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In vitro propagation, phytochemical profiling and biological activities (antioxidant, anti-inflammatory and cytotoxicity) of *Aerides odoratum* lour: An indigenous medicinal orchid of Bangladesh

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Abstract
Aerides odoratum Lour., an epiphytic medicinal orchid native to Bangladesh, holds significant therapeutic and ornamental value but faces severe population decline due to overharvesting and habitat destruction. This study established an optimized *in vitro* propagation protocol and comprehensively evaluated quantitative phytochemical profiles, antioxidant, anti-inflammatory and cytotoxic activities across its naturally grown leaf, stem and root extracts. A robust propagation system utilizing varied basal medium supplemented with plant growth regulators (PGRs) successfully promoted seed germination, protocorm-like bodies (PLBs) proliferation and shoot-root differentiation and *ex vitro* acclimatization. Qualitative phytochemical screening revealed abundant secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids and quinones, distributed differentially across organs. Biological efficacies demonstrated concentration-dependent responses. In the DPPH free radical scavenging assay, the leaf extract exhibited superior antioxidant activity with an IC_{50} of 181.25 µg/ml compared to stem (IC_{50} = 135.05 µg/ml) and root (IC_{50} = 57.138 µg/ml) extracts (standard ascorbic acid IC_{50} = 26.08 µg/ml). Anti-inflammatory evaluation *via* egg albumin denaturation inhibition revealed exceptional potency in the leaf extract (IC_{50} = 89.81 µg/ml), root extract (IC_{50} = 82.80 µg/ml), stem extract (IC_{50} = 40.56 µg/ml) and relative to standard acetylsalicylic acid (IC_{50} = 30.24 µg/ml). Cytotoxic evaluation using the brine shrimp lethality bioassay indicated moderate safety thresholds with median lethal concentration (LC_{50}) values of 234.84 µg/ml (stem), 143.05 µg/ml (leaf) and 292.88 µg/ml (root) and relative to standard (LC_{50} = 43.50 µg/ml). These quantitative findings scientifically validate the traditional ethno-medicinal uses of *A. odoratum* and confirm its pharmacological potential, while the established tissue culture protocol offers a sustainable conservation framework.

Keywords: *Aerides odoratum* Lour., Anti-inflammatory, Antioxidant, Cytotoxicity, *In vitro* propagation, Phytochemical profiling

Introduction

The family Orchidaceae represents one of the most taxonomically advanced, diverse and widespread families of flowering plants, comprising over 25,000 accepted species distributed across varied global ecosystems (Chase *et al.* 2015) [1]. Beyond their immense ornamental appeal in the global horticultural trade, orchids have been recognized as profound reservoirs of therapeutic secondary metabolites. For centuries, traditional medical systems, including Ayurveda and Traditional Chinese Medicine, have extensively utilized orchid derived formulations to treat an array of physiological disorders (Pant 2013) [2]. The production of diverse phytochemicals such as alkaloids, bibenzyl derivatives, phenanthrenes and flavonoids dictates their widespread pharmacological applicability, including antimicrobial, anti-inflammatory, neuroprotective and antioxidant activities (Gantait *et al.* 2021) [3].

Bangladesh, characterized by its tropical and subtropical climate, harbors a rich diversity of indigenous orchids, prominently distributed in the evergreen and semi evergreen forests of the Chittagong Hill Tracts, Sylhet and the Sundarbans (Hossain 2009) [4]. Among these, *Aerides odaoratum* Lour. (syn. *Aerides odorata*), a monopodial epiphytic orchid, stands out due to its profound medicinal utility. Various tribal communities in Bangladesh and neighboring regions have historically relied on *A. odoratum* to treat severe ailments. The root paste is applied externally for rheumatism and snake bites, while the leaves are utilized in decoctions to treat dyspepsia, epilepsy, cuts and bone fractures due to their localized antibacterial and wound healing properties (Huda and Kashem 2021) [5].

Despite its recognized medicinal value, *A. odoratum* is currently classified among the threatened flora. The highly specialized ecological requirements of orchids including pollinator specificity, mandatory mycorrhizal associations for seed germination and extreme susceptibility to habitat disturbance render them particularly vulnerable (Swarts and Dixon 2009) [6]. Consequently, unchecked commercial harvesting of wild *A. odoratum* for horticultural and traditional medicine markets has drastically depleted its natural populations (Bhatnagar *et al.* 2017) [7]. To alleviate this ecological pressure, the development of optimized *in vitro* plant tissue culture techniques is imperative. Asymbiotic seed germination and micropropagation utilizing plant growth regulators (PGRs) facilitate rapid, mass scale clonal multiplication, providing a sustainable biomass supply for both conservation and pharmacological extraction (Chugh *et al.* 2009) [8].

While the traditional uses of *A. odoratum* are well documented, there exists a critical research gap concerning

comprehensive phytochemical profiling and quantitative evaluation of pharmacological activities across its specific vegetative parts (leaf, stem and root). Validating biological efficacy through structured *in vitro* assays specifically for free radical scavenging, protein denaturation inhibition and cytotoxicity is necessary to position this species as a viable candidate for modern phyto-medicine (Saleh-e-In *et al.* 2016) [9].

Therefore, the present study was meticulously designed with explicit objectives: (i) to standardize *in vitro* propagation protocols for *A. odoratum* utilizing varied basal media and PGRs; (ii) to induce robust rooting and successfully acclimatize seedlings under *ex vitro* conditions; (iii) to qualitatively screen secondary metabolites present in wild-grown leaf, stem and root tissues; and (iv) to systematically evaluate and compare their antioxidant (DPPH), anti-inflammatory (albumin denaturation) and cytotoxic (brine shrimp lethality) potentials.

Materials and Methods

1. Plant material and morphological identification

Capsules and vegetative segments of the indigenous epiphytic orchid *Aerides odoratum* Lour. were collected from the natural habitat of Bengchari, Kaptai, Rangamati, Bangladesh. Taxonomic identification was confirmed using standard literature. The plant is characterized by an elongated, scandent, densely leafy stem and obliquely notched, thick, fleshy leaves. The inflorescence is racemose and multi-flowered with pendulous yellow-green blooms.

