

Antioxidant, Anti-inflammatory, Cytotoxic Activities of *In vitro* and Naturally Grown Plant Parts of *Dendrobium farmeri* Paxton, an Indigenous Medicinal Orchid Species of Bangladesh

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Abstract

Dendrobium farmeri Paxton, an endangered epiphytic orchid native to Bangladesh is traditionally used for possesses significant traditional medicinal importance. However, its pharmacological potential remains under explored. This study was designed to evaluate the antioxidant, anti-inflammatory and cytotoxic bioactivities of methanolic crude extracts derived from naturally grown plant parts (leaves, pseudo bulbs, roots) and *in vitro* developed plantlets of *D. farmeri*. The antioxidant capacity, assessed *via* the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, revealed that the leaf extract exhibited the highest scavenging efficiency ($71.13 \pm 0.007\%$ at $250 \mu\text{g/ml}$) compared to standard ascorbic acid ($80.81 \pm 0.003\%$ at $250 \mu\text{g/ml}$). Anti-inflammatory potential, determined by the *in vitro* egg albumin denaturation inhibition assay, demonstrated maximum protection in the leaf extract ($54.4 \pm 0.17\%$ at $250 \mu\text{g/ml}$), while standard acetylsalicylic acid yielded $94.3 \pm 0.02\%$ at $250 \mu\text{g/ml}$ concentration. Furthermore, the Brine Shrimp Lethality Bioassay (BSLA) indicated strong cytotoxic potential across all extracts, with the root extract demonstrating the highest toxicity ($\text{LC}_{50} = 32.547 \mu\text{g/ml}$) compared to the standard potassium dichromate ($\text{LC}_{50} = 16.685 \mu\text{g/ml}$). Notably, *in vitro* derived plantlets also exhibited substantial bioactivities, confirming their viability as an alternative sustainable source for medicinal compounds. These findings provide rigorous empirical validation for the traditional therapeutic uses of *D. farmeri* and highlight its potential as a natural source for antioxidant, anti-inflammatory and cytotoxic agents, reinforcing the urgent need for its conservation in Bangladesh.

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Introduction

The Orchidaceae family is one of the most naturally and aesthetically diverse groups of flowering plants, representing the world's second-largest plant family with over 779 genera and 25,000 species distributed globally (Mabberley 2008, Chen et al. 2009). While cosmopolitan in distribution, orchids are exceptionally abundant and adaptable in the tropical and subtropical forests of Asia, including India, Nepal, Bhutan and Bangladesh. In Bangladesh, orchids naturally thrive as epiphytes on the trunks of ancient tropical trees in the rainforests of Chittagong, Cox's Bazar, Sylhet and the Sundarbans. Unfortunately, due to rapid habitat fragmentation and uncontrolled wild harvesting, the Orchidaceae family is in a highly vulnerable position. The loss of numerous orchid species from Bangladesh's flora over the past century highlights an escalating ecological crisis that requires immediate conservation actions (Rashid et al. 2017).

Beyond their profound horticultural and ornamental significance, orchids have been utilized in traditional medicine systems for millennia. Records dating back to 2800 B.C. indicate the extensive use of orchids in Chinese herbal remedies, where the genus *Dendrobium* (traditionally known as "shi-hu") is highly prized (Bulpitt 2005, Jalal et al. 2008). In Bangladesh, orchids are also recognized for their ethno-medicinal importance, particularly among indigenous communities of the Chittagong Hill Tracts. Several orchid species have traditionally been used for the treatment of fever, wounds, gastrointestinal disorders and inflammatory conditions. The continued use of these plants in folk medicine underscores their cultural significance and therapeutic potential in the country (Hossain 2009, Hossain 2011). The therapeutic efficacy of medicinal orchids is largely attributed to their rich reservoirs of plant secondary metabolites. Previous phytochemical studies on *Dendrobium* species have resulted in the discovery of diverse bioactive chemical groups, including alkaloids, flavonoids, polysaccharides, bibenzyls and phenanthrenes (Wang et al. 2009). These natural secondary metabolites act as defense mechanisms for the plant and offer intriguing pharmacological properties for human health, such as antioxidant, anti-inflammatory and cytotoxic activities (Zhang et al. 2005, Liu et al. 2011).

