J. Orchid Soc. India, 39:1-8, 2025 *ISSN 0971-5371*
https://doi.org/10.64873/JOSI.v39.i1-2. 1-8

BIOLOGICAL ACTIVITIES OF *ACAMPE PRAEMORSA* (ROXB.) BLATT. & MCCANN, AN EPIPHYTIC MEDICINAL ORCHID OF BANGLADESH UNDER *IN VIVO* AND *IN VITRO* CONDITIONS

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Abstract

The seeds of *Acampe praemorsa* (Roxb.) Blatt. & McCann successfully germinated *in vitro* on 0.8% (w/v) agar solidified KC, MS, MVW, and PM basal media and developed into seedlings. Both *in vitro* raised seedlings and *in vivo* obtained plant parts (leaf, stem, and root) were investigated for antioxidant, anti-inflammatory, and cytotoxic activities. The moderate antioxidant activity was showed in methanolic crude extract of leaf (IC_{50}= 229.55 µgml^{-1}), stem (IC_{50}= 103.86 µgml^{-1}), root (IC_{50}= 222.22 µgml^{-1}), and *in vitro* seedlings (IC_{50}= 208.47 µgml^{-1}) samples. Strong anti-inflammatory activity was observed on leaf (IC_{50}= 95.15 µgml^{-1}), stem (IC_{50}= 63.87 µgml^{-1}), and *in vitro* developed seedlings (IC_{50}= 80.39 µgml^{-1}), whereas root sample showed the moderate (IC_{50}= 137.50 µgml^{-1}) anti-inflammatory activity. The cytotoxic activity of methanolic crude extract of leaf, stem, root, and *in vitro* raised seedlings exhibited moderate activity with the LC_{50} values as 265.20, 224.13, 357.73, and 160.21 µgml^{-1}, respectively.

Introduction

ORCHID FLOWERS are beautiful and highly fascinating, displaying an exceptional range of diversity in form, size, colour, and texture, along with long-lasting floral qualities. In ecological studies, it is often necessary to assess ecosystem integrity quickly, and indicator species can be used for this purpose. Orchids, in particular, are considered indicators of a region's healthy climate (Barua *et al*., 2019). There are over 28,481 species of orchids in the world, making them the captivating group of flowering plants in nature (WFO, 2023). According to Chase *et al*. (2015), the family Orchidaceae accounts for nearly 8% of the diversity of angiosperm species. Due to their beauty and high demands, orchids are particularly vulnerable to illegal trafficking, which has put them in danger of going extinct (Adhikari *et al.,* 2019). In addition to their ornamental uses, many orchid species are also being used commercially for their therapeutic benefits. In Asia, there are 105 genera and 419 species of orchids which possess therapeutic benefits (Teoh, 2016). With 177 species identified under 70 genera, Bangladesh is home to a diverse and extensive variety of orchids (Huda, 2007). The tribal people of Bangladesh use about 26 species of orchids to heal various illnesses (Huda *et al*., 2006).

Acampe praemorsa, an epiphytic orchid, is a popular medicinal plant. Communities of the Chakma and Marma tribes produce a decoction from the plant leaves or whole plant that works well to cure injuries, fever, and earaches. In Bangladesh, it is prevalent in the hilly regions, particularly in Bandarban, Cox's Bazar, Rangamati, and Khagrachari (Huda and Kashem, 2021). This species contains a diverse array of secondary metabolites and exhibits several beneficial bioactivities, including anti-inflammatory, antioxidant, and antibacterial effects (Hoque *et al*., 2024; Rahman *et al*., 2023). The present study was aimed to evaluate the biological characteristics such as antioxidant, anti-inflammatory, and cytotoxic effects of plant parts (leaf, stem, and root) obtained *in vivo* and *in vitro* raised seedlings.

Material and Methods

Collection of Plant Material

The plants of *A. praemorsa* and its capsules were collected from Bengchari, Kaptai, and Rangamati regions of Bangladesh.