2. Sterilization and preparation of explants

Healthy, un-dehisced capsules of *A. odoratum* were initially washed under running tap water to eliminate adhering dust, followed by a sterile distilled water wash. Capsules were swabbed with a dilute antiseptic (Savlon) and subsequently surface-sterilized using a 0.2% (w/v) mercuric chloride ($HgCl_2$) solution for 5 minutes. Inside a pre-sterilized laminar airflow cabinet, capsules were subjected to a final disinfection step utilizing 70% ethanol for 1 minute and thoroughly rinsed with double sterile distilled water. Surface sterilized capsules were longitudinally dissected with a sterile surgical blade and powdery seeds were aseptically inoculated onto germination media. For vegetative micropropagation, *in vitro* grown seedlings attaining 3-4 cm in height was excised into leaf and nodal segments (1.0-1.5 cm) for subsequent sub-culturing.

3. Preparation of culture media

Four distinct basal media were formulated for aseptic seed germination and micropropagation: MS (Murashige and Skoog 1962) [10] with 3% (w/v) sucrose; PM: Phytamax (Arditti 1977) [11] with 2% (w/v) sucrose; MVW (Vacin and Went 1949) [12] with 2% (w/v) sucrose and KC (Knudson 1946) [13] with 2% (w/v) sucrose.

4. Plant growth regulators (PGRs) and culture conditions

Basal medium was supplemented with varied concentrations and combinations of specific auxins Naphthaleneacetic acid (NAA) and cytokinin 6-Benzylaminopurine (BAP). Stock solutions of PGRs were prepared by dissolving 10 mg of the respective hormone in 1 ml of specific solvent and adjusting volume to 100 ml with distilled water. All inoculated culture vessels were incubated in a highly controlled culture room maintained at 25 ± 2°C with a photoperiod of 14 hours of continuous cool-white fluorescent light and 10 hours of darkness.

5. Acclimatization and *ex vitro* hardening

In vitro regenerated seedlings manifesting well developed roots (2-3 cm) were subjected to a gradual acclimatization protocol. Culture vessels were uncapped for 24 hours within the culture room, then transitioned to ambient conditions for incrementally longer intervals. Seedlings were gently extracted and agar was meticulously washed from roots under running water. They were then transplanted into pots containing a sterilized, moistened matrix of coconut coir, coir dust, brick pieces and charcoal and monitored for 2-3 months to record survival and establishment.

6. Phytochemical extraction and qualitative screening

Fresh leaf, stem and root tissues from wild grown *A. odoratum* were air dried and pulverized into fine powder. Extracts were prepared by macerating specific ground plant parts in high purity methanol. Extracts were subjected to qualitative phytochemical screening to identify therapeutically active secondary metabolites, following established methodologies (Harborne 1973, Sofowora 1993) [14-15]. Specific spot tests for alkaloids were performed employing five distinct reagents: Dragendorff's, Hager's, Mayer's, Wagner's and Tannic acid reagent. Tests for other metabolites included ethyl acetate/ammonia test for flavonoids, foaming test for saponins, ferric chloride test for tannins and phenols, sulfuric acid interface test for terpenoids and steroids and magnesium acetate test for anthraquinones. Relative abundance was recorded qualitatively based on precipitate density or color intensity.

7. Bioactivity Assays

7.1 Antioxidant activity (DPPH assay)

Free radical scavenging potential was assessed utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams *et al.* 1995) [16]. Briefly, 3 ml of methanolic plant extract at varying concentrations (50, 100, 150, 200 and 250 µg/ml) was mixed with 3 ml of 0.004% DPPH methanolic solution. The reaction mixture was incubated in total darkness for 30 minutes. Absorbance was measured at 517 nm against a blank using a UV visible spectrophotometer. Ascorbic acid served as the positive standard. Scavenging activity percentage was calculated as:

$$\text{Scavenging Activity (\%)} = \left[\frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}}\right] \times 100$$

IC_{50} values were computed utilizing linear regression analysis.

7.2 Anti-inflammatory activity (albumin denaturation assay)

Anti-inflammatory efficacy was evaluated via inhibition of egg albumin denaturation (Mizushima and Kobayashi 1968) [17]. Reaction mixtures comprised 1 ml of varying sample

concentrations (50-250 µg/ml) and 1 ml of 5% aqueous egg albumin solution. pH was carefully adjusted to 5.6 ± 0.2. Mixtures were incubated at 37°C for 20 minutes, followed by controlled heat induction at 57°C for 20 minutes. Post-cooling, turbidity was recorded spectrophotometrically at 660 nm. Acetylsalicylic acid was utilized as the standard reference. Inhibition percentage was calculated and IC_{50} values were derived.

7.3 Cytotoxic activity (brine shrimp lethality bioassay)

Cytotoxicity was screened employing the brine shrimp (*Artemia salina*) lethality bioassay (Meyer *et al.* 1982) [18]. Artificial seawater (3.8% NaCl w/v) was prepared to hatch shrimp eggs under constant aeration for 48 hours. Methanolic plant extracts were prepared at concentrations of 10, 100, 250 and 500 µg/ml. For each concentration, 20 active nauplii were transferred into test tubes containing respective extracts. Potassium dichromate ($K_2Cr_2O_7$) was evaluated concurrently as the positive standard (at 10, 20, 30 and 50 µg/ml). After 24 hours of incubation, surviving nauplii were counted using a magnifying glass. Mortality percentage was computed and median lethal concentration (LC_{50}) was extrapolated from regression curves.

8. Statistical analysis

All biological assays were executed in triplicate (n=3). Results were expressed as Mean ± Standard Error of Mean (SEM). Linear regression models were generated to calculate precise IC_{50} and LC_{50} values across all evaluated parameters.

Results

1. *In vitro* propagation and acclimatization

Seed germination, protocorm formation and propagation utilizing varied basal media and selective combinations of auxins and cytokinins (0.5 mg/l BAP and 0.5 mg/l NAA) demonstrated successful *in vitro* generation of *A. odoratum*. Subsequent sub-culturing onto elongation and specific rooting media yielded study, well developed root architectures. During the critical phase of *ex vitro* acclimatization, generated seedlings exhibited strong morphogenic resilience, achieving an 75% survival rate when transplanted to composed potting matrices.

2. Phytochemical screening

Extensive qualitative profiling identified the presence of diverse secondary metabolites across tissues of naturally grown *A. odoratum*.

