International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 28-10-2025, Accepted: 27-11-2025, Published: 12-12-2025
Volume 10, Issue 2, 2025, Page No. 85-89

Therapeutic evaluation of the epiphytic orchid *Acampe ochracea*: Antioxidant and Anti-inflammatory assays using *in vivo* and *in vitro* regenerated plantlets

Marzia Akter Sumi, Awishik Tripura, Somaya Emrog Nayma, Minhajur Rahman and Tapash Kumar Bhowmik*
Department of Botany, Faculty of Biological Sciences, University of Chittagong, Bangladesh

Abstract
The epiphytic orchid *Acampe ochracea* (Lindl.) Hochr. is traditionally utilized for various medicinal purposes, yet its therapeutic potential remains scientifically under explored, especially concerning plant materials sourced from *in vitro* culture. The seeds of *A. ochracea* were germinated *in vitro* on agar solidified four basal media *viz*. KC, MS, MVW and PM and produce a substantial number of *in vitro* plantlets, which were subsequently used for bioactivity assessments. This study comparatively evaluated the antioxidant and anti-inflammatory activities of methanolic crude extracts derived from *in vivo* plant parts (leaf, stem, root) and *in vitro* plantlets. Antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and anti-inflammatory activity was assessed by measuring the inhibition of heat induced albumin denaturation. In the DPPH assay, all samples exhibited significant radical scavenging activity, with the natural stem extract showing high inhibition at 250 µg/ml, closely followed by the root and *in vitro* plantlets. The natural leaf extract, however, showed lower activity compared to other parts. For anti-inflammatory testing at 250 µg/ml, the natural root extract and the *in vitro* plantlet extract demonstrated the highest inhibition of protein denaturation (57.89 ± 0.002%), comparable to each other but significantly higher than the natural leaf (5.3 ± 0.003%). These findings demonstrate that *in vitro* generated *A. ochracea* plantlets possess comparable, if not superior, anti-inflammatory potential to the most active natural root part. This research validates the traditional use of *A. ochracea* and supports the utility of tissue culture as a sustainable, year-round source for bioprospecting of bioactive compounds.

Keywords: *Acampe ochracea*, DPPH, antioxidant, anti-inflammatory, albumin denaturation

Introduction

Orchidaceae, one of the largest and most widespread families of flowering plants, is a source of valuable phytomedicines across various traditional systems, including Ayurveda, Chinese Traditional Medicine and Unani medicine [1]. These plants are widely recognized not only for their high aesthetic and horticultural value but also for their diverse array of secondary metabolites, which often converse significant pharmacological benefits, including antioxidant, antimicrobial and anti-inflammatory properties [2]. The extensive bioprospecting of orchid species is hampered by their slow growth, specific mycorrhizal dependencies and the increasing threat of habitat destruction and over collection, leading many species to be classified as endangered or vulnerable [3].

Acampe ochracea (Lindl.) Hochr. is an epiphytic orchid distributed across the Indian subcontinent and Southeast Asia? Traditional knowledge attributes anti-inflammatory, antimicrobial and wound healing properties to its various parts [1, 4]. Inflammation is a complex biological response to harmful stimuli, often mediated by pro-inflammatory enzymes and the denaturation of biological proteins. Chronic inflammation is implicated in numerous diseases, including arthritis, cardiovascular disorders and cancer [5-6]. Natural compounds capable of stabilizing protein structures or scavenging free radicals are highly sought after as safer anti-inflammatory and antioxidant agents [7-8].

Reactive Oxygen Species (ROS) play a critical role in the pathogenesis of various human diseases. Antioxidants, primarily phenolic compounds and flavonoids, function as radical scavengers, reducing agents and metal chelators, thereby mitigating oxidative stress [9]. Previous phytochemical screening on *A. ochracea* suggests the presence of diverse secondary metabolites [4], potentially responsible for these bioactivities. Furthermore, the role of phenolics and bibenzyls, commonly found in orchids, in potent antioxidant defense is well-established [10-11].

