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ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF AN EPIPHYTIC AND ENDANGERED ORCHID, *DENDROBIUM MOSCHATUM* (BUCH.-HAM.) SW.

Tahli Sanjana, M M Hoque, and M K Huda

Department of Botany, University of Chittagong, Chattogram- 4331, Bangladesh

Abstract

Antioxidant and anti-inflammatory potential of *Dendrobium moschatum* fractions of root, stem, and leaf was investigated. The dried samples of these parts were extracted with Butanol-1, DCM (dichloromethane), n-Hexane, and Methanol fractions. Both Methanol fraction of root [IC_{50} (inhibition concentration 50 μgml^{-1}) = 30.21 μgml^{-1}] and leaf (IC_{50} = 31.54 μgml^{-1}) possessed the most efficient antioxidant potential (free radical scavenging assay of DPPH) which was closely followed by DCM fraction of leaf with an IC_{50} value of 33.78 μgml^{-1}. The least antioxidant activity (albumin denaturation technique) was seen in the n-Hexane fraction of leaf and IC_{50} value was 94.74 μgml^{-1}. The lowest anti-inflammatory potential was shown by n-Hexane fraction of both root (IC_{50} = 71.80 μgml^{-1}) and stem (IC_{50} = 71.21 μgml^{-1}) while the highest anti-inflammation was demonstrated in root by Methanol fraction with an IC_{50} value of 39.01μgml^{-1} closely followed in leaf by Butanol-1 fraction whose IC_{50} value was 39.00 μgml^{-1}, n-hexane fraction (IC_{50} = 39.21 μgml^{-1}), and methanol fraction (IC_{50} = 39.79 μgml^{-1}). Hence, the results indicated that leaf part showed the most prospective anti-inflammatory activity. From the present study, it may be concluded that the plant has great medicinal potential and further researches are needed to explore its potential.

Introduction

ORCHIDS BEING the most diverse group among the angiosperms are nature's most extravagant flowering plants distributed throughout the world from tropics to high alpine (White and Sharma, 2000). It comprises about 28,484 species in approximately 750 genera (Govaerts *et al.,* 2017). Bangladesh, a depository of orchids is reported to have 177 orchid species under 70 genera (Huda, 2007), of which 26 species are used for curing different ailments by its tribal people (Huda *et al.,* 2006). Pharmacological studies conducted on orchids indicate the immense potentiality of these plants in treating different diseases such as in reducing free radical-induced tissue injury (Pourmorad *et al*., 2006; Stajner *et al.,* 2009).

Dendrobium moschatum (Buch.-Ham.) Sw. is a traditionally used, medicinally important, and an endangered epiphytic orchid species (Akter *et al*., 2017; Huda, 2007). Moscatilin isolated from stems of this orchid showed anti-tumour activity against various human cancer cell lines (Mittraphab *et al*., 2016; Prasad and Koch, 2014). Rotundatin and Moscatin (Phenanthrenes) isolated from this species were reported to be efficient in the inhibition of aggregation of platelets by collagen and arachidonic acid (Chattopadhyay *et al*., 2012). Several metabolites have been extracted and isolated from the stems of different *Dendrobium* spp., provided with immunomodulatory, hepatoprotective, antioxidant, antimalarial, and anticancer activities (Chanvorachote *et al.,* 2013; Liu *et al.,* 2011; Sukphan *et al.,* 2014). Various studies have been carried out in determining the antioxidant and anti-inflammatory properties of different *Dendrobium* species (Akter *et al*., 2020; Moretti *et al*., 2013; Xing *et al*., 2013). However, there is yet no published document on the antioxidant and anti-inflammatory efficacy of fractionated (n-Hexane, Dichloromethane, Methanol, and Butanol-1) parts (root, stem, and leaf) of *D. moschatum* extract in Bangladesh. So the present investigation was undertaken so as to determine the antioxidant and anti-inflammatory potential of fractionated (n-Hexane, Dichloromethane, Methanol, and Butanol-1) parts of *D. moschatum* root, stem, and leaf extracts.

Material and Methods

Dendrobium plant materials have been collected from Bandarban hill tracts. The plant species have been identified consulting relevant taxonomic literature and following standard taxonomic methods. It was also identified by comparing the prevailing herbarium specimens and consulting experts of Department of Botany, University of Chittagong. A voucher specimen (accession no. HCUK215) has been deposited at the University of Chittagong herbarium for future reference. After the separation of the plant parts into root, stem, and leaf, all the parts were washed with tap water. They were then cut into small pieces and dried at 65°C for 48 hrs. All the parts were macerated separately into fine powder and stored in air tight containers. The extraction was done with Methanol for which around 50 g of plant sample from each part were taken. After filtering the

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extract using Whatman no. 1 filter paper, it was rotavaporated and kept as crude.

