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Estimation of phenolic contents and anti-oxidant activity of *Begonia trichocarpa*

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ABSTRACT

This work was designed to give a taxonomical description, estimation of anthocyanin content, phenol content and antioxidant activity of Begonia trichocarpa dazll belong to Begoniaceae. The taxonomical description shows leaves are 8-10 x 3-5 cm, ovate-oblong, acuminate, obliquely cordate at base, hirtus, petiole 3-4 cm long, reddish, succulent, male and female flower with perianth segments, Ovary Subglobose, to 3.5 mm long, with semi-obovate wings on either sides. The leaf and stem contain 23.71% and44.08% of anthocyanin content respectively. The phenolic content of methanol extract was found to be high with 46.96% followed by chloroform 17.5%, ethyl acetate 12.89% and petroleum ether 8.12%. A dose dependent anti oxidant activity was found in both DPPH free radical scavenging method and Nitric acid scavenging method. DPPH assay of methanol extract and ethyl acetate extract shows maximum % inhibition 53.0% and 50.93% at the concentration of 400µg/ml respectively, where as ascorbic acid exhibit 70.36% and Ic50 values were 335.23µg/ml, 370.74µg/ml and 16.8407 µg/ml respectively. Nitric acid scavenging activity of methanol extract and ethyl acetate extract shows maximum % inhibition 46.53 % 27.36 % and ascorbic acid exhibit 53.34% ,Ic25 values were found 150.87 µg/ml,509.16 µg/ml and ascorbic acid 0.633 µg/ml.

Key words: Perianth segments, Anthocyanin content, Phenol content, *Begonia trochocarpa* Dalzell

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INTRODUCTION

Human had been aware of importance and relationship between plant and human body, effects of herbs on mind and emotions. In recent years there has been renewed interest in Herbal medicine has been increasing daily due to its preventive nature, it may be due its anti oxidant property of phytoconstituents which is present in medicinal plants. The body provides with antioxidants like proteases, lipase, transferase, and DNA repair enzymes to repair free radical damage. Over production of free radical lead in sufficiency of endogenous antioxidants and here increased importance of exogenous antioxidants. Endogenously enzymes defenses the body from oxidative stress besides this, exogenous antioxidants are also defenses the cells from oxidant stress. Non- enzymatic (exogenous) anti-oxidants are supporters of enzymatic anti oxidants, carotenoids, Vitamin C, vitamin E, plant polyphenols like flavanoids, anthocyanins are supplied though food and suspend free radical chain reactions.[1-2]

Begonia trochocarpa Dalzell (Family: *Begoniaceae*) is a genus of perennial flowering plants, the genus contains 1,795 different plant species [3]˙ Ethnomedically, the leaves of *Begonia trichocarpa* plant is used by local

people for the treatment of various infections with avoidance of any scientific evidence. Traditionally it is used for the treatment of throat infection such as sore throat, opacity of the eyes, wound healing, skin infections. This is the Rare, Endangered and Threatened (RET) plants of Kerala compiled by The Kerala Forest Research Institute (KFRI), Peechi, Kerala with information from various institutions and scientists [4-5].

The present study aim to gives an identity for this plant through Taxonomical description and estimation of phenolic principle of *Begonia trichocarpa* Dalzell related to its anti oxidant activity

MATERIALS AND METHODS

Plant collection and identification

Whole plant of *Begonia trichocaropa* was collected from the rural areas of Kottayam district

Kerala, India. The whole plant material was identified, authenticated by Head of the department, Post Graduate and Research Department of Botany, supported by FIST (DST, Govt. of India) & SARD (KSCSTE, Govt. of Kerala), St Thomas College, Pala, Kottayam.

Evaluation of taxonomical characters

The fresh plant was observed for various taxonomical characters such as leaf, stem and flowers as per the procedure [6].

Preparation of extracts

Begonia trichocarpa leaf was washed, shade dried, powered and successively extracted with Hexane, petroleum ether, chloroform, ethyl acetate and methanol and water (in the order of increasing polarity).The solvents were removed completely under reduced pressure and a semi solid mass was obtained[7].

Estimation of Total phenol content

Total phenol content of different extracts of *Begonia trichocarpa* was determined by using spectro photometric method (Singleton et.al.,1999)[8].Stock solution of 1mg/ml solution of sample was as prepared. The reaction mixture of different extracts were prepared by mixing 0.5ml of methanol solution of extract, 2.5ml of 10% Folin-ciocalteu's reagent dissolved in water and 2.5ml 7.5% $NaHCo_3$.The samples were there after incubated in a thermostat at 45 for 45mts.The absorbance was measured at λ max 765nm.The same procedure was repeated for standard solution of gallic acid and the calibration curve was construed. Based on the measured absorbance, the concentration of phenol was read (mg/ml) from the calibration curve, then the content of phenols in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).The blank was prepared in the same procedure without the sample.

