

Phytochemical Investigation, *In Vitro* Radical-Scavenging Capacity, and Brine Shrimp Toxicity Profile of the Wild Orchid *Dendrobium tortile* Lindl. from Bangladesh

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Research Article

Keywords: *Dendrobium tortile*, Antioxidant activity, total phenolic content, brine shrimp lethality, Bangladesh

Posted Date: October 22nd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11447387/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Dendrobium orchids have long been used in traditional medicine, partly for their phytochemical richness. We examined the phytochemical profile, antioxidant capacity and brine shrimp toxicity of *Dendrobium tortile* Lindl. leaves, stems and roots, using crude extracts and n-hexane, dichloromethane, butanol and methanol fractions. Screening found alkaloids, flavonoids, phenols, tannins, terpenoids, steroids and other metabolites in every plant part. Roots had the highest total phenolic content (96.76 ± 1.17 mg GAE/g), and stems the highest total flavonoid content (34.43 ± 0.26 mg QE/g). DPPH scavenging rose with concentration (50–250 $\mu\text{g/mL}$). Of the crude extracts, root was the most potent ($\text{IC}_{50} = 32.33 \pm 0.14$ $\mu\text{g/mL}$), ahead of leaf (39.11 ± 0.29 $\mu\text{g/mL}$) and stem (45.29 ± 1.54 $\mu\text{g/mL}$); ascorbic acid gave 27.30 ± 0.03 $\mu\text{g/mL}$. The lowest fraction IC_{50} values were stem n-hexane (44.59 ± 2.82 $\mu\text{g/mL}$), root methanol (55.36 ± 0.46 $\mu\text{g/mL}$), leaf methanol (57.81 ± 0.50 $\mu\text{g/mL}$) and root dichloromethane (58.08 ± 0.55 $\mu\text{g/mL}$). All differed significantly from ascorbic acid ($p < 0.01$) except stem n-hexane. In the brine shrimp assay, mortality increased with concentration (100–1000 $\mu\text{g/mL}$). Potassium dichromate gave an LC_{50} of 22.54 ± 1.07 $\mu\text{g/mL}$ (extremely toxic), while every *D. tortile* extract was moderately toxic (LC_{50} 172.79–351.69 $\mu\text{g/mL}$); stem crude extract and its dichloromethane and methanol fractions were most lethal. Total phenolic content correlated strongly with IC_{50} ($r = -0.99$). *D. tortile* appears to be a promising source of natural antioxidants and bioactive compounds, but its safety needs further evaluation. Bioavailability and compound isolation studies are recommended.

1. Introduction

The family Orchidaceae is one of the most taxonomically diverse and evolutionarily advanced lineages of flowering plants. Among its members, the genus *Dendrobium* is one of the largest, comprising nearly 1,400 species valued for their ornamental appeal and ethnopharmacological relevance [1]. Bangladesh harbors about 188 orchid species in 72 genera, including 28 *Dendrobium* taxa [2]. Orchids have long been used in traditional medicine as tonics and to treat respiratory, gastrointestinal, inflammatory and metabolic disorders [3, 4].

Dendrobium tortile Lindl. (known locally as “Torriam” among ethnic communities) is an epiphytic perennial orchid with flat, sharply pointed pseudobulbs, thin tubular leaf sheaths, and fragrant pale lilac to purple flowers with twisted petals and a dilated, yellow, hood-like lip [5, 6]. *Dendrobium* species produce diverse secondary metabolites, including alkaloids (notably dendrobine), phenanthrenes, bibenzyls, fluorenones, sesquiterpenoids and flavonoids [7–9]. A chemotaxonomic survey of foliar flavonoids in 142 orchid species showed that flavone C-glycosides and flavonols are widespread and function as structural antioxidants and endogenous stress-response elements [10]. Coumarins, stilbenoids, saponins and polysaccharides from related taxa, such as *D. devonianum*, *D. loddigesii*, *D. tosaense* and *D. huoshanense*, show immunomodulatory, neuroprotective, anticancer, hepatoprotective, antibacterial and antidiabetic activities [11–13].

Reactive oxygen species (ROS), including hydroxyl radicals, superoxide anions, hydrogen peroxide and nitric oxide, are generated by physiological processes and by exposure to toxins and ultraviolet radiation [14]. Excess ROS damage lipids and proteins and cause DNA strand breaks, contributing to cancer, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, diabetes and premature aging [15, 16]. Antioxidants neutralize free radicals by donating hydrogen atoms or single electrons, thereby arresting chain-propagation reactions [17]. Because synthetic antioxidants such as butylated hydroxytoluene (BHT) have been linked to hepatotoxicity and potential carcinogenic risks, interest in natural antioxidants from medicinal plants has grown worldwide [18]. Plant phenolics, flavonoids and tannins have favorable redox potentials that make them effective radical scavengers, hydrogen donors and singlet oxygen quenchers [1]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a well-established, mechanistic method for evaluating this capacity; it is based on the concentration-dependent reduction of the stable purple DPPH radical to the pale yellow, non-radical hydrazine DPPH-H [19]. Marked radical-scavenging activity has been reported in related orchids, for example in successive extracts of *Eulophia gracilis* [20] and in *Dendrobium ochreatum* [21].

Natural product research also seeks new chemotherapeutic candidates against cancer, a leading cause of global mortality. Multidrug resistance and the severe side effects of conventional antineoplastic drugs make the continued search for plant-derived cytotoxic agents necessary [22]. Antiproliferative compounds from *Dendrobium* species, such as moscatilin, dendrofalconerol A, denbinobin, dendroflorin and gigantol, have shown apoptotic and antimigratory effects in several human cancer cell lines [23, 24]. The brine shrimp (*Artemia salina*) lethality assay is a cost-effective and reliable preliminary screen for bioactive compounds, because toxicity at microgram concentrations suggests pharmacological activity [25, 26]. It has been applied to *Swertia chirayita* and to the epiphytic orchids *Dendrobium amoenum*, *Eulophia nuda* and *Vanda roxburghii* [27–29]. Previous phytochemical work on *D. tortile* is limited to a study of commercially sourced material, which isolated a new compound, 4-(2-hydroxypropyl)-2(5H)-furanone, together with six additional constituents showing α -glucosidase inhibitory activity [30].

Given the ethnomedicinal use of *Dendrobium* species and the scarce documentation of other native medicinal orchids in Bangladesh, further pharmacological studies of *Dendrobium* species are warranted. To our knowledge, comprehensive quantitative profiling and bioactivity testing of *D. tortile* have not been reported. This study therefore analyzed the phytochemical profile of wild *D. tortile* leaves, stems and roots from Bangladesh and evaluated the *in vitro* DPPH radical-scavenging potential and brine shrimp lethality of their crude extracts and solvent fractions.