In Vitro Seed Germination and Seedling Development

Immature seeds were used for experimentation. For this purpose, the green capsules of *A. praemorsa* were procured, washed with running tap water, and then three to four times with sterile distilled water. Following a savlon-soaked cotton rubdown, the capsules were rinsed with distilled water 2-3 times. These were then cleaned 2-3 times with double distilled water after being treated with 0.2% (w/v) $HgCl_2$ for 5 mins so as to sterilize their surface. Finally, the surface was disinfected in laminar airflow by treating these with 70% ethanol for 1 min and then washing these 2-3 times with double sterile distilled water. Different nutrient

Received: January 01, 2025; Accepted: March 02, 2025

media such as MS (Murashige and Skoog, 1962), PM (Phytamax-Arditti, 1977), MVW (Modified Vacin and Went, 1949), and KC (Knudson, 1946) fortified with 0.8% (w/v) of agar were used and 2-3% sucrose was used as carbon source. Before combining the dissolved agar, the pH of the media were adjusted at 5.8 in MS and 5.4 in KC, PM, and MVW media using 0.1N NaOH or HCl. Agar was dissolved in the mixture by boiling it in a water bath. Then, 50 ml of medium was poured into 100 ml of each culture vessel and the vessels were autoclaved at 121°C for 30 min at 15 psi pressure. The seeds were then carefully inoculated inside the vessels. The whole process was performed under the clean air laminar airflow chamber. The cultures were consistently kept at an ambient temperature of 25±2°C and given a 14 hr photoperiod with an intensity of 4000-5000 lux.

Preparation of Crude Extract

The seedlings grown *in vitro*, along with plant parts obtained *in vivo* (such as leaf, stem, and root), were initially cut into small segments and dried. The samples were then pulverized into fine powder. A conical flask containing 60 g of powder was filled with 120 ml of methanol and allowed to macerate for 7-8 days. After then, Whatman filter paper No. 1 was used to filter the soaked samples. The extracts were boiled in a water bath at 60°C until the crude extracts separated and the methanol evaporated.

Test of Antioxidant Activity

According to Brand-Williams *et al*. (1995) method with a minor modifications, antioxidant activity was evaluated on the basis of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) to scavenge free radicals. In this process, five concentrations (50, 100, 150, 200, and 250 $\mu g ml^{-1}$) were utilized.

Using the following equation, the scavenging activity against DPPH was estimated:

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

Where,

A= Absorbance of control (DPPH solution with same volume of methanol)

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

Test of Anti-Inflammatory Activity

The inhibition of albumin denaturation approach was examined in accordance with Mizushima and Kobayashi (1968) and Sakat *et al*. (2010) methods with slight modification, with a view to study anti-inflammatory activity. The reaction mixture contained 1% aqueous egg albumin solution and test extracts of 50, 100, 150, 200, and 250 $\mu g ml^{-1}$. The pH of the entire reaction mixture was set at 5.60±0.2.

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

Where,

A= Absorbance of control (5% egg albumin solution and respective solvent)

B= Absorbance of test group (5% egg albumin solution and plant extract) or Absorbance of standard solution (5% egg albumin solution and acetyl salicylic acid).

Test of Cytotoxicity Activity

The cytotoxic activity of methanolic crude extracts of *in vitro* seedlings and *in vivo* obtained plant parts (leaf, stem, and root) of *A. praemorsa* was assessed using a slightly modified method of Meyer *et al*. (1982) named brine shrimp lethality assay. The plant extract was taken at concentrations of 10, 100, 250, and 500 $\mu g ml^{-1}$ and the standard solution was taken at concentrations of 10, 20, 30, and 50 $\mu g ml^{-1}$.