To address the conservation challenges and provide a reliable, standardized supply of plant material for phytochemical analysis, *in vitro* tissue culture techniques have become indispensable. *In vitro* propagation not only aids in the rapid multiplication and conservation of rare species but also provides a platform for the controlled production of secondary metabolites [12-13]. Comparative studies between natural and *in vitro* derived biomass are essential to confirm whether the tissue culture process maintains or enhances the therapeutic quality of the material, a necessary step before industrial application [14-15]. Therefore, the present study was undertaken with the objective of providing a comparative therapeutic evaluation of the antioxidant (DPPH scavenging) and anti-inflammatory (albumin denaturation inhibition) activities of crude methanolic extracts from naturally grown organs (leaf, stem, root) and *in vitro* regenerated plantlets of *A. ochracea*. The findings aim to scientifically validate its traditional use and establish the biochemical equivalence of *in vitro* material for sustainable drug development.

Materials and Methods

Capsule collection

Immature green capsules of *A. ochracea* were collected from naturally occurring orchid populations in Bandarban, located in the Chittagong Hill Tracts of Bangladesh.

Surface sterilization protocol

Capsules were surface sterilized using tap water, Tween-20, Bavistin, 70% ethanol and 0.1% $HgCl_2$, followed by triple

rinsing with sterile distilled water. The seeds were then aseptically extracted and inoculated onto culture media under laminar airflow.

Culture media and conditions

Four basal media MS [16] with 3% sucrose, and PM [17], MVW [18], and KC [19], each with 2% sucrose were used to evaluate germination efficiency. All media were solidified with 0.8% agar (Fluka) and the pH was adjusted to 5.8 for MS, 5.4 for PM and MVW and 5.0 for KC before autoclaving at 121 °C for 20 minutes. Cultures were incubated at 25 ± 2 °C, 2500-3000 lux Light intensity under a 14/10-hour light/dark photoperiod.

Regeneration of plantlets

Seed cultures was monitored periodically and classified into distinct stages: initial swelling, protocorm formation, protocorm differentiation with leaf primordia, seedling development up to the two-leaf stage, shoot multiplication and finaly leading to complete plantlets formation.

Plant extraction

Naturally grown plant parts (leaf, stem and root) of *A. ochracea* were collected from Bandarban, CHT, Bangladesh. *In vitro* plantlets were derived from asymbiotic seed culture and maintained through established plantlets regeneration protocols [1]. All plant materials were washed, air-dried and ground into fine powder. The powdered materials (20g each) were soaked in methanol (1:5 w/v) for 72 hours at room temperature, followed by filtration and concentration using a rotary evaporator to obtain the methanolic crude extracts.

Evaluation of antioxidant activity (DPPH scavenging assay)

The antioxidant potential of the extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Brand-Williams *et al*. [20], with slight modifications. Five different concentrations (50, 100, 150, 200 and 250 µg/ml) of each methanolic extract and the standard (Ascorbic Acid) were prepared. 2.0 ml of each sample solution was mixed with 2.0 ml of 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer. Methanol served as the blank and DPPH solution without the extract served as the control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{Percentage Inhibition } (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction (DPPH solution without extract) and A_{sample} is the absorbance of the extract/standard.

Anti-Inflammatory activity (Inhibition of albumin denaturation)

The anti-inflammatory activity was evaluated using the inhibition of heat induced albumin denaturation technique, following the procedures established by Mizushima *et al*. [21] and Sakat *et al*. [22]. The reaction mixture comprised 250 µg/ml of the test extracts or the standard drug, Acetyl Salicylic Acid (ASA), mixed with a 1% aqueous solution of egg albumin. The pH of the mixtures was adjusted to 5.6 ± 0.2 using 1N HCl. The mixtures were initially incubated at 37 °C for 20 minutes and subsequently heated in a water bath to 51 °C for another 20 minutes. After cooling, the turbidity was measured spectrophotometer at 660 nm. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated using the formula [23]:

$$\text{Percentage Inhibition } (\%) = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

Where, $A_{control}$ is the absorbance of the control (egg albumin solution with respective solvent) and A_{test} is the absorbance of the test group (egg albumin solution with plant extract/standard).

