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PRELIMINARY STUDIES ON PHYTOCHEMICAL PROPERTIES AND ANTIMICROBIAL ACTIVITY OF *BEGONIA TRICHOCARPA*

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ABSTRACT

In the present study, the *Begonia trichocarpa* plant was collected, dried in shade and observed for various morphological characters. Transverse sections of leaf, petiole, stem and fine powder of leaf were observed for their microscopical characters. Evaluation of physical characters of leaf powder was carried out. Coarse powder of *Begonia trichocarpa* leaf and stem were extracted with solvents such as Hexane, Petroleum ether, Chloroform, Ethyl acetate Methanol and Water. The extracts were subjected to preliminary phytochemical screening. TLC of different extracts was carried out and flavonoid content of the leaf extracts was estimated. Antimicrobial activity of leaf extracts was evaluated against four strains of bacteria, *Psudeomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and two fungi viz *Candida albicans* and *Aspergillus niger*. Significant results were obtained from the morphological, microscopical and fluorescence evaluation. In the preliminary phytochemical evaluation, the presence of flavonoids, steroids, lipids, carbohydrates, amino acids, coumarin and oxalic acid were found. Estimation of flavonoid content revealed the presence of significant quantity of flavonoids in petroleum ether extract. In the antimicrobial evaluation, a dose dependent inhibitory activity was found. In the TLC analysis, three to five spots were observed in different extracts. These results are useful for the further investigation in the future.

Key words: *Begonia trichocarpa*, Phytochemical evaluation, Antimicrobial activity

INTRODUCTION

For thousands of years, cultures throughout the world have used plants or plants extracts for medicinal purpose. Many drugs commonly used today were developed from plants used in traditional medicinal systems. Several drugs have been developed following ethno botanical leads and a large proportion of commercial drugs used today to treat a variety of diseases including cancer were originally discovered through research on plants[1, 2]. The ethno medicinal plants may provide unique bio active compounds[3]. *Begonia trichocarpa* Dalzell is one among them, belongs to the family Begoniaceae generally distributed in the evergreen and semi evergreen forests is commonly found in the Western Ghats. It is also widely found in the Idukki, Kottayam, Kollam and Thiruvananthapuram districts of Kerala. It is included in the list of rare endangered and threatened (RET) plants by Kerala forest research institute (KFRI), Peechi, Kerala[4]. Traditionally, the rural population of Kerala uses the leaf extract of this plant for the management of throat infections. In Malayalam, it is known as 'Kalle puli'. It is known in the name of 'Khatadya' among the Pawra tribe of Satpura hills, Maharashtra, India. They use the leaf juice of this plant for the opacity of eyes[5]. It was found that this plant has not yet been evaluated for phytochemistry and bio-efficacy properties. Therefore, the present study was designed to evaluate the phytochemial properties and antimicrobial activity of this plant, an attempt to provide a direction for further research.

MATERIALS AND METHODS

Plant collection and identification

Whole plant of *Begonia trichocarpa* was collected from the rural areas of Kottayam District, and taxonomic identification was done in the Department of Botany, St. Thomas College, Pala, Kottayam, Kerala, South India.

Evaluation of morphological characters

The collected plant was dried in shade and observed for various morphological characters such as size, shape, surface characteristics, texture, fracture and appearance of cut surfaces including organoleptic characters.

Evaluation of microscopical characters

The plant parts were soaked in water overnight. Transverse sections of the leaf, petiole and stem were taken and stained with phloroglucinol & Hcl (1:1) solution and mounted on a glass slide by using glycerin and covered with cover slip. Different microscopical characters were observed under microscope.

Evaluation of powder characters

The fine leaf powder of *Begonia trichocarpa* was evaluated for powder microscopical characters. The powder sample stained with phloroglucinol & Hcl (1:1) solution was mounted on a glass slide using glycerin and covered with a cover slip was observed under the microscope.

Evaluation of physical characters

Ash values, extractive values, loss on drying, swelling and foaming index were determined by the procedure of[6]. Analysis of fluorescence of leaf powder was carried out as per the procedure of [7, 8].

Preparation of extracts and preliminary phytochemical screening

Coarse powder of *Begonia trichocarpa* leaf and stem were extracted in soxhlet apparatus assembly successively for 72 h

with Hexane, Petroleum ether, Chloroform, Ethyl acetate Methanol and Water. The extracts were subjected to preliminary phytochemical screening according to the method of [9].

Estimation of flavonoid content

Flavonoid content of the leaf extracts of *B. trichocarpa* was estimated by the procedure of [10].

