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ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *IN VIVO* GROWN PLANTS AND *IN VITRO* RAISED PLANTLETS IN *DENDROBIUM CREPIDATUM* LINDL. & PAXT. OF BANGLADESH

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Abstract

The present investigation was conducted in both *in vivo* grown plants and *in vitro* raised plantlets of *Dendrobium crepidatum* Lindl. & Paxt. with a view to assessing antioxidant and anti-inflammatory activities. Antioxidant activity was performed for methanolic crude extracts of leaf, stem, and root parts with DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay. The methanolic root extract of *in vivo* grown plants showed the highest scavenging activity (87.22%) as compared to the highest scavenging activity of standard antioxidant Ascorbic acid (98.59%); whereas *in vitro* raised plantlets showed 55.52% of scavenging activity at 250 μgml^{-1} concentration. Anti-inflammatory activity was evaluated using heat induced egg albumin denaturation assay. Amongst different parts of *in vivo* grown plants, methanolic leaf extract showed the highest inhibition of egg albumin (92.86%) as compared to standard anti-inflammatory agent, Acetylsalicylic acid which showed 94.74% inhibition. On the other hand, *in vitro* developed plantlets showed 85.71% inhibition. The results focussed on the potential of *D. crepidatum* as the source for both biological activities including antioxidant and anti-inflammatory actions.

Introduction

ORCHIDS WITH over 28,484 species in approximately 850 genera represent one of the most advanced and largest families of angiosperms with innumerable hybrids and varieties (Govaerts *et al.,* 2017). These orchids are distributed around the globe except for the freezing Antarctic region and deadly hot desert areas. They have extremely high floricultural appeal because of the extraordinary beauty of highly enchanting flowers with their an incredible range of variations in floral shape, size, colouration, and fragrance. *Atharva Ved* was the first to present documentary evidence for medicinal uses of orchids followed by *Charak Samhita*, *Sushrut Samhita*, and *Kashyap Samhita*. A copy of *Charak Samhita* was sent to Bagdad for the princess of Barmecides tribe, from where it went to China (Kaushik, 1983, 1985, 2019). These are also used as source of food, glues, gums, narcotics, essences, and perfumes (Chinsamy *et al*., 2014; Lawler, 1984). Heptacosane and Octacosanol isolated from *Vanda roxburghii* roots were found to have anti-inflammatory activity (Chawla *et al*., 1992). Various phytochemicals with high biological activity including carotenoids, flavonoids, phenolics, and their derivatives were found in orchid extracts that showed powerful antioxidant activities (Hoque *et al*., 2021; Joseph *et al*., 2018; Sanjana *et al.,* 2021; Sharma and Pathak, 2020; Stajner *et al*., 2010). The antioxidant activities of these compounds are based on scavenging diverse reactive oxygen species (ROS) including peroxyl radicals, hydroxyl radicals, hypochlorous acid, superoxide anions and peroxynitrite, thus protecting the human body against oxidative damages (Chao *et al*., 2014).

Dendrobium crepidatum Lindl. & Paxt. is an epiphytic orchid, confined to an altitude range of 600-1400 m. This species has medicinal as well as ornamental value (Chen and Ji, 1998); it is widely used in traditional Chinese medicine for the treatment of cancer, diabetes, cataracts, and fever (Lam *et al*., 2015). The present study was carried out so as to determine antioxidant and anti-inflammatory potential in both *in vivo* grown plants and *in vitro* raised plantlets of *D. crepidatum.*

Material and Methods

Collection of Plant Material

In vivo grown plants of *D. crepidatum* were collected from Remakri, Bandarban, Bangladesh and *in vitro* raised plantlets were obtained from Plant Tissue Culture Laboratory, Department of Botany, University of Chittagong, Bangladesh.

Drying Samples and Preparation of Extracts

All collected plant samples were washed under running tap water to remove dust and other extra particles. Further washing with double distilled water was done followed by separation of different plant parts (leaf, stem, and root). The samples were cut into small pieces and dried in the oven. The dried samples were ground into a fine powder and soaked in methanol. The plant extract was filtered using Whatman no. 1 filter papers followed by placing into water bath (60°C) for evaporation

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of methanol. A crude extract was obtained gradually which was used for antioxidant and anti-inflammatory tests.

