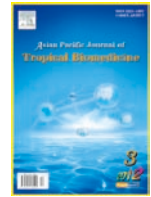




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In vitro antioxidant studies of *Begonia malabarica* Lam. and *Begonia floccifera* Bedd

Velusamy Kalpanadevi, Veerabahu Ramasamy Mohan*

Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin–628008, Tamil Nadu, India.

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ABSTRACT

Objective: To evaluate the total phenolic, flavonoid contents and *in vitro* antioxidant activity of methanol extracts of *Begonia malabarica* Lam. and *Begonia floccifera* Bedd. whole plant. **Methods:** Total phenolic content was estimated using the Folin Ciocalteu method. The flavonoid content was determined using aluminium chloride. *In vitro* antioxidant activities and reducing power capacity were determined using standard methods. **Results:** The total phenolics and flavonoids were estimated. The methanol extracts of whole plants of *Begonia malabarica* and *Begonia floccifera* showed potent *in vitro* antioxidant activities using various models viz, DPPH, hydroxyl, superoxide and ABTS radical scavenging activity. **Conclusions:** The present study confirmed that methanol extracts have potential *in vitro* antioxidant activity. The phytochemical phenolics and flavonoids could be the reason for its antioxidant activity.

1. Introduction

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. In recent years, natural antioxidants, particularly those present in fruits and vegetables have gained increasing interests among consumers and the scientific community. Epidemiological studies have demonstrated that frequent intake of fruits and vegetables are associated with a lower risk of age-related disease such as coronary heart diseases and cancer [1,2]. Natural food usually contains dietary antioxidants that can scavenge free radicals. Some studies have indicated that phenolic substances, such as flavonoids, phenolic acids and tannins are much more potent antioxidants than vitamin C and vitamin E [3]. These phenolic compounds also possess diverse biological functions including antioxidative, antidiabetic, anticarcinogenic, antimicrobials, antiallergic, antimutagenic and antiinflammatory activities [4,5].

In addition to antioxidants present in fruits and vegetables,

another important source of antioxidants is herbs including those derived from traditional medicines, which may possess more potent antioxidant activity than common dietary plants. *Begonia malabarica* Lam (Narayananasanjivi) and *Begonia floccifera* Bedd (Kalthannare) belongs to the family Begoniaceae. Five or six fresh leaves or five to ten grams of powder prepared from the shade dried aerial part of the *Begonia malabarica* is taken as an astringent by the Palliyar tribals of Saduragiri hills, Western Ghats, Tamil Nadu [6]. Kanikkar tribals of Agasthiarmalai Biosphere Reserve, Tamil Nadu, used the fresh leaf juice of *Begonia malabarica* with salt to treat giddiness [7]. The paste prepared from 10 gram of fresh leaves *Begonia malabarica* is applied externally once in a day for one week to treat foot sores by Kanikkar tribals of Agasthiarmalai Biosphere Reserve, Tamil Nadu [8]. The juice prepared from 10 grams of the fresh leaves of *Begonia floccifera* with 200mL of water is taken orally in empty stomach for a period of two days in a single dose to relieve stomach pain by the Kanikkar tribals of Agasthiarmalai Biosphere Reserve, Tamil Nadu [9].

The objective of this study is to find antioxidant activities of whole plant of *Begonia malabarica* and *Begonia floccifera* extracts using various *in vitro* models.

*Corresponding author: V. R. Mohan, Ethnopharmacology Unit, Research Department of Botany, V. O. Chidambaram College, Tuticorin–628008, Tamil Nadu, India.

Tel: +91 9487279902

E-mail: vrmohanvoc@gmail.com

2. Materials and Methods

The whole plant samples of *Begonia malabarica* Lam. and *Begonia floccifera* Bedd. were collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The collected samples were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

2.1. Preparation of Extracts

Ten grams of powdered whole plants of *Begonia malabarica* and *Begonia floccifera* were extracted separately with methanol (100mL) in shaker for 24 h at room temperature. Extract was filtered through Whatman filter paper. The filtrates were subjected to analysis for total phenolic, flavonoid contents and *in vitro* antioxidant activities.

2.2. Estimation of Total phenolic content

Total phenolic content was estimated using the Folin–Ciocalteu method [10]. Samples (100 μ L) were mixed thoroughly with 2 mL of 2% Na₂CO₃. After 2 min. 100 μ L of Folin–Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 gram of dry weight (g/100g–1DW) of the plant samples.