Thin layer chromatography

TLC of different extracts of *B. trichocarpa* was carried out as per the procedure of [11]

In vitro antimicrobial activity

Extracts of *B. trichocarpa* were tested against four human pathogenic bacteria viz. *P. aeruginosa, E. coli, K. pneumoniae, S. aureus* and two fungi viz *C. albicans* and *A. niger*. The microbes were procured from MTCC, Chandigarh, India. Muller Hinton Agar and Sabouraud Dextrose Agar were used as the media for bacteria and fungi respectively. Antibacterial and antifungal activity of 25, 50 and 100 µgm/ml of the extracts of *B. trichocarpa* was evaluated by well plate method. Control plates were also maintained. Gentamycin and Clotrimazole were used as control drugs for bacteria and fungi respectively. Plates for antibacterial activity were incubated at 37°C for 24h whereas the plates for antifungal activity were kept at room temperature for 48h. After incubation, zones of inhibition were measured in mm and recorded.

RESULTS

Whole plant of *Begonia trichocarpa* was collected (Figure 1) and dried in shade and evaluated for various morphological characters. The results are shown in table 1. Results of analysis of loss on drying, foreign matter, ash values, extractive values, foaming and swelling index are shown in table 2. Results of fluorescence analysis of leaf powder of *B. trichocarpa* are shown in table 3. In the preliminary phytochemical evaluation, the hexane, petroleum ether and chloroform extracts of leaves and stems of *B. trichocarpa* showed the presence of lipids. The hexane, chloroform and ethyl acetate extracts of leaves and stems showed the presence of steroids. The ethyl acetate and methanol extracts of leaves showed the presence of lipids and the methanol extracts of leaves indicated the presence of steroids. The methanol and water extracts of leaves and stems indicated the presence of coumarin. All extracts except the hexane and petroleum ether extracts of stem indicated the presence of flavonoids. Gum, mucilage, alkaloids, volatile oil, glycosides, saponins and tannins were not found in the preliminary phytochemical evaluation. Presence of oxalic acid, non reducing sugar and amino acids were observed in methanol and water extracts of stem and leaves (Table 4). Evaluation of flavonoid content in the leaf extracts revealed that chloroform and ethyl acetate extract showed the content of 1319.5µg/ml individually and petroleum ether and methanol extracts indicated the quantity of 2842 and 1624 µg/ml respectively (Table 5). Results of TLC analysis of leaf extracts were shown in Table 6.

Transverse sections of the leaf, petiole and stem were evaluated. The transverse section of leaf showed a multi-stranded vascular system. Totally six, three larger and three smaller size vascular bundles were observed. Larger vascular bundles were located along the basal part and smaller vascular bundles were located on the upper part. The vascular bundles were collateral and consist of wide circular, thin walled xylem elements which rarely located near the meta-xylem and the rest were phloem elements. The phloem elements located near meta- xylem elements were 20 µm wide. The lamina was thick and simple in structure. The mesophyll tissue consists of a single layer of short, wide conical palisade cells and an ad axial layer of small spherical mesophyll cells. The marginal part of the lamina was thick, blunt and straight. The epidermal cells of the lamina were appeared highly dilated.

Petiole is appeared in planoconvex in sectional view. The petiole was 900 µm in vertical plane and 2.3 µm in the horizontal plane, adaxial side was flat and semicircular. The epidermal layers of the petiole consist of smaller thin walled square shaped cells. The ground tissues are parenchyma cells which were evenly arranged. The petiole consists of six independent vascular strands arranged in a circle.

Results of analysis of the transverse section of stem showed that the epidermis consists of single layer, straight walled, elongated parenchyma cells with thick cuticle. Cork and cortex contain parenchyma cells, calcium crystals were seen in cortex region. Cambium and open collateral vascular bundles were observed. Larger pith consists of parenchyma cells with prism type calcium oxalate crystals and abundant starch grains were observed (Figure 2).