The crude was then separated into four different solvent (n-Hexane, Dichloromethane, Methanol, and Butanol-1) by following modified Kupchan (Kupchan, 1969) method. The separated n-Hexane, Dichloromethane, Methanol, and Butanol-1 fraction were completely dried by rotavaporator. The extracts were then refrigerated at 4°C. Determination of antioxidant activity of Methanolic, n-Hexane, Butanol-1, and Dichloromethane was done by free radical scavenging assay of DPPH (Brand-Willams *et al.*, 1995). The absorbance of all the solutions including DPPH (control solution "A") was measured against blank at 517 nm using UV-visible spectrophotometer (Shimadzu, Japan) where standard was Ascorbic acid. The experiment was performed in triplicates.

The DPPH scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{A-B}{A}\right)X100$$

Where, A = Absorbance of control (DPPH solution without the sample) and B = Absorbance of DPPH solution in the presence of the sample (extract/Ascorbic acid).

The anti-inflammatory activity was studied by using inhibition of albumin denaturation technique (Mizushima and Kobayashi, 1968). Standard was Acetylsalicylic acid and 5% egg albumin solution was used as the control. Absorbance was measured at 660 nm (Spectrophotometer). The experiment was performed in triplicate. The anti-inflammatory activity was calculated by using the following equation:

$$\text{\% of inhibition} = \left(\frac{A-B}{A}\right)X100$$

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

In the antioxidant and anti-inflammatory assay, the % scavenging activity and % of inhibition respectively was plotted against log concentration and the IC_{50} (inhibition concentration 50 μgml^{-1}) value was determined by linear regression analysis.

Results and Discussion

Antioxidant Activity

The results of DPPH free radical scavenging activity of Ascorbic acid which was used as standard showed scavenging of 52.09, 60.76, 80.89, 87.12, and 95.65% at the five concentrations *i.e.* 50, 100, 150, 200, and 250 μgml^{-1} respectively. All extracts showed scavenging capacity against the DPPH free radicals. The scavenging percentage of DPPH free radicals varied from 42.51 (n-Hexane at 50 μgml^{-1}) to 89.04 (Methanol at 250 μgml^{-1}) for the leaf fractions (Fig. 1). The highest scavenging activity of n-Hexane, Butanol-1, and DCM were 70.12, 78.34, and 80.11% respectively for leaf (Fig. 1). For root fractions, it varied from 48.02% (n-Hexane at concentration 50 μgml^{-1}) to 93.00% (Methanol at 250 μgml^{-1}), whereas the maximum scavenging activity of DCM, n-Hexane, and Butanol-1 were 75.21%, 78.07%, 78.23% respectively (Fig. 2). For stem, the highest % of scavenging was 91.63

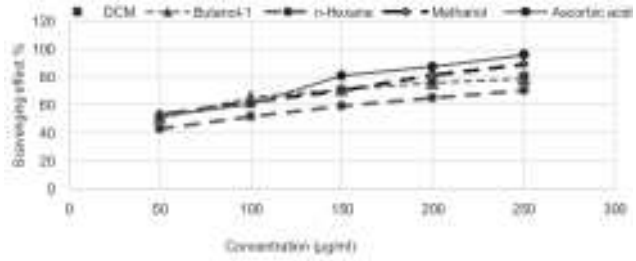

Fig. 1. Relative % scavenging effect of standard Ascorbic acid and fraction sample (DCM, Butanol-1, n-Hexane, Methanol) of *D. moschatum* leaf for antioxidant assay.

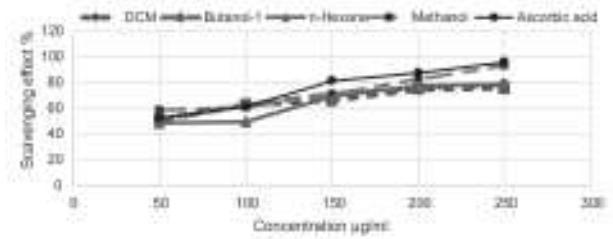

Fig. 2. Relative % scavenging effect of standard Ascorbic acid and fraction samples (DCM, Butanol-1, n-Hexane, Methanol) of *D. moschatum* root for antioxidant assay.

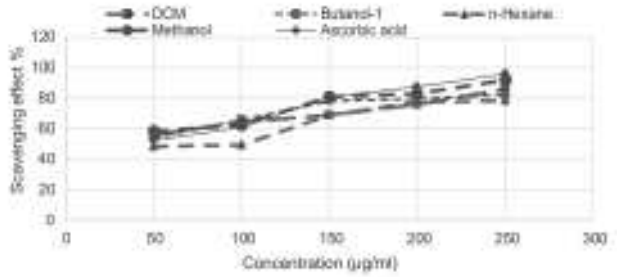

Fig. 3. Relative % Scavenging effect of standard Ascorbic acid and fraction samples (DCM, Butanol-1, n-Hexane, Methanol) of *D. moschatum* stem for antioxidant assay.