The results detailed in Table 1 reveal the organ-specific distribution of alkaloids across leaf, stem, root and *in vitro* shoots tissues utilizing five distinct analytical reagents. Dragendorff's reagent (D) and Hager's reagent (H) demonstrated intense orange-red and yellow precipitates respectively in leaf extracts (recorded as +++), whereas Mayer's reagent (M) exhibited moderate precipitation (++) and Tannic acid (T) and Wagner's reagent (W) showed strong positive responses (+++). In *in vitro* shoots extracts, Dragendorff's, Mayer's and Wagner's reagent yielded moderate positive precipitation (++), while Hager's and Tannic acid reagent showed minimum positive precipitation (+). Whereas, in stem extracts, Dragendorff's reagent yielded maximum positive precipitation (+++), while Hager's reagent showed a complete absence (---) and Mayer's, Tannic acid and Wagner's reagents indicated moderate-to-high presence (++). Root extracts prominently reacted with Mayer's and Wagner's reagents (+++), confirming that alkaloidal compounds are heavily synthesized and translocate across all vegetative organs, providing a strong chemical basis for their traditional use in neurological and somatic disorders.

The comprehensive screening of twelve distinct phytochemical groups summarized in Table 2 highlights significant metabolic partitioning among the leaf, stem, root and *in vitro* shoots tissues. Flavonoids, In *in vitro* shoots extracts terpenoids, quinones and cardiac glycosides showed moderate response (++); tannins, phlobatannins, anthraquinones and coumarins showed minimum responses (+) while saponins and steroid showed a complete absence (---). Saponins were moderately detected (++) in leaf and stem extracts, but showed lower presence (+) in roots. Tannins, terpenoids and quinones were exceptionally abundant (+++) across all three plant organs, underscoring their baseline contribution to the plant's robust defense and antioxidant mechanisms. Flavonoids showed moderate response in leaves and stems (++) but peaked in root tissues (+++). Interestingly, phlobatannins were entirely absent (---) in leaves, whereas they were heavily concentrated in stems (+++) and moderately present in roots (++). Steroids and coumarins were highest in leaves (+++), while anthraquinones were prominently concentrated in stems (+++). Cardiac glycosides were abundant in both leaves and roots (+++) but moderate in stems (++). This clear biochemical heterogeneity confirms that different plant parts accumulate specialized secondary metabolites tailored for distinct ecological and pharmacological roles.

Table 1: Qualitative phytochemical profiling (alkaloids) of naturally grown leaf, stem and root of *Aerides odoratum*.

Plant parts Used	Qualitative estimation of alkaloids				
	D	H	M	T	W
Leaf	+++	+++	++	+++	+++
Stem	+++	---	++	++	++
Root	++	+	+++	++	+++
In vitro Shoots	++	+	++	+	++

Note: D= Dragendorff's, H= Hager's, M= Mayer's, T= Tannic acid, W= Wagner's reagent. +++ = high, ++ = medium, + = mild, --- = absent.

Table 2: Qualitative test of twelve other phytochemicals of *Aerides odoratum.*

	Name	Plant parts used of *A. odoratum*			
		Leaf	**Stem**	**Root**	***In vitro* Shoots**
Secondary Metabolites (% of coloration)	Sap.	++	++	+	---
	Tan.	+++	+++	+++	+
	Flv.	++	++	+++	++
	Str.	+++	++	+	---
	Ter.	+++	+++	+++	++
	Phl.	---	+++	++	+
	Qui.	+++	+++	+++	++
	Ant.	+	+++	++	+
	Cou.	+++	+++	++	+
	Car. gly.	+++	++	+++	++

Note: +++ = highest response, ++ = medium response, + = lowest response, --- = absent.

3. Antioxidant activity

The DPPH free radical scavenging capability of methanolic extracts exhibited concentration dependent increases across all tested samples. As shown in Figure 1, the reference standard ascorbic acid demonstrated exceptionally high radical scavenging efficiency. Scavenging activity increased progressively from 52.30 ± 0.72% at 50 µg/ml to a maximum of 94.67 ± 0.23% at 250 µg/ml, yielding a baseline half maximal inhibitory concentration (IC_{50}) of 26.08 µg/ml. This establishes the benchmark for evaluating plant extract potency.

The concentration dependent antioxidant profiles of the leaf, stem and root extracts are detailed in Figure 2, Figure 3 and Figure 4 respectively, with comparative IC_{50} values consolidated in Figure 5. Leaf extracts (Figure 2) showed scavenging activity ranging from 15.54 ± 0.34% at 50 µg/ml to 60.65 ± 0.33% at 250 µg/ml, corresponding to an IC_{50} of 181.25 µg/ml. Notably, the stem extract (Figure 3) outperformed both, demonstrating superior radical scavenging capacity ranging from 34.54 ± 0.24% at 50 µg/ml to a peak of 67.49 ± 0.38% at 250 µg/ml, yielding a significantly lower and more potent IC_{50} value of 135.05 µg/ml. Root extracts (Figure 4) exhibited comparable activity, rising from 47.47 ± 0.76% to 71.08 ± 0.37% with an IC_{50} of 57.138 µg/ml. This superior antioxidant efficacy in stems directly correlates with their high concentration of tannins, terpenoids and quinones identified during phytochemical screening.

4. Anti-inflammatory activity

Inhibition of thermally induced egg albumin denaturation demonstrated strong *in vitro* anti-inflammatory properties across all extracts. As presented in Figure 6, the reference standard acetylsalicylic acid exhibited potent inhibition of protein denaturation, increasing from 51.22 ± 0.27% at 50 µg/ml to 92.33 ± 0.49% at 250 µg/ml, with a calculated IC_{50} of 30.24 µg/ml.

The anti-inflammatory efficacy of the extracts evaluated *via* protein denaturation inhibition is detailed in Figure 7 (Leaf), Figure 8 (Stem) and Figure 9 (Root), with comparative IC_{50} summaries in Figure 10. Leaf extracts (Figure 7) demonstrated higher efficacy, with inhibition rising from 46.23 ± 0.41% to 74.91 ± 0.55% (IC_{50} = 89.81 µg/ml). Strikingly, the stem extract (Figure 8) exhibited the most potent anti-inflammatory activity, achieving 50.55 ± 0.31% inhibition even at the lowest concentration of 50 µg/ml and reaching a maximum inhibition of 95.55 ± 0.50% at 250 µg/ml, yielding a remarkably strong IC_{50} value of 40.56 µg/ml. Root extracts (Figure 9) showed moderate inhibition ranging from 42.22 ± 0.63% to 87.77 ± 0.81%, corresponding to an IC_{50} of 82.80 µg/ml. These findings provide robust empirical validation for the traditional utilization of *A. odoratum* leaf and stem pastes in treating rheumatism and inflammatory joint conditions.