B

A

2. Materials and Methods

2.1. Plant Material Collection and Identification

Fresh wild specimens of *Dendrobium tortile* Lindl. were collected from the Dudpukuria–Dhopachari Wildlife Sanctuary, Chandanaish, Chattogram, Bangladesh (22.471553° N, 92.08640° E). The plant was authenticated by an expert orchidologist, and a voucher specimen (accession no. CTGUHS18101) was deposited at the Chittagong University Herbarium (CTGUH), Department of Botany, University of Chittagong.

2.2. Sample Preparation, Extraction and Fractionation

Leaves, stems and roots were washed, cut into small pieces, oven-dried at 60°C and ground separately. The powders were stored in airtight containers until extraction. Each powder (100 g) was extracted with 1L methanol at room temperature for 48–72 h with frequent shaking, followed by 10 min of sonication, and the extraction was repeated. The filtrates were evaporated under reduced pressure at below 51°C temperature with a rotary evaporator and dried to obtain the crude extracts. Each methanolic crude extract (MCD) was partitioned by a modified Kupchan liquid–liquid partition procedure into n-hexane (NHE), dichloromethane (DME), n-butanol (BE) and methanol-derived (ME) fractions. All fractions were dried under reduced pressure, weighed and stored at 4°C [31, 32].

2.3. Qualitative Phytochemical Screening

Screening for secondary metabolites was conducted using established colorimetric and precipitation protocols [33, 34]. Alkaloids were evaluated using 2% HCl followed by Dragendorff's, Hager's, Mayer's, Wagner's, and tannic acid reagents. Flavonoids were identified via the ammonia test. Phenols and tannins were detected with 10% and 5% aqueous FeCl₃, respectively. Glycosides and cardiac glycosides were assessed using Fehling's and Keller–Killiani tests. Steroids and terpenoids were tested using the chloroform reaction, while quinones and coumarins were identified using H₂SO₄ and 10% NaOH. Biuret and foam tests were employed to identify proteins and saponins.

2.4. Quantitative Phytochemical Estimation

2.4.1. Total Phenolic Content (TPC)

Total Phenolic Content (TPC) was quantified using the Folin–Ciocalteu method [35]. Extract solutions (1 mL, 1 mg/mL) were combined with 10% Folin–Ciocalteu reagent (5 mL) and 7.5% Na₂CO₃ (5 mL). After 20 min incubation at 25°C, absorbance was measured at 760 nm. TPC was expressed as mg gallic acid equivalents (GAE)/g dried extract.

2.4.2. Total Flavonoid Content (TFC)

TFC was determined by the aluminum chloride colorimetric method [36]. Extract (1.0 mL, 1 mg/mL) was mixed with 4 mL of distilled water and 0.30 mL of 10% NaNO₂. After 5 min, 0.30 mL of 10% AlCl₃ and 2.0 mL of 1% NaOH were added. Absorbance was measured at 510 nm against a quercetin calibration curve (25–150 µg/mL), and results were expressed as mg quercetin equivalents (QE) per gram of dried extract.

2.5. DPPH Radical-Scavenging Assay

Antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay [37]. 3ml of Crude extracts and fractions (50–250 µg/mL in methanol) were mixed with 0.004% methanolic DPPH solution (3 mL) and incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm against a methanol blank. Ascorbic acid served as the positive reference standard. Scavenging activity (%) was calculated as $[(A - B)/A] \times 100$, where A is the absorbance of the control and B is the sample absorbance. All tests were conducted in triplicate.

2.6. Brine Shrimp Lethality Assay

General toxicity was screened using the brine shrimp (*Artemia salina*) lethality assay [25]. Eggs were hatched in artificial seawater (3.8% sodium chloride, pH 8.5) under aeration for 48 h at room temperature to obtain nauplii. Extracts (1 mg/mL stock) were diluted to 100, 150, 250, 500 and 1000 µg/mL in seawater containing dimethyl sulfoxide (DMSO, < 0.01% in 5 mL). Active nauplii (10 per vessel) were transferred in containing 5 mL of test solution for 24 h, after which the survivors were counted. Potassium dichromate (1 mg/mL stock) diluted to (10–50 µg/mL) served as the positive control, and alongside a seawater blank. Mortality (%) was calculated as $(N_t/N_0) \times 100$, where N_t is the number of dead nauplii and N_0 is the total number of nauplii (10). Percent mortality was recorded in triplicate.

2.7. Statistical Analysis

Data are presented as mean $\pm$ SEM (n = 3). Quantitative phytochemical data were analyzed by Fisher's LSD test ($p < 0.01$). DPPH IC₅₀ value (the concentration giving 50% inhibition) of each replicate was calculated by linear interpolation fitting curve of percentage inhibition against concentration, 50% inhibition at the lowest concentration 50 µg/mL they were extrapolated and compared against ascorbic acid via one-way ANOVA followed by Dunnett's test using GraphPad Prism 8.0.2. The correlation matrix, heatmap and graphs were prepared with GraphPad Prism 8.0.2. Brine shrimp (LC₅₀) were estimated by probit analysis with a probit calculator and in SPSS version 26.0 [38, 39].

3. Results and Discussion

3.1. Qualitative Phytochemicals

Qualitative screening (Table 1) detected ten of the twelve phytochemical classes tested in the leaves, stems and roots of *D. tortile*: alkaloids (positive with Dragendorff's, Hager's, Mayer's, Wagner's and tannic acid reagents), flavonoids, phenols, tannins, terpenoids, steroids, cardiac glycosides, quinones, coumarins and proteins. Glycosides were present in leaves and stems only, and saponins in stems only. These results agree with the alkaloids, phenolics, tannins and flavonoids reported for *D. amoenum* [27] and with the alkaloids, bibenzyls, phenanthrenes and polyphenols that characterize the genus [12, 40].

Table 1
Qualitative phytochemical screening of leaves, stems and roots of *D. tortile* Lindl.

Phytochemical class (test)	Leaves	Stems	Roots
Alkaloids (Dragendorff's)	+	+	+
Alkaloids (Hager's)	+	+	+
Alkaloids (Mayer's)	+	+	+
Alkaloids (Wagner's)	+	+	+
Alkaloids (tannic acid)	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Glycosides	+	+	–
Cardiac glycosides	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Quinones	+	+	+
Coumarins	+	+	+
Proteins	+	+	+
Saponins	–	+	–

+, present; –, absent.

3.2. Total Phenolic and Flavonoid Contents

TPC ranged from 38.63 ± 0.64 to 96.76 ± 1.17 mg GAE/g of dried extract (Table 2; calibration curves in Fig. 2A). Roots contained significantly more phenolics than leaves (42.36 ± 1.53) and stems (38.63 ± 0.64) ($p < 0.01$). TFC varied over a narrower range (30.48 ± 0.31 to 34.43 ± 0.26 mg QE/g), and the organs differed significantly in the order stems > leaves (31.35 ± 0.19) > roots (Table 2, Fig. 2B). The ranking of plant parts thus reversed between the two assays, suggesting that the root phenolic pool consists largely of non-flavonoid phenolics such as tannins, phenolic acids, bibenzyls and phenanthrenes [27]. Phenolic compounds, including phenols and tannins, are known to contribute to the free-radical scavenging and antioxidant activities of plants [41]. Phenolic acids (ferulic, *p*-coumaric and sinapic) were likewise concentrated in the roots of *Phalaenopsis* hybrids [42], which may reflect a protective role of root tissue in constant contact with substrate and microorganisms, although this was not tested here. The appreciable contents agree with reports for *D. sonia* [43].