Dendrobium farmeri Paxton is a sympodial, epiphytic orchid characterized by pendulous racemes of pinkish-mauve and yellow flowers. Distributed across the lowland evergreen forests of the Himalayas, Myanmar and Bangladesh (specifically the Cox's Bazar and Bandarban region), *D. farmeri* is currently classified as rare and endangered due to extreme over exploitation (Singh et al. 2001, Rahman et al. 2017). Despite its known traditional medicinal applications and the presence of potent phytochemicals, there is a distinct lack of comprehensive, quantitative pharmacological validation regarding its bioactivity profile.

Therefore, the explicit objectives of this study were to scientifically evaluate and validate the antioxidant, anti-inflammatory and cytotoxic potencies of the methanolic crude extracts derived from the leaves, pseudo bulbs and roots of naturally occurring

D. farmeri, as well as from *in vitro* regenerated plantlets. By comparing these bioactivity profiles against established pharmaceutical standards, this study aims to substantiate the ethno-medicinal claims of *D. farmeri*, provide a baseline for future drug discovery and advocate for the commercial micropropagation and conservation of this threatened indigenous orchid species.

Materials and Methods

D. farmeri Paxton plant materials (capsules used for *in vitro* plantlet development, leaves, pseudo bulbs and roots) were collected from Manikpur, Chakaria, Cox's Bazar, Bangladesh. Taxonomic identification and verification were conducted prior to processing (Huda 2008). Whole plant parts of *in vitro* plantlets were used for bio-activity test developed after 3-4 months of culture using established aseptic *in vitro* techniques, serving as a secondary source of plant material for comparative bioactivity evaluation.

For the preparation of methanolic crude extracts, fresh, finely chopped and ground plant materials (leaves, pseudo bulbs, roots and *in vitro* derived plantlets) were used. The ground biomass was soaked in 100% methanol to facilitate cold maceration. Following adequate incubation, the mixtures were filtered. The resulting methanolic filtrates were then completely concentrated using a Vacuum Rotary Evaporator (WiseVen® WRE-10, DAIHAN Scientific Co., Ltd., Republic of Korea) under reduced pressure and controlled temperature. The dried crude extracts were collected, weighed and stored in sterile vials at 4°C in dark until required for quantitative bioassays.

The antioxidant capacity of the methanolic crude extracts was evaluated quantitatively based on their efficiency in scavenging the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to a modified protocol described by Brand-Williams et al. (1995).

A stock solution of DPPH (0.004% w/v) was prepared in methanol. The methanolic crude extracts and the standard reference antioxidant, Ascorbic Acid, were prepared in a range of serial concentrations (50, 100, 150, 200 and 250 µg/ml). For the assay, 3 ml of the prepared DPPH solution was combined with 3 ml of each extract concentration or standard solution in separate test tubes. The reaction mixtures were thoroughly vortexed and incubated in total darkness for 30 min at room temperature. The reduction of the DPPH radical (indicated by a color shift from deep purple to yellow) was measured spectrophotometrically by recording the absorbance at 517 nm against a methanol blank. The percentage of DPPH free radical scavenging activity was calculated using the following equation:

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

Where A is the absorbance of the control (DPPH solution with an equal volume of methanol) and B is the absorbance of the test sample or standard.

The *in vitro* anti-inflammatory potential was assessed using the inhibition of egg albumin protein denaturation method, as described by Mizushima and Kobayashi (1968),

with minor modifications. The reaction mixture consisted of the test extracts at a concentration of 250 µg/ml combined with a 1% aqueous solution of egg albumin. The pH of all reaction mixtures was carefully adjusted to 5.6 ± 0.2 using 1N HCl.

Only the samples were initially incubated at 37°C for 20 min to allow for binding and interaction, followed by a heat shock treatment at 51°C for 20 min to induce protein denaturation. After rapid cooling to arrest the reaction, the turbidity of the mixtures was measured spectrophotometrically at 660 nm. Acetylsalicylic acid (ASA) was utilized as the standard reference drug. The percentage of inhibition of protein denaturation was calculated as follows:

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

Where A represents the absorbance of the control (5% egg albumin without extract) and B represents the absorbance of the test group or the standard drug.