The following equation can be used to get the mortality rate:

$$\text{Percent (\%) of mortality} = \left(\frac{Nt}{No}\right) \times 100$$

Where,

N_t = Number of dead nauplii

N_o = Number of taken nauplii

Results and Discussion

In Vitro Seed Germination and Seedling Development

The immature seeds of *Acampe praemorsa* procured from green capsules were cultured on full strength 0.8% (w/v) agar solidified four basal nutrient media namely KC, MS, MVW, and PM. The seeds successfully germinated on all the nutrient media; PM basal medium, however, proved the best for seed germination followed by MS, MVW, and KC media. Similarly, some earlier studies were made on successful *in vitro* propagation of a few orchid species representing diverse habits and habitats (Anuprabha and Pathak *et al*., 2012; Arora *et al*., 2014; Bhowmik and Rahman, 2023; Dhillon and Pathak, 2023; Hossain *et al*., 2009; Jaryal *et al*., 2025a,b; Kaur *et al*., 2017; Kirti *et al*., 2023; Kumari and Pathak, 2021; Mutum *et al*., 2022; Pathak *et al*.,

2001, 2017, 2022, 2023; Sunita *et al*., 2021; Thakur and Pathak, 2020, 2021; Tripura *et al*., 2022; Vasundhra *et al.,* 2021).

Antioxidant Activity

In the present study, the results of DPPH free radical scavenging activity of *in vivo* sourced leaf, stem, and root, and *in vitro* seedlings extracts of *A. praemorsa* and ascorbic acid at different concentrations (50, 100, 150, 200, 250 μgml^{-1}). Amongst the five different concentrations *i.e.* showed varied scavenging activity [(33.97±0.34, 38.25±0.23, 43.87±0.32, 48.30±0.11, 51.11±0.33)%, (42.18±0.24, 52.24±0.09, 55.72±0.44, 59.68±0.16, 63.82±0.38)%, (35.30±0.76, 40.47±0.65, 45.49±0.40, 48.30±0.29,51.85± 0.37)%, (36.91±0.34, 37.20±0.23, 45.77±0.32, 48.45±0.11, 54.51±0.33)% and (52.30±0.72, 67.13±0.47, 78.12±0.56, 85.30±0.49, 94.67±0.23)% at 50, 100, 150, 200, 250 μgml^{-1} respectively (Table 1 and Fig. 1)]. The IC_{50} values of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings extracts and ascorbic acid were found as 229.55 μgml^{-1}, 103.86 μgml^{-1}, 222.22 μgml^{-1}, 208.47 μgml^{-1}, and 26.19 μgml^{-1} (Fig. 2). The plant parts *i.e.* leaf, stem, root, and the *in vitro* grown sample showed moderate antioxidant activity.

Methanolic leaf extract of *A. praemorsa* showed moderate antioxidant activity in an experiment conducted by Rahman and Huda (2021), with an IC_{50} value of 181.06 μgml^{-1}. By using *in vitro* and *in vivo* investigations, Kumar *et al*. (2021) assessed the antioxidant activity of *A. praemorsa*. On free radicals including superoxide, hydroxyl DPPH, hydrogen peroxide and evaluation of reducing power, *in vitro* antioxidant activity was conducted. The findings of the antioxidant activity tests showed that the extracts have concentration-dependent action against the studied free radicals. Nevertheless, ethyl acetate extract has greater potential against hydroxyl free radicals than hydroalcoholic extract. Hydroalcoholic extract is more protective against superoxide, DPPH, H_2O_2 free radicals and reducing power. Suja and Williams (2016) conducted an experiment on *A. praemorsa*, varying the scavenging activity of ethanol extracts from the lowest (51.01±0.015%) at a 25 µl concentration to the highest (54.93±0.010%) at 100 μgml^{-1} concentration.

