Research Article

Antimicrobial and *In vitro* Antioxidant Potential of B*egonia dipetala* Graham

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ABSTRACT

The present investigation evaluates the antimicrobial and *in vitro* antioxidant potential of extracts of *Begonia dipetala*. Antimicrobial activity, DPPH free radical scavenging activity, Superoxide anion scavenging activity, Nitric oxide scavenging activity and Ferric reducing antioxidant power assay were carried out on different concentration of the extracts. Antimicrobial activity was found to be appreciable in ethanolic extract. The DPPH radical scavenging activity was higher (93.3%) when the concentration was increased. The reducing power of the extract increased with the increasing concentration. The *in vitro* antioxidant studies clearly indicate that the ethanolic extract of *Begonia dipetala* has significant antioxidant activity.

Keywords: Antimicrobial, Antioxidant, *Begonia dipetala*, DPPH, Reducing power.

INTRODUCTION

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals and reactive oxygen species can be involved in several numbers of diseases.[1,2] As plants produces a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to development of a phytomedicines to act against microbes. Plant-based antimicrobials have enormous therapeutic as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.[3]

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. Antioxidant activities of polyphenols have been suggested to exert beneficial pharmacological effects on neurological disorders on the basis of *in vitro* studies.[4,5]

Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggest that plant based antioxidants have beneficial effect on prevention of chronic diseases.[6,7] As a result, there has been a keen interest in evaluating the role of bioactive constituents from medicinal plants in reducing the risk of diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others.

Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential.[8]

MATERIALS AND METHODS

Collection of plant material and preparation of extract

The plant materials were collected from Western Ghats of Salem. The plants were washed thoroughly and shade dried for about 15 days, powdered and extracted using different solvents. About 10 g of the plant powder was extracted with 250 ml solvents by using Soxhlet apparatus for 18 hours at a temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No. 1 filter paper and then concentrated by using an evaporator. The residual extracts were stored in refrigerator at 4^0C in small sterile glass bottles.

Antimicrobial screening

About 100mg of ethanolic, petroleum ether and chloroform extracts were dissolved in 1mL of Dimethyl sulphoxide (DMSO) separately which served as the test extracts for antibacterial and antifungal assay.

Test microorganisms

The bacterial cultures used in the study were *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and fungal cultures were *Epidermophyton floccosum, Microsporum gypseum and Trichophyton mentagrophytes*

were procured from Department of Microbiology, Sri Ramachandra University, Porur, Chennai.

Culture medium

Mueller Hinton Agar (MHA) medium was used to study the antibacterial activity and Potato Dextrose Agar (PDA) was used to study the antifungal activity.

Antibacterial activity assay

Antibacterial activity of solvent extracts was determined by well diffusion method on MHA medium. The bacterial culture to be tested was inoculated as lawn culture using sterile swab. Wells were made on to the agar plate using sterile cork borer (5 mm). The extracts were applied to different wells in serially increasing volumes 20µl, 35µL and 50µL using a micro litre syringe. DMSO (Dimethyl Sulphoxide) served as negative control and Ampicillin (10 µg) was used as the reference. The plates were labelled, covered and incubated at 37°C for 24 h.

Anti fungal activity assay

The fungal mycelial suspension was spread on PDA plates and wells were made with 5mm cork borer. The extracts were applied to different wells in serially increasing volumes 20µl, 35µL and 50µL using a micro litre syringe. DMSO served as negative control whereas nystatin (10 µg) was used as the reference. The plates were labelled, covered, and incubated at 28°C for 48 h.

***In vitro* antioxidant activity**

DPPH radical scavenging activity[9]

DPPH scavenging activity was measured by the Spectrophotometric method. To a methanolic solution of DPPH (20 µM), 0.05 mL of the test extracts dissolved in respective solvents at different concentration (50µg, 100µg, 200µg, 400µg). An equal amount of ethanol was added to the control. After 20 min. the decrease in the absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula. BHT was used as standard.

Inhibition (%) = (Control - Test)/control X 100

Superoxide radical scavenging activity[10]

Superoxide radical was generated from the photo reduction of riboflavin and was detected by NBT reduction method. The reaction mixture contained EDTA (6 µM), with 3 µg NaCN, riboflavin (2 µM), NBT (2µM), KH_2PO_4 - Na_2HPO_4buffer (67 mM, pH 7.8) and various concentrations of the extracts in a final volume of 3.0 mL. The tubes were illuminated under incandescent lamp for 15 min. The optical density at 530nm was measured before and after illumination the inhibition of superoxide radical was determined by comparing the absorbance values of the control with that of tests. Ascorbic acid was used as standard.

Nitric oxide radical scavenging activity[11]

Nitric oxide was generated from sodium nitroprusside and measured by Greiss reaction. Sodium nitroprusside in standard phosphate buffer solution was incubated with different concentration (50µg, 100µg, 200µg, 400µg) of the ethanol extract dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 h. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL of Griess reagent (1% sulphanilamide, 2% ortho phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm. The experiment was repeated in triplicates and percentage inhibition was calculated. Ascorbic acid was used as standard.