2.3. Estimation of Flavonoids

The flavonoids content was determined according to Eom et al [11]. An aliquot of 0.5mL of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

2.4. DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH–H [12].

The free radical scavenging activity of all the extracts was evaluated by 1, 1–diphenyl–2–picryl–hydrazyl (DPPH) according to the previously reported method [12]. Briefly,

an 0.1mm solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (62.5,125,250,500 &1000 μ g/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbances were measured at 517 nm using a UV–VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation).Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_0 - A_1)/A_0\} * 100\}$$

Where, A₀ is the absorbance of the control reaction, and A₁ is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.5. Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of [13]. Stock solutions of EDTA (1mM), FeCl₃ (10mM), Ascorbic Acid (1mM), H₂O₂ (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of FeCl₃,0.1mL H₂O₂, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (62.5,125,250,500 &1000 μ g/mL)dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37°C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

$$\text{Hydroxyl radical scavenging activity} = \{(A_0 - A_1)/A_0\} * 100\}$$

Where, A₀ is the absorbance of the control reaction, and A₁ is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.6. Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [14]. The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, PH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (62.5,125,250,500 &1000 μ g/mL), and 0.5 mL Tris – HCl buffer (16mM, PH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the

following equation

Superoxide radical scavenging activity = $\{(A_0 - A_1)/A_0\} \times 100$
 Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the test were performed in triplicates and the results were averaged

2.7. Antioxidant Activity by Radical Cation (ABTS. +)

ABTS assay was based on the slightly modified method of Re et al [15]. ABTS radical cation (ABTS.+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use . The ABTS.+ Solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of $100 \mu\text{L}$ of sample or trolox standard to 3.9 mL of diluted ABTS.+ solution ,absorbance was measured at 734 nm by Genesys 10S UV–VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).
 ABTS radical cation activity = $\{(A_0 - A_1)/A_0\} \times 100$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.8. Reducing Power

The reducing power of the extract was determined by the method of Singh et al [16] with minor modification to Oyaizu [17]. 1.0mL of solution containing 62.5,125,250,500 & $1000 \mu\text{g/mL}$ of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980gm (10 minutes at 50°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0mL of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

2.9. Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

3. Results

The total phenolic content of *Begonia malabarica* and

Begonia floccifera were found to be $0.23 \text{ g } 100\text{g}^{-1}$ and $0.70 \text{ g } 100\text{g}^{-1}$ respectively. The flavonoid content of *Begonia malabarica* and *Begonia floccifera* were found to be $2.52 \text{ g } 100\text{g}^{-1}$ and $1.43 \text{ g } 100\text{g}^{-1}$ respectively. *Begonia malabarica* and *Begonia floccifera* whole plant extracts (methanol) exhibited potent *in vitro* antioxidant activity in DPPH radical scavenging, hydroxyl radical scavenging, superoxide radical scavenging, ABTS radical cation scavenging and reducing power in comparison to the known antioxidants, such as ascorbic acid and trolox. The results of the DPPH scavenging activity of *Begonia malabarica* and *Begonia floccifera* whole plant extracts are shown in figure 1. It was observed that methanol extracts of whole plant of *Begonia malabarica* had higher activity than that of the whole plant extract of *Begonia floccifera*. At a concentration of $1000 \mu\text{g/mL}$, the scavenging activity of methanol extract of the whole plant of *Begonia malabarica* reached 96.14% while at the same concentration, that of the *Begonia floccifera* was 63.51%. The results of the hydroxyl radical scavenging activity of *Begonia malabarica* and *Begonia floccifera* whole plant extracts are shown in figure 2. At a concentration of $1000 \mu\text{g/mL}$, the scavenging activity of methanol extract of the whole plant of *Begonia malabarica* exhibited higher activity than ascorbic acid. Superoxide radical scavenging activity of *Begonia malabarica* and *Begonia floccifera* whole plant extracts was studied and compared with ascorbic acid (Figure 3). The superoxide radical scavenging activity of *Begonia malabarica* and *Begonia floccifera* extracts increased with increasing concentration. At a concentration of $1000 \mu\text{g/mL}$, the superoxide radical scavenging activity of methanol extracts of *Begonia malabarica* and *Begonia floccifera* were found to be 81.55% and 62.56% respectively. Results of the ABTS radical cation scavenging activity are present in figure 4. Among the studied plant extracts, *Begonia malabarica* exhibited higher activity (79.11%) at a concentration of $1000 \mu\text{g/mL}$ than trolox. As shown in Figure 5, the reducing power of extracts increased with increase in concentration. The reducing power values of the methanol extracts of *Begonia malabarica* whole plant were slightly higher than that of ascorbic acid. The IC_{50} value of DPPH, hydroxyl, superoxide and ABTS–radical scavenging activities are shown in figure 6.