Antioxidant Activity

DPPH Assay

The antioxidant activities of the methanolic crude extracts of plant parts of natural and *in vitro* grown plants, as well as the standard antioxidant Ascorbic acid, were assessed on the basis of the free radical scavenging effect of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity according to the method described by Brand-Williams *et al*. (1995) with slight modification. DPPH is composed of stable free radical molecules, a well-known radical and a trap (scavenger) for other radicals.

Preparation of Reagents

Methanolic crude extracts of the leaf, stem, and root of naturally grown and *in vitro* raised plantlets of *D. crepidatum* were used to prepare a range of concentrations (50, 100, 150, 200, and 250 µgml^{-1}). Ascorbic acid with different concentrations (50, 100, 150, 200, and 250 µgml^{-1}) used as standard and 0.004 % (0.004 g) DPPH solution was prepared with methanol. The experiment was performed in triplicates.

Procedure

DPPH solution (3 ml) was mixed with 3 ml of extract solution and standard solution separately. These solution mixtures were kept in the dark for 30 min. The degree of DPPH purple decolourization to yellow indicated the scavenging efficiency of the extracts. The absorbance of DPPH solution (control solution A) was measured at 517 nm using a UV visible spectrophotometer; Ascorbic acid served as the positive control. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The scavenging activity against DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{A-B}{A}\right) \times 100$$

Table 1. DPPH free radical Scavenging assay of methanolic crude extract of leaf, stem, root parts and *in vitro* raised plantlets with Ascorbic Acid (as standard).

Scavenging activity (%)		Concentration (µgml^{-1})				
		50	100	150	200	250
Ascorbic acid (Standard)		95.44	96.93	97.84	98.09	98.59
Plants grown *in vivo*	Leaf	52.03	52.28	53.61	55.10	55.19
	Stem	52.86	57.09	60.49	60.25	61.08
	Root	66.80	79.59	79.92	81.99	87.22
Plantlets raised *in vitro*	Plantlets	54.36	54.77	54.85	55.27	55.52

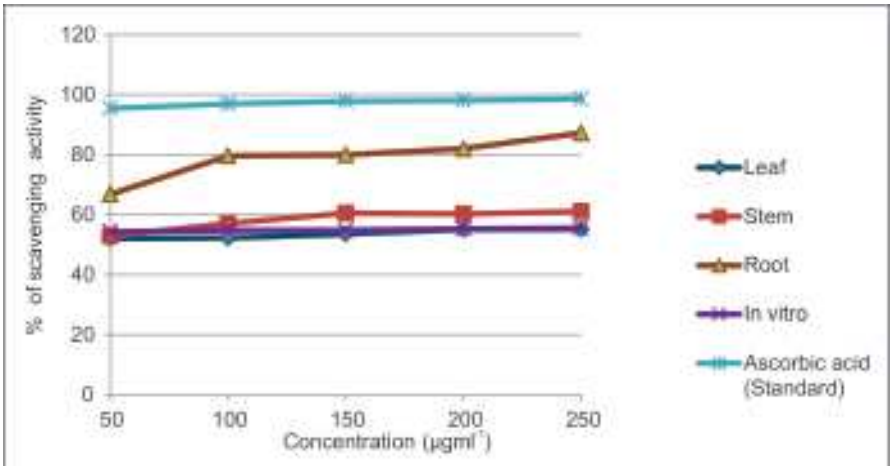

Fig. 1. Relative % scavenging activity of methanolic crude extracts of leaf, stem, and root parts of *in vivo* grown plants and *in vitro* raised plantlets of *Dendrobium crepidatum* with standard antioxidant Ascorbic acid.

Where, A = Absorbance of control (DPPH solution with the same volume of methanol) and

B = Absorbance of DPPH solution in the presence of the sample (extract/ Ascorbic acid).