Estimation of Anthocyanins content from stem and leaf *Begonia trichocarpa*

Estimation of total Anthocyanine content was carried by pH differential method(Aswathy J M 2015)[9-10].1gm of leaf was digested and homogenized with 3ml methanol and 3ml 1%HCl .The homogenized mixture was vortexed for 30sec.and kept it in water bath at 60^0C for 20minutes.The mixture was centrifuged for 10 minutes at 1000 rpm, The supernatant was separated in a 10 ml volumetric flask. Repeat the centrifugation with 3ml methanol till the methanol get colourless, mixed all the supernatant together and made up to 10ml with methanol.1ml of the sample was diluted with for preparing two dilutions of the sample, one volume with adjusted with potassium chloride buffer pH 1.0 and the other with sodium acetate buffer pH 4.5.kept the dilution for 15 minutes. Readings was taken at 510 nm and700 nm and blank was done with water. All the measurement were made in 15 and 1 hr. Calculate the reading of absorbance of the diluted sample (A) using the formula

$$A = (A_{510}-A_{700})\ pH1.0-(A_{510}-A_{700})\ pH4.5$$

The Monomeric anthocyanins content was calculated as mg/L from the formula

$$\text{Total Anthacyanins content} == (A \times MW \times DF \times 1000)/(\varepsilon x1)$$

Where MW is molecular weight (449,2),DF is dilution factor, / is the molecular absorptivity (26.900) and converted to mg/g

Estimation of anti oxidant activity in vitro methods
Estimation of anti oxidant activity of methanol and ethyl acetate was performed by the method of DPPH radical scavenging activity and Nitric oxide radical scavenging activity

DPPH free radical scavenging activity [11]
Stock solution of 60M DPPH was prepared in methanol. Different concentrations of 50µ/ml, 100 µ/ml, 200µ/ml, 400µ/ml sample solutions of methanol extract and ethyl acetate extract was prepared by dissolving in DMSO. 3.9ml of DPPH solution was mixed with 100 µl of test solution at different concentrations. The sample was kept for 15 minute at room temperature and decrease in absorbance was measured. Ascorbic acid is used as standard and Methanol was used as blank. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula.

$$\% \text{ Inhibition} = [\ (B-T)\ ] / C \times 10$$

B = absorption of blank sample (t= 0 min)

C = absorption of test extracts solution (t=15 minutes) T = absorption of test solution

Nitric oxide radical scavenging activity[12]
Determination of nitric oxide radical scavenging activity was determined by sodium nitroprusside method (marcocci et al., 1994.) Two ml of 10mM sodium nitroprusside dissolved in 1.0 ml phosphate buffer saline (pH 7.4)is was prepared and mixed with 1.0 ml of sample at various concentration(100-400µgm/ml). The mixture was incubated at 29^{o}C, after 150 minute of incubation.0.5ml of the incubated solution was mixed with 1.0 ml of Griess reagent and incubated at room temperature for 30 minutes, the absorbance at 546nm was measured. Ascorbic acid was used as standard. The amount of nitric oxide radical inhibition was calculated by using following this equation:

$$\% \text{ inhibition of NO radical } [A_0 - A_1]/A_0\ 100$$

Where A_0 is the absorbance before reaction and A_1 absorbance after reaction has taken place with Griess reagent.

RESULTS AND DISCUSSION

I. Taxonomical evaluation
Taxonomical studies of *Begonia trichocarpa* was carried out to study detailed taxonomical characters of the *Begonia trichocarpa*, through it gives an detailed description about the plant. The informations are given below.
Habitat: Evergreen and semi-evergreen forests at an altitude of 800-1200 meters.

Description: Annual sub erect hispid herbs; stem reddish brown, flaccid, sub-erect, hirtus.

Leaves are 8-10 x 3-5 cm, ovate-oblong, acuminate, obliquely cordate at base, hirtus, membranous, sinuate-serrate, 5-nerved from the base, ribs prominent at ventral side, depressed at dorsal side; petiole 1-3 cm long, hispid; stipule 6 x 2 mm, lanceolate; petiole 3-4 cm long, reddish, succulent

Flowers: in few flowered cymes, 2 cm across; peduncle 1-2 cm long. Male and female flowers are seen together in the same peduncle.

Male flower: solitary, perianth segments 4, glabrous, ovate, outer segments 0.5 × 1 cm, inner one 5-6 mm.

Stamens numerous, 42-49, yellow, monadelphous, in short staminal tube, filaments short, anthers yellow, extrose, basifixed.

Female flowers: Perianth segments 3, unequal, oblong or obovate, obtuse, acute or acuminate at apex, pinkish-red, smaller one 3-5mm, other two 6 mm-1cm. Styles 3, rather stout, to 2.5 mm long, glabrous; stigma broad, crenate at margins.

Ovary: Subglobose, to 3.5 mm long, with semi-obovate wings on either sides, united below the ovary and tapering into the pedicel, abruptly apiculate at apex, one face furnished with a keel, decurrent along the united wings, minutely glandular hairy, 2-celled; placenta 2 in each cell. Capsules: 1-2 cm, obovate, truncate at apex, wings are acute, seeds minute, many, hexagonal. Flowering and fruiting: August-December.