Reducing power radical scavenging activity[12]

About50µg, 100µg, 200µg, 400µg of the extracts in 1.0mL of ethanol were mixed with phosphate buffer (2.5 mL, 0.2M, pH 6.6) and potassium ferrocyanide (2.5 mL, 1%). The mixture was incubated at 50°C for 20min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000g for 10 min. The upper layer of the solution (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

RESULTS AND DISCUSSION

The petroleum ether, chloroform and ethanol extracts of *B. dipetala* were tested for growth inhibiting activity against three bacterial strains in three varying concentration. The results (Table 1,2 &3, Figure 1) show that the plant possess appreciable amount of antibacterial activity against the strains tested. The ethanol extract of the plant was found to be more active than the rest of the extracts. The zone of inhibition was much comparable to that of the standard. The activity was found to be more on the Gram positive organism (*Staphylococcus aureus*) than the Gram negative organisms tested.

The chloroform extract of the plant was found to inhibit the organism only at the higher concentration (50µl). The activity of both ethanol and chloroform extracts on *Staphylococcus aureus, Salmonella typhi, Escherichia coli* was found to correlate with the earlier reports in the antibacterial activity of *B. malabarica*[13], *B. floccifera.*[14]

The petroleum ether extract was found to be active only against *Staphylococcus aureus* and *Escherichia coli* at the concentration of 50µl. The activity of the extract can be attributed to the presence of higher polar constituents as the ethanol and chloroform extracts were found to be more active than the petroleum ether extract.

Table 1: Antimicrobial activity of the ethanolic extract of *B. dipetala*

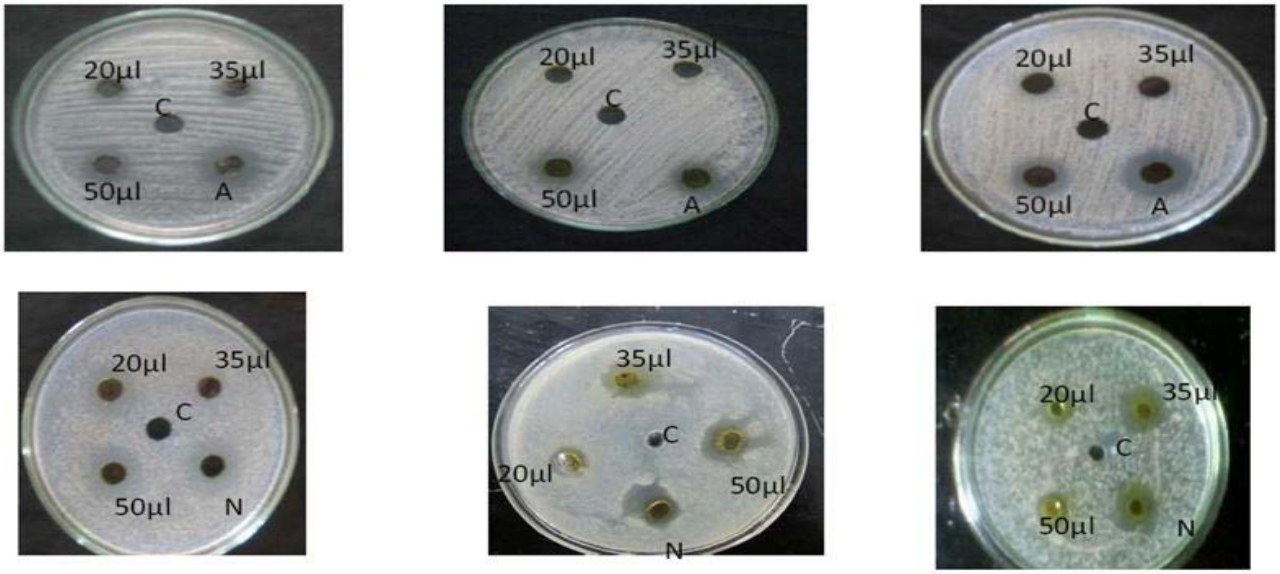
Test Organisms	Zone of inhibition in mm (Ethanolic extract)				
	20µl	35µl	50µl	Ampicilin10µg/mL/ Nystatin10µg/mL	Control DMSO
Staphylococcus aureus	13	21	25	30	-
Salmonella typhi	11	19	20	22	-
Escherichia coli	11	20	22	25	-
Epidermophytonfloccosum	10	11	14	20	-
Microsporumgypseum	9	12	15	20	-
Trichophytonmentagrophytes	10	11	13	16	-

Table 2: Antimicrobial activities of the chloroform extract of *B. dipetala*

Test Organisms	Zone of inhibition in mm (Petroleum ether extract)				
	20µl	35µl	50µl	Ampicilin10µg/mL/ Nystatin10µg/mL	Control DMSO
Staphylococcus aureus	13	17	21	30	-
Salmonella typhi	9	13	16	22	-
Escherichia coli	9	14	15	25	-
Epidermophytonfloccosum	9	10	11	20	-
Microsporumgypseum	11	11	12	20	-
Trichophytonmentagrophytes	11	11	13	16	-