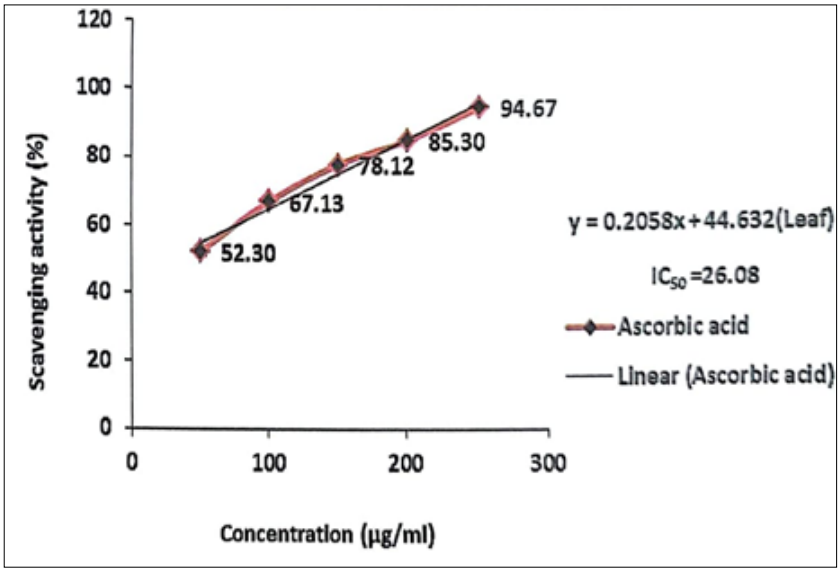
5. Cytotoxic activity (Brine shrimp lethality bioassay)

General toxicity mechanisms against *Artemia salina* nauplii revealed concentration-dependent mortality rates across all evaluations. As detailed in Figure 11, the positive reference standard potassium dichromate induced rapid, concentration-dependent mortality in *Artemia salina* nauplii. Mortality increased from 40% (8 dead out of 20 nauplii) at 10 µg/ml to 100% (20 dead out of 20) at 50 µg/ml, yielding a highly potent LC_{50} value of 43.50 µg/ml.

The brine shrimp lethality metrics for leaf, stem and root extracts across logarithmic concentrations (10 to 500 µg/ml) are presented in Figure 12, Figure 13 and Figure 14 respectively. Leaf extracts (Figure 12) showed moderate toxicity, with mortality increasing from 10% to 75% (LC_{50} = 143.05 µg/ml). Stem extracts (Figure 13) demonstrated the highest relative cytotoxicity among the plant parts, with mortality rising from 15% (3 dead, 17 alive at 10 µg/ml) to 90% (18 dead, 2 alive at 500 µg/ml), yielding an LC_{50} of 234.84 µg/ml. Root extracts (Figure 14) exhibited the lowest toxicity, with mortality ranging from 15% at 10 µg/ml to 60% at 500 µg/ml, resulting in an LC_{50} of 292.88 µg/ml. According to established toxicological benchmarks, LC_{50} values between 100-500 µg/ml designate moderate toxicity, indicating that the extracts maintain biological safety at lower thresholds while possessing bioactive constituents capable of cellular inhibition at elevated concentrations.

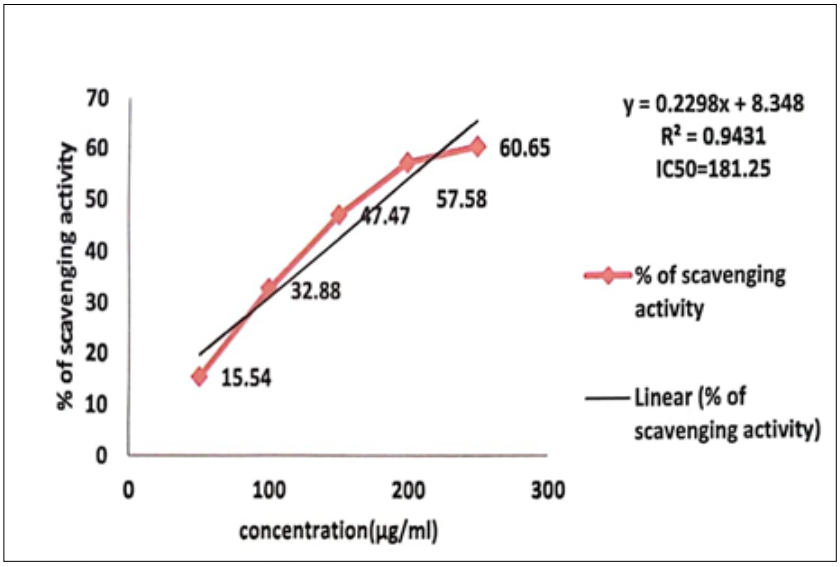
Discussion

Exhaustive *in vitro* propagation and acclimatization techniques documented in this study offer a definitive, sustainable strategy for countering rapid ecological depletion of *Aerides odoratum*. Because terrestrial and epiphytic orchids rely heavily on intricate mycorrhizal relationships in natural habitats, *in vitro* replication relies fundamentally on complex, asymbiotic nutrient provisions (Chugh *et al.* 2009) [8]. Transition from controlled micropropagation into successful *ex vitro* acclimatization observed here mirrors established protocols applied to endangered Orchidaceae genera, verifying viability of scalable commercial cloning for therapeutic harvesting without disturbing indigenous populations (Pant 2013) [2].



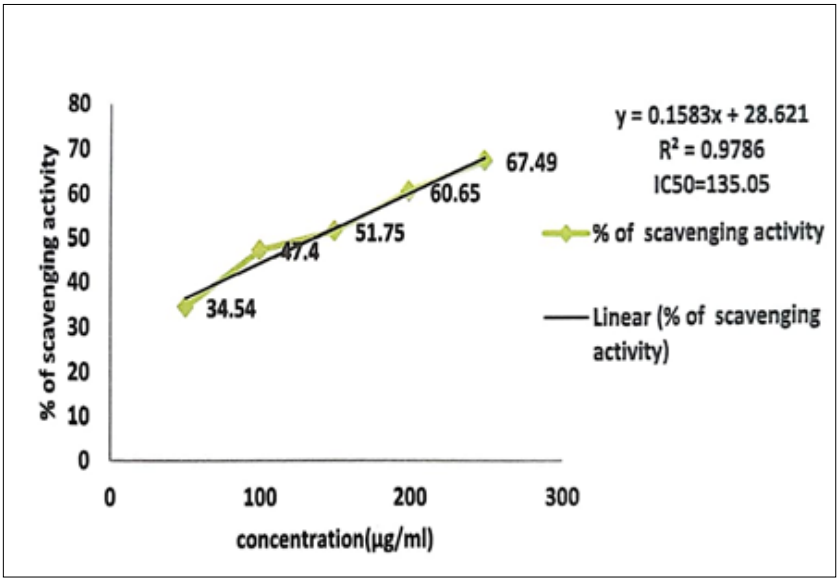
Note: Results are presented as mean ± SEM (n = 3)