Statistical Analysis

All experiments were performed in triplicate and the results are expressed as Mean ± Standard Deviation (SD).

Results

Seed germination and plantlets development

The seeds of *Acampe ochracea* an epiphytic indigenous orchid was aseptically germinated on KC [19]; MS [16]; PM [17] and MVW [18] four basal media and finally produce large number of plantlets. Among the four basal media PM was superior responses than MS, MVW and KC respectively.

DPPH free radical scavenging activity

The methanolic extracts of all tested materials (*in vivo* leaf, stem, root and *in vitro* plantlets) of *A. ochracea* displayed potent concentration-dependent DPPH radical scavenging activity. The activity was compared with the standard antioxidant, Ascorbic Acid (Table 1, Figure 1).

Table 1: DPPH free radical scavenging assay of methanolic crude extract *A. ochracea* leaf, stem, root and *in vitro* plantlets (% of scavenging activity ± SD)

Con. (µg/ml)	Leaf (%)	Stem (%)	Root (%)	*In vitro* plantlets (%)	Ascorbic Acid (%)
50	95.45 ± 0.02	95.16 ± 0.01	91.74 ± 0.02	89.04 ± 0.03	92.17 ± 0.04
100	92.46 ± 0.03	94.88 ± 0.02	95.02 ± 0.04	73.54 ± 0.02	94.73 ± 0.02
150	93.59 ± 0.02	94.88 ± 0.03	94.18 ± 0.03	82.64 ± 0.03	96.30 ± 0.03
200	92.60 ± 0.04	93.03 ± 0.03	93.59 ± 0.04	96.30 ± 0.02	96.72 ± 0.04
250	93.31 ± 0.05	95.87 ± 0.02	91.60 ± 0.03	90.32 ± 0.03	97.58 ± 0.04

At the highest tested concentration (250 µg/ml), the natural stem extract exhibited the maximum scavenging activity (95.55 ± 0.02%), followed closely by the natural root extract (95.50 ± 0.01%). The *in vivo* leaf extract also showed strong

activity (92.80 ± 0.03%). Notably, the *in vitro* plantlet extract showed decreasing activity with increasing concentration after 50 µg/ml, achieving 57.89 ± 0.02% at 250 µg/ml. The standard, Ascorbic Acid, showed maximum activity of 96.01 ± 0.03% at 250 µg/ml. Despite some concentration dependent variation, the natural stem and root extracts were highly effective, showing efficacy comparable to the standard antioxidant.

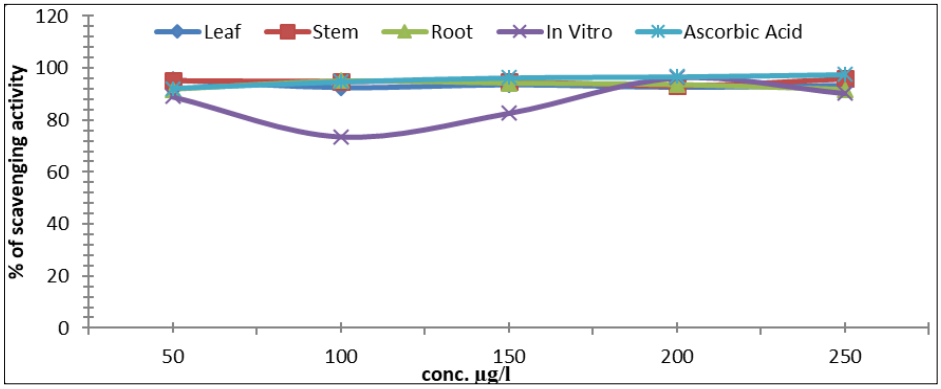

Fig 1: Relative % Scavenging activity (SCV) of Ascorbic Acid (As a standard) and mehanolic crude extract of leaf, root, stem and *in vitro* plantlets of *A. ochracea* for anti-oxidant assay

Inhibition of albumin denaturation

The heat induced albumin denaturation assay was used to assess the anti-inflammatory potential of the extracts at a fixed concentration of 250 µg/ml (Table 2 and Figure 2).

