainvillea leaves. The FRAP activity is correlated to catechin, ferulic acid and total phenols which are present in bougainvillea [16, 19].

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [20]. Reducing power is to the measure of the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of extracts [21].

Table 3: Antioxidant activity of *Bougainvillea spectabilis* leaf extracts

Tests	Standard Equivalent in Methanolic Extract (mg/g)	Standard Equivalent in Aqueous Extract (mg/g)
DPPH Scavenging Assay	226.66±2.5	79.16±0.83
NO Radical Scavenging Assay	77.16±0.58	58.66±0.52
FRAP Assay	27.83±1.52	43.5±0.258
Reducing Power Assay	6.30±0.3	4.13±0.321
SO Radical Scavenging Assay	299.16±3.80	321.66±2.64
H_2O_2 Radical Scavenging Assay	11.2±0.17	7.9±0.17
Linoleic Acid Assay	44±0.5	39.66±0.76

(The results obtained were expressed as Mean ± S.D. of triplicates)

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [12]. The RP in 30 g bougainvillea leaves was found to be higher in methanolic extract than in aqueous extract. The reducing power is mainly correlated to the presence of reductones like ascorbic acid [22].

Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [20]. The SO scavenging activity in bougainvillea leaves was estimated to be

299.16 ±3.8 ascorbic acid equivalent in methanolic extract and 321.6 ± 2.6 mg ascorbic acid equivalent in aqueous extracts of bougainvillea leaves. SO scavenging activity is correlated to total flavanoids [12, 23].

Human beings exposed to H_2O_2 indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure. Hydrogen peroxide enters the human body through inhalation of vapor or mist and through eye or skin contact. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. Antioxidants scavenge hydroxyl radicals [12]. The H_2O_2 scavenging activity in 1 gram bougainvillea leaves was estimated to be 11.2 ±0.17 mg ascorbic acid equivalent in methanolic extract and 7.9 ±0.17 mg ascorbic acid equivalents in aqueous extract indicating a significant scavenging activity in bougainvillea leaves. Hydrogen peroxide radical scavenging activity is correlated to the presence of total phenols [24].

The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid. The free radicals then will oxidize the highly unsaturated beta carotene. Consequently, the orange colored chromophore of beta carotene would be degraded. The antioxidant activity in bougainvillea leaves was estimated to be 44 ± 0.5 mg ascorbic acid equivalent in methanolic extract and 39.6 ± 0.76 mg ascorbic acid equivalent in aqueous extract indicating better activity in the methanolic extract. Total antioxidant activity is correlated to TPC [25].

CONCLUSION

The current research shows that *Bougainvillea* leaf extracts possess significant amount of phytochemicals and *in vitro* antioxidant activity. A significant linear relationship between antioxidant activity and phytochemicals, that are responsible for the *in vitro* antioxidant property of the leaf extracts, supported this observation. The methanolic extract demonstrated to be a better candidate in terms of its antioxidant capacity. Analyzing the results, it is clear that the plant is a good candidate for development of new nutraceuticals for treatment of oxidative stress related diseases and the extracts can be prepared in bulk for commercial purpose. Further investigations are on to locate the active principle from the extracts and for their phytopharmaceutical studies.

REFERENCES

[1] Kangralar VA, Patil SD, Bandivadekar RM. International J Pharmaceutical Applications 2010; 1: 38-45.
[2] Rackova L, Kostalova D, Oblozinsky M. J Inflammation 2007; 4: 1-7.
[3] Valko M, Moncol J, Mazur M. Chemico-Biological Interactions 2006; 160: 1-40.
[4] Nagavani V, Rao D, Madhavi Y. Elec J Env Agricult Food Chem 2010; 9: 1471-1484.
[5] Miladi S, Damak M. Journal de la Société Chimique de Tunisie 2008; 10: 101-109.
[6] Deshraj C, Balakrishnan B, Jayakar B. Pak J Pharm Sci 2009; 22: 368-372.

[7] Adebayo J, Adesokan A. Biokemistri 2005; 17: 45-50.
[8] Umamaheswari A, Nuni A. Advances in Biological Research 2008; 2: 1-5.
[9] Chopra RN, Nayar SL, Chopra IC. Council of Scientific and Industrial Research 1956, New Delhi.
[10] Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Biyani MK, Mohan H. Phytochemistry 2003; 63: 97-104.
[11] Barlow SM. Toxilogical aspects of antioxidants used as food additives. In:Food antioxidants, 1st ed. 1990, Elsevier, London, pp 253-307.
[12] Chandha S, Dave R. African J Microbiology Research 2009; 3: 981-996.
[13] Ferruzzi M, Courtney P, Bohm V. J Food Sci 2002; 67: 2589-2595.
[14] Slater A, Scott W. Plant Biotechnology: The genetic manipulation of plants 2008. Oxford University Press, London, pp 229.
[15] Ypuwei Z, Jinlian Z, Yonghong P. LWT Food Sci Technol 2008; 41: 1586-1591.
[16] Thaipong K, Byrne D, Crosby K. J Food Comp Anal 2006; 19: 669-675.
[17] Khalaf N, Ashok K, Shakya A. Turk J Biol 2008; 32: 51-55.
[18] Sonawane I, Dhasade V, Nirmal S. International Journal of Pharmaceutical Sciences and Research 2010; 1: 57-60.
[19] Adebayo J, Ayoade A, Adesokan A. Biokemistri 2005; 17: 45-50.
[20] Blazovics A, Lugasi A, Szentmihalyi K, Kery A. Acta Biological Szegediensis 2003; 47: 99-102.
[21] Gulcin I, Oktay M, Kirecci E, Kufrevioglu I. Food Chem 2003; 83: 371-382.
[22] Li H, Li Y, Wang X. J Food Drug Anal 2008; 16**:** 1-7.
[23] Chen H, Yen G. J Agric Food Chem 2006; 2: 686-694.
[24] Elmastas M, Ibao K, Gulcin I. J Iran Chem Soc 2006; 3: 258-266.
[25] Anwar F, Qayyum H, Iqbal S. Grasas Y. Aceites 2010; 61: 237-243.
[26] McDonald S, Prenzler P, Robards K. Food Chem 2001; 73: 73-84.
[27] Chang C, Wen H, Yang M. J Food Drug Anal 2002; 10: 178-182.
[28] Mohun AF, Cook IY. J Clin Path 1962; 15: 169-180.
[29] Jayaraman J. Estimation of Chlorophyll and Carotenoid. In Laboratory Manual in Biochemistry 2011, New Age International, New Delhi,
[30] McCune L, Johns T. J Ethno Pharmacol 2002; 82:197-205.
[31] Green L, Wagner D, Young V. PNAS 1981; 78: 7764-7768.
[32] Athukorala Y, Jeon Y, Kim K. Food Chem Toxicol 2006; 44: 1065-1074.
[33] Robak J, Gryglewski. J Biochem Pharmacol 1998; 37: 837-841.
[34] Ruch R, Cheng S, Klaunig J. Carcinogenesis 1989; 10: 1003-1008.
[35] Lingnert H. J Food Process Pres 1979; 3: 87-103.