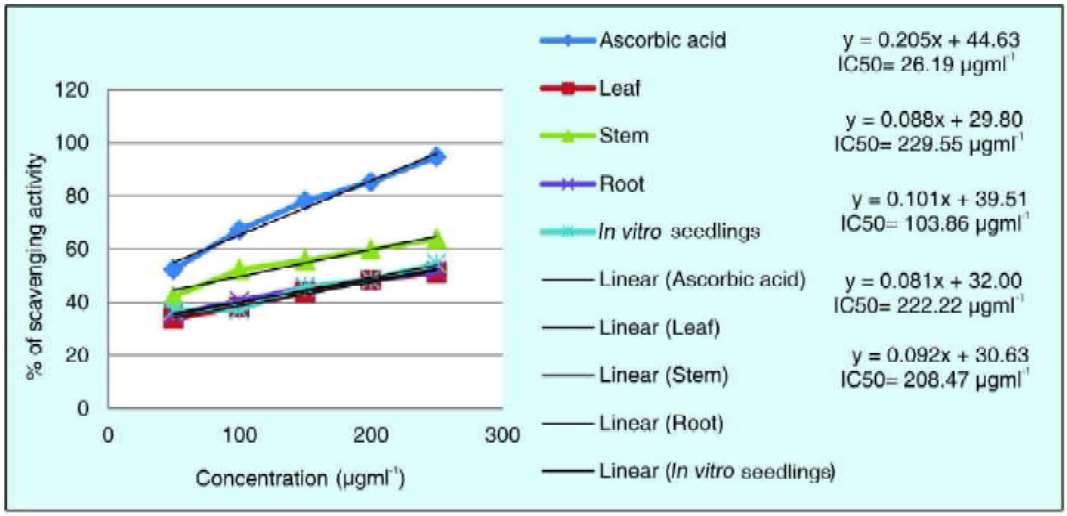

Fig. 1. Scavenging activity of *in vivo* sourced leaf, stem, root, and *in vitro* plantlets of *Acampe praemorsa* and Ascorbic acid (Standard).

Anti-Inflammatory Activity

The results of anti-inflammatory activity of leaf, stem, root, *in vitro* extract of *A. praemorsa* and acetyl salicylic acid at the five different

Table 1. Antioxidant activity of *in vivo* sourced leaf, stem, root, *in vitro* plantlets extracts of *Acampe praemorsa* and Ascorbic acid at different concentrations.

Concentration (μgml^{-1})	Per cent (%) of scavenging activity				
	Leaf	Stem	Root	*In vitro* seedlings	Ascorbic acid
50	33.97±0.34	42.18±0.24	35.30±0.76	36.91±0.42	52.30±0.72
100	38.25±0.23	52.24±0.09	40.47±0.65	37.20±0.19	67.13±0.47
150	43.87±0.32	55.72±0.44	45.49±0.40	45.77±0.85	78.12±0.56
200	48.30±0.11	59.68±0.16	48.30±0.29	48.45±0.15	85.30±0.49
250	51.11±0.33	63.82±0.38	51.85±0.37	54.51±0.26	94.67±0.23
IC_{50} value	229.55	103.86	222.22	208.47	26.19

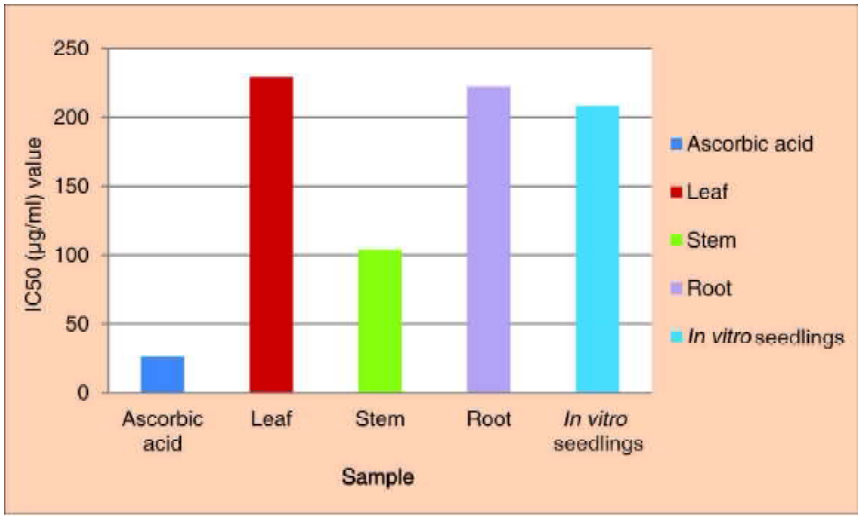

Fig. 2. IC_{50} (μgml^{-1}) values of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Ascorbic acid