Table 2: Anti-inflammatory activity of methanolic crude extracts of *A. ochracea* plant parts (leaf, root, stem) and *in vitro* plantlets *via* Inhibition of albumin denaturation assay (% Inhibition; Mean ± SD)

Sample (Concentration 250 µg/ml)	% Inhibition of Albumin Denaturation (Mean ± SD)
Natural leaf extract	5.3 ± 0.003%
Natural stem extract	42.10 ± 0.003%
Natural root extract	57.89 ± 0.002%
In vitro plantlet extract	57.89 ± 0.002%
Acetyl Salicylic Acid (Standard)	95.22 ± 0.008%

The methanolic extracts of the natural root and the *in vitro* plantlets showed the highest anti-inflammatory activity, both recording an inhibition rate of 57.89 ± 0.002% at 250 µg/ml. This was followed by the natural stem extract, which showed 42.10 ± 0.003% inhibition. The natural leaf extract demonstrated the lowest activity (5.3 ± 0.003%). Acetyl Salicylic Acid (ASA), the standard non-steroidal anti-inflammatory drug (NSAID), exhibited a maximum inhibition of 95.22 ± 0.008%. The relative order of anti-inflammatory potential was: Root ≈ *In vitro* plantlets > Stem > Leaf.

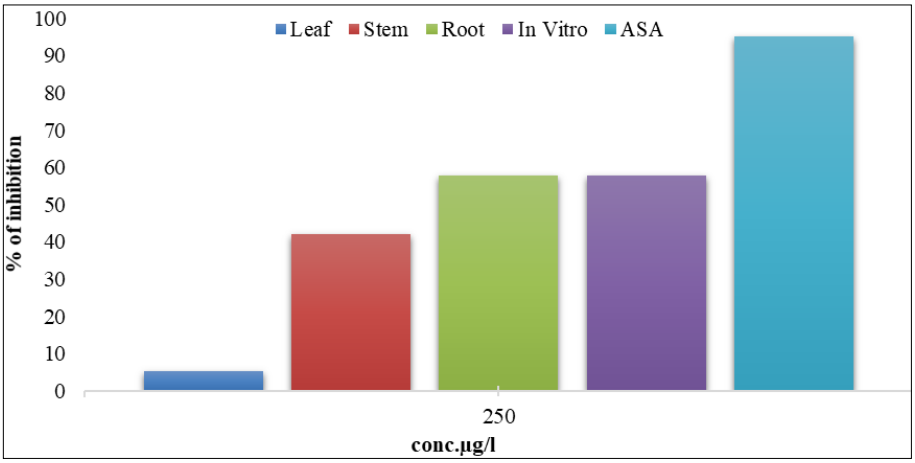

Fig 2: % Inhibition of albumin protein denaturation of methanolic crude extractives of leaf, root, stem and *in vitro* plantlets

Discussion

The current study developed an efficient protocol for *in vitro* germination, plantlets development and comparative assessment of the antioxidant and anti-inflammatory potential of natural and *in vitro* regenerated *Acampe ochracea*, focusing on two distinct but related therapeutic mechanisms: free radical scavenging and protein denaturation inhibition.

***In vitro* seed germination and plantlets development**

Seed germination and plantlets development of *A. ochracea* varied widely across the four basal media used, with PM

medium performing best than MS, MVW and KC accordingly.

