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Unveiling the Bioactive Potential and Pharmacological Spectrum of *Dendrobium tortile*Lindl.

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Abstract

Dendrobium tortile Lindl. is a poorly studied orchid whose organ-level pharmacology is unexplored. Total alkaloids, tannins and proteins were quantified in leaves, stems and roots, and methanolic extracts were evaluated for *in vitro* anti-inflammatory activity (albumin denaturation) and for *in vivo* analgesic (acetic acid-induced writhing), aphrodisiac (days 1 and 7) and central nervous system (CNS) depressant (hole-board) activities in Swiss albino mice. Data were analyzed by one-way ANOVA followed by Tukey's or Dunnett's test. Alkaloids were highest in leaves (66.47 ± 1.60 mg/g), whereas roots contained the most tannins (22.39 ± 0.22 mg TAE/g) and proteins (37.24 ± 0.41 mg BSAE/g). In the albumin denaturation assay, IC_{50} values were 52.81 ± 0.48 µg/mL for acetylsalicylic acid (ASA), 74.52 ± 0.39 for leaf, 76.71 ± 0.32 for root and 95.42 ± 0.64 µg/mL for stem extracts. All extracts reduced writhing significantly ($p < 0.001$); the leaf extract was most effective (61.09% at 200 and 63.95% at 400 mg/kg) and comparable to diclofenac sodium (10 mg/kg; 59.44%). Leaf and stem extracts at both doses significantly improved all sexual behaviour parameters ($p < 0.001$), the 400 mg/kg stem extract being most effective (day-1 mount and intromission frequencies, 15.17 ± 0.31 and 13.50 ± 0.72), whereas root extracts were weaker. At 400 mg/kg, leaf, root and stem extracts reduced head dipping to 16.83 ± 0.83, 15.50 ± 1.16 (both $p < 0.001$) and 20.50 ± 1.40 ($p < 0.05$), respectively, against 25.17 ± 0.95 in controls. *D. tortile* is a promising source of bioactive constituents that merit bioactivity-guided isolation.

1. Introduction

Plant products remain a cornerstone of drug discovery, supplying many of the scaffolds of modern therapeutics [1]. Orchidaceae is among the largest flowering plant families, with about 28,000 species in some 736 genera [2]. Orchids have long been used in Ayurvedic, Unani and Chinese medicine against inflammation, muscular pain, rheumatism and sexual weakness, although most preparations have never been scientifically validated [3, 4]. Within the family, *Dendrobium* is regarded as one of the most prized genera [5]; its stems have been prescribed in Traditional Chinese Medicine for over two millennia as a Yin-nourishing tonic [6, 7]. Antioxidant, anti-inflammatory, neuroprotective, antidiabetic, immunomodulatory and anticancer activities have been reported and ascribed to alkaloids, bibenzyls, phenanthrenes, flavonoids, tannins and polysaccharides [8, 9]. *Dendrobium tortile* Lindl., an epiphytic orchid of tropical Asia, has received little attention; the only phytochemical study isolated a new furanone and six known compounds from the whole plant, of which dendrofalconerol A was the strongest α-glucosidase inhibitor [10].

Inflammation is an adaptive response whose persistence underlies many chronic diseases and is tightly coupled to nociception [11]. Injured tissue releases histamine, kinins and prostaglandins, which increase vascular permeability and sensitize nociceptors [12]. Inhibition of protein denaturation is an accepted *in vitro* indicator of anti-inflammatory potential [13, 14], whereas acetic acid-induced writhing is a standard visceral pain model in which peritoneal prostaglandins and lipoxygenase products trigger abdominal constrictions [15]. Orchids have performed well in such assays: *Vanda roxburghii* roots and *V. tessellata* leaves attenuated writhing in mice [16, 17], *D. macrostachyum* showed *in vitro* anti-inflammatory activity

[18], and several South African orchids inhibited cyclooxygenase [19]. CNS depressants reduce neuronal excitability; the ethanolic leaf extract of *Cymbidium aloifolium*, for example, diminished locomotor and exploratory behaviour in mice [20]. Male sexual dysfunction, including erectile dysfunction, premature ejaculation and hypogonadism, affects a considerable proportion of men worldwide [21], and the adverse effects of synthetic phosphodiesterase-5 inhibitors sustain interest in botanicals. *Dendrobium nobile* is a traditional Chinese tonic and aphrodisiac [6], and aphrodisiac activity has also been reported for *V. tessellata* and *Dactylorhiza hatagirea* [22, 23].

Combining organ-wise phytochemical analysis with bioassays can show where active constituents accumulate; in *D. lindleyi*, alkaloid and tannin levels in leaf, stem and root extracts correlated closely with anti-inflammatory activity [24]. The *in vivo* pharmacology of the separate organs of *D. tortile* appears not to have been quantified, and because the species is a wild epiphyte, identifying the most active organ is also relevant to sustainable harvesting. This study was therefore designed to quantify the total alkaloid, tannin and protein contents of crude extracts from the leaves, stems and roots of wild-collected *D. tortile* and to assess their *in vitro* anti-inflammatory potential and *in vivo* analgesic, aphrodisiac and CNS depressant activities, thereby establishing a pharmacological baseline for targeted isolation and further preclinical work.