concentrations (50, 100, 150, 200, 250 μgml^{-1}) showed varied inhibition activity (46.23±0.41, 50.70±0.20, 54.35±0.15, 61.75±0.30, 74.91±0.55%), (50.53±0.31, 53.51±0.47, 56.14±0.60, 59.21±0.43, 71.05±0.50%), (45.61±0.63, 47.73±0.36, 49.56±0.45, 53.56±0.55, 57.89 ±0.81%), (49.22±0.31, 51.50±0.47, 55.18±0.60, 57.12±0.43, 70.48±0.50%) and (51.22±0.27, 66.36±0.21, 75.46±0.34, 82.91±0.22, 92.33±0.49%), respectively (Table 2; Fig. 3). The IC_{50} values of methanolic crude extracts of leaf, stem, root, *in vitro* and acetyl salicylic acid was found as 95.15, 63.87, 137.50, 80.39, and 30.36 μgml^{-1} (Fig. 4). *In vivo* sourced leaf, stem, and *in vitro* plantlet extracts of the presently studied species revealed strong anti-inflammatory activity. Rahman and Huda (2021) earlier demonstrated mild anti-inflammatory effect for methanolic leaf extracts of *A. praemorsa*. The anti-inflammatory properties of the whole plant extracts of *A. praemorsa* were confirmed by Vibha *et al*. (2019). In methanolic crude extracts, the highest anti-inflammatory activity was 76.87% in stem and the least (44.89%) in the leaf extracts. The comparison of aqueous extract to ethanolic extracts revealed that the former had significantly more anti-inflammatory efficacy.

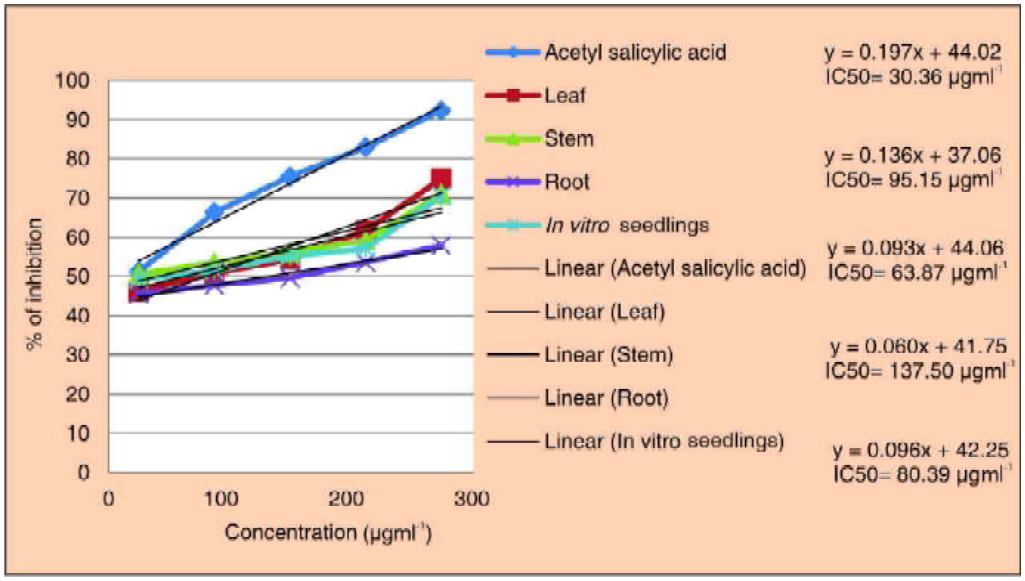

Fig. 3. Per cent (%) of inhibition and IC_{50} (μgml^{-1}) value of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Acetyl salicylic acid.