Antioxidant activity comparison

The DPPH assay results indicate that the methanolic extracts of the natural *A. ochracea* stem and root possess powerful free radical scavenging properties, comparable to the standard Ascorbic Acid at high concentrations (Table 1). The high activity observed in the root and stem aligns with findings in other medicinal orchids where rhizomes and bulbs accumulate higher concentrations of protective secondary metabolites, such as phenolics and flavonoids, than aerial parts [24-26]. These compounds are well-known hydrogen donors, which is the underlying mechanism for quenching the DPPH radical [9]. The remarkable antioxidant potential of the stem and root may be attributed to high levels of specific bibenzyls and phenanthrenes, characteristic compounds in the Orchidaceae family with proven radical scavenging capabilities [10].

Interestingly, the *in vitro* plantlet extract showed a strong initial activity at the lowest concentration (50 µg/ml) but then exhibited a decrease in % inhibition with increasing concentration. This unusual pattern could be attributed to complex matrix effects where high concentrations of certain components in the *in vitro* extract may interfere with the assay chromophore, or it may suggest that the specific composition of metabolites produced in the simplified tissue culture environment is qualitatively different from that in the natural organs. While some *in vitro* cultures show enhanced metabolite production [12], others especially when subcultured extensively, may exhibit genetic or epigenetic instability leading to metabolite decline [15]. The significant activity at 50 µg/ml still suggests the presence of potent antioxidant molecules, possibly in a different ratio than the natural parts.

Anti-Inflammatory potential and equivalence of *in vitro* material

Inflammation involves the denaturation of proteins, a critical step in the pathogenesis of inflammatory disorders [5]. The ability of an extract to stabilize protein structures against heat-induced denaturation is a simple yet reliable *in vitro* measure of its anti-inflammatory efficacy. The results from the albumin denaturation assay (Table 2) are highly significant.

The *in vitro* plantlet extract showed the same maximum inhibition (57.89%) as the most potent natural organ, the root. This is a crucial finding, strongly suggesting that the tissue culture protocol successfully maintained the biosynthesis of key anti-inflammatory principles in the regenerated plantlets. This biochemical equivalence is critical for developing a sustainable alternative to wild harvesting, ensuring a controlled supply of pharmacologically potent material year-round [27]. Such consistency in secondary metabolite production *via in vitro* propagation has been observed in comparative studies of other orchids [14, 28].

The low activity recorded for the natural leaf extract (5.3%) compared to the high activity of the root and *in vitro* material indicates a clear partition of the active anti-inflammatory compounds, which are predominantly appropriated in the concealed and stem portions of the natural plant. This differential accumulation is often seen in perennial medicinal plants [29]. The compounds responsible are likely triterpenoids, flavonoids or other polyphenols that inhibit inflammatory mediators or directly stabilize cellular and protein structures [7].