Anti-inflammatory Activity

The anti-inflammatory activity of *D. crepidatum* was studied by using the inhibition of the albumin denaturation technique which was done according to Mizushima and Kobayashi (1968) and Sakat *et al*. (2010). The reaction mixture comprised of test extracts (250 µgml^{-1}) and 5% aqueous solution

of egg albumin, and the pH (5.6±0.2) of all reaction mixtures was adjusted by 1N HCl. The sample extracts were incubated at 60°C for 5 min, after cooling and filtering the samples, the turbidity was measured by a spectrophotometer at 660 nm. The experiment was performed thrice for each sample. The anti-inflammatory activity was calculated by using the following equation:

$$\% \text{ of inhibition} = \left(\frac{A - B}{A}\right) \times 100$$

Where, A= Absorbance of control (5% egg albumin solution and respective solvent); B= Absorbance of the test group (5% egg albumin solution and plant extract) /or B= Absorbance of standard solution (5% egg albumin solution and Acetylsalicylic acid).

Results and Discussion

Antioxidant Activity

DPPH free radical scavenging method was used for the determination of the scavenging activities of methanolic crude extracts of leaf, stem, and root parts of naturally grown and *in vitro* raised plantlets of *Dendrobium crepidatum*. The results are presented in Table 1 and Fig. 1. Ascorbic acid used as standard showed scavenging of 95.44, 96.93, 97.84, 98.09, and 98.59%, whereas methanolic crude extract of leaf showed 52.03, 52.28, 53.61, 55.10, and 55.19% at the five concentrations *i.e.* 50, 100, 150, 200, and 250 μgml^{-1}, respectively. The methanolic crude extract of stem and root revealed scavenging of 52.86, 57.09, 60.49, 60.25, and 61.08%; and 66.80, 79.59, 79.92, 81.99, and 87.22%, respectively. On the other hand, methanolic extract of *in vitro* developed plantlets showed 54.36, 54.77, 54.85, 55.27, and 55.52% scavenging activity at the above mentioned concentration, respectively. Amongst these, the average highest scavenging activity of standard Ascorbic acid (98.59%), leaf extract (55.19%), stem extract (61.08%), root extract (87.22%), and *in vitro* developed plantlets (55.52%) was observed at concentration 250 μgml^{-1}. The lowest scavenging activity recorded 95.44, 52.03, 52.86, 66.80, and 54.36% for Ascorbic acid, leaf, stem, root and *in vitro* raised plantlets, respectively at a concentration of 50 μgml^{-1}. All extracts showed scavenging capacity against the DPPH free radical whereas root extract showed the highest rate of antioxidant properties.

Similar types of findings were noted by Giri *et al.* (2012), in *Habenaria edgeworthii*. These results are also in the agreement with Rashmi *et al.* (2015) who worked on the free radical scavenging activity of selected orchids of Karnataka, India; the authors (Rashmi *et al.*, 2015) tested four epiphytic orchids namely, *Coelogyne breviscapa, Dendrobium nutantiflorum, Luisia zeylanica*, and *Pholidota pallida* for antioxidant activity. Extracts of *Luisia zeylanica* exhibited stronger radical scavenging activity when compared to other orchid extracts. Paudel *et al.* (2019) assessed antioxidant activities of extracts of *D. crepidatum* by DPPH assay. Ethanol and Acetone extracts scavenged 94.69±0.10% and 93.41±0.86% of DPPH free radicals, respectively. They showed 50% inhibition of DPPH free radicals (IC_{50})

Table 2. Albumin protein denaturation assay of anti-inflammatory activity for methanolic crude extract of leaf, stem, root parts of *in vivo* grown plants and *in vitro* raised plantlets.

