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Research Article

CHEMICAL CHARACTERIZATION AND FREE RADICAL SCAVENGING ACTIVITY OF *DENDROBIUM THONGCHAI* EXTRACTS: A PROMISING SOURCE FOR NEUROPROTECTIVE AND ANTIMICROBIAL AGENTS

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ABSTRACT

Objectives: This study assessed the potential of *Dendrobium thongchai* (gold) root extracts as sources of neuroprotective and antimicrobial compounds by characterizing their phytochemical constituents and antioxidant activity.

Methods: Methanol and ethanol were used to macerate root powders, and the extraction yield was quantified using gravimetric analysis. Gas chromatography-mass spectrometry analyzed the methanolic (MeOH) extract, identifying compounds based on National Institute of Standards and Technology (NIST) library matches. Ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl assays measured antioxidant activity at five concentrations (0.8–4.0 mg/mL). Each assay was performed in triplicate, with statistical analysis using one-way analysis of variance and Tukey's *post hoc* test to determine significant differences ($p<0.05$).

Results: Gas chromatography-mass spectrometry (GC-MS) profiling of the MeOH extract identified 45–47 compounds, including indole derivatives, fatty acid esters, alkaloids, and glycosides. Several metabolites showed moderate to strong NIST match factors, indicating antioxidant and neuroprotective potential, such as indole, 3-[2-(3-methylphenyl)ethenyl]-, oleic acid eicosyl ester, and an α-tocopherol derivative. Antioxidant assays showed concentration-dependent activity in both extracts; the ethanolic extract showed higher radical scavenging efficiency (IC_{50}=3.43±0.12 mg/mL) compared to the MeOH extract (IC_{50}=3.74±0.09 mg/mL). FRAP results confirmed ethanol as more effective for phenolic extraction.

Conclusion: This analysis reveals the chemical diversity and antioxidant properties of *D. thongchai* (gold). The presence of indole and glycosidic compounds suggests neuroprotective and antimicrobial potential, positioning this orchid for phytopharmaceutical research. Further work is needed to isolate and validate bioactive constituents using advanced analytical and biological assays.

Keywords: *Dendrobium thongchai*, Gas chromatography-mass spectrometry, Antioxidant activity, Indole derivatives, Phenolics, Neuroprotection, Antimicrobial.

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INTRODUCTION

The escalating occurrence of both infectious and chronic diseases poses a critical global health issue, with oxidative stress and antibiotic resistance emerging as two interconnected challenges [1]. Neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's, are frequently associated with oxidative stress, which results from an imbalance between the body's antioxidant defenses and the production of reactive oxygen species (ROS) [2]. Elevated ROS levels can inflict damage on proteins, nucleic acids, and lipids in neurons, potentially leading to apoptosis and subsequent neurodegeneration [3]. Consequently, there is a growing body of research focused on natural antioxidants that can neutralize ROS, exploring their potential as neuroprotective agents [4].

Simultaneously, the pressing demand for innovative antimicrobial strategies is compelled by the developing threat of antibiotic resistance. The potency of traditional antibiotics is being undermined by multidrug-resistant bacteria, which poses a risk of negating decades of medical advancements [5]. Historically, natural products have been instrumental in the development of antibiotics, and there is a growing interest in plant-derived metabolites as either direct antimicrobial agents or as adjuvants that can enhance antibiotic effectiveness [6]. These two challenges – oxidative stress-induced neurodegeneration and the global antibiotic resistance crisis – emphasize the critical need to explore medicinal plants for bioactive compounds with multiple therapeutic applications [7].

Orchids (Orchidaceae) stand out among natural resources for their notable aesthetic and therapeutic attributes [8]. In the realm of traditional Chinese and Ayurvedic medicine, various species within the Dendrobium genus have been historically utilized to address conditions such as fever, infections, and neurological disorders [9]. These Dendrobium