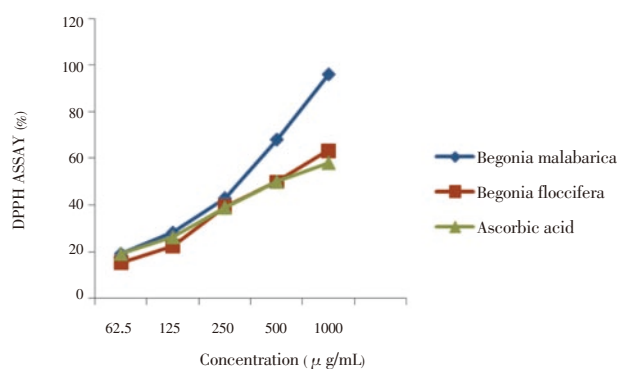


Fig 1: DPPH radical scavenging activity of methanol extract of *Begonia malabarica* and *Begonia floccifera*

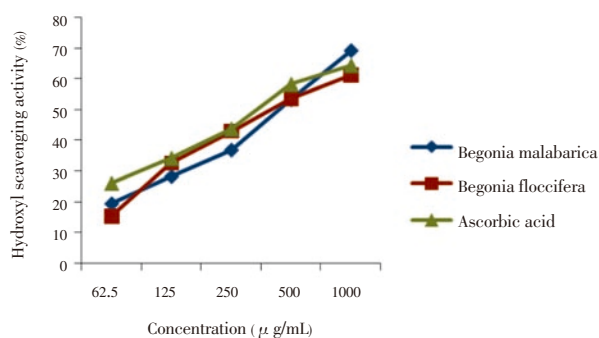


Fig 2: Hydroxyl radical scavenging activity of methanol extract of *Begonia malabarica* and *Begonia floccifera*

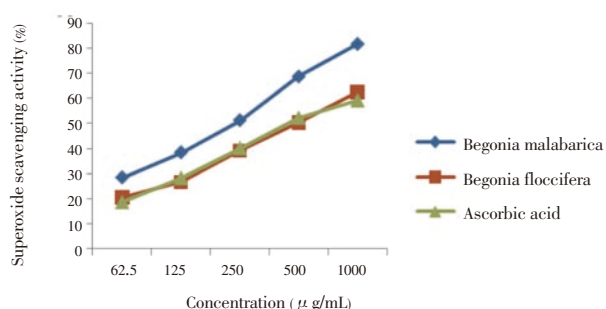


Fig 3: Superoxide radical scavenging activity of methanol extract of *Begonia malabarica* and *Begonia floccifera*

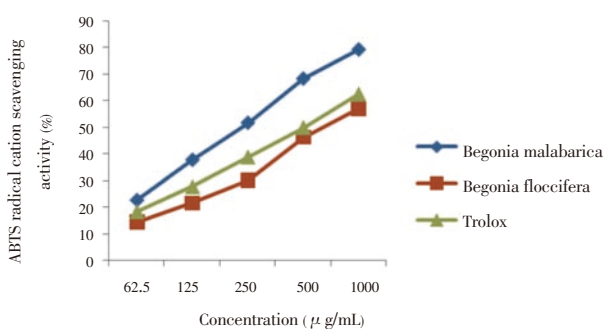


Fig 4: ABTS radical cation scavenging activity of methanol extract of *Begonia malabarica* and *Begonia floccifera*

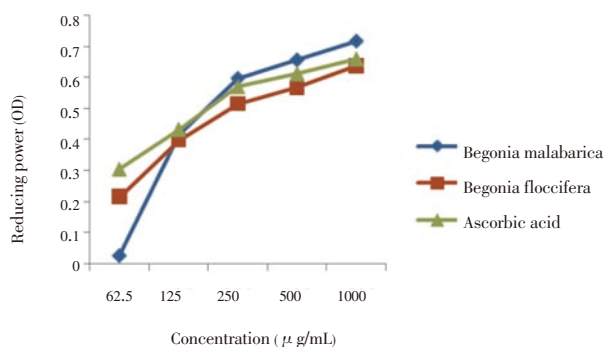


Fig 5: Reducing power ability of methanol extract of *Begonia malabarica* and *Begonia floccifera*