Table 2. Anti-inflammatory activity of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Acetyl salicylic acid.

Concentration (μgml^{-1})	Per cent (%) of inhibition				
	Leaf	Stem	Root	*In vitro* seedlings	Acetyl salicylic acid
50	46.23±0.41	50.53±0.31	45.61±0.63	49.22±0.93	51.22±0.27
100	50.70±0.20	53.51±0.47	47.73±0.36	51.50±0.55	66.36±0.21
150	54.35±0.15	56.14±0.60	49.56±0.45	55.18±0.84	75.46±0.34
200	61.75±0.30	59.21±0.43	53.56±0.55	57.12±0.10	82.91±0.22
250	74.91±0.55	71.05±0.50	57.89±0.81	70.48±0.87	92.33±0.49
IC_{50} value	95.15	63.87	137.50	80.39	30.36

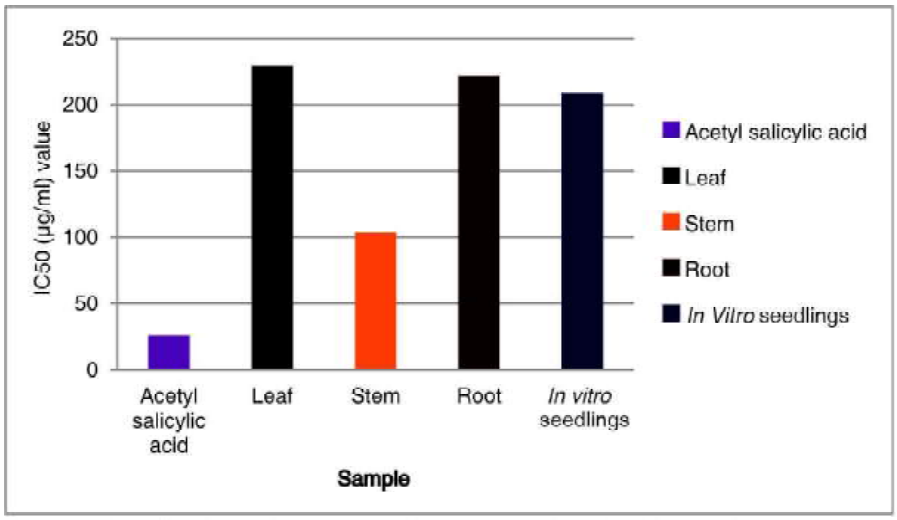

Fig. 4. IC_{50} (μgml^{-1}) value of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Acetyl salicylic acid.

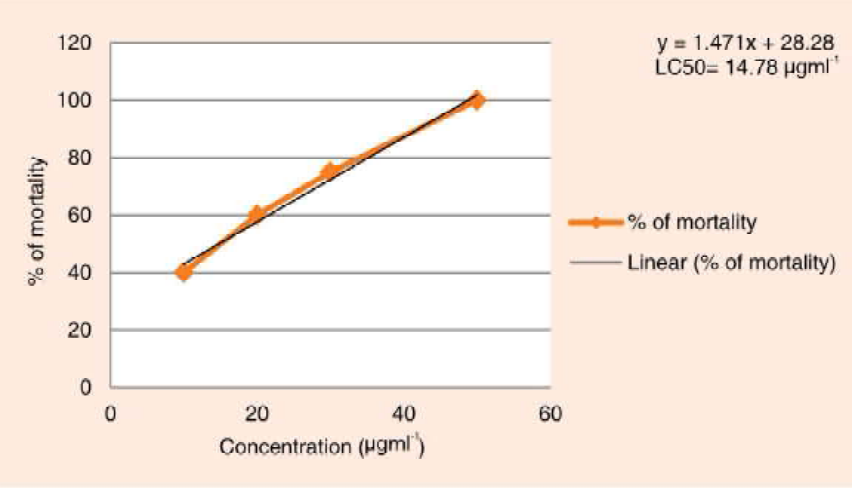

Fig. 5. Per cent mortality and LC_{50} (μgml^{-1}) value of potassium dichromate (Standard).