The general cytotoxicity profile of the methanolic extracts was determined using the highly reliable Brine Shrimp Lethality Bioassay (BSLA) modeled after Meyer et al. (1982). Artificial sea water was prepared by dissolving 38g of sodium chloride (NaCl) in 1000 ml of distilled water (3.8% w/v) and filtered to obtain a clear solution. *Artemia salina* (brine shrimp) cysts were added to the artificial sea water and hatched under constant oxygenation and room temperature for 48 to 72 hrs to yield active nauplii.

Stock solutions of the methanolic extracts (1 mg/ml) were prepared in distilled water. Serial dilutions were performed to achieve final testing concentrations of 10, 100, 250 and 500 µg/ml. For each concentration, 5 ml of artificial sea water containing exactly 10 live nauplii was transferred into experimental vials and the respective extract doses were added. A parallel series utilizing potassium dichromate ($K_2Cr_2O_7$) at concentrations of 10, 20, 30 and 50 µg/ml served as the positive standard, while vials containing only sea water and nauplii served as the negative control. After a 24 hrs incubation period, the surviving nauplii in each vial were counted using a magnifying glass. The percentage of mortality was calculated for each concentration.

All biological assays were performed in triplicate ($n = 3$) to ensure reproducibility. Data were presented as Mean \pm Standard Error of Mean (SEM). For the Brine Shrimp Lethality Bioassay, the mortality data were subjected to regression analysis to calculate the median lethal concentration (LC_{50}) utilizing probit analysis algorithms (Finney 1952) and straight-line graph equations.

Results and Discussion

The antioxidant efficiency of the methanolic crude extracts of *D. farmeri* (leaves, pseudo bulbs, roots and *in vitro* plantlets) was evaluated at varying concentrations (50, 100, 150, 200 and 250 µg/ml) against standard Ascorbic Acid using the DPPH radical scavenging model. The data, detailed as Mean \pm SEM %, demonstrated concentration dependent free radical neutralization. The results of the DPPH free radical scavenging activities for the methanolic crude extracts and the standard antioxidant are presented comprehensively in Table 1.

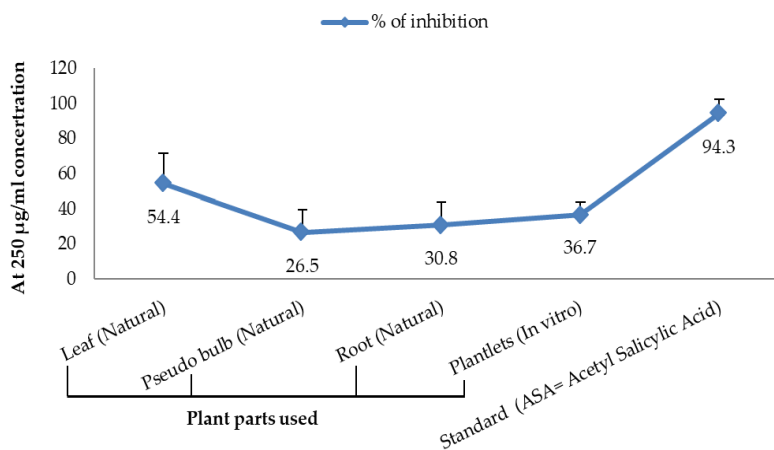
Table 1. DPPH free radical scavenging assay for methanolic crude extract of *D. farmeri* (leaf, pseudo bulb, root and *in vitro* plantlet) with Ascorbic Acid (as standard).

Plant parts used and standard	Concentration ($\mu\text{g/ml}$)				
	50	100	150	200	250
	% of Scavenging activity				
Ascorbic Acid (Standard)	77.72 \pm 0.014	80.93 \pm 0.006	81.79 \pm 0.009	81.73 \pm 0.003	80.81 \pm 0.003
Natural Leaf	45.59 \pm 0.029	40.84 \pm 0.020	59.34 \pm 0.015	67.70 \pm 0.008	71.13 \pm 0.007
Natural Pseudo-bulb	38.83 \pm 0.029	41.29 \pm 0.017	63.97 \pm 0.006	68.04 \pm 0.008	70.22 \pm 0.002
Natural Root	54.98 \pm 0.008	62.26 \pm 0.003	65.98 \pm 0.003	64.55 \pm 0.003	66.49 \pm 0.006
<i>In vitro</i> Plantlets	57.33 \pm 0.003	54.41 \pm 0.002	59.05 \pm 0.004	61.28 \pm 0.002	60.37 \pm 0.005

Note: Results are presented as Mean \pm SEM (n=3).