Table 2
Total phenolic and flavonoid contents of leaves, stems and roots of *D. tortile* Lindl.

Organ	TPC (mg GAE/g dried extract)	TFC (mg QE/g dried extract)
Leaves	42.36 ± 1.53 ^a	31.35 ± 0.19 ^a
Stems	38.63 ± 0.64 ^a	34.43 ± 0.26 ^b
Roots	96.76 ± 1.17 ^b	30.48 ± 0.31 ^c

Values are mean ± SEM (n = 3). TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; QE, quercetin equivalents. Different superscript letters within a column indicate significant differences (LSD, p < 0.01).

3.3. DPPH Radical-Scavenging Activity of Crude Extracts

All crude extracts scavenged DPPH over 50–250 µg/mL, with activity increasing with concentration in the leaf and stem extracts (Table 3). Ascorbic acid showed consistently high activity, increasing from 97.11 ± 0.08% at 50 µg/mL to 98.57 ± 0.05% at 250 µg/mL. Leaf extract activity rose from 72.55 ± 0.47% to 94.26 ± 1.01%, the highest extract value at 250 µg/mL, and stem extract activity rose from 62.12 ± 1.64% to 90.40 ± 0.32%. The root extract was most active showed scavenging (83.38 ± 0.09% to 94.93 ± 2.37%).

Ascorbic acid had the lowest IC₅₀ (27.30 ± 0.03 µg/mL; Table 4). Among the crude extracts, the root extract had the lowest IC₅₀ (32.33 ± 0.14 µg/mL), followed by the leaf (39.11 ± 0.29 µg/mL) and stem (45.29 ± 1.54 µg/mL) extracts, giving a potency order of ascorbic acid > root > leaf > stem. The IC₅₀ values of all three extracts differed significantly from that of the standard (leaf and stem, p < 0.0001; root, p < 0.01).

Table 3
DPPH radical-scavenging activity (%) of ascorbic acid and crude extracts of *D. tortile* Lindl.

Sample	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
Ascorbic acid	97.11 ± 0.08	97.57 ± 0.12	98.02 ± 0.05	98.30 ± 0.05	98.57 ± 0.05
Leaf	72.55 ± 0.47	75.57 ± 0.29	78.98 ± 0.86	89.00 ± 0.08	94.26 ± 1.01
Stem	62.12 ± 1.64	74.93 ± 4.50	81.31 ± 3.55	89.85 ± 0.46	90.40 ± 0.32
Root	83.38 ± 0.09	87.60 ± 0.89	88.78 ± 0.61	91.26 ± 0.62	94.93 ± 2.37

Values are mean ± SEM (n = 3).

Table 4
DPPH IC₅₀ values (µg/mL) of ascorbic acid and crude extracts of *D. tortile* Lindl.

Sample	IC ₅₀ (µg/mL)
Ascorbic acid	27.30 ± 0.03
Leaf crude extract	39.11 ± 0.29 ****
Stem crude extract	45.29 ± 1.54 ****
Root crude extract	32.33 ± 0.14 **

Values are mean ± SEM (n = 3). One-way ANOVA with Dunnett's test versus ascorbic acid: **** p < 0.0001; ** p < 0.01.

3.4. DPPH Radical-Scavenging Activity of Solvent Fractions

The solvent fractions showed clear polarity-dependent differences in radical-scavenging activity (Figs. 3–5; Table 5). In leaves, the dichloromethane (DME) and methanol-derived (ME) fractions were the most active at 250 µg/mL (89.82 ± 1.15% and 89.42 ± 1.34%, respectively; Fig. 3). In stems, the DME fraction was the most active (53.11 ± 1.57% to 94.52 ± 1.11% across the concentration range), whereas the ME fraction was the least active (27.33 ± 0.78% to 64.14 ± 0.79%; Fig. 4). Among the root fractions, the ME fraction was the most active (50.98 ± 1.33% to 91.43 ± 0.73%; Fig. 5). The IC₅₀ values of all fractions were higher than that of ascorbic acid (Table 5); that is, all fractions were less potent than the standard.

Figures 3–5. Relative DPPH radical-scavenging activity (%) of ascorbic acid (As.A) and solvent fractions of *Dendrobium tortile* Lindl. leaves (Fig. 3), stems (Fig. 4) and roots (Fig. 5). NHE, n-hexane; DME, dichloromethane; BE, n-butanol; ME, methanol-derived fractions. Values are mean ± SEM (n = 3).

In leaves, the methanol-derived fraction was the most potent (IC₅₀ = 57.81 ± 0.50 µg/mL, p < 0.001), closely followed by the dichloromethane fraction (61.72 ± 3.87 µg/mL, p < 0.0001), whereas the n-hexane (130.13 ± 12.50 µg/mL) and n-butanol least active (156.07 ± 3.23 µg/mL) (Table 5).

Stems showed the reverse pattern: the n-hexane (44.59 ± 2.82 µg/mL) and dichloromethane (52.34 ± 1.73 µg/mL, p < 0.01) fractions were the most active, whereas the n-butanol (162.06 ± 4.88 µg/mL) and methanol-derived (191.11 ± 4.27 µg/mL) fractions were the weakest (both p < 0.0001). The stem n-hexane fraction did not differ significantly from ascorbic acid.

In roots, the methanol-derived (55.36 ± 0.46 µg/mL, p < 0.0001) and dichloromethane (58.08 ± 0.55 µg/mL, p < 0.001) fractions were more active than the n-hexane (74.06 ± 0.39 µg/mL) and n-butanol (107.86 ± 2.86 µg/mL) fractions.

Table 5
DPPH IC₅₀ values (µg/mL) of solvent fractions of leaves, stems and roots of *D. tortile* Lindl.

Plant part	n-Hexane	Dichloromethane	n-Butanol	Methanol-derived
Leaf	130.13 ± 12.50 ****	61.72 ± 3.87 ****	156.07 ± 3.23 ****	57.81 ± 0.50 ***
Stem	44.59 ± 2.82 ^{ns}	52.34 ± 1.73 **	162.06 ± 4.88 ****	191.11 ± 4.27 ****
Root	74.06 ± 0.39 ****	58.08 ± 0.55 ***	107.86 ± 2.86 ***	55.36 ± 0.46 ****

Values are mean ± SEM (n = 3). Ascorbic acid (standard): 27.30 ± 0.03 µg/mL. Dunnett's test versus ascorbic acid: **** p < 0.0001; *** p < 0.001; ** p < 0.01; ns, not significant.