2. Materials and Methods

2.1. Plant collection and authentication

Wild *D. tortile* Lindl. was collected in March 2018 from the Dudpukuria–Dhopachari Wildlife Sanctuary, Chattogram, Bangladesh (22.471553° N, 92.08640° E) (Fig. 1). Identification was assisted by Prof. Dr. Mohammed Kamrul Huda (Department of Botany, University of Chittagong) and authenticated at the Chittagong University Herbarium (CTGUH), where a voucher specimen was deposited (accession no. CTGUHS18101).

2.2. Preparation of crude methanol extracts

Leaves, stems and roots were separated, washed, air-dried (25 ± 2°C) and oven-dried to constant weight at 60°C, then ground to a fine powder. Powder (50 g per organ) was macerated in 500 mL of 95% (v/v) methanol for 48 h with shaking, followed by 10 min of sonication [25]. After filtration (Whatman No. 1), the filtrates were evaporated under reduced pressure (rotary evaporator, ≤ 50°C) to give the crude methanol extracts of leaf (MCL), stem (MCS) and root (MCR), which were stored at 4°C.

2.3. Quantitative phytochemical analysis

Total alkaloids. Alkaloids were determined gravimetrically [26]. Fresh tissue (5 g per organ) was macerated in 200 mL of 10% (v/v) acetic acid in ethanol for 4 h. The filtrate was concentrated to one-

quarter of its volume, and concentrated NH_4OH was added dropwise until precipitation was complete. The precipitate was washed with dilute NH_4OH, dried at 60°C and weighed.

Total tannins. Tannins were quantified by the Folin–Denis method [27]. Extract solution (1 mg/mL; 1 mL) was mixed with 0.5 mL of Folin–Denis reagent and 1 mL of 35% (w/v) Na_2CO_3, made up to 10 mL with distilled water and incubated for 30 min at room temperature. Absorbance was read at 700 nm against a reagent blank, using a tannic acid calibration curve (40–200 µg/mL; Appendix 1A). Tannin content was expressed as mg tannic acid equivalents per g of dry extract (mg TAE/g).

Total proteins. Proteins were determined by the Lowry method [28]. Extract solution (1 mg/mL; 0.2 mL) was mixed with 4.5 mL of reagent I (2% Na_2CO_3 in 0.1 N NaOH, 1% sodium potassium tartrate and 0.5% $CuSO_4 \cdot 5H_2O$, 48:1:1, v/v) and incubated at 25°C for 10 min. Reagent II (0.5 mL; Folin–Ciocalteu reagent diluted 1:1 with water) was added, and incubation continued for 30 min. Absorbance was read at 660 nm against a bovine serum albumin (BSA) curve (40–200 µg/mL; Appendix 1B), and protein was expressed as mg BSA equivalents per g of dry extract (mg BSAE/g).

2.4. In vitro anti-inflammatory assay

Inhibition of heat-induced egg albumin denaturation was assayed as described previously [13, 14, 29]. Sample extract (1mg/1 mL; 50, 100, 150, 200, 250 or 300 µg/mL) was mixed with 0.2 mL of 1% (w/v) egg albumin solution (pH 5.6 ± 0.2), incubated at 37°C for 20 min, heated at 57°C to 70 °, cooled, and read at 660 nm. Acetylsalicylic acid (ASA) was the reference standard, and each concentration was assayed in triplicate. Inhibition (%) was calculated as $(A_{control} - A_{test})/A_{control} \times 100$, and IC_{50} was derived for each replicate by linear regression of inhibition (%) on $\log_{10}$ concentration.

2.5. In vivo studies

2.5.1. Animals and treatments. Swiss albino mice (25–30 g) were obtained from the Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram, and housed six per cage at 25 ± 2°C under a natural light–dark cycle with free access to a standard diet and water. Mice were acclimatized for 7 d (10 d for the aphrodisiac test), with males and females kept apart; either sex was used in the analgesic and CNS tests and males in the aphrodisiac test. Animal handling followed the institutional ethical guidelines of the Faculty of Biological Sciences Ethical committee, University of Chittagong (EACUBS2018) [30]. Extracts were suspended in 1% (v/v) Tween-80 in saline and administered orally at 200 and 400 mg/kg (10 mL/kg); control animals received the vehicle. Animals were randomly allocated to groups of six (n = 6).

2.5.2. Acetic acid-induced writhing test. Analgesic activity was assessed by the writhing test [15]. Mice were fasted for 12 h and received the vehicle, diclofenac sodium (10 mg/kg) or an extract 30 min before intraperitoneal injection of 0.6% (v/v) acetic acid (10 mL/kg). Writhes were counted for 15 min, starting 5 min after injection [31]. Inhibition (%) was calculated as [(A − B)/A] × 100, where A and B are the mean numbers of writhes in the control and treated groups, respectively.

2.5.3. Aphrodisiac activity. Males received an extract (200 or 400 mg/kg) or the vehicle as a single dose or daily for 7 consecutive days. One hour after dosing, a non-oestrous female was placed with each male in a transparent cage. Mount frequency (MF), intromission frequency (IF), mount latency (ML) and intromission latency (IL) were recorded on days 1 and 7; latencies were timed from introduction of the female to the first mount and first intromission, respectively. A mount was scored when the male assumed the copulatory posture without penetration, and an intromission when penetration with pelvic thrusting occurred [32, 33]. Single-dose animals were observed for 4 h (09:00–13:00).