Fig 1: Calculation of IC_{50} (µg/ml) value of ascorbic acid (Standard)



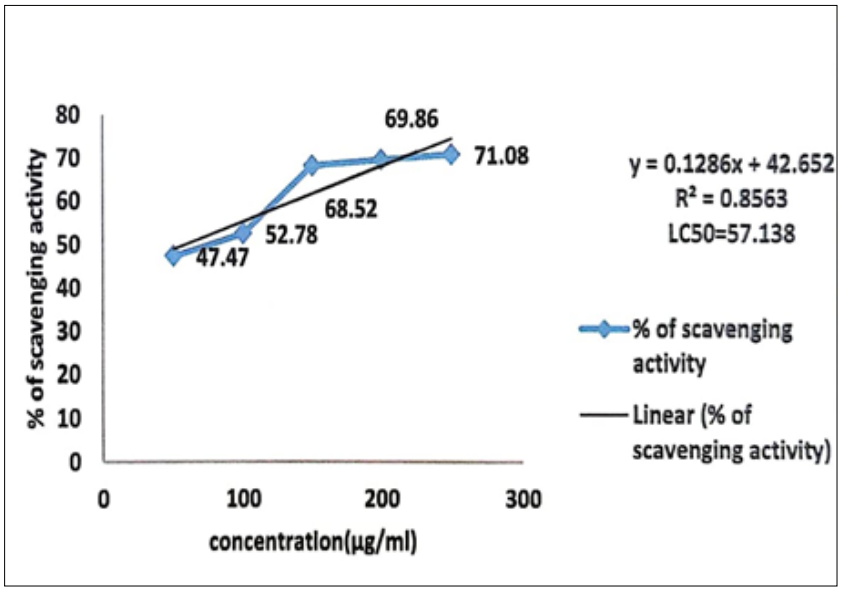
Note: Results are presented as mean ± SEM (n = 3)

Fig 2: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Leaf)



Note: Results are presented as mean ± SEM (n = 3)

Fig 3: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Stem)



Note: Results are presented as mean ± SEM (n = 3)

Fig 4: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Root)

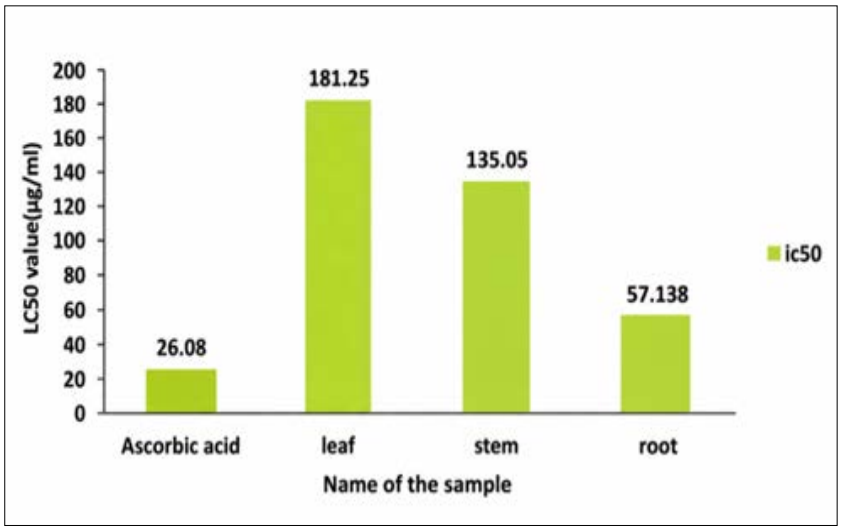
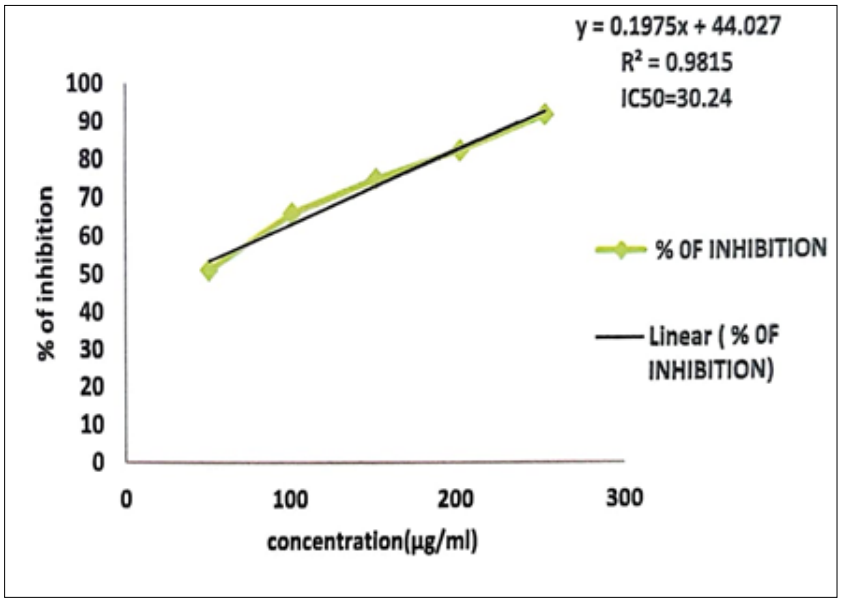
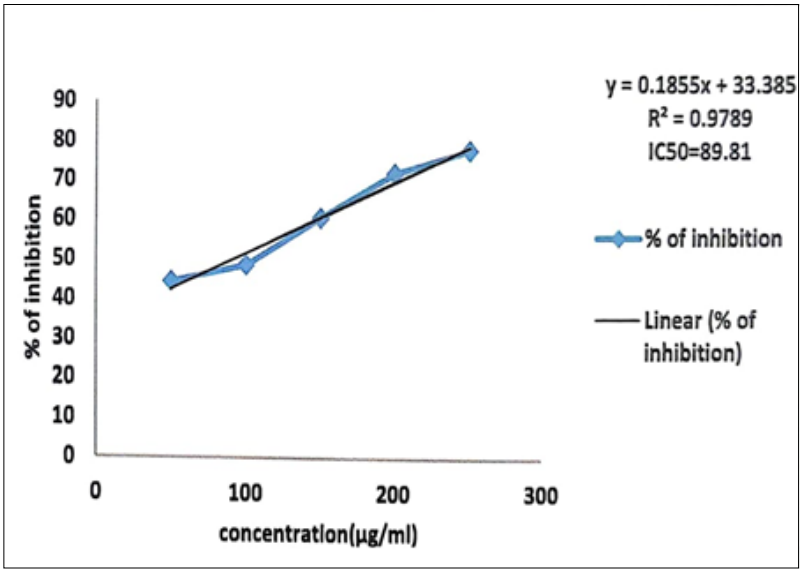


