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ISSN 6299 -0975

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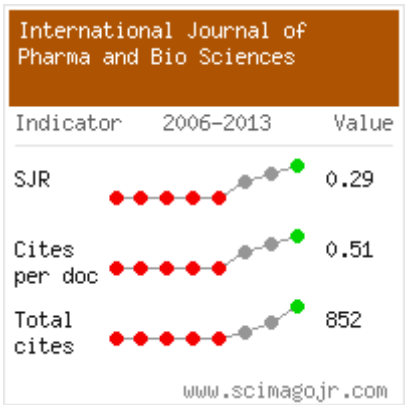

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Int J Pharm Bio Sci 2016 Jan; 7(1): (P) 1 - 6

Research Article **Novel drug delivery sys**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTHOCYANIN FROM *BEGONIA* CULTIVARS AND ITS ANTIOXIDANT POTENTIALITIES

ASWATHY JM[1], DINESH BABU KV[2] AND MURUGAN K[1]*

[1]Plant Biochemistry & Molecular Biology Laboratory, Department of Botany, University College, Thiruvananthapuram, Kerala- 695034
[2]Department of Chemistry, Govt. College for Women, Thiruvananthapuram, Kerala- 695014

ABSTRACT

Plant based antioxidants from fruits, vegetables and flowers have fascinated pharmaceutical industries. Anthocyanins are proven nutraceutical health. *Begonia* species forms diverse hyper group and are distinguished on the basis of morphological parameters. *Begonia rex-cultorum* (Baby rainbow) and *Begonia malabarica* exhibited the highest antioxidant activities. The anthocyanin concentration positively correlates with the antioxidant potentialities among the cultivars. The IC_{50} value related with DPPH and metal chelating activities of the *B. rex-cultorum* (Baby rainbow) extract are 32.3 µg/mL and 18.7 µg/mL respectively. Remarkable scavenging potentialities are displayed against metal chelating, β-carotene bleaching, ABTS radical, FRAP assays and the results are comparable with synthetic antioxidant like BHT. The diversity in radical scavenging in these assays may be due to factors like stereo selectivity of the radicals or due to the differential solubility of anthocyanin molecules in the crude extract. Further studies are warranted to isolate and fractionate the major anthocyanins in the cultivars.

KEY WORDS: ***Begonia*, Antioxidant potential, Anthocyanin, Oxidative stress.**

MURUGAN K
Plant Biochemistry & Molecular Biology Laboratory, Department of Botany, University College, Thiruvananthapuram, Kerala- 695034

INTRODUCTION

Begonia is represented Ca. 1600 species and forms one of the largest plant genus distributed along the wet tropics. *Begonia,* a perennial flowering plant belongs to Begoniaceae. The large size of the genus and its variation makes it as the ideal sample for speciation studies [1]. The highly diverse and hyper genus is featured by diverse morphometric characteristics but, share similar floral morphology with caudex stem base. At the generic level *Begonias* are easily distinguished by its asymmetry of leaf form, succulent petioles, unisexual flowers that are borne within the same inflorescence and winged capsules. Divergent natural selection has promoted speciation in a wide range of taxa. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl 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 pharmaceutical 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 [2,3]. 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 [4]. These phenolic compounds also possess diverse biological functions including antioxidative, antidiabetic, anticarcinogenic, antimicrobics, antiallergic, antimutagenic and antiiflammatory activities [5,6]. 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. Significant demand was noticed related with the natural pigments such as chlorophylls, carotenoids, anthocyanins and betalains colorants among the consumers as ingredients in food. Anthocyanins are water soluble pigments that occur naturally. In plants, they give protection against the harmful UV irradiations, attractants for seed dispersal and pollination and also provide antimicrobial and antiviral activities. Anthocyanins have been used as part of the human diet throughout the history; however, they have gained renewed attention due to their positive health benefits. Naturally, anthocyanin is dominating over the flowers but *Begonia* is an exception where the leaves are brightly coloured with this unique pigment. Anthocyanin content usually varies with the genotypes. Thus, in this scenario the present study aims to evaluate the antioxidant caliber among 5 selected cultivars belonging to three species with their anthocyanin content.