(Methanol at concentration 250 μgml^{-1}) and lowest was 48.02 (n-Hexane at concentration 50 μgml^{-1}). The greatest % scavenging activity demonstrated by Butanol-1, n-Hexane, and DCM were 81.85%, 78.07%, 85.57% respectively (Fig. 3) for stem part. It was seen that the per cent of scavenging

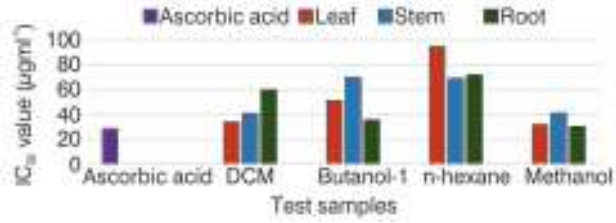

Fig. 4. Comparison of IC_{50} value of standard with four fraction extractives of *D. moschatum* leaf, stem, and root.

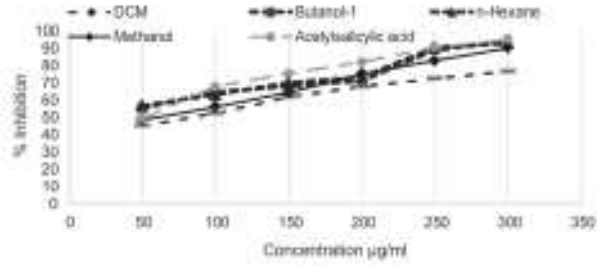

Fig. 5. Per cent Inhibition of albumin protein denaturation of different fraction parts of *D. moschatum* leaf and standard Acetylsalicylic acid.

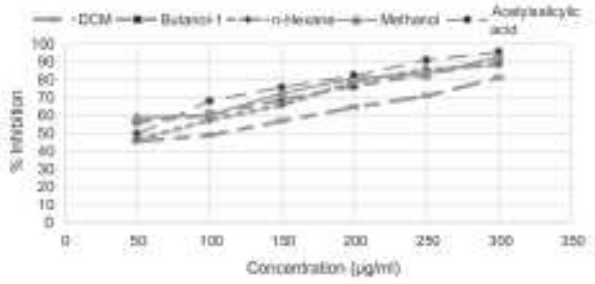

Fig. 6. Per cent Inhibition of albumin protein denaturation of different fraction parts of *D. moschatum* root and standard Acetylsalicylic acid.

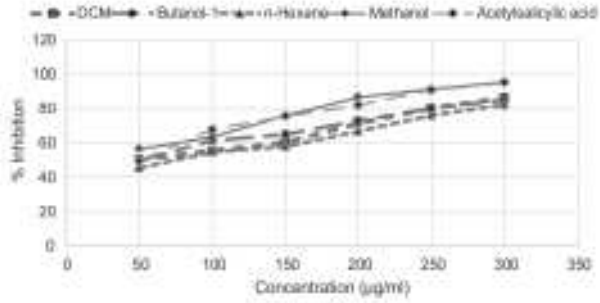

Fig. 7. Per cent Inhibition of albumin protein denaturation of different fraction parts of *D. moschatum* stem and standard Acetylsalicylic acid.

increased as the concentration of the doses of all the extracts increased. At the same concentration, the scavenging of standard was always higher than the scavenging of plant extracts. According to Mukherjee *et al*. (2012), a study of the *in vitro* aqueous extract of *Dendrobium aqueum* showed that the increase of free radical scavenging activity was in a dose dependent manner where the highest activity of 49% was found at

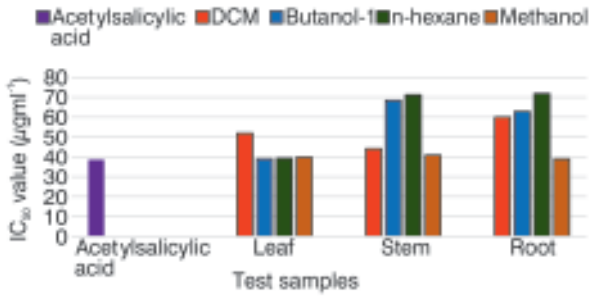

Fig. 8. Comparison of IC_{50} value of standard Acetylsalicylic acid with four fraction extractives of *D. moschatum* leaf, stem, and root for anti-inflammatory test.

a dose of 100 µgml^{-1}; whereas the standard showed scavenging of 93.3% at 100 µgml^{-1} which was significantly higher than the plant extract at the same concentration (which can be compared to the present work).