IUCN status: Vulnerable.

fig .1. B.trichocarpa with flowers

fig 2.Female flower

fig.3. Perianth segments and ovary

fig .4.splited ovary with stigma

fig .5.Split capsule

fig.6. Male flower

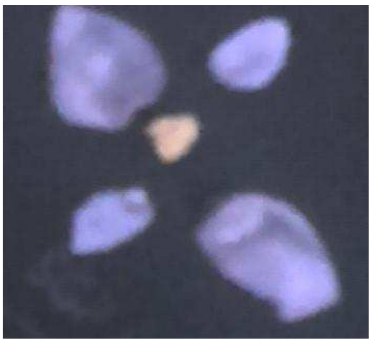

fig.7 .Perianth segments

fig.8.Monadelphous stamen

fig.9.Anther

II.Phenolic content of different extracts of B.trichocarpa

The phenol content of the B.trichocarpa was estimated spectrophotometric methods and reading are expressed in mg/of gallic acid in grams. The estimation of MEBT showed the presences of 49.96 mg of phenol content, followed by CHBT contain 17.63 mg and 12.89 mg, PEBT with 8.12mg of phenol present in the leaf. Methanol showed high phenol content as compare with PEBT. High phenol content of methanol gives in

signal for its corresponding antioxidant activity.

Table 1 .Total Phenol content of B.trichocarpa leaf

Extracts	Optical density at max 765 nm	Concentration of phenol in mg of GA/g of extract
MEBT	0.444	49.96 ±0.005774
CHBT	0.194	17.63 ±0.088191
EABT	0.162	12.89 ±0.005774
PEBT	0.123	8.11 ±0.0120

III. Estimation of anthocyanin content of stem and leaf of b.trichocarp

The anthocyanine content was estimated by differential pH method and found in the leaf contain 23.71mg/gm and in the stem 44.08mg/gm of anthocyanins. The morphological comparison of leaf shows very light purple colour over the greenish colour of leaf, but the stem was appeared as purplish in colour The anthocyanin content present in the stem was more when compared with leaf. The anthocyanin content of the leaf was hidden under the chlorophyll of the leaf.

Table 2. Observations of estimation of Anthocyanins of stem and leaf Begonia trichocarpa

Samples in buffers	Absorbance at 510nm buffer pH1.0 pH4.5	Absorbance at 700nm buffer pH1.0 pH 4.5	Calculated A value	Concentrations in mg/L	Total Anthocyanins in mg/g
leaf	0.203 0.059	0.050 .048	0.122	237.1241	23.71
stem	0.293 0.083	0.0.54 0.054	0.239	449.450	44.08

IV.Anti oxidant activity of B.trichocarpa

1. DPPH radical scavenging activity of methanol extract and ethyl acetate extracts of B.trichocarpa

DPPH radical scavenging activity of methanol extract (MEBT) and ethyl acetate(EABT) was given.(Table 3) The methanol extract and ethyl acetate extract shows maximum % inhibition 53.0% and 50.93% at the concentration of 400µg/ml respectively, where as ascorbic acid exhibit 70.36%. Ic50 values were 335.23µg/ml, 370.74µg/ml and 16.84 µg/ml respectively.

Table 3.DPPH radical scavenging activity of MEBT and EABT

Concentration(µg/ml)	Percentage inhibition of MEBT	Percentage inhibition of EABT	Percentage inhibition of Ascorbic acid
50	21.47± 0.2273	10.56± 0.29759	35.56± 0.2275
100	34.45± 0.3095	31.35± 0.22729	43.55± 0.3934
200	42.43± 0.2272	37.45± 0.22727	57.73± 0.2273
400	53.00± 0.2279	50.77± 0.1488	70.36± 0.1488

2. NITRIC ACID SCAVENGING ACTIVITY OF MEBT and EABT

Nitric acid Radical scavenging activity of MEBT and EABT was performed using Griess reagent was listed in table below. The concentration depend activity was observed. The methanol extract and ethyl acetate extract shows maximum % inhibition 46.53% and 27.36% at the concentration of 400µg/ml respectively,where as ascorbic acid exhibit 53.08%. Ic25 values were found 150.87 µg/ml, 509.16 µg/ml and ascorbic acid 0.633 µg/ml.

Table 4 Nitric oxide radical scavenging activity of MEBT AND EABT

Concentration(µg/ml)	Percentage inhibition of MEBT	Percentage inhibition of EABT	Percentage inhibition of Ascorbic acid
50	12.31± 0.111	9.45± 0.72789	17.40± 0.05160
100	21.14± .0.01172	14.24± .18321	29.25± 0.08406
200	33.79± 0.07280	21.01± 18721	42.11± 0.04093
400	46.53± 0.145611	27.36± .17833	53.34± 0.04093

CONCLUSION

Methanol and ethyl acetate extract of *Begonia trichocarpa* has shown marked dose dependent Anti oxidant activity in both DPPH free radical scavenging method and Nitric acid scavenging method. Total phenol content of different extracts of *Begonia trichocarpa* was estimated; out of this methanol extracts contain 49.96% of phenol content and 23.71% anthocyanin content present in the leaf. The anti oxidant activity of *Begonia trichocarpa* may be due the high phenol content and the presence of anthocyanin in the leaf give a supporting evidence for this. The taxonomical description gives an identity for *Begonia trichocarpa*

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