MATERIALS AND METHODS

Plant materials

For the whole attempted work, the fresh healthy plants belonging to three *Begonia* species such as *Begonia heracleifolia* Cham. & Schltdl. and *Begonia malabarica* Lam. and three cultivars of *Begonia rex* (*Begonia rex* 'baby rainbow' L.H.Bailey*, Begonia rex* 'black beauty' & *Begonia rex*'Sir Percy') [7,8] (P.C. 100006, 100007, 100008, 100009,100010) were collected from the department garden. Leaf sample at specific growth stage was selected for the analytical and biochemical analysis.

Estimation of anthocyanin content

1g leaf sample homogenized in 3 ml methanol with 1% HCl and the anthocyanin content was quantified by the standard protocol of Sutharut and Sudarat [9].The absorbance of each dilutions was read at 510 and 700 nm against blank distilled water.

Hydrogen Peroxide Scavenging

Hydrogen peroxide scavenging activity was determined according to a previously described method of Ruch *et al* [10].

The DPPH assay

Antioxidant activity was determined by DPPH assay using the standard protocol of Saeed *et al* [11].

Metal chelating activity

The chelation of ferrous ions was estimated by the method of Dinis *et al* [12]. The absorbance of the solution was thereafter measured at 562 nm.

ABTS radical scavenging activity

ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid assay was performed according to the protocol of Delgado-Andrade *et al* [13].

FRAP assay

The FRAP (Ferric reducing antioxidant power assay) assay was carried as per the proven protocol of Benzie and Strain [14]. The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to ferrous colored form in the presence of antioxidants.

β-carotene bleaching (BCB) test

Antioxidant activity of anthocyanin was determined according to slightly modified version of the β-carotene bleaching method [15].

RESULTS

Anthocyanin content

As the first phase of the study anthocyanin was quantified. *Begonia rex* 'baby rainbow' and *Begonia rex* 'black beauty' possessed highest anthocyanin content compared to other cultivars and is morphologically distinguishable (Table 1). The anthocyanin content among the *Begonia* cultivars ranged between 21.3 to 92.6 mg/g.

Antioxidant Activity
Antioxidant potentialities of the cultivars were evaluated using different assays.

DPPH radical Scavenging activity
DPPH radical Scavenging is due to its hydrogen donating ability. The anthocyanin molecules was able to significantly reduce the stable radical DPPH to yellow coloured DPPH and was comparable to the synthetic standard antioxidants such as trolox and BHT. Highest scavenging activity was observed in *B.rex* cultorum 'Baby rainbow' and least for *Begonia malabarica* (Table 2).

Metal Chelating activity
Metal chelating activity also increased at par with the increase in concentrations of anthocyanin content. The percentage of inhibition of metal chelating varied from 12-90% among the cultivars with different concentrations. The percentage of inhibition of free radicals by various concentrations of sample was more or less equal to that of the respective standard like Trolox and BHT (Table 3).

FRAP Assay
FRAP also repeated similar trend to that of other antioxidant potentialities (Table 4). *Begonia rex* cultorum 'Baby rainbow', *B.malabarica* and *B.rex* cultorum 'black beauty' possessed highest activities.

Hydrogen peroxide scavenging activity
Hydrogen peroxide scavenging activity of anthocyanin at 10 µg/ml concentration was found to be highest for *Begonia rex* 'Balck beauty' ie., 21.8 % followed by *Begonia rex* 'Baby rainbow' and highest activity was observed at 100 µg/ml concentration(74.5%) lower than the standard Trolox (Table 5).

ABTS radical scavenging activity
Anthocyanin showed effective scavenging of ABTS radical cation in a concentration dependent manner. Thus, results in significant decrease in the concentration of $ABTS^{\cdot}$ radical due to the scavenging potentiality of anthocyanin (Table 6).

β- carotene bleaching assay
B. malabarica possessed highest β- carotene bleaching activity with 100 µg concentration (53.8%) and least for *B. heracleifolia* (Table 7).