2.5.4. CNS depressant activity (hole-board test). Mice were treated with an extract, diazepam (1 mg/kg; positive control) or the vehicle. After 30 min, each mouse was placed on a wooden hole-board (40 × 40 × 25 cm) with 16 holes of 3 cm diameter [34], and head dips (head entering a hole up to eye level) were counted for 5 min [35, 36].

2.6. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Group differences were tested by one-way analysis of variance (ANOVA), followed by Tukey's test for comparisons among groups (phytochemical contents and IC_{50} values) [37] and Dunnett's test for comparisons with the control in the *in vivo* studies [38]. Differences were considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$. Calculations were performed with Microsoft Excel 2019, GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA) and SPSS 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Total alkaloid, tannin and protein contents

Alkaloid, tannin and protein contents differed significantly among organs (Table 1; calibration curves in Appendix 1). Leaves contained the most alkaloids (66.47 ± 1.60 mg/g), followed by roots (49.07 ± 1.05) and stems (31.27 ± 1.29 mg/g). Tannins were highest in roots (22.39 ± 0.22 mg TAE/g), followed by leaves (15.23 ± 0.38) and stems (12.56 ± 0.55). Protein followed the order roots > leaves > stems (37.24 ± 0.41 > 26.49 ± 0.40 > 12.45 ± 0.51 mg BSAE/g). Stems were thus lowest in all three constituents.

Table 1
Total alkaloid, tannin and protein contents of *D. tortile* leaves, stems and roots.

Plant part	Alkaloids (mg/g fresh tissue)	Tannins (mg TAE/g dry extract)	Proteins (mg BSAE/g dry extract)
Leaves	66.47 ± 1.60[a]	15.23 ± 0.38[a]	26.49 ± 0.40[a]
Stems	31.27 ± 1.29[b]	12.56 ± 0.55[b]	12.45 ± 0.51[b]
Roots	49.07 ± 1.05[c]	22.39 ± 0.22[c]	37.24 ± 0.41[c]

Values are mean ± SEM (n = 3). Different superscript letters within a column indicate significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$). TAE, tannic acid equivalents; BSAE, bovine serum albumin equivalents.

3.2. In vitro anti-inflammatory activity

All extracts inhibited albumin denaturation in a concentration-dependent manner (Table 2; Fig. 2). At 50 µg/mL, inhibition ranged from 38.55% (stem) to 41.06% (root), compared with 48.72% for ASA; at 300 µg/mL it reached 88.62% (leaf), 88.50% (root) and 83.45% (stem), compared with 95.48% for ASA. IC_{50} values differed significantly among samples (one-way ANOVA, $F(3, 8) = 1365$, $p < 0.001$; regression R^2 = 0.906–0.994), and a nonlinear fit gave values within about 2 µg/mL of these. ASA was the most potent (52.81 ± 0.48 µg/mL), followed by the leaf (74.52 ± 0.39), root (76.71 ± 0.32) and stem (95.42 ± 0.64 µg/mL) extracts. ASA was thus about 1.4-fold more potent than the leaf and root extracts, and the stem extract was about 1.3-fold weaker than the leaf extract; the leaf–root difference was small (2.2 µg/mL; $p = 0.045$).

Table 2
IC_{50} values of *D. tortile* extracts and ASA in the albumin denaturation assay.

Sample	IC_{50} (µg/mL)	Fold vs ASA	R^2 (range)	Inhibition at 300 µg/mL (%)
ASA (standard)	52.81 ± 0.48^{a}	1.00	0.927–0.994	95.48 ± 0.36
MCL (leaf)	74.52 ± 0.39^{b}	1.41	0.988–0.993	88.62 ± 0.32
MCR (root)	76.71 ± 0.32^{c}	1.45	0.979–0.986	88.50 ± 0.44
MCS (stem)	95.42 ± 0.64^{d}	1.81	0.906–0.919	83.45 ± 0.65

Values are mean ± SEM (n = 3). Fold = IC_{50} of sample ÷ IC_{50} of ASA. Different superscript letters indicate significant differences (one-way ANOVA followed by Tukey's test, $p < 0.05$).

3.3. Analgesic activity (acetic acid-induced writhing test)

Acetic acid produced marked writhing in controls (65.66 ± 0.80), and every treatment reduced it significantly ($p < 0.001$; Table 3). Diclofenac sodium inhibited writhing by 59.44%. The leaf extract was most effective (61.09% at 200 mg/kg and 63.95% at 400 mg/kg), numerically similar to or slightly above the reference drug, followed by the root (53.55% and 57.36%) and stem extracts (45.67% and 55.83%). Inhibition increased with dose for all extracts, and potency ranked leaf > root > stem at both doses.