Results of powder drug evaluation showed that the presence of anisocytic stomata, phloem fibre, xylem vessels of both spiral and annular thickening spongy mesophyll, calcium oxalate crystals of squares and prism type. It appeared as single squares or along with parenchyma cells (Figure 3). Regarding with antimicrobial activity, a dose dependent activity was found. The zone of inhibition was increased with increase in concentration of drug extract. All extracts in the quantity of 100 µl showed a higher inhibition. Petroleum ether showed a maximum growth inhibition of 18 mm against *P. aeruginosa,* which was followed by methanol extract 17 mm, Chloroform extract 15 mm and ethyl acetate extract 14 mm zone of inhibition. Both ethyl acetate and chloroform extracts showed a zone of inhibition of 15 mm against *E. coli* followed by methanol and petroleum ether extract showed 12 mm zone of inhibition. *K. pneumoniae* was highly inhibited by methanol extract with a zone of inhibition of 15 mm followed by ethyl acetate 13 mm, petroleum ether and chloroform extract showed 12 mm zone of inhibition individually. The *S. aureus* was highly inhibited by ethyl acetate extract with zone of inhibition of 16 mm. The methanol extract showed 14 mm and the petroleum ether and chloroform extracts showed 12 mm zone of inhibition individually. In case of fungi, the petroleum ether extract showed a zone of inhibition of 26 mm followed by chloroform, methanol and ethyl acetate extracts showed 25 mm, 24 mm and 18 mm respectively against *A. niger*. The methanol and petroleum ether extract showed 20 mm zone of inhibition individually against *C. albicans* which was followed by chloroform and ethyl acetate extracts showed 18 mm and 16 mm zone of inhibition respectively against the same organism (Table 7).

Table 1: Morphological parameters of *B. trichocarpa*

Parameters	Plant parts			
	Stem	**Leaf**	**Root**	**Flower**
Colour	Pink	Upper: Waxy green	White/Pink	M: Pinkish white
		Lower: Baby pink		F: Whitish pink
Odour	Odourless	Odourless	Odourless	Indistint
Taste	Sour	Sour	Slightly bitter	Slightly bitter
Size	2-4 m	Length: 10 - 11 cm	Length: 20 cm	M: 3-4 mm
		Width: 5.5 - 6 cm	Width: 0.5-1 cm	F: 7-9mm
Shape	Cylindrical	Cordate, Acute, Asymmetrical, Unicostate venation	Straight/Sucker	Perianth segments M: 4 No F: 3 No
Touch	Smooth	Waxy	Fiberous	Smooth
Fracture	Fibrous	-	Fibrous	-

M- Male flower; F- Female flower; No- Number

Table 2: Physical evaluation of *B. trichocarpa*

Parameters	Values (%w/w)	
	Leaf	**Stem**
Foreign matter	Absent	Absent
Loss on drying	9.3038 ± 7350	10.2267 ± 0.1446
Ash values		
Total ash	5.2028 ± 2952	9.9995 ± 0.5326
Acid insoluble ash	1.5670 ± 0.0168	1.9836 ± 0.0081
Water soluble ash	3.6133 ± 0.0315	2.5529 ± 0.0139
Sulphated ash	11.5837 ± 0.0736	12.0756 ± 0.1049
Extractive values		
Petroleum ether	1.2266 ± 0.1101	0.8742 ± 0.1150
Chloroform	0.7701 ± 0.0100	0.6400 ± 0.0916
Methanol	1.3682 ± 0.1126	0.9057 ± 0.0050
Ethyl acetate	0.5344 ± 0.0110	0.3741 ± 0.0255
Swelling index	Absent	Absent
Foaming index	Absent	Absent

Table 3: Fluorescence analysis of *B. trichocarpa* leaf powder

Treatment	Observation		
	Day light	**Short wavelength UV light**	**Long wave length UV light**
Powder as such	Green	Dark green	Dark green
Powder+Con.HCL	Yellowish green	Dark green	Green
Powder+Con.HNO_3	Green	Light green	Brown
Powder+Con.H_2SO_4	Green	Dark green	Brownish green
Powder+NaOH (1N) in water	Olive green	green	Green
Powder+ Powder+NaOH (1N) in Methanol	Dark green	Olive green	Dark green

Table 4: Preliminary phytochemical evaluation of *B. trichocarpa* extracts

Tests	Hexane		P. ether		Chloroform		Ethyl acetate		Methanol		Water	
	L	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**
Carbohydrate	-	-	-	-	-	-	-	-	+	+	+	+
Reducing sugar	-	-	-	-	-	-	-	-	-	-	-	-
Non reducing sugar	-	-	-	-	-	-	-	-	+	+	+	+
Oxalic acid	-	-	-	-	-	-	-	-	+	+	+	+
Amino acids	-	-	-	-	-	-	-	-	+	+	+	+
Coumarin	-	-	-	-	-	-	-	-	+	+	+	+
Gum	-	-	-	-	-	-	-	-	-	-	-	-
Mucilage	-	-	-	-	-	-	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	-	-	-	-	-	-	-
Saponin	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-	-	-	-	-
Lipids	+	+	+	+	+	+	+	-	+	-	-	-
Steroids	+	+	-	-	+	+	+	+	+	-	-	-
Flavonoid	+	-	+	-	+	+	+	+	+	+	+	+