Name of the plant		Plant parts used	% inhibition
Dendrobium crepidatum	*In vivo* grown plants	Leaf	92.86
		Stem	71.43
		Root	78.57
	In vitro raised plantlets	Plantlets	85.71
Standard (ASA= AcetylSalicylic Acid)			94.74

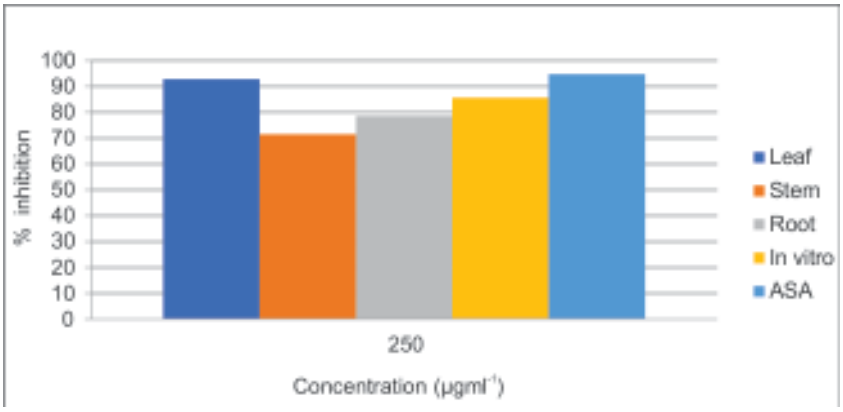

Fig. 2. Relative % inhibition of methanolic crude extracts of leaf, stem, root parts of naturally grown and *in vitro* raised plantlets of *Dendrobium crepidatum* with standard Acetyl Salicylic Acid.

at concentrations of 73.90 µgml^{-1} and 99.44 µgml^{-1}, which were found to be statistically similar to that of Ascorbic acid (control).

Anti-inflammatory Activity

Inflammation is a complex biological response of vascular tissues to harmful stimuli and is triggered by mechanical injuries, microbial infections, burns, allergens, and other noxious stimuli (Wang *et al*., 2014). The inflammatory processes involve a complex interplay between cells of the blood, the blood vessels and the cells of the involved tissue. Denaturation of proteins is a well-documented cause of inflammation. Regarding the mechanism of the anti-inflammation activity, the ability of the extract to inhibit egg albumin denaturation was studied. Egg albumin denaturation method for anti-inflammatory activities of methanolic crude extracts of leaf, stem, and root parts of naturally grown and *in vitro* developed plantlets of *D. crepidatum* was used. The results are presented in Table 2 and Fig. 2. The highest inhibition of egg albumin denaturation of methanolic leaf extract was recorded at 92.86% followed by root extract (78.57%) at the concentration of 250 µgml^{-1}. The lowest inhibition of egg albumin denaturation was found in stem extract *i.e*. 71.43%. On the other hand, *in vitro* raised plantlets showed an inhibition rate of 85.71%. The inhibition for all the methanolic crude extracts of leaf, stem, root, and *in vitro* samples were compared with standard Acetyl Salicylic Acid (94.74%). Present results indicate that leaf extract responds to the highest rate of anti-inflammatory activity amongst all other parts of the natural sample whereas *in vitro* raised plantlets also possessed a significant rate of anti-inflammatory properties. Our observations are in line with the findings of Hossain *et al*. (2020) on anti-inflammatory effect of three epiphytic orchids from Bangladesh. They reported the highest inhibition of egg albumin denaturation in *Papilionanthe teres* (88.22±0.205), followed by *Luisia zeylanica* (82.33±0.144) and the lowest inhibition was found in the roots of *Rhynchostylis retusa* (71.32±0.151). Moderate anti-inflammatory activity was found in the leaf extract of *R. retusa*. Sukumaran and Yadav (2016) worked on anti-inflammatory potential of *Dendrobium macrostachyum* and observed that the ethanol and water extract were highly effective as albumin denaturation inhibitors with IC_{50} value of 114.13 and 135.818 µgml^{-1}, respectively and proteinase inhibitors with IC_{50} value of 72.49 and 129.68 µgml^{-1}, accordingly.

Conclusion

From the results of the present study, it may be concluded that both *in vivo* grown plants and *in vitro* raised plantlets of *D. crepidatum* have remarkable potential for antioxidant and anti-inflammatory activities. Root and Leaf extract of naturally grown plants are more effective for antioxidant and anti-inflammatory activities, respectively.

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