DISCUSSION

The results of the present study suggested the health-promoting properties of anthocyanin in *Begonia* cultivars in terms of their antioxidant activity, stable over time. Literature data relieved strong correlation between polyphenols and antioxidant activity. For measuring the total antioxidant activity, DPPH, FRAP, ABTS, Beta carotene bleaching, hydrogen peroxide and metal chelating assays were used. Interestingly, remarkable antioxidant nature was noticed among the cultivars with varied levels. The results are comparable with five dietary plants used as medicine for the treatment of inflammatory diseases. Among the five selected plants *Mentha aquatica* was highly active which contained the highest amount of phenolics (337 mg/g) and flavonoids (15.75 mg/g) [16]. Dehpour *et al* [17] proved nutraceutical potential bythe antioxidant properties of *Ferula asafoetida* by various in *vitro* assays such as DPPH radical scavenging, nitrous oxide scavenging showed good nitric oxide scavenging and Fe^{2+} chelating activity. The extracts showed weak reducing power which was not comparable with vitamin C ($p < 0.001$). Similarly, DPPH and TBA assays in four species of *Prangos* using water and methanolic extracts showed marginal antioxidant activity with DPPH assay in both the extracts mean while, high activity was observed in methanolic extract in TBA test compared to the water extract [18]. Further, the ethanolic extracts of *Pimpinella anisoides* were characterized by 23 major compounds. DPPH assay exhibited significant IC_{50} value. NO scavenging potential in terms of IC_{50} values was 102.7 µg/ml [19]. DPPH scavenging activity of butanolic extract of *Ammi visnaga* of Apiaceae was investigated by Bencheraiet *et al* [20]. The extract possessed an equivalent high amount of antioxidant activity of 78.7% at 200µg/ml extract.The FRAP (ferric reducing ability of plasma) assay of some medicinal plants of Lamiaceae and Apiaceae assessed by Gohari *et al* [21] concluded that the antioxidant activity might be due to the presence of flavanoids, rosmarinic acid and coumarins in the plant extracts. The extracts showed considerable antioxidant effect from 16.36 mmol of $FeSO_4$/100 g dry plant equivalents in *Scutellaria tornefortii* (ethyl acetate extract) to 404.12 mmol of $FeSO_4$/100 g dried plant in *Salvia macrosiphon* (methanol extract). *In vitro* studies of antioxidant potentialities in three species of *Annona* suggest that *A.muricata* possessed potent antioxidant activity than the leaf extracts of *A.squamosa* and *A.reticulata. A.muricata* showed maximum scavenging activity (90.05%) of ABTS radical cation. *A.reticulata* showed better activity in quenching DPPH(89.37%). *A.squamosa* showed least inhibition except in hydroxyl radical (79.79%) [22]. Ethanolic extract of *Spaeranthus indicus* showed maximum cation radical scavenging (ABTS) up to 41.99% followed by DPPH (33.27%), superoxide dismutase (25.14%) and nitric oxide radical (22.36%) [23]. Results indicated that alcoholic extracts of *Medicago sativa* is an effective antioxidant in relation with the presence of polyphenols and flavanoids in the extracts [24]. *In vitro* antioxidant studies in methanolic extracts by various methods proved that *Mentha arvensis, Moringa oleifera* plant materials are potent. *Trigonella foenum-graecum, Tamarindus indica* have moderate and *Amaranthus viridis, Spinacia oleracea* have mild antioxidant activity or free radical scavenging activity [25]. Guava (*Psidium guajava* L.) fruit methanolic extract exhibited comparable antioxidant activity with ABTS ((3-ethyl-benzothiazoline-6-sulfonic acid)), DPPH (2,2'- diphenyl-1-picrylhydrazyl), FRAP (Ferric reducing antioxidant power assay) and ORAC (oxygen radical absorption capacity) assays. Antioxidant activity measured in dichloromethane extract in guava fruit extract was low (2% of total) compared to antioxidant activity measured in methanol extract. Ascorbic acid and

phenolics were the major contributors to antioxidant activity in guava fruit [26]. Antioxidant potentialities of *Arachis hypogaea* in the peal using DPPH and ABTS assays revealed highest activity in acetone and methanolic extracts respectively [27]. The results obtained suggest that anthocyanins in *Begonia* cultivars are responsible for the antioxidant activity.