DPPH scavenging is attributed to hydrogen-atom or electron donation by phenolic hydroxyl groups. The IC₅₀ ranking of the crude extracts follows TPC, so the larger root phenolic pool probably explains the superior activity of the root extract at low concentration, in agreement with the stronger DPPH activity of roots than leaves in *Phalaenopsis* hybrids [42]. In contrast, an aqueous extract of *D. aqueum* showed dose-dependent scavenging with a maximum of only 49% at 100 µg/mL, significantly below the standard (93.3% at the same concentration) [44]. The IC₅₀ values compare favorably with those reported for *D. moniliforme* stem extracts (42.39–58.77 µg/mL for chloroform, acetone, hexane and ethanol extracts; 223.15 µg/mL for the methanol extract) [45]. A stem ethanolic extract of *D. macrostachyum* Lindl. showed IC₅₀ values of 10.21, 31.54 and 142.97 µg/mL in the DPPH, ABTS and reducing-power assays, respectively [46].

The variation in antioxidant capacity among plant parts and solvent fractions can be attributed to the polarity-dependent extraction of bioactive secondary metabolites. Polar methanol and semi-polar dichloromethane probably concentrated hydrogen-donating hydroxyl groups, enhancing DPPH scavenging. The significant differences observed (p < 0.0001) highlight the influence of solvent polarity on phytochemical yield and bioactivity; in, higher solvent polarity improved antioxidant extraction, with moderately polar fractions from Malvaceae seeds were most active hydrogen-donating phenolic aglycones [47]. The weak activity of the n-butanol fractions, which were among the least active in every plant part, is unexpected because butanol usually concentrates polar phenolics. It may result from co-extraction of glycosides (present in leaves and stems), while glycosylation masks hydroxyl groups. The stem pattern suggests largely lipophilic antioxidants, as expected for bibenzyls and phenanthrenes, and bibenzyl content correlated significantly with DPPH activity in a survey of *Dendrobium* stems [48].

3.5. Brine Shrimp Lethality

Mortality of *Artemia* nauplii increased with extract concentration (100–1000 µg/mL) for every crude extract and fraction of leaves, stems and roots (Fig. 6). The potassium dichromate used as positive control, produced 96.67% mortality at 50 µg/mL with a median lethal concentration LC₅₀ of 22.54 ± 1.07 µg/mL (95% CI: 15.08–30.44 µg/mL), categorizing it as extremely toxic.

The brine shrimp lethality test is a rapid, simple screen for general acute toxicity; its LC_{50} values are typically governed by combined effects of membrane interaction, neurotoxicity and broad metabolic disruption rather than by a single target mechanism [25, 49]. Recent interpretive categories place $LC_{50} < 1000 \mu\text{g/mL}$ as an indication of bioactivity or toxicity that justifies follow-up in cell-based cytotoxicity assays, but not as specific evidence of anticancer activity [50]. In this assay, extracts with LC_{50} values of 0–100 $\mu\text{g/mL}$ are considered extremely toxic, 100–500 $\mu\text{g/mL}$ moderately toxic and 500–1000 $\mu\text{g/mL}$ poorly toxic [51].

LC_{50} values estimated by probit analysis are given in Table 6. All test extracts were moderately toxic, with LC_{50} values ranged 172.79–351.69 $\mu\text{g/mL}$. In leaf extracts, LC_{50} ranged from $200.99 \pm 6.23 \mu\text{g/mL}$ (n-hexane, lowest) to $351.69 \pm 8.75 \mu\text{g/mL}$ (n-butanol, highest). For stem extracts, LC_{50} increased in the order dichloromethane (172.79 ± 3.49) < methanol (186.38 ± 7.72) < crude (218.21 ± 7.10) < n-hexane (266.31 ± 6.72) < n-butanol ($319.28 \pm 6.43 \mu\text{g/mL}$). Root fraction's the methanol solvent had the lowest LC_{50} ($212.47 \pm 2.15 \mu\text{g/mL}$) and the dichloromethane fraction the highest ($313.59 \pm 8.09 \mu\text{g/mL}$). Among the crude extracts, LC_{50} increased in the order stem (218.21 ± 7.10) < leaf (226.73 ± 7.00) < root ($241.95 \pm 8.63 \mu\text{g/mL}$).

Table 6
LC₅₀ values and 95% confidence intervals of crude extracts and fractions of
D. tortile Lindl. in the brine shrimp lethality assay.

Organ	Extract	LC ₅₀ (µg/mL)	95% CI (µg/mL)
Leaf	Crude (MCD)	226.73 ± 7.00	129.60–396.56
Leaf	n-Hexane (NHE)	200.99 ± 6.23	74.72–355.25
Leaf	Dichloromethane (DME)	230.79 ± 5.01	137.29–387.31
Leaf	Methanol (ME)	222.48 ± 5.24	133.78–369.60
Leaf	n-Butanol (BE)	351.69 ± 8.75	196.26–628.04
Stem	Crude (MCD)	218.21 ± 7.10	118.65–351.92
Stem	n-Hexane (NHE)	266.31 ± 6.72	151.94–466.78
Stem	Dichloromethane (DME)	172.79 ± 3.49	81.48–267.42
Stem	Methanol (ME)	186.38 ± 7.72	96.89–287.84
Stem	n-Butanol (BE)	319.28 ± 6.43	181.93–560.33
Root	Crude (MCD)	241.95 ± 8.63	149.90–390.54
Root	n-Hexane (NHE)	260.12 ± 1.19	165.23–409.52
Root	Dichloromethane (DME)	313.59 ± 8.09	182.44–539.03
Root	Methanol (ME)	212.47 ± 2.15	131.47–343.38
Root	n-Butanol (BE)	275.43 ± 7.07	149.30–508.14
Standard	Potassium dichromate	22.54 ± 1.07	15.08–30.44

LC₅₀ calculated by probit analysis. MCD, crude methanolic extract; NHE, n-hexane; DME, dichloromethane; BE, n-butanol; ME, methanol-derived fraction. Values are mean ± SEM (n = 3).

Moderate lethality (LC₅₀ of 173–352 µg/mL) in an invertebrate screen flags the need for dedicated safety work before any food or medicinal use; the brine shrimp assay has been reported to track toxicity in higher animals [49]. Ethanol gave the highest activity in Siamese bamboo (LC₅₀ = 80.78 ± 0.62 µg/mL), whereas less polar chloroform gave much lower activity (926.40 ± 4.27 µg/mL), reflecting poor recovery of cytotoxic and redox-active polar and mid-polar compounds [51]. Discreetly polar solvents probably extracted alkaloid free bases, phenanthrenes and bibenzyls, consistent with the lethality of the stem dichloromethane and methanol extracts (172.79 and 186.38 ± 7.72 µg/mL). The contrast between the stem and root dichloromethane fractions suggests specific toxic constituents, and the lethality of leaf n-hexane suggests lipophilic terpenoids and steroids, since several metabolite classes may contribute additively or synergistically to lethality in a whole-organism model. The lethality of *D. tortile* may be due

to its bioactive constituents; earlier studies likewise associate phenolics, flavonoids and tannins with cytotoxic, antitumor and anticancer activity, possibly by acting on xenobiotic-metabolizing enzymes and hormones [24, 30]. Several *Dendrobium* species have shown cytotoxicity: *D. nobile* against human lung and ovarian carcinoma cells, *D. chrysanthum* against HL-60 cells, and *D. longicornu* and *D. gratiosissimum* against U251 cells [52, 53].