Table 3
Effect of *D. tortile* leaf, stem and root extracts on acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg)	No. of writhes (mean ± SEM)	Inhibition (%)
Control (vehicle)	10 mL/kg	65.66 ± 0.80	—
Diclofenac sodium	10	26.63 ± 0.67***	59.44
Leaf (MCL)	200	25.55 ± 2.11***	61.09
	400	23.67 ± 1.33***	63.95
Stem (MCS)	200	35.67 ± 1.05***	45.67
	400	29.00 ± 0.58***	55.83
Root (MCR)	200	30.50 ± 2.11***	53.55
	400	28.00 ± 1.33***	57.36

Values are mean ± SEM (n = 6). ***$p < 0.001$,**$p < 0.01$, *$p < 0.05$ versus control (one-way ANOVA followed by Dunnett's test).

3.4. Aphrodisiac activity

3.4.1. Sexual behaviour after single and repeated dosing. Leaf and stem extracts markedly enhanced sexual behaviour at both doses, whereas root extracts had limited effects (Table 4; Fig. 3). On day 1, MF rose from 8.33 ± 0.42 in controls to 12.67–15.17 and IF from 5.50 ± 0.43 to 11.50–13.50 (all $p < 0.001$), while ML and IL fell by 25–39% and 45–55%, respectively. The 400 mg/kg stem extract gave the strongest response (MF 15.17 ± 0.31; IF 13.50 ± 0.72; IL − 55%).

Table 4
Effects of *D. tortile* leaf, stem and root extracts on the sexual behavior of male mice on days 1 and 7.

Group	Dose (mg/kg)	ML (s)	IL (s)	MF	IF
Day 1					
Control	—	102.34 ± 4.96	222.77 ± 14.53	8.33 ± 0.42	5.50 ± 0.43
Leaf (MCL)	200	67.05 ± 2.43***	111.64 ± 9.19***	12.67 ± 0.42***	11.50 ± 0.43***
	400	76.62 ± 5.14***	121.35 ± 8.56***	14.67 ± 0.33***	12.17 ± 0.60***
Stem (MCS)	200	62.12 ± 2.62***	103.84 ± 4.19***	13.33 ± 0.49***	11.67 ± 0.42***
	400	61.98 ± 3.38***	99.68 ± 5.27***	15.17 ± 0.31***	13.50 ± 0.72***
Root (MCR)	200	101.60 ± 5.39	170.67 ± 11.68*	7.67 ± 0.49	6.50 ± 0.43
	400	92.32 ± 3.54	166.37 ± 12.79*	9.50 ± 0.43	8.50 ± 0.43**
Day 7					
Control	—	99.51 ± 5.45	216.13 ± 6.92	8.83 ± 0.31	6.67 ± 0.33
Leaf (MCL)	200	56.28 ± 4.16***	83.87 ± 2.14***	14.33 ± 0.56***	12.17 ± 0.31***
	400	66.14 ± 5.21***	94.25 ± 2.02***	16.17 ± 0.40***	14.00 ± 0.26***
Stem (MCS)	200	60.60 ± 2.70***	92.30 ± 2.06***	15.17 ± 0.40***	12.67 ± 0.42***
	400	56.51 ± 2.92***	89.28 ± 2.74***	16.33 ± 0.56***	14.33 ± 0.21***
Root (MCR)	200	97.82 ± 4.18	149.89 ± 8.58***	9.17 ± 0.48	7.17 ± 0.48
	400	89.85 ± 5.52	158.87 ± 10.72***	9.83 ± 0.31	8.83 ± 0.31***

Values are mean ± SEM (n = 6). ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ versus control (Dunnett's test). ML, mount latency; IL, intromission latency; MF, mount frequency; IF, intromission frequency.

After 7 days of dosing the effects persisted (the day-7 observation window differed from that on day 1): MF reached 14.33–16.33 and IF 12.17–14.33 (controls, 8.83 ± 0.31 and 6.67 ± 0.33; $p < 0.001$), and ML

and IL fell by 34–43% and 56–61%. By contrast, the root extract did not alter ML or MF at either time point (MF 7.67–9.83). It shortened IL (− 23% to − 25%, $p < 0.05$ on day 1; −26% to − 31%, $p < 0.001$ on day 7) and increased IF only at 400 mg/kg (8.50 ± 0.43 on day 1, $p < 0.01$; 8.83 ± 0.31 on day 7, $p < 0.001$).

3.4.2. Four-hour observation. Hourly analysis showed that the stimulatory effect was front-loaded and transient (Fig. 4). In the first hour, leaf and stem extracts significantly increased MF and IF at both doses ($p < 0.001$), whereas the root extract did so only for IF at 400 mg/kg ($p < 0.01$). In the second hour, MF remained elevated for all leaf and stem treatments ($p < 0.05$–0.001), as did IF for the stem extract at both doses and the leaf extract at 200 mg/kg ($p < 0.01$–0.001). In the third hour, the effect was confined to MF with leaf (both doses) and stem 200 mg/kg ($p < 0.05$–0.01) and to IF with the stem extract ($p < 0.01$–0.001). No group differed from control in the fourth hour.