Fig 5: IC_{50} values (antioxidant) of standard and plant extracts of *A. odoratum*



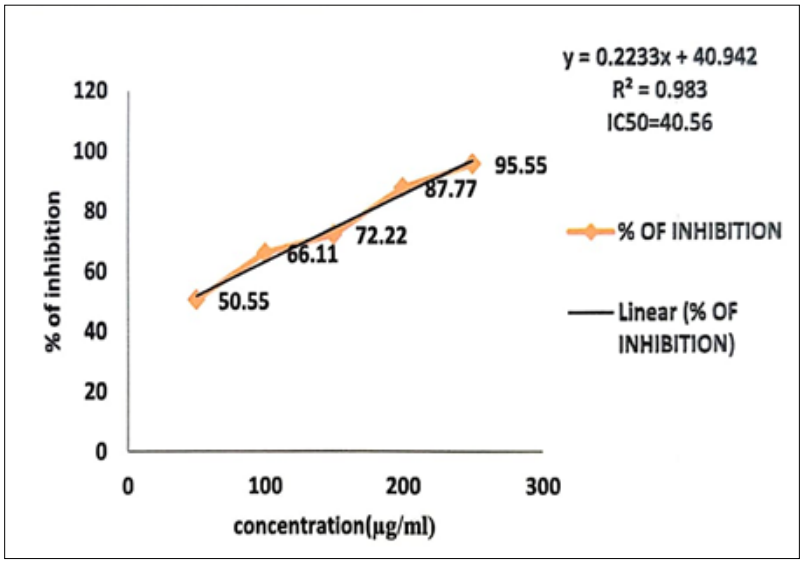
Note: Results are presented as mean ± SEM (n = 3)

Fig 6: Calculation of IC_{50} (µg/ml) value of acetyl salicylic acid (Standard)



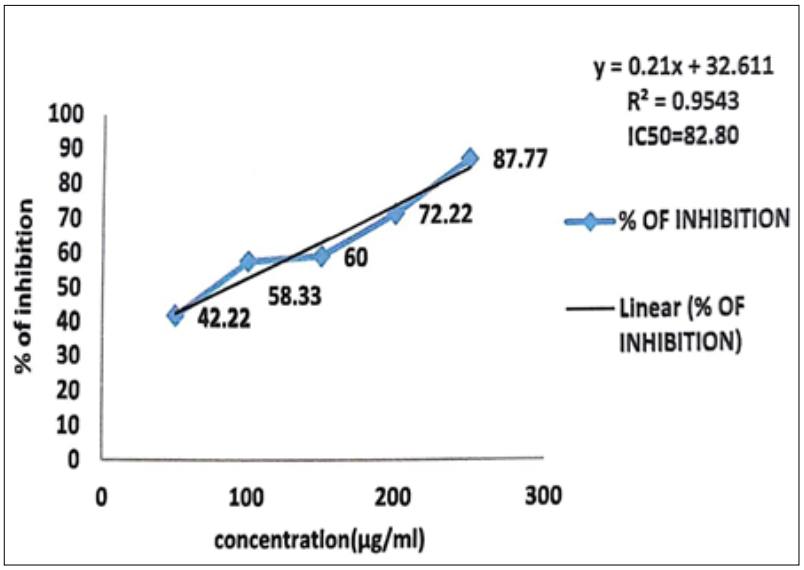
Note: Results are presented as mean ± SEM (n = 3)

Fig 7: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Leaf)



Note: Results are presented as mean ± SEM (n = 3)

Fig 8: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Stem)



Note: Results are presented as mean ± SEM (n = 3)

Fig 9: Calculation of IC_{50} (µg/ml) value of *A. odoratum* (Root)

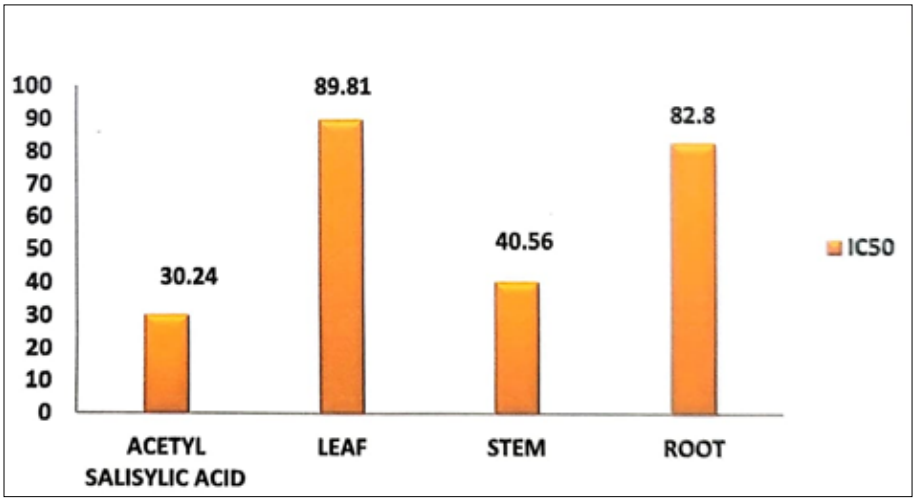


Fig 10: IC_{50} values of standard and crude extract of *A. odoratum* (Anti-inflammatory)