3.6. Correlation Matrix and Heatmap Analysis

The Pearson correlation matrix and heatmap (Fig. 7) relate TPC and TFC to DPPH IC₅₀ and brine shrimp LC₅₀. TPC showed a near-perfect negative correlation with IC₅₀ ($r = -0.99$). Because a lower IC₅₀ indicates higher antioxidant potency, this suggests that phenolic compounds are the main contributors to radical scavenging, in line with observations in *Dendrobium amoenum* stem extracts, where phenolic levels correlated strongly with antioxidant capacity [54]. In contrast, TFC correlated positively with IC₅₀ ($r = +0.92$); that is, higher flavonoid content was associated with suggesting that flavonoids play a minor or different role from total phenolics. This trend mirrors *Dendrobium crepidatum* hexane extracts, in which the highest flavonoid content (82.62 µg QE/mg) coincided with the weakest IC₅₀ (306.77 µg/mL) among the extracts tested [54]. Scavenging in the present extracts may therefore be driven by non-flavonoid phenolics or by low-reactivity flavonoid species such as glycosylated derivatives. In the brine shrimp assay, TPC correlated positively with LC₅₀ ($r = +0.97$); because higher LC₅₀ values denote lower lethality, extracts richer in phenolics were less toxic to *A. salina*. In contrast, TFC correlated negatively with LC₅₀ ($r = -0.89$), indicating that flavonoid-rich extracts were more cytotoxic.

4. Conclusions

This is the first combined phytochemical, antioxidant and brine shrimp toxicity study of wild *Dendrobium tortile* Lindl. from Bangladesh. Ten of the twelve phytochemical classes we tested for were present in leaves, stems and roots. Roots held the most phenolics while stems held the most flavonoids. In the DPPH assay the crude root extract was the strongest scavenger came close to ascorbic acid. Among the fractions, the stem n-hexane fraction, Leaf and stems methanol and dichloromethane fraction were most active for antioxidant activity. Every extract was moderately toxic to *Artemia salina* and the stem dichloromethane and methanol fractions were the most active. The work was needed because *D. tortile* is poorly documented. The only earlier chemical study used commercially bought material, and nothing quantitative had been reported for wild plants from Bangladesh. Our results aimed to give a baseline for future use or conservation planning. Overall, these findings validate the pharmacological potential of *Dendrobium tortile* as a sustainable natural source of antioxidant compounds. The strong correlations established between major phytochemical groups (phenolics and flavonoids) with radical scavenging capacity and cytotoxic metrics which provides a rational framework for standardized botanical drug development. However, full clinical translation requires addressing current limitations, Subsequent investigations should prioritize high-resolution profiling (e.g., LC-MS/MS) to isolate key compounds, alongside *in vivo* toxicological and pharmacokinetic studies to confirm mammalian safety and efficacy.

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

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Mohammed Kamrul Huda conceived the research and supervised and reviewed the study. Selina Akther Lipa designed and conducted the research, analyzed and interpreted the data, and prepared and organized the manuscript. Kazi Mohammad Mesbaul Alam supervised the work and reviewed the results and the whole manuscript.

Acknowledgements

Co-author Professor Dr. Mohammed Kamrul Huda, Department of Botany, University of Chittagong, passed away unexpectedly on 6 February 2021; the authors mourn his passing. The authors are grateful to the Ministry of Education (MoE), Government of the People's Republic of Bangladesh, for support and logistic facilities, and to the Departments of Botany, University of Chittagong for laboratory facilities.

Data Availability

All data supporting this study are included in the article. Additional data are available from the corresponding author on reasonable request.

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Figures



Figure 1

Photographs of (A) flowers and (B) whole plant of *Dendrobium tortile* Lindl. from Dhopachari, Bangladesh.

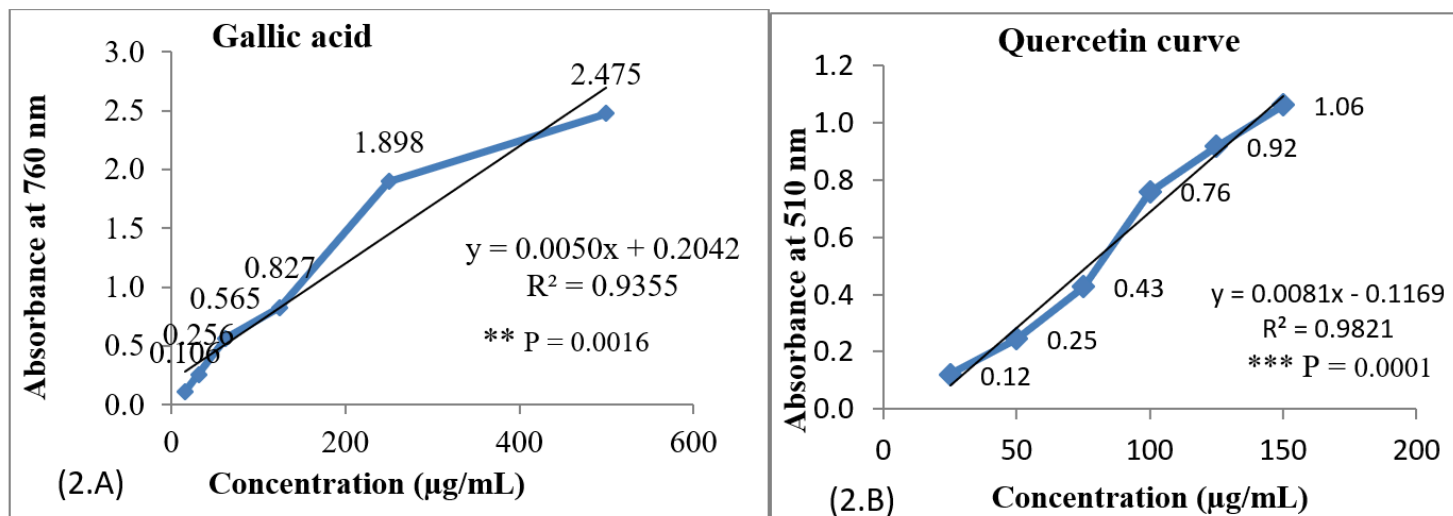


Figure 2

Calibration curves of the standards: (A) gallic acid for total phenolic content and (B) quercetin for total flavonoid content.