3.5. CNS depressant activity (hole-board test)

Diazepam (1 mg/kg) significantly increased head dipping relative to the vehicle (37.67 ± 1.87 vs 25.17 ± 0.95; +49.7%, $p < 0.001$), validating the assay (Table 5). The extracts altered head dipping in a dose- and organ-dependent manner. At 400 mg/kg, the root, leaf and stem extracts reduced head dips by 38.4% ($p < 0.001$), 33.1% ($p < 0.001$) and 18.5% ($p < 0.05$), respectively, indicating a sedative, CNS depressant-like effect that was strongest for root and leaf. At 200 mg/kg, MCL (+ 11.2%) and MCS (+ 4.6%) did not differ from control, whereas the root extract increased head dips by 22.5% ($p < 0.01$), an anxiolytic-like effect. Root extracts therefore showed a biphasic response: anxiolytic-like at the lower dose and sedative like at the higher dose.

Table 5
Effect of *D. tortile* crude extracts on head dipping in the hole-board test in mice.

Treatment	Dose (mg/kg)	Head dips (mean ± SEM)	Change vs control (%)
Control (vehicle)	10 mL/kg	25.17 ± 0.95	—
Diazepam	1	37.67 ± 1.87***	+ 49.7
MCL	200	28.00 ± 1.06	+ 11.2
MCL	400	16.83 ± 0.83***	−33.1
MCS	200	26.33 ± 1.43	+ 4.6
MCS	400	20.50 ± 1.40*	−18.5
MCR	200	30.83 ± 0.76**	+ 22.5
MCR	400	15.50 ± 1.16***	−38.4

Values are mean ± SEM (n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control (one-way ANOVA followed by Dunnett's test). Leaf (MCL), stem (MCS) and root (MCR) of *D. tortile* extracts.

4. Discussion

The study asked whether the pharmacologically relevant chemistry of *D. tortile* is evenly distributed and whether any unevenness is reflected in bioactivity. Leaves held the most alkaloids, roots the most tannins and proteins, and stems the least of all three (Table 1), and the extracts behaved differently in each bioassay: leaves led in the anti-inflammatory and analgesic tests, leaf and stem extracts stimulated sexual behaviour, and the root extract had the strongest sedative-like effect. No single parts of *D. tortile* was best across all assays. Heat-induced albumin denaturation is a simple *in vitro* screen in which aspirin-like drugs protect albumin from heat denaturation [13, 14]. The leaf, root and stem values (74.52, 76.71 and 95.42 µg/mL) were lower than those reported for ethanol and water extracts of *D. macrostachyum* (114.13 and 135.82 µg/mL) [18], although differences in solvent and protocol limit the comparison. The strong leaf activity is consistent with its high alkaloid content, and a similar relationship between alkaloid and tannin levels and anti-inflammatory activity has been described across the organs of *D. lindleyi* [24]. The root matched the leaf with fewer alkaloids but more tannins, suggesting that tannins, also phenolics content found highest for roots explained in earlier study of *D. tortile* [39]. Alkaloids, bibenzyls, phenanthrenes and flavonoids from *Dendrobium* have all been linked to anti-inflammatory effects and Antioxidant potential noted with lowest IC_{50} values in stem n-hexane of *D. tortile* [8, 9, 39], but individual compounds were not profiled, so these links remain speculative. Notably, the stem, used in Chinese medicine but in this study other parts showed enriched potential, so traditional organ choice in other *Dendrobium* species may not transfer to *D. tortile* [6].

Acetic acid writhing releases prostaglandins and other mediators in the peritoneal cavity that sensitize nociceptors [12, 15], so reduced writhing points to a peripheral anti-inflammatory action. The leaf extract reduced writhing by 61.09% at 200 mg/kg and 63.95% at 400 mg/kg, similar to diclofenac (59.44%) and well above the 42–45% reported for *V. tessellata* leaf extracts at 400 mg/kg [17]. Root (53.55–57.36%) and stem (45.67–55.83%) extracts were less effective, and inhibition rose with dose in all three. Cyclooxygenase inhibition, known for other orchid extracts [19], may be one route, but it was not tested in this study. In addition, sedatives and muscle relaxants also reduce writhing, and the leaf and root extracts that performed best also suppressed exploratory behaviour at 400 mg/kg, so part of the effect may be central; hot-plate and tail-flick tests would help resolve this, since *V. roxburghii* shows both peripheral and central antinociception [16]. The stem extract was weakest *in vitro* yet clearly active *in vivo*, showing that albumin protection alone does not predict analgesic effect.

Head dipping reflects exploratory drive: anxiolytics raise it and sedatives lower it [34, 40]. Diazepam raised head dips by about 50%, confirming assay sensitivity, and at 400 mg/kg all extracts reduced them (root − 38.4%, leaf − 33.1%, stem − 18.5%). This sedative-like profile agrees with reports on *Cymbidium aloifolium* [20] and *Acampe papillosa* [35]. The root extract increased head dips by 22.5% at 200 mg/kg, an anxiolytic-like change, before turning sedative at the higher dose. A shift from anxiolysis to sedation with increasing dose is typical of drugs acting at the benzodiazepine site of the $GABA_A$ receptor, and flavonoids are known ligands of this site [41, 42]. *Dendrobium* species also contain several classes of

neuroactive compounds, Flavonoid's content was richly found in *D. tortile* previously analyzed by this author [39, 43].