L-Leaf; S-Stem; + Present; - Absent

Table 5: Flavonoid content of leaf extracts of *B. trichocarpa*

Extracts	Absorbance	Quantity (µgm/ml)
Chloroform	0.090	1319.5
Petroleum ether	0.180	2842
Methanol	0.109	1624
Ethyl acetate	0.092	1319.5

Table 6: TLC profile of different extracts *B. trichocarpa* leaf

Extracts	Solvent system	Number of spots observed			Rf values
		UV	Day light	I_2	
Petroleum ether	Ch : Ea (7 : 3)	2	3	3	0.7, 0.90, 0.98
Chloroform	Bz : Ea (8.5 : 1.5)	4	5	5	0.06, 0.41, 0.35, 0.51,0.96
Ethyl acetate	Ch : Ea (7 : 3)	4	5	5	0.18, 0.30, 0.75, 0.85, 0.98
Methanol	Ch : Ea (7 : 3)	3	4	4	0.38, 0.77, 0.94, 0.98

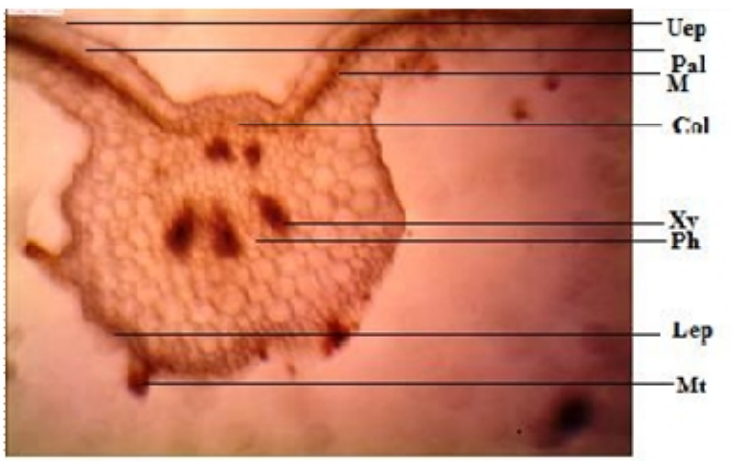
Ch – Chloroform; Ea – Ethyl acetate; Bz - Benzene

Table 7: Antimicrobial activity of different extracts of *B. trichocarpa* leaf

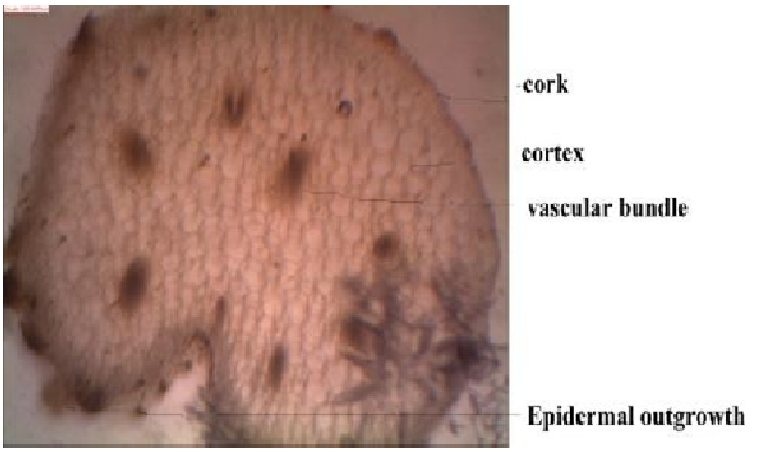
Microorganism	Methanol extract (µg/ml)				Ethyl acetate extract (µg/ml)				Petroleum ether extract (µg/ml)				Chloroform extract (µg/ml)			
	25	50	100	C	25	50	100	C	25	50	100	C	25	50	100	C
A. niger	11	14	24	28	10	12	18	20	10	23	26	11	11	15	25	21
E. coli	10	11	12	20	10	11	15	25	10	11	12	27	11	13	15	27
S. aureus	10	11	14	26	10	11	16	25	NA	NA	12	28	10	11	12	27
K. pneumoniae	11	12	15	40	11	12	13	36	10	11	12	40	NA	11	12	38
P. aeruginosa	11	14	17	20	11	12	14	40	12	14	18	39	11	13	15	41
C. albicans	12	14	20	24	11	12	16	24	11	13	20	26	12	15	18	31