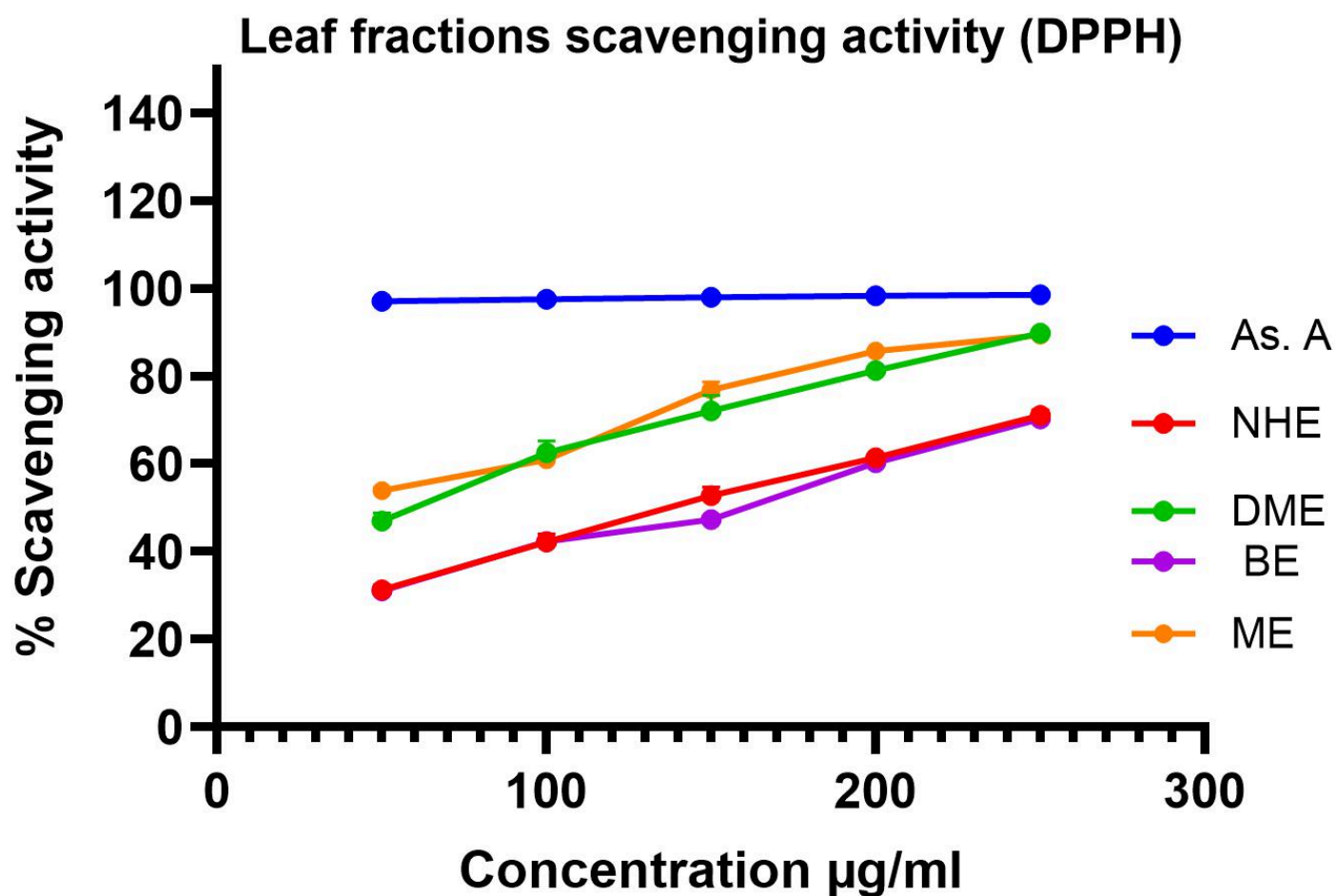


Figure 3

Relative DPPH radical-scavenging activity (%) of ascorbic acid (As.A) and solvent fractions of *Dendrobium tortile* Lindl. leaves (Figure 3), stems (Figure 4) and roots (Figure 5). NHE, n-hexane; DME, dichloromethane; BE, n-butanol; ME, methanol-derived fractions. Values are mean $\pm$ SEM (n = 3).