Table 1
Anthocyanin content among Begonia cultivars

Sl.no.	Varieties	Monomeric anthocyanin pigment (mg/g)
1	*Begonia rex* 'baby rainbow'	69.64
2	*Begonia heracleifolia*	42.74
3	*Begonia rex*'Sir Percy'	21.37
3	*Begonia malabarica*	22.88
5	*Begonia rex* 'black beauty'	92.67

Table 2
DPPH radical - scavenging activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex -cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
5	17.2	24.3	27.5	10.5	16.4
10	23.6	38.6	42.2	22.3	25.9
20	40.1	45.7	59.5	30.6	32.5
40	50.8	59.6	68.3	41.5	45.6
60	67.6	73.4	79.7	50.6	52.7
80	70.3	80.2	83.6	60.3	63.7
100	80. 7	87.4	90.3	68.9	70.2
Trolox (100 µg/ml)	96				
BHT(100 µg/ml)	94				

Table 3
Metal chelating activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex -cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex – cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
5	22.8	27.0	26	12	18
10	33.7	39.0	35	24	26
15	41.9	46.5	45	29	35
20	48.4	54.9	51	34	41
25	56.5	61.7	57	40	47
30	60.2	70.5	62	45	51
35	65.5	79	66	52	58
40	70.8	83	70	58	64
45	74.7	88	78	61	71
50	79	90	83	70	75
Trolox (100 µg/ml)	98				
BHT(100 µg/ml)	97				

Table 4
FRAP assay activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex -cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex – cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
1	12.1	24.2	21	15	19
2	20.4	37. 6	32	22	24
3	33.5	48	39	28	37
4	45	59	45	34	40
5	54.5	64	55	45	51
6	62.5	70	63	57	59
7	70.5	76	70	60	67
8	79.1	82	74	68	72
9	80.2	85	80	71	74
10	92.9	100	92	85	90
Trolox (100 µg/ml)	90				
BHT (100 µg/ml)	100				

Table 5
Hydrogen peroxide scavenging activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex -cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex – cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
5	8.3	15.3	17.5	5.8	7.3
10	14.4	20.5	21.8	11	10
20	19.8	25.8	29.4	14.3	15.5
40	20.5	33.4	38.5	17.5	19.8
60	25.6	47.8	53.4	19.3	21.4
80	39.8	59.3	67.8	23.7	29.5
100	45.5	66.4	74.5	32.2	37.8
Trolox (100 µg/ml)	77.9				
BHT(100 µg/ml)	77.5				

Table 6
ABTS radical - scavenging activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex – cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex – cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
10	12.8	10.5	9.3	3.5	8.9
20	20.7	15.8	12.4	8.7	13.4
40	29.8	22.5	20.3	14.6	20.8
60	35.5	30.3	27.6	22.8	33.4
80	44.6	40.2	35.3	30.5	40.2
100	53.8	49.5	42.6	38.7	50.5
Trolox (100 µg/ml)	60.9				
BHT (100 µg/ml)	67.5				

Table 7
β- carotene bleaching activities of Begonia cultivars

Concentrations (µg/ml)	*B.malabarica*	*B.rex -cultorum* 'baby rainbow'	*B.rex-cultorum* 'black beauty'	*B.rex – cultorum* 'sir percy'	*B.heracleifolia*
	% inhibition	% inhibition	% inhibition	% inhibition	% inhibition
10	12.8	10.5	9.3	6.2	3.5
20	20.7	15.8	12.4	11.3	8.7
40	29.8	22.5	20.3	19.2	14.6
60	35.5	30.3	27.6	25.5	22.8
80	44.6	40.2	35.3	31.2	30.5
100	53.8	49.5	42.6	40.3	38.7
Trolox (100 µg/ml)	50.9				
BHT(100 µg/ml)	57.5				

CONCLUSION

Morphologically the cultivars showed variation among each other and showed variation in anthocyanin content, with the *Begonia rex* 'baby rainbow' and *Begonia rex* 'black beauty' possessing highest anthocyanin content which is morphologically distinguishable. Anthocyanin was found to be an effective antioxidant in different *in vitro* assays when compared to the standard antioxidants. This preventive effectiveness of anthocyanin may be related to the existence of a relationship between the content of anthocyanin to the antioxidant activity resulting in cellular defenses. The extracts of *Begonia* may have excellent potential as functional ingredients representing potential source of natural antioxidant. Further studies are warranted to fractionate and identify the lead molecule responsible for this.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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