Leaf and stem extracts shortened mount and intromission latencies and raised mount and intromission frequencies; the effect persisted after seven days of dosing but faded within four hours of a single dose (Table 4; Figs. 3 and 4). Similar effects have been reported for *Vanda tessellata* [22], *Dactylorhiza hatagirea* [23], *Orchis anatolica* [44] and *Eulophia macrobulbon* [45]. In *Eulophia macrobulbon*, phenanthrenes were linked to phosphodiesterase-5 (PDE5) inhibition, and nitric oxide-mediated erection depends on testosterone [46]. Intromission frequency more than doubled in the leaf and stem groups, which hints at improved erectile function, but testosterone, nitric oxide and PDE5 were not measured, so the mechanism remains open but D. tortile had not shown acute toxicity [39]. The root pattern is informative: it was the strongest sedative and the weakest aphrodisiac, whereas the stem was the mildest sedative and the most effective aphrodisiac. which also explain the 400 mg/kg leaf dose did no better than 200 mg/kg on latencies, although this idea is untested.

In this study Crude extracts were tested, though the aphrodisiac test had no positive control, and head dipping was not complemented by locomotor tests, so sedation is inferred. Priorities include bioassay-guided fractionation with LC-MS and NMR characterization of leaf and stem constituents; carrageenan-edema, hot-plate and naloxone studies for pain; locomotor, thiopental sleeping-time for CNS effects; hormonal and PDE5 assays for aphrodisiac action. Because *D. tortile* is a wild epiphyte, leaf-based preparations would also be less destructive than stem or root-based ones.

5. Conclusion

This study provides the first *in vivo* pharmacological assessment of *Dendrobium tortile*, revealing clear anatomical compartmentalization of bioactive metabolites. Leaf possessed the highest alkaloid density, whereas roots were predominant in tannins and proteins. Methanolic extracts from all organs showed anti-inflammatory activity in the albumin denaturation assay and marked analgesic activity, with the leaf extract giving writhing inhibition numerically similar to that of diclofenac sodium. Leaf and stem extracts enhanced male sexual behaviour, the stem extract being the most effective, whereas the root extract had little aphrodisiac effect but the strongest sedative-like and a biphasic effect on head dipping. These findings confirm that biological efficacy in *D. tortile* is organ-dependent. As typical for plant-based pharmacological evaluations, mapping organ-wise bioactivity provides a crucial foundation for selective drug discovery. The leaf material emerges as the most viable candidate for downstream therapeutic application, balancing potent anti-inflammatory and analgesic properties with a sustainable, non-destructive harvest that protects vulnerable wild epiphytic populations. Bioassay-guided fractionation, mechanistic characterization, and comprehensive in vivo safety evaluations are now warranted to isolate active compounds.

Declaration of Generative AI and AI-Assisted Technologies in Scientific Writing

Google Gemini and Claude were used for language editing and to improve grammatical clarity. The authors reviewed and verified all content and take full responsibility for the accuracy and integrity of the manuscript.

Declarations

Ethical approval.

The animal experiments were approved by the Department of Botany, Faculty of Biological Sciences, University of Chittagong, Chattogram, Bangladesh (EACUBS2018).

Conflict of interest.

No conflict of interest was declared.

Authors' contributions.

The research was conceived, supervised and reviewed by M. K. Huda, and the study was designed by M. K. Huda and S. A. Lipa. The experimental work, data analysis and interpretation, and manuscript preparation and organization were carried out by S. A. Lipa, K. M. M. Alam and M. M. Hoque.

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Data availability.

All data are included in the article; additional data are available from the corresponding author on reasonable request.

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47. Appendices
48. Appendix 1 Calibration curves of standard chemicals: (A) tannic acid (Folin–Denis method, 700 nm) and (B) bovine serum albumin (Lowry method, 660 nm)

Figures



Figure 1

Wild-collected *Dendrobium tortile* Lindl., Chattogram, Bangladesh.