Table 3: Antimicrobial activity of the petroleum ether extract of *B. dipetala*

Test Organisms	Zone of inhibition in mm (chloroform extract)				
	20µl	35µl	50µl	Ampicilin10µg/mL/Nystatin10µg/mL	Control DMSO
Staphylococcus aureus	9	14	18	30	-
Salmonella typhi	-	10	11	22	-
Escherichia coli	11	13	15	25	-
Epidermophytonfloccosum	11	11	12	20	-
Microsporumgypseum	9	10	11	20	-
Trichophytonmentagrophytes	-	-	9	16	-



A: *Staphylococcus aureus;* **B:** *Salmonella typhi;* **C:** *Escherichia coli;* **D:** *Epidermophyton floccosum;* **E:** *Microsporum gypseum;* **F:** *Trichophyton mentagrophytes (A – Ampicilin, C – Control, N – Nystatin).*

Figure 1: Antimicrobial activity of ethanolic extract of B. dipetala

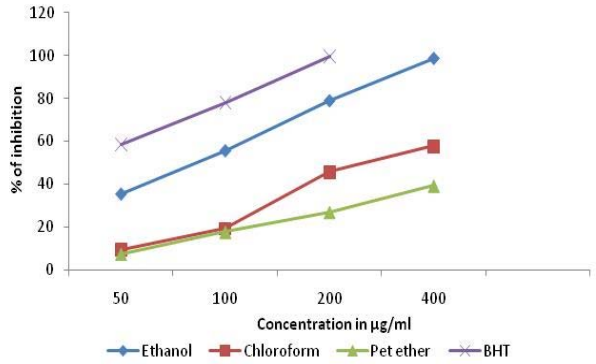


Figure 2: DPPH scavenging activity of extracts of *B. dipetala*

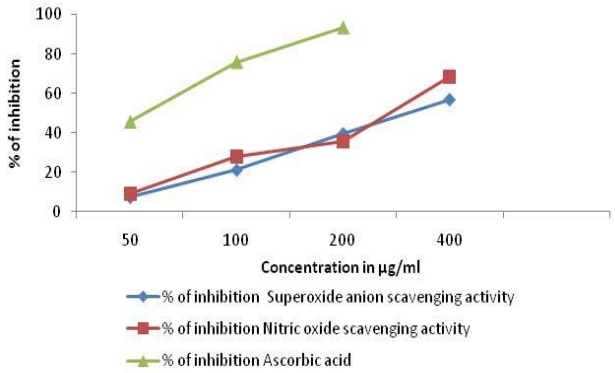


Figure 3: Scavenging activity of ethanolic extract of *B. dipetala*

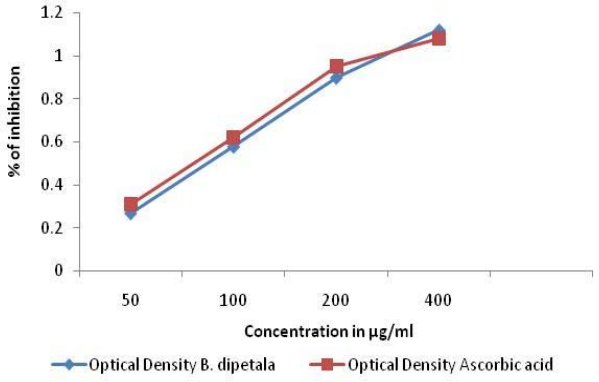


Figure 4: Reducing power assay of ethanolic extract of *B. dipetala*

The petroleum ether, chloroform and ethanol extracts of *B. dipetala* were tested for activity against three fungal strains in three varying concentration. Nystatin was employed as standard to compare the activity of the extracts The ethanol extracts of the plant was found to be active against all the strains and was found to inhibit the growth of *Trichophytonment agrophytes* more than the other two strains.

The chloroform and petroleum ether extract was found to exert only a minimal activity on the fungal strains tested. The petroleum ether extract did not show any inhibition on *Microsporum gypseum* at 20µl and 35µl. On the whole the antifungal activity of the extracts was found to be less significant when compared to the antifungal activity as reported by Suresh.[15]

The reducing power assay of ethanolic extract showed reduction at various concentrations similar to that of standard ascorbic acid (Figure 4). DPPH scavenging activity of the ethanolic extract showed IC_{50} value of 32.34 when compared to that of standard BHT which was 20.3 (Figure 2). Scavenging activity showed IC_{50} Value of 165.45, nitric oxide scavenging activity showed IC_{50} value of 134.20 when compared to that of standard ascorbic acid which was 32.14 (Figure 3).

Antioxidants have been widely used in the food industry to prolong shelf life. However, there is a widespread agreement that some synthetic antioxidants such as butyl hydroxyl anisole and butyl hydroxyl toluene (BHA and BHT, respectively) need to be replaced with natural antioxidants because of their potential health risks and toxicity. Thus, the search for antioxidants from natural resources has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds.[16] Phenolics from natural source such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation. Antioxidants that scavenge free radicals play an important role in cardiovascular disease, aging, cancer, and inflammatory disorders.[17]

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that ethanolic extract of *Begonia dipetala* exhibits high antioxidant and free radical scavenging activities. It also chelates iron and has reducing power. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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