Ascorbic acid consistently exhibited the highest antioxidant power, peaking at 81.79 \pm 0.009% at 150 $\mu\text{g/ml}$ and showing 80.81 \pm 0.003% at 250 $\mu\text{g/ml}$. Among the plant extracts, the methanolic crude extract of the natural leaves displayed the most potent scavenging activity, initiating at 45.59 \pm 0.029% (50 $\mu\text{g/ml}$) and reaching a maximum of 71.13 \pm 0.007% inhibition at the highest concentration of 250 $\mu\text{g/ml}$. The pseudo bulb extract followed closely, yielding a maximum scavenging activity of 70.22 \pm 0.002% at 250 $\mu\text{g/ml}$. The root extract showed steady, robust neutralization, ranging from 54.98 \pm 0.008% at 50 $\mu\text{g/ml}$ to 66.49 \pm 0.006% at 250 $\mu\text{g/ml}$. Notably, the methanolic extract of the *in vitro* generated plantlets also exhibited considerable antioxidant stability, with a maximum scavenging activity of 61.28 \pm 0.002% recorded at 200 $\mu\text{g/ml}$.

Inflammation processes are fundamentally linked to the denaturation of tissue proteins. The capacity of *D. farmeri* extracts to inhibit the heat induced denaturation of egg albumin was quantified at a fixed dose of 250 $\mu\text{g/ml}$ to ascertain anti-inflammatory potential. The findings indicating the percentage of inhibition are presented in Fig. 1.



Note: Results are presented as Mean \pm SEM (n=3).

Fig. 1. Relative % of inhibition of methanolic crude extract of leaves, pseudo bulbs, roots and *in vitro* plantlets of *D. farmeri* with Standard Acetyl Salicylic Acid (ASA).

The standard reference, Acetylsalicylic Acid (ASA), demonstrated near complete protection with an inhibition rate of $94.3 \pm 0.02\%$ at $250 \mu\text{g/ml}$ concentration. The methanolic leaf extract of *D. farmeri* provided the most substantial plant based protection, registering a $54.4 \pm 0.17\%$ inhibition of albumin denaturation in leaf sample. The *in vitro* plantlet extract exhibited an inhibition rate of $36.7 \pm 0.07\%$, surpassing both the natural root extract ($30.8 \pm 0.13\%$) and the pseudo bulb extract, which showed the lowest inhibition capacity at $26.5 \pm 0.13\%$.

The general toxicity screening of the *D. farmeri* extracts was executed *via* the Brine Shrimp Lethality Bioassay. The percentage mortality of nauplii escalated correlatively with increasing extract concentrations (10, 100, 250 and $500 \mu\text{g/ml}$), while the negative control group registered zero mortality. According to established toxicity thresholds, LC_{50} values below $100 \mu\text{g/ml}$ indicate strong cytotoxic activity. The results of the brine shrimp lethality test for the methanolic crude extract of leaves, pseudo bulbs, roots and *in vitro* plantlets with Standard Acetyl Salicylic Acid (ASA) are presented in Table 2.

Table 2. Result of Brine Shrimp Bioassay of methanolic crude of *D. farmeri* and standard potassium dichromate.