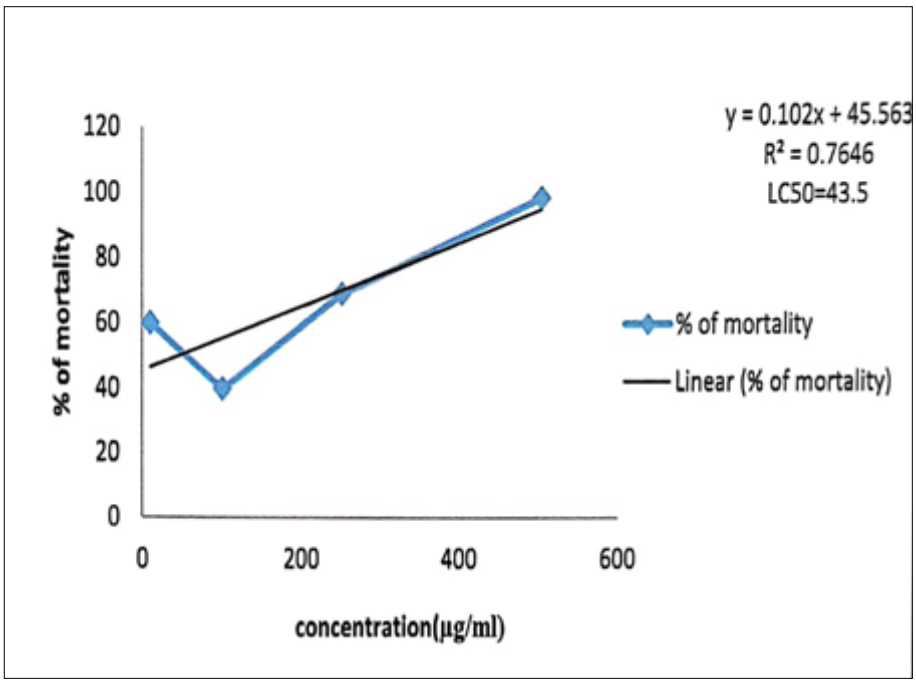


Fig 11: Calculation of LC_{50} (µg/ml) value of potassium dichromate (Standard)

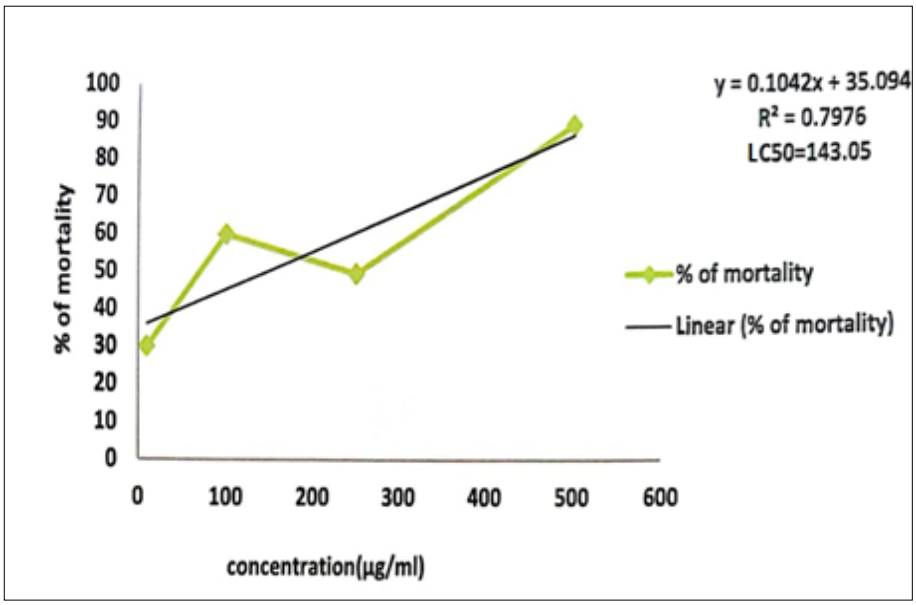


Fig 12: Calculation of LC_{50} (µg/ml) value of leaf in cytotoxicity test of *A. odoratum*

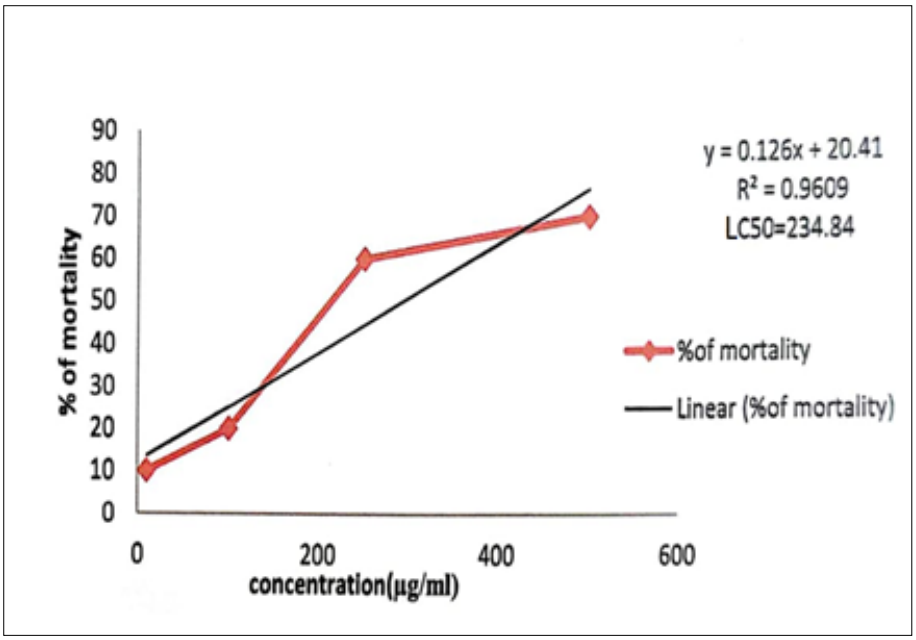


Fig 13: Calculation of LC_{50} (µg/ml) value of stem in cytotoxicity test of *A. odoratum*