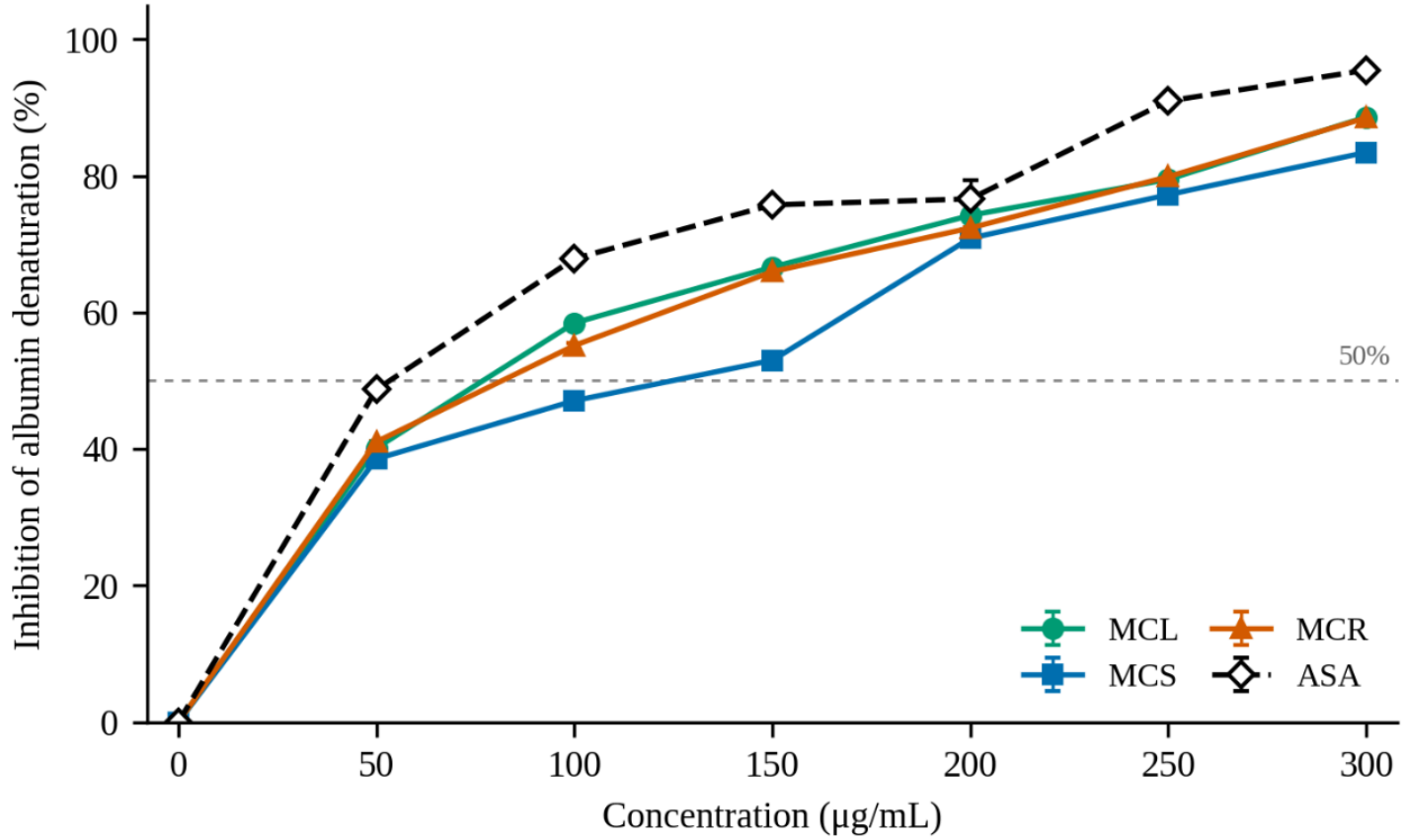


Figure 2

Inhibition (%) of albumin denaturation by methanolic crude extracts of *D. tortile* leaf (MCL), stem (MCS) and root (MCR) and by acetylsalicylic acid (ASA) (mean ± SEM, n = 3). The dashed line marks 50% inhibition.