All values are expressed in mm

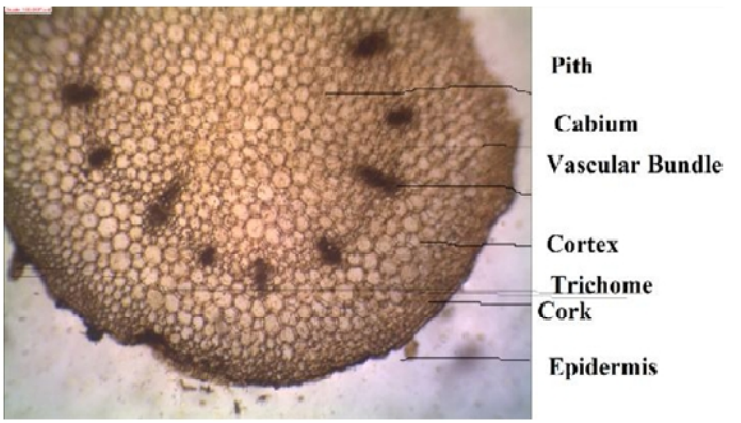
Figure 1: Whole plant of *Begonia trichocarpa*



Leaf

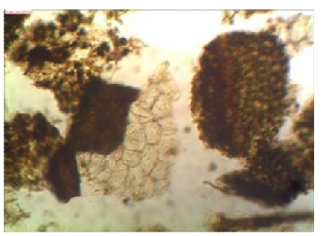


Petiole

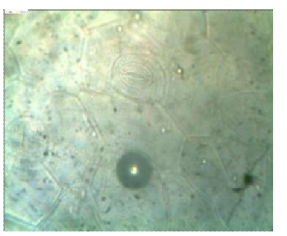


Stem

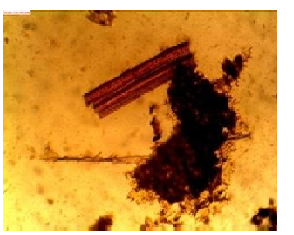
Figure 2: Transverse section of *B. trichocarpa*



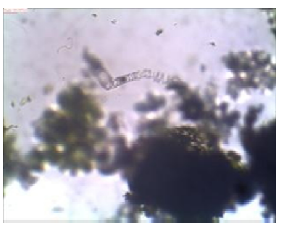
Stomata with epidermal cells



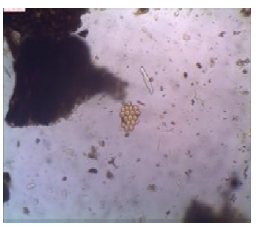
Stomata



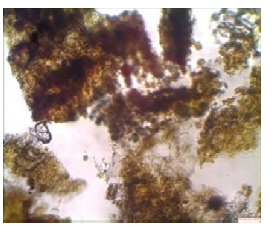
Xylem vessels (annular)



Xylem vessels (spiral)



Spongy mesophyll



Calcium oxalate crystals

Phloem fibre

Figure 3: Powder drug evaluation of *B. trichocarpa*

DISCUSSION

In the present study, morphological, physical, phytochemical and antimicrobial properties of *B. trichocarpa* were evaluated. Various significant data were obtained from the results of present study. Analysis of previous literature reports [3, 5, 12] indicated the ethno botanical importance, phytochemical and pharmacological properties of different *Begonia* species. In the present study, the phytochemical evaluation of leaf extracts of *B. trichocarpa* revealed the presence of flavonoids in the majority of selected extracts. Steroids and lipids were also present significantly. Presence of carbohydrate of non reducing type, oxalic acid, amino acids and coumarin were also identified. Analysis of flavonoid content showed the presence of flavonoid in all the selected extracts. But the petroleum ether extract contains a significant quantity comparing with other extracts. In the antimicrobial evaluation, a dose dependent inhibitory activity was found. All the selected extracts in 100 µg/ml dose showed a significant anti microbial activity. In case of antibacterial activity, the petroleum ether, methanol and chloroform extracts showed a significant activity against the bacteria, *P. aeruginosa*. In case of antifungal activity, the petroleum ether extract showed a significant activity against the selected fungi, *A. niger* and *C. albicans*.

CONCLUSION

Begonia trichocarpa is a noted herb having a significant ethno medical importance in central and southern parts of Kerala and also in the certain tribal community belongs to Maharashtra. In the present study, morphological, physical, phytochemical and antimicrobial properties of this herb were evaluated. Of course, the outcome of this study would provide a good platform for further research which may give more valuable results.

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