Cytotoxic Activity

In case of methanolic crude extracts of *in vivo* sourced leaf, stem, root, *in vitro* seedlings and potassium dichromate, the mortality rate of nauplii varied at the four different concentrations *i.e.* 10, 100, 250, 500 μgml^{-1}. These were observed as 10, 35, 55, 75 % at 10 μgml^{-1}; 15, 40, 50, 90 % at 100 μgml^{-1}; 15, 30, 45, 60% at 250 μgml^{-1}; 35, 45, 60, 80 % at 500 μgml^{-1}, and 40, 60, 75, 100 % in potassium dichromate (Table 3; Fig. 5). LC_{50} values of methanolic extracts of *in vivo* sourced leaf, stem, root, *in vitro* seedlings and potassium dichromate were 265.20 μgml^{-1}, 224.13 μgml^{-1}, 357.73 μgml^{-1}, 160.21 μgml^{-1} and 14.78 μgml^{-1}, respectively (Figs. 6-7). After correlating with the standard, it was observed that all the plant parts, leaf, stem, and root of *A. praemorsa* revealed moderate cytotoxic activity along with *in vitro* seedlings.

Jhansi and Khasim (2018) evaluated the *in vitro* cytotoxic activity of *A. praemorsa*, which demonstrated the maximum zone of inhibition against *Candida albicans* fungus at 17.00 mm. Joshi *et al.* (2020) experimented with various wild orchids where the extract of *Dendrobium transparens* (DTs) and *Vanda cristata* (VCw) showed high cytotoxic effect towards the HeLa and U251 cell lines (IC_{50} of DTs: 382.14 μgml^{-1} and 75.84

Table 3. Cytotoxic activity of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Potassium dichromate.

Treatment	Concentration (μgml^{-1})	Number of Nauplii taken	Number of Dead Nauplii	Mortality (%)	LC_{50} (μgml^{-1})
Leaf	10	20	2	10	265.20
	100		7	35	
	250		11	55	
	500		15	75	
Stem	10		3	15	224.13
	100		8	40	
	250		10	50	
	500		18	90	
Root	10		3	15	357.73
	100		6	30	
	250		9	45	

Table 3. Cytotoxic activity of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Potassium dichromate.(contd.)

Treatment	Concentration (μgml^{-1})	Number of Nauplii taken	Number of Dead Nauplii	Mortality(%)	LC_{50} (μgml^{-1})
	500		16	80	
In vitro seedlings	10		7	35	160.21
	100		9	45	
	250		12	60	
	500		16	80	
Potassium dichromate	10		8	40	14.78
	20		12	60	
	30		15	75	
	50		20	100	