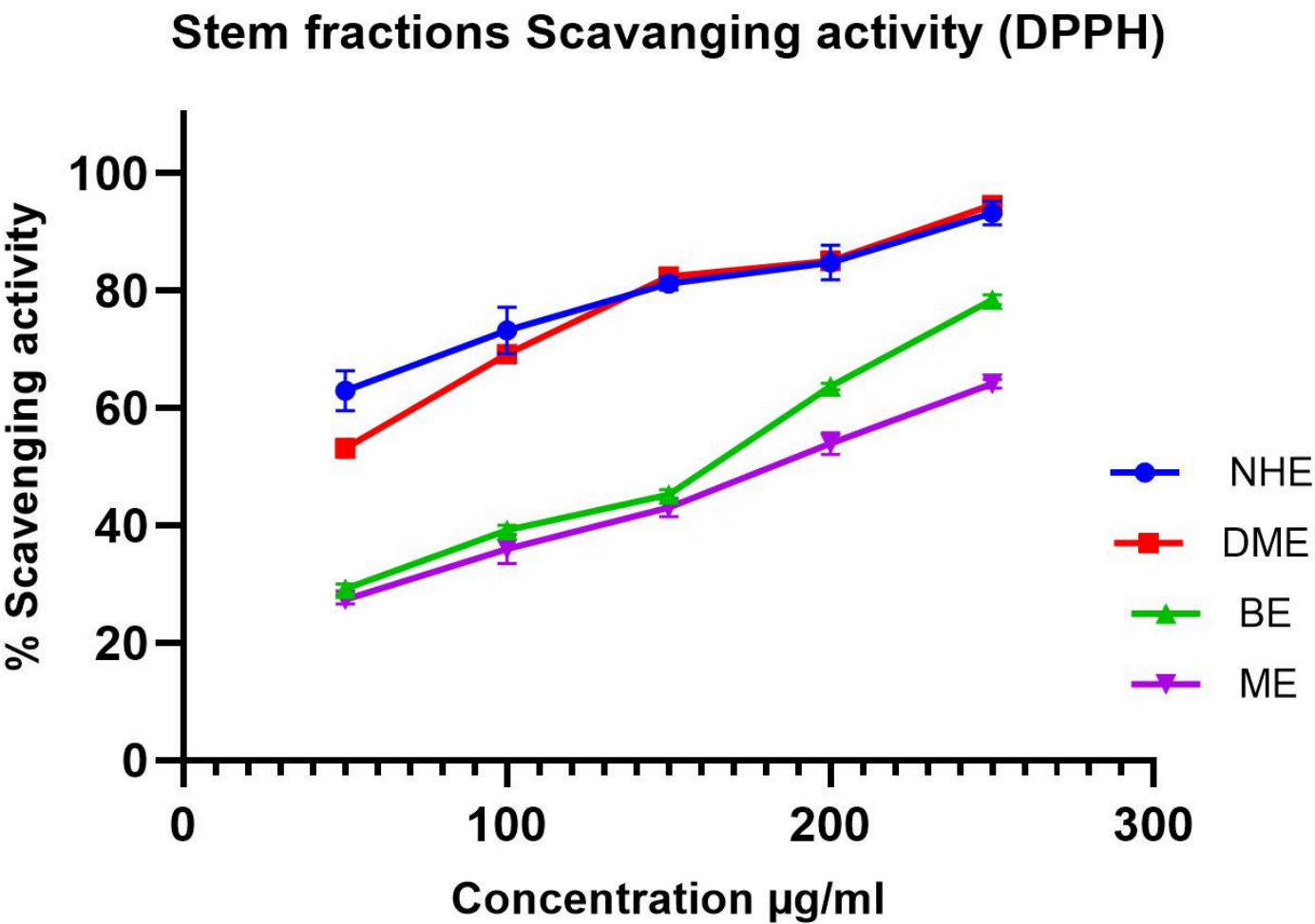


Figure 4

Relative DPPH radical-scavenging activity (%) of ascorbic acid (As.A) and solvent fractions of *Dendrobium tortile* Lindl. leaves (Figure 3), stems (Figure 4) and roots (Figure 5). NHE, n-hexane; DME, dichloromethane; BE, n-butanol; ME, methanol-derived fractions. Values are mean $\pm$ SEM (n = 3).

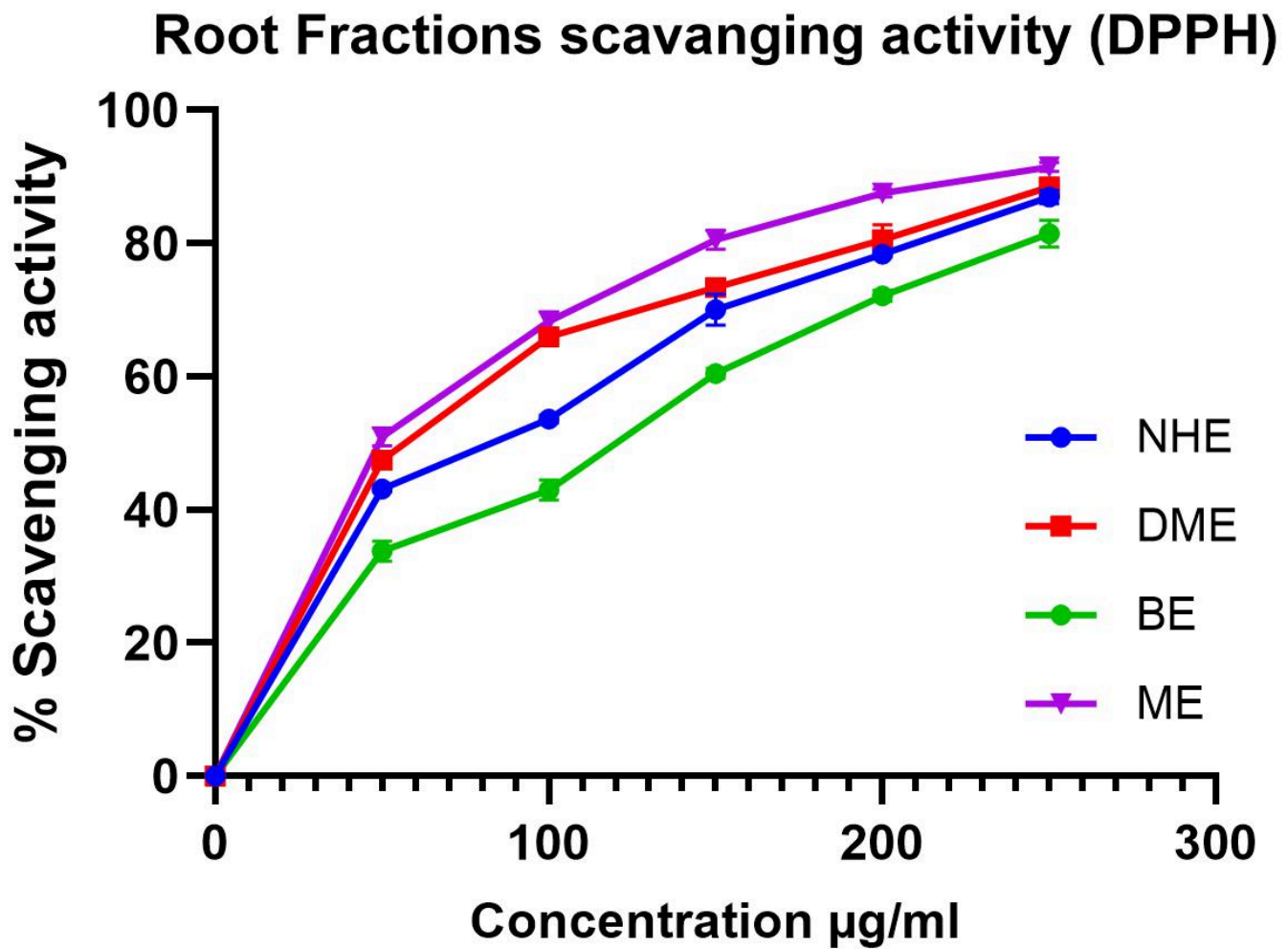


Figure 5

Relative DPPH radical-scavenging activity (%) of ascorbic acid (As.A) and solvent fractions of *Dendrobium tortile* Lindl. leaves (Figure 3), stems (Figure 4) and roots (Figure 5). NHE, n-hexane; DME, dichloromethane; BE, n-butanol; ME, methanol-derived fractions. Values are mean $\pm$ SEM (n = 3).