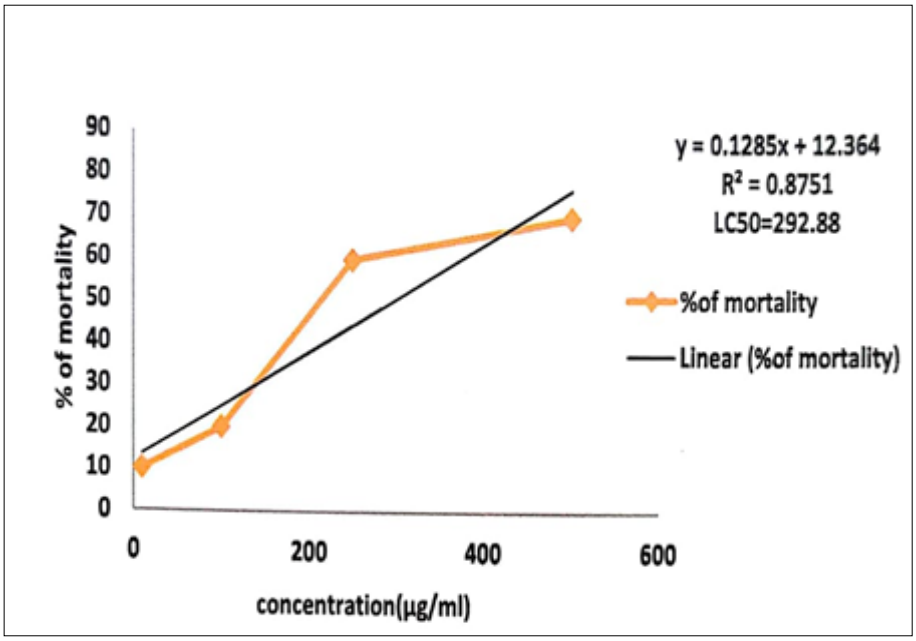


Fig 14: Calculation of LC_{50} (µg/ml) value of root in cytotoxicity test of *A. odoratum*

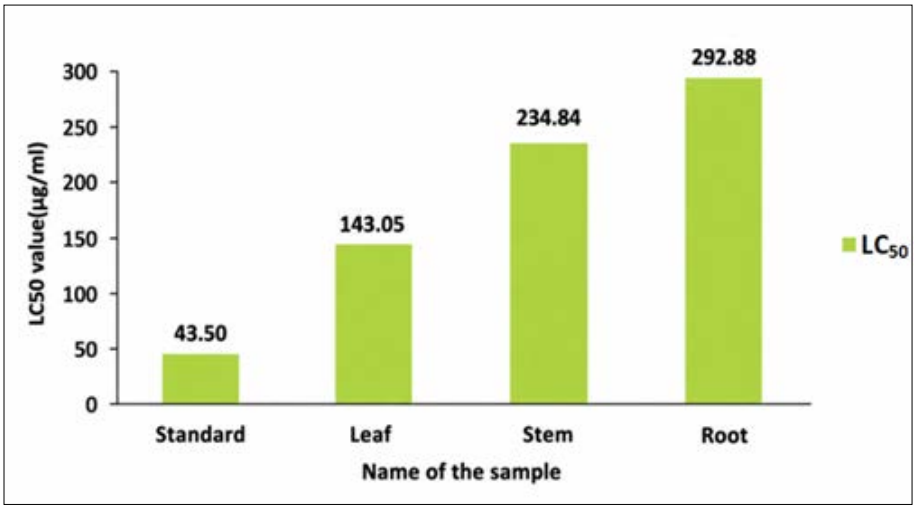


Fig 15: LC_{50} values of standard and crude extract of *A. odoratum* (Cytotoxicity)

Potent pharmacological value of orchids is intrinsically tied to their rich reservoir of secondary metabolites, serving evolutionary roles in interspecies defense mechanisms and possessing structural geometries highly compatible with human pharmacological targets (Gantait *et al.* 2021) [3]. Phytochemical profiling of *A. odoratum* robustly corroborates this. Differential accumulation of metabolites observed such as massive abundance of flavonoids in roots, steroids and glycosides in leaves and phlobatannins and anthraquinones in stems provides critical biochemical rationale for varied ethnomedicinal applications (Huda and Kashem 2021) [5]. Recent findings by Thant *et al.* (2026) [19] on *Aerides odorata* phenolic constituents further highlight their significant anti-neuroinflammatory properties.

The DPPH radical scavenging assay relies on reduction of stable DPPH radicals by hydrogen-donating antioxidants (Brand-Williams *et al.* 1995) [16]. In our study, *A. odoratum* extracts demonstrated moderate antioxidant ranges. Significantly, leaf extract yielded strongest antioxidant activity (IC_{50} = 181.25 µg/ml). This correlates perfectly with qualitative data showing high abundance of tannins, terpenoids and quinones in stems. Tannins and phenolic derivatives in Orchidaceae are renowned for effective free radical stabilization capacity, buffering cellular architecture from acute oxidative stress (Gantait *et al.* 2021) [3].

Inflammation, characterized by localized protein denaturation, is a primary physiological response to injury or pathogenic insult (Mizushima and Kobayashi 1968) [17]. *A. odoratum* displayed remarkably strong capacity to inhibit thermally induced egg albumin denaturation, effectively mimicking physiological mechanisms of standard non-steroidal anti-inflammatory drugs (NSAIDs) like acetylsalicylic acid. Stem extract (IC_{50} = 40.56 µg/ml) and leaf extract (IC_{50} = 89.81 µg/ml) exhibited potent anti-inflammatory properties. This empirical finding solidly substantiates historical use of *A. odoratum* leaf and stem pastes by tribal communities for treating arthritis, rheumatism and acute joint swellings (Bhatnagar *et al.* 2017) [7]. High concentrations of flavonoids, coumarins and alkaloids strongly suggest synergistic suppression of inflammatory mediators (Saleh-e-In *et al.* 2016) [9].

Cytotoxicity screening *via* brine shrimp lethality bioassay provides reliable parameters for preliminary detection of broad spectrum biological activity, particularly antitumor and localized cytotoxic phenomena (Meyer *et al.* 1982) [18]. According to established pharmacognostic guidelines, an LC_{50} between 100-500 µg/ml is defined as moderately toxic, implying biological safety at low therapeutic thresholds while retaining potential cellular inhibition capacities at higher thresholds (Baravaliya and Chanda 2012) [20]. All tested vegetative extracts of *A. odoratum* fell comfortably within this moderate range (Stem: 234.84 µg/ml, Leaf: 143.05 µg/ml, Root: 292.88 µg/ml). This progressive lethality indicates presence of bioactive threshold inhibitors likely identified alkaloids and specific cardiac glycosides that could be isolated for targeted neoplastic studies in future research.

Conclusion

This extensive investigation validates *Aerides odoratum* Lour. as a profound source of biologically active secondary metabolites with robust antioxidant, anti-inflammatory and moderate cytotoxic properties. Exceptional anti-inflammatory and radical scavenging capability of stem and leaf extracts provides strong empirical backing for historic ethno-medicinal integration against rheumatologic and topical inflammatory disorders. Concurrently, establishment of a standardized *in vitro* propagation protocol offers a highly scalable and sustainable solution to meet commercial and pharmaceutical demands, effectively alleviating wild-harvesting pressures and conserving this critical indigenous orchid species.

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