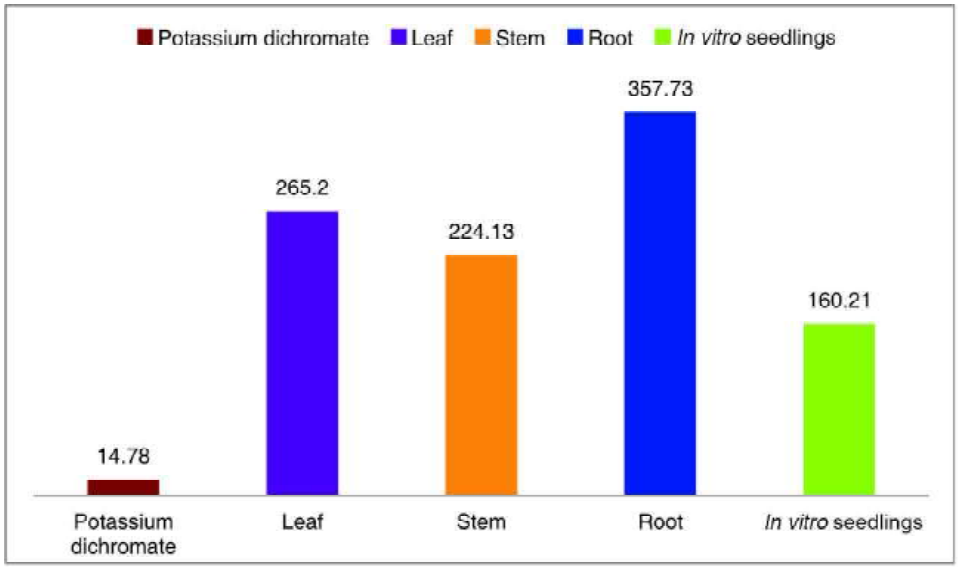

Fig. 7. LC_{50} (μgml^{-1}) value of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa* and Potassium dichromate.

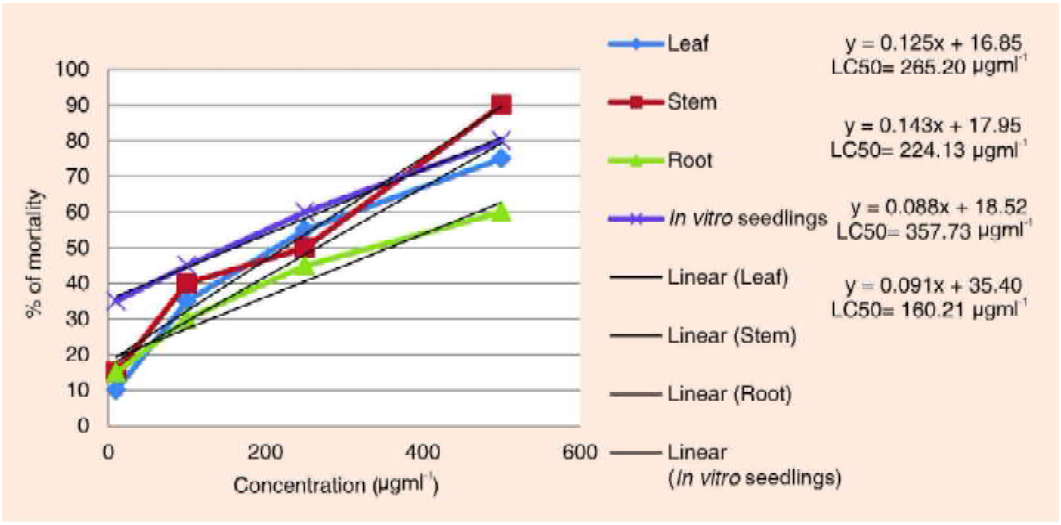

Fig. 6. Per cent mortality and LC_{50} (μgml^{-1}) value of *in vivo* sourced leaf, stem, root, and *in vitro* seedlings of *Acampe praemorsa*.

μgml^{-1}, respectively and IC_{50} of VCw: 317.23 μgml^{-1} and 163.66 μgml^{-1}, respectively).

Conclusion

The moderate antioxidant activity of *A. praemorsa* was found in the *in vivo* sourced leaves, stems, roots, and *in vitro* seedlings. The *in vitro* sample as well as the *in vivo* sourced leaf and stem exhibited potent anti-inflammatory effects. Both *in vitro* and *in vivo* sourced plant parts of this epiphytic orchid species exhibited a moderate level of cytotoxicity. Hence, *Acampe praemorsa,* an epiphytic orchid species found in Bangladesh, holds significant potential for widespread use in pharmaceutical applications due to its medicinal properties.

Acknowledgement

We would like to thank the Laboratory of Plant Tissue Culture and Biotechnology, Department of Botany, University of Chittagong, Bangladesh for providing all laboratory facilities during our research.

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