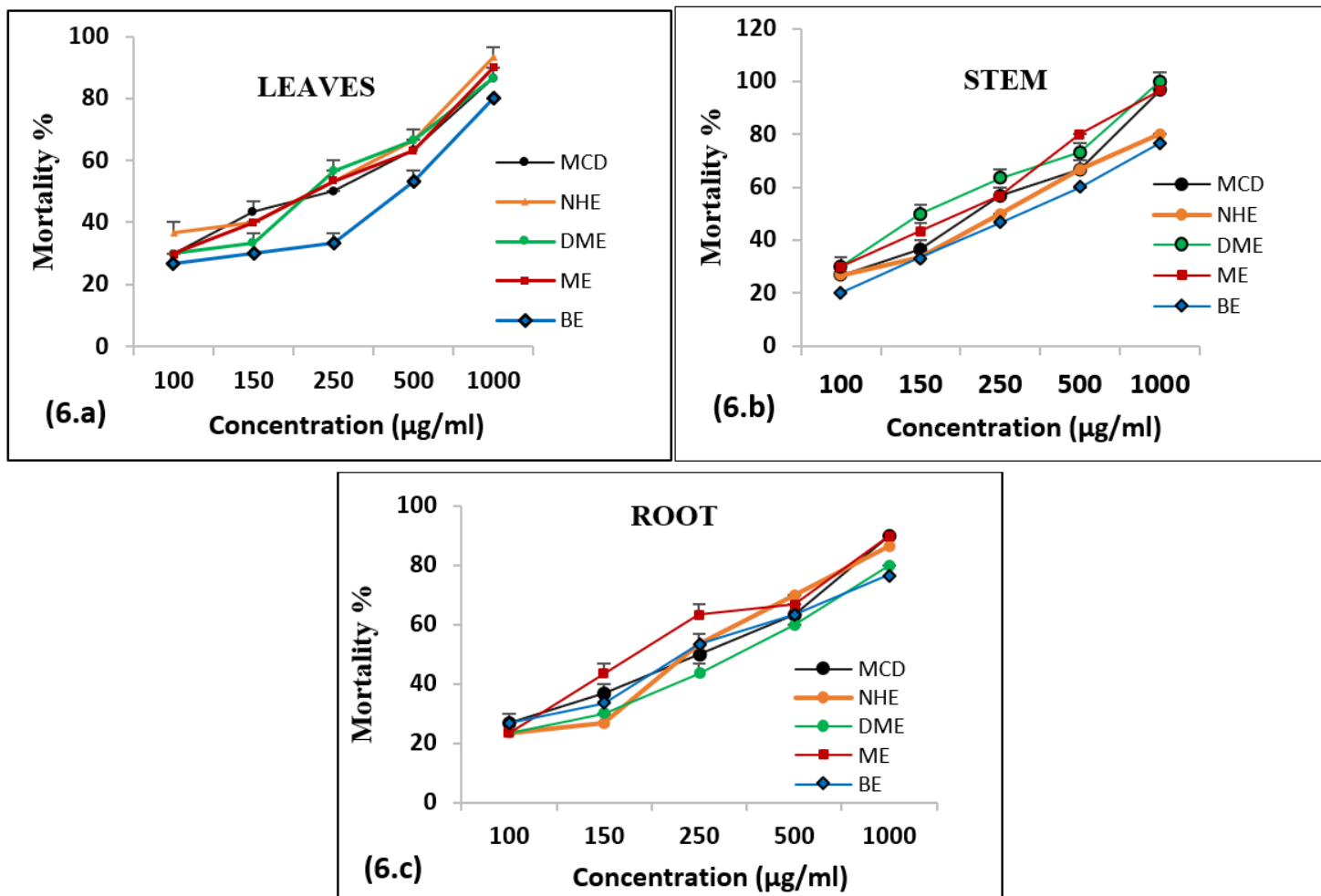


Figure 6

Brine shrimp lethality (% mortality) at different concentrations of crude extracts (MCD) and fractions of *Dendrobium tortile* Lindl.: leaves (6.a), stems (6.b) and roots (6.c). NHE: n-hexane; DME: dichloromethane; ME: methanol-derived; BE: n-butanol. Values are mean $\pm$ SEM.

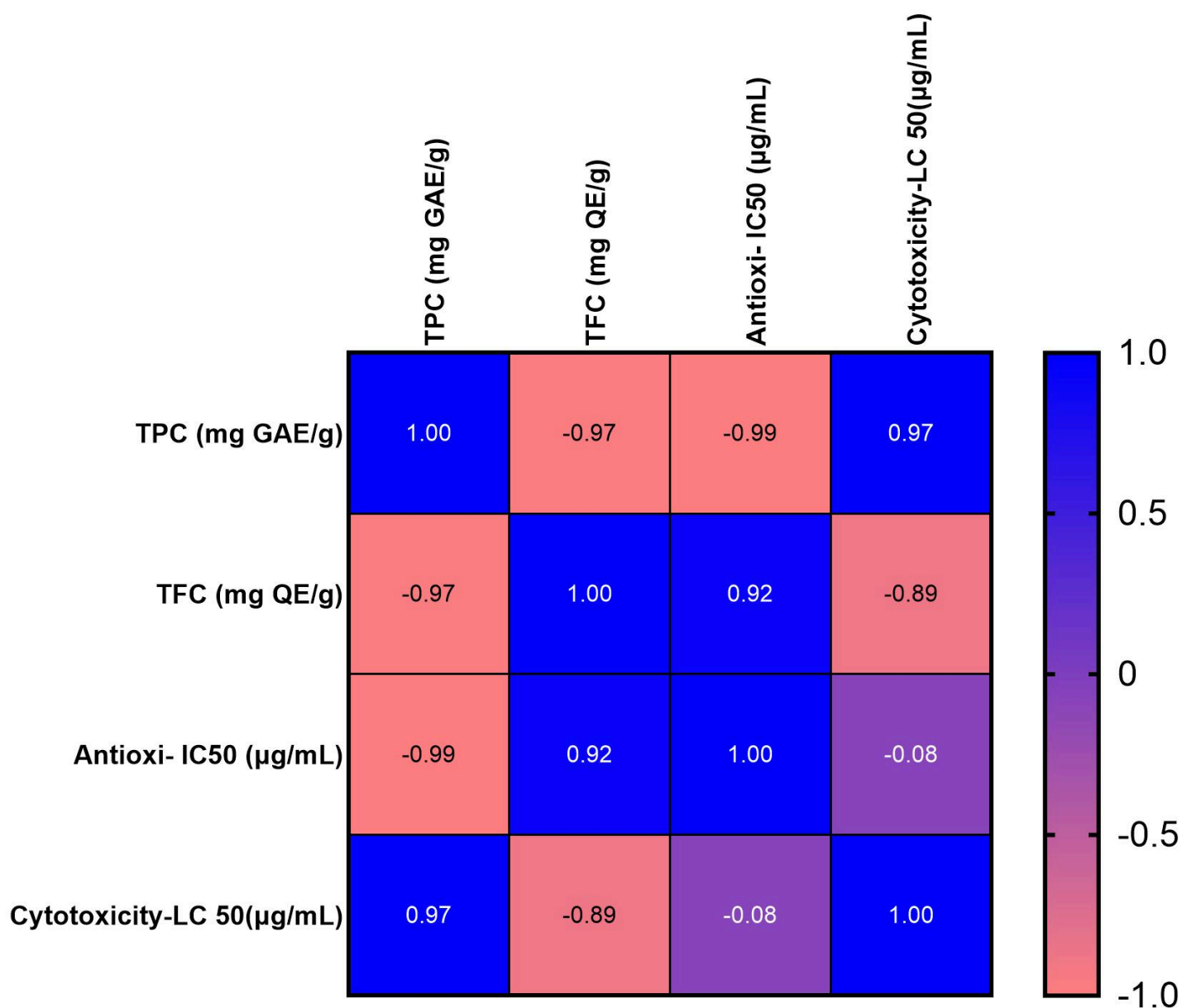


Figure 7

Pearson correlation matrix and heatmap relating total phenolic content (TPC), total flavonoid content (TFC), DPPH IC₅₀ and brine shrimp LC₅₀.