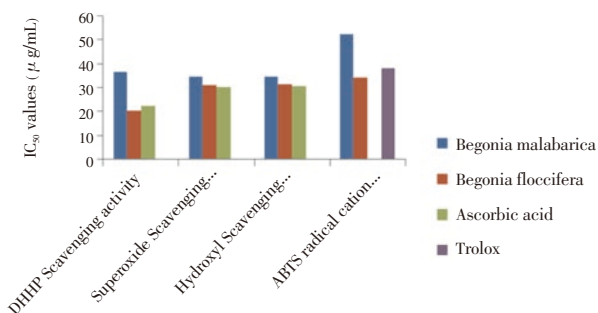


Fig 6: IC₅₀ values of methanol extract of *Begonia malabarica* and *Begonia floccifera*

4. Discussion

The screening of the whole plant of *Begonia malabarica* and *Begonia floccifera* indicates that presence of total phenolic and flavonoid contents which are known to possess antioxidant activities [18–20]. Flavonoids, the major group of phenolics compounds are reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoid health-promoting functions in organism. They are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity [21–23]. The DPPH test provided information on the reactivity of test compounds with a stable free radical. Because of its add electron, 2,2-Diphenyl-Picryl Hydrazyl radical (DPPH) gives a strong absorption band at 517 nm in visible spectroscopy. The efficacies of antioxidants are often associated with their ability to scavenge stable free radicals [24]. It has been shown that the scavenging effects on the DPPH radical increase sharply with the increasing concentration of the samples and standards to a certain extent [20] and hence are said to be strongly dependent on the extract concentration. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acids moieties to cell membrane phospholipids and causes damage to cell [25]. In the present study *Begonia malabarica* and *Begonia floccifera* whole plant extract exhibited concentration dependent scavenging activity against hydroxyl radical in a reaction system. Numerous biological reactions generate superoxide radical which is highly toxic species. Although they cannot directly initiate lipid oxidation, superoxide radical anions are potential precursors

of highly reactive species such as hydroxyl radical and thus study of the scavenging of this radical is important. Superoxide radicals were generated in a PMS–NADH system and assayed by the reaction of NBT [26]. In the present study, whole plant extracts of *Begonia malabarica* showed the highest superoxide radical scavenging activity than the whole plant extracts of *Begonia floccifera* and ascorbic acid. The reduction of the 2, 2'-azinobis (3-ethylbenzothiazoline sulphonate) radical cation (ABTS⁺) has been widely used to measure the antioxidant capacity of natural extracts [27,28]. The reduction of ABTS⁺ with free radical scavengers present in the tested sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734nm. From the result, the methanol extract of whole plant of *Begonia malabarica* possessed the highest ABTS⁺ scavenging capacity whereas the methanol extract of *Begonia floccifera* showed the lowest ABTS scavenging activity. Several reports indicated that the reducing power of bioactive compounds was associated with antioxidant activity [29–32]. Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power. The reducing power of methanol extracts of *Begonia malabarica* and *Begonia floccifera* appeared to be higher. The result was well correlated with amount of phenolic and flavonoid contents present in the plant extracts.

Quantitative phytochemical analysis indicated that the plant contains significant amounts of phenolics compounds such as total phenolic and flavonoids. These classes of compounds were responsible for antioxidant and free radical scavenging effect of plant materials [33–35]. The determination of antioxidant activity of plant extracts is an unresolved problem. The results from different antioxidant assays are even difficult to compare because of the difference in substrates, probes, reaction conditions and quantification methods [36]. There are 20 different indices of antioxidant activity which are currently in use and no single index is considered sufficient to quantify its antioxidant activity [37]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen, flavonoids are wide spread in all natural compounds and possess a broad spectrum of biological activities. The chemical composition of *Begonia malabarica* and *Begonia floccifera* indicated the presence of phenolic compounds including flavonoids, which are known to possess antioxidant activities [32]. The high phenolic and flavonoid contents in *Begonia malabarica* and *Begonia floccifera* may be responsible for its free radical scavenging activity.

On the basis of the results it is concluded that the extracts contain higher quantities of phenolic compounds, which exhibit antioxidant and free radical scavenging activity. *In vitro* assay systems confirm *Begonia malabarica* and *Begonia floccifera* whole plants as natural antioxidants

but it is doubtful that specific components responsible for antioxidant activity. Further *in vivo* assessment also needed to confirm antioxidant assay of *Begonia malabarica* and *Begonia floccifera* whole plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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