Fig. 4 comprises the comparison of IC_{50} value of fractioned sample of root, stem, and leaf. The IC_{50} value of standard Ascorbic acid was 28.51 µgml^{-1}. In case of leaf, Methanol fraction showed the most potential IC_{50} value of 31.54 µgml^{-1}. The least antioxidant potential was shown by n-Hexane extract of leaf which was 94.74 µgml^{-1}. For stem, the highest antioxidant activity was shown by DCM fraction with an IC_{50} value of 40.84 µgml^{-1}. The IC_{50} value of root fractioned sample Methanol topped at value 30.21 µgml^{-1}. Sukumaran and Yadav (2016) in their study on the antioxidant potential of *Dendrobium macrostachyum* stem and leaf extracts found that the stem ethanolic extracts exhibited significant IC_{50} value of 10.21, 31.54, and 142.97 µgml^{-1} respectively for DPPH, ABTS radical scavenging, and reducing power activity. Paudel *et al*. (2019) while working on assessment of antioxidant and cytotoxic activities of *Dendrobium crepidatum* extracts found that the extract showed 50% inhibition of DPPH free radicals (IC_{50}) at concentrations of 73.90 µgml^{-1}, which was found to be statistically similar to that of Ascorbic acid.

Anti-inflammatory Activity

The results of anti-inflammatory activity of Acetylsalicylic acid used as standard showed inhibition activity as 49.55%, 67.88%, 75.56%, 82.18%, 90.89%, and 95.22% at the six concentrations *i.e.* 50, 100, 150, 200, 250, and 300 µgml^{-1} respectively (Fig. 5). The IC_{50} value of Acetylsalicylic acid was 38.51 µgml^{-1}.

The anti-inflammatory activity of four fractions of root (Fig. 6) revealed that DCM fraction had the highest inhibition of 80.91% whereas n-Hexane had the utmost inhibition of 88.34%. Again the inhibition of Butanol-1 fraction varied from 56.32-90.01% and Methanol fraction varied from 58.56-92.67%. The IC_{50} values of DCM,

Butanol-1, n-Hexane, and Methanol of root were 59.93, 62.76, 71.8, 39.01 µgml^{-1}, respectively (Fig. 8).

For different fractionated parts of stem (Fig. 7), the maximum anti-inflammation shown by DCM, Butanol-1, n-Hexane, and Methanol fractions were 86.35, 85.23, 82.34, 95.34% respectively. The IC_{50} value of DCM, Butanol-1, n-Hexane, Methanol of stem were 44.12, 68.45, 71.21, 40.84 µgml^{-1} respectively (Fig. 8).

Amongst the four fractionated parts of leaf for anti-inflammatory assay, DCM showed highest inhibition of 76.90% at 300 µgml^{-1} (Fig. 5), Butanol-1 fraction showed the maximum inhibition of 94.34% and n-Hexane showed the utmost inhibition of 93.18% at the highest concentration of 300 µgml^{-1}. On the other hand, the inhibition varied from 48.76-90.45% for the Methanol fraction. The IC_{50} value of DCM, Butanol-1, n-Hexane, and Methanol of leaf were 51.87, 39.00, 39.21, 39.79 µgml^{-1}, respectively (Fig. 8).

Abdullah *et al*. (2014) worked on anti-inflammatory activity of selected plants from Saudi Arabia and amongst these, *Trichodesma trichodesmoides* var. *tomentosum* exhibited the highest anti-inflammatory activity. The most potent fraction was the n-Butanol fraction which is comparable to the present work where Butanol-1 fraction of leaf showed the most potential anti-inflammatory activity. Sukumaran and Yadav (2016) on their work on the anti-inflammatory potential of *Dendrobium macrostachyum* observed that the Ethanol and water extract was highly effective as albumin denaturation inhibitors (IC_{50} = 114.13 and 135.818 µgml^{-1} respectively) and proteinase inhibitors (IC_{50} = 72.49 and 129.681 µgml^{-1} respectively). Membrane stabilization was also noticeably inhibited by the stem ethanolic extract among other extracts (IC_{50} = 89.33 µgml^{-1}) but comparatively lower to aspirin standard (IC_{50} = 83.926 µgml^{-1}).

Based on the results of the present study, it can be concluded that in *D. moschatum* leaf, the Methanol fraction indicated the most potential antioxidant activity and Butanol-1 fraction showed the highest anti-inflammatory activity. For stem, it was seen that Methanol fraction showed utmost inflammation activity and DCM fraction revealed the most efficient antioxidant activity. The tests of root fraction revealed that Methanol fractionated parts showed the highest anti-inflammatory and antioxidant activity.

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