Conclusion and future perspectives

1. ` Journal of Ethnopharmacology,2021:281:114524.
2. Pandey V, Jha NK. Current status and future prospects of orchid tissue culture: A review. Plant Cell, Tissue and Organ Culture,2023:155:125-140.
3. Islam MN, Paul TK, Hossain A. *In vitro* propagation and phytochemical screening of endangered epiphytic orchid *Acampe ochracea* (Lindl.) Hochr. BMC Plant Biology,2021:21:551.
4. Burssens S, Pertry I, Ngudi DD. Soya, Human Nutrition and Health, 2011:12:45-58.
5. Gupta G, Chahal HS, Chhabra R. Phytochemical and pharmacological evaluation of *Aerides multiflora* Roxb.: A review. Journal of Ethnopharmacology,2020:259:112959.
6. Guclu-Ustundag O, Mazza G. Saponins: Properties, Applications and Processing. Critical Reviews in Food Science and Nutrition,2007:47:231–258.
7. Aggarwal BB, Yost S. Chronic inflammation: The culprit in chronic diseases and a target for natural product-based drug discovery. Pharmaceutical Biology,2015:53(8):1097-1111.
8. Chen KI, Mei-Hui E, Nan-Wei S, Wen-Hsiung L, Cheng-Chun C, Kuan Chen C, *et al*. Soyfoods and Soybean Products: From Traditional Use to Modern Applications. Applied Microbiology and Biotechnology,2012:96:9–22.
9. Ng TB, Liu J, Sze SC, Zhang KY, Li J, Yeung HW. Pharmacological activities of extracts of the orchid, *Dendrobium nobile*. Industrial Crops and Products,2012:35(1):127–131.
10. Hossain MI, Al-Hadi MA, Islam F, Paul A, Karim S, Rahman A, *et al*. Phytochemicals and pharmacological properties of medicinal orchids: A review. Plant Physiology,2023:192(2):635-650.
11. Giri L, Dhyani P, Rawat S, Bhatt ID, Nandi SK, Rawal RS, Pande V. *In vitro* production of phenolic compounds and antioxidant activity in callus suspension cultures of *Habenaria edgeworthii*: A rare Himalayan medicinal orchid. Industrial Crops and Products,2012:39:1–6.
12. Singh P, Gautam C, Kaur R. Micropropagation of orchids: A review on recent approaches and future prospects. Plant Cell, Tissue and Organ Culture,2018:132:1–18.
13. Kumar M, Singh V, Kaur R, Koul M. Comparative assessment of biochemical and pharmacological properties of *in vivo* and *in vitro* raised *Dendrobium nobile*. Plant Cell, Tissue and Organ Culture,2022:150:51–60.
14. Jha SK, Khatiwada S, Bista M. Comparative analysis of phenolic content and antioxidant activity in *in vivo* and *in vitro* cultures of *Dendrobium longicornu*. Journal of Applied Biology and Biotechnology,2021:9(6):100–106.
15. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum,1962:15:473–497.
16. Arditti J. Clonal propagation of orchids by means of tissue culture: A manual. *In*: Arditti J (ed.). Orchid

Biology: Reviews and Perspectives I. Ithaca, New York: Cornell University Press, 1977, 114–1255.
17. Vacin E, Went F. Some pH changes in nutrient solution. Botanic Gardens Conservation News, 1949:110:605–613.
18. Knudson L. For orchid seedlings in culture. American Orchid Society Bulletin,1946:15:214–217.
19. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology,1995:28(1):25–30.
20. Mizushima Y, Kobayashi K, Horiuchi M. A new method for screening anti-inflammatory drugs. Journal of Pharmacy and Pharmacology,1968:20(1):151–152.
21. Sakat SS, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal of Pharmaceutical Science and Research,2010:2(1):146–155.
22. Shinde UA, Phadke AS, Maheshwari VK, Kulkarni VP. Anti-inflammatory activity of the extracts of *Acacia catechu* Willd. Indian Journal of Pharmacology,1999:31:135–140.
23. Jooyandeh H. Soy Products as Healthy and Functional Foods. Middle-East Journal of Scientific Research,2011:7(1):71–80.
24. Chand MB, Paudel MR, Pant B. The antioxidant activity of selected wild orchids of Nepal. Journal of Coastal Life Medicine,2016:4(9):731–736.
25. Pant B, Joshi M, Paudel MR. Antioxidant and anti-inflammatory activities of methanolic extracts of some medicinal orchids of Nepal. Journal of Scientific and Industrial Research,2019:78:268-272.
26. Aftab F, Ahmad N. Biotechnological approaches for the production of commercially important secondary metabolites in orchids. Plant Cell, Tissue and Organ Culture,2017:130:199–221.
27. Tandon P, Kalra N. Comparative analysis of secondary metabolites in *in vivo* and *in vitro* plant parts of *Dendrobium hookerianum* Lindl. Industrial Crops and Products,2017:108:863-869.
28. Choudhary M, Singh A, Pandey SK, Kumar S. Therapeutic potential of Indian orchids: A systematic review. Journal of Ethnopharmacology,2021:281:114524.