Plant parts used	Concentration ($\mu\text{g/ml}$)	Log 10 dose	Total	(Mean \pm SEM) % Mortality	Mortality (%) in Probits	LC_{50} ($\mu\text{g/ml}$)	95% Fiducial CL. (Lower to Upper) $\mu\text{g/ml}$	LC_{50} Standard ($\mu\text{g/ml}$)	Chi-test (χ^2) Sig	Toxicity profile
Leaves	10	1	10	32 ± 0.66	4.53	34.622	2.249 to 95.982	16.685	0.99	Strong
	100	2		64 ± 0.51	5.36					
	250	2.4		76 ± 0.51	5.71					
	500	2.7		92 ± 0.37	6.41					
Pseudo bulbs	10	1	10	26 ± 0.51	4.36	43.588	3.413 to 122.376	16.685	0.996	Strong
	100	2		68 ± 0.37	5.47					
	250	2.4		72 ± 0.37	5.58					
	500	2.7		86 ± 0.51	6.08					
Roots	10	1	10	32 ± 0.66	4.53	32.547	1.525 to 92.793	16.685	0.9985	Strong
	100	2		68 ± 0.86	5.47					
	250	2.4		76 ± 0.51	5.71					
	500	2.7		90 ± 0.45	6.28					
<i>In vitro</i> plantlets	10	1	10	32 ± 0.97	4.53	66.56	7.626 to 177.992	16.685	0.9988	Strong
	100	2		44 ± 0.75	4.85					
	250	2.4		64 ± 0.51	5.36					
	500	2.7		80 ± 0.71	5.84					
Standard potassium dichromate	10	1.00	10	32 ± 0.58	4.53	16.685	7.537 to 24.549	-	0.98	Strong
	20	1.30		50 ± 0.71	5.00					
	30	1.48		76 ± 0.68	5.71					
	50	1.70		92 ± 0.37	6.41					

The standard cytotoxic agent, potassium dichromate, yielded mortalities ranging from $32 \pm 0.58\%$ (at $10 \mu\text{g/ml}$) to $92 \pm 0.37\%$ (at $50 \mu\text{g/ml}$), resulting in a calculated baseline LC_{50} of $16.685 \mu\text{g/ml}$. All methanolic fractions of *D. farmeri* demonstrated highly potent, statistically significant cytotoxicity profiles ($\text{LC}_{50} < 100 \mu\text{g/ml}$). The mortality rates and regression curve for the natural leaf extracts which yielded highest mortality $92 \pm 0.37\%$ (at $500 \mu\text{g/ml}$) and LC_{50} of $34.622 \mu\text{g/ml}$ (Table 2). The specific mortality outcomes for the pseudo bulb fraction, which yielded highest mortality $86 \pm 0.51\%$ (at $500 \mu\text{g/ml}$) and LC_{50} of $43.588 \mu\text{g/ml}$. The methanolic root extract exhibited the highest cytotoxic potential among the plant parts, killing $90 \pm 0.45\%$ of nauplii at $500 \mu\text{g/ml}$ and generating an LC_{50} of $32.547 \mu\text{g/ml}$. Furthermore, the toxicity profile for the extract derived from the *in vitro* cultured plantlets, highest mortality $80 \pm 0.71\%$ (at $500 \mu\text{g/ml}$) and exhibiting an LC_{50} of $66.56 \mu\text{g/ml}$. A comprehensive comparison among all the calculated LC_{50} values against the standard potassium dichromate is visualized and clearly demonstrating that the methanolic crude extract of the root has the most significant cytotoxic potential at 5% level of significance (Fig. 2).

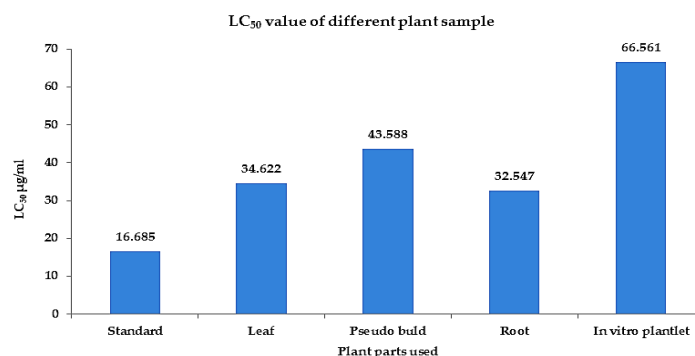


Fig. 2. Comparison in all LC_{50} values found in cytotoxic assay including standard (Potassium dichromate) and methanolic crude extracts of leaf, pseudo bulb, root and *in vitro* plantlet of *D. farmeri*.