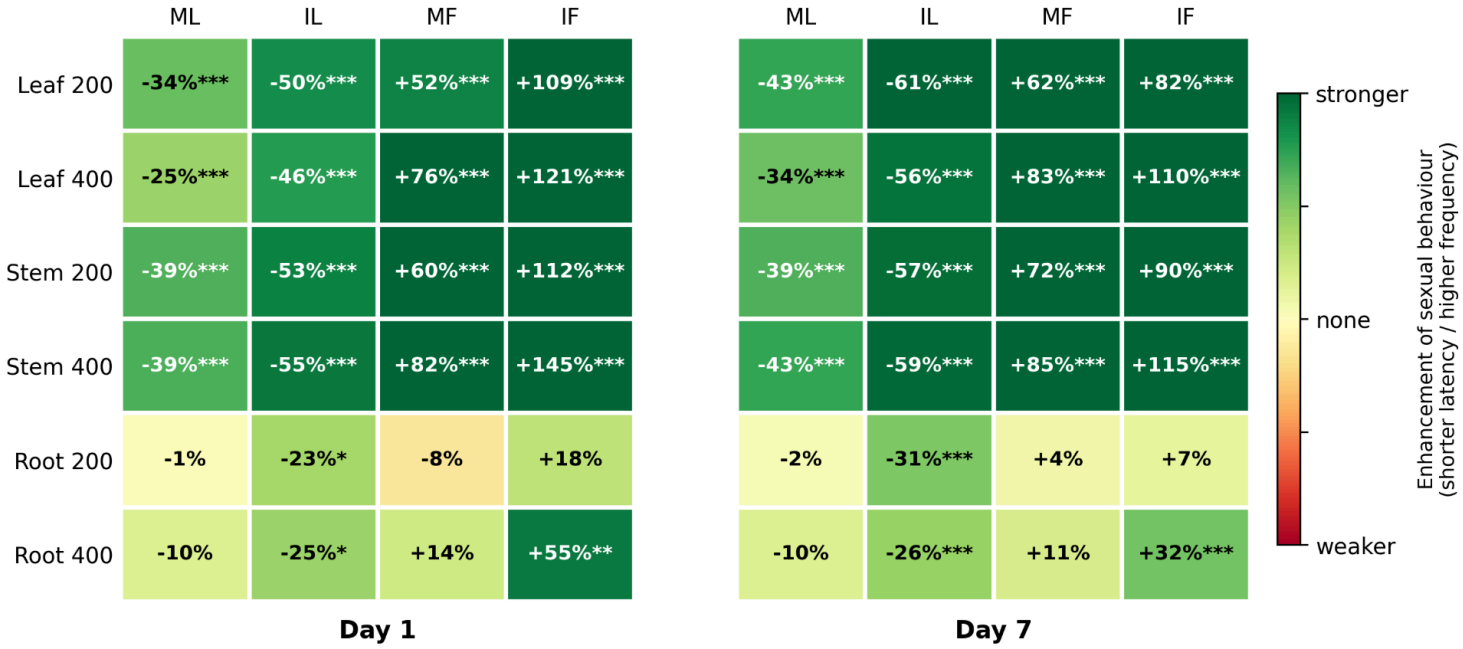


Figure 3

Percentage change in sexual behavior parameters relative to control on days 1 and 7. Shorter latencies (ML, IL) and higher frequencies (MF, IF) indicate enhanced behavior. *$p < 0.05$, **$p< 0.01$, ***$p < 0.001$ versus control.

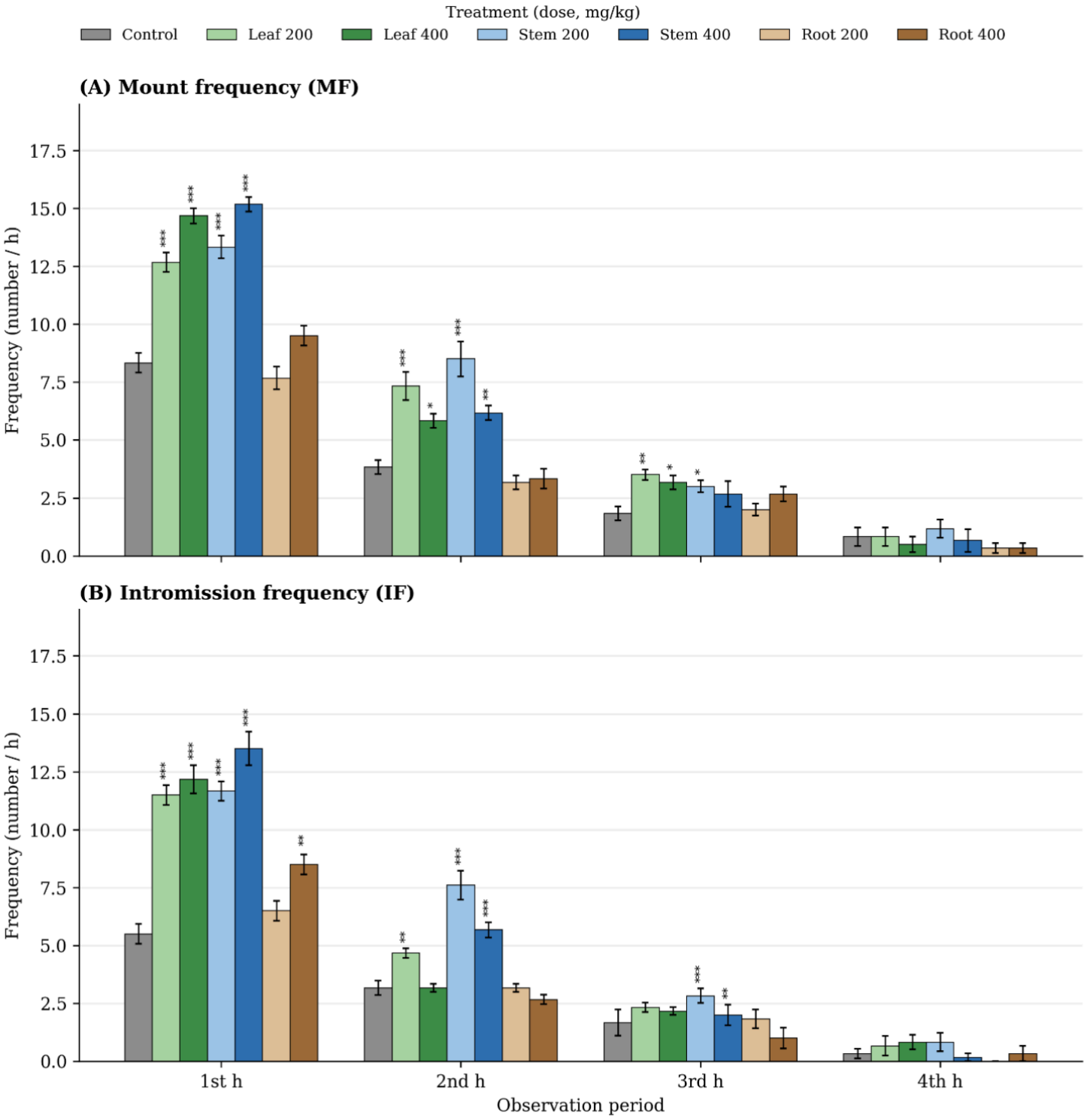


Figure 4

Hourly (A) mount frequency (MF) and (B) intromission frequency (IF) in mice treated with *D. tortile* extracts (200 and 400 mg/kg) over 4 h. Data are mean ± SEM (n = 6). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control (Dunnett's test).

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