The empirical results of this investigation strictly validate the pharmacological potency of *D. farmeri* Paxton, highlighting robust antioxidant, anti-inflammatory and cytotoxic capacities. Plant secondary metabolites, specifically polyphenols and flavonoids, act as powerful electron donors that terminate the chain reactions of lipid peroxidation and mitigate oxidative stress (Lobo et al. 2010, Ahmed et al. 2014). In the current study, the methanolic leaf extract of *D. farmeri* achieved an exceptional DPPH scavenging rate of 71.13%, which correlates directly with the high qualitative abundance of phenolic and flavonoid compounds (Mumu et al. 2026) typically concentrated in the foliar tissues of *Dendrobium* species (Do et al. 2014, Azwanida 2015). These findings parallel global literature; for instance, Paudel et al. (2019) demonstrated massive free radical neutralization in *D. crepidatum*, while Yang et al. (2007) isolated specific potent antioxidant derivatives like cis-melilotoside from the stems of *D. aurantiacum*. The ability of *D. farmeri* to stabilize reactive oxygen species firmly supports its traditional application in treating age-related and degenerative cellular disorders.

The anti-inflammatory assay further supported the therapeutic versatility of the leaf extracts, inhibiting protein denaturation by 54.4%. Protein denaturation is a major precipitating factor for inflammatory autoimmune diseases such as rheumatoid arthritis (Modak et al. 2021, Silvestrini and Silvestrini 2023). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) like Acetylsalicylic Acid exert their effects similarly, though often with gastro-intestinal side effects (Pradhan et al. 2021). The moderate-to-high inhibition achieved by *D. farmeri* extracts points to the presence of active secondary metabolites such as; terpenoids and steroids (Mumu et al. 2026) capable of stabilizing cellular membranes and preventing mediator release. This aligns with prior ethnobotanical reports by Lin et al. (2001), who isolated anti-inflammatory phenanthraquinones from *Dendrobium moniliforme*, proving that the genus is a rich repository for cyclooxygenase (COX) inhibiting or membrane-stabilizing phytochemicals.

Cytotoxicity profiling *via* the brine shrimp lethality assay provides a crucial baseline indicator for cytotoxicity capabilities. Standard pharmacognostic criteria dictate that crude extracts exhibiting an LC_{50} lower than 100 $\mu\text{g/ml}$ are classified as possessing strong cytotoxic potential (Saleh-e-In et al. 2016). Remarkably, all extracts tested in this study fell well below this threshold. The root extract showed extreme potency ($LC_{50} = 32.547 \mu\text{g/ml}$), closely followed by the leaves ($LC_{50} = 34.622 \mu\text{g/ml}$). This intense lethality suggests the presence of specific bioactive alkaloids or bibenzyl-phenanthrene derivatives capable of inducing apoptosis in rapidly dividing cells. Similar potent cytotoxicity has been documented in other Nepalese and Asian wild orchids; Joshi et al. (2020) confirmed that methanolic extracts of *D. transparens* exhibit highly lethal actions against human cervical carcinoma and glioblastoma cell lines.

Equally vital to the pharmacological discoveries of this study are the conservation implications. The extract from *in vitro* propagated plantlets showed significant DPPH scavenging (61.28%), protein stabilization (36.7%) and robust cytotoxicity ($LC_{50} = 66.56 \mu\text{g/ml}$). This proves that tissue cultured clones of *D. farmeri* retain the genetic competence to synthesize active secondary metabolites *ex situ*. Because wild *D. farmeri* is critically endangered in Bangladesh due to illegal trafficking and habitat loss, shifting commercial pharmaceutical exploitation from wild harvested specimens to mass scale *in vitro* bioreactors is an urgent and viable reality.

This study provides comprehensive empirical validation of the medicinal efficacy of *D. farmeri* Paxton. The methanolic extracts of its natural leaves and roots exhibited profound antioxidant free-radical scavenging, exceptional anti-inflammatory protein stabilization and potent cytotoxicity profiles ($LC_{50} < 35 \mu\text{g/ml}$), indicating strong cytotoxicity potential. Crucially, the parallel bioactivities observed in *in vitro* generated plantlets establish a benchmark for the sustainable pharmaceutical formulation and commercial exploitation of this species, mitigating the ecological pressure on wild populations and supporting the immediate conservation of Bangladesh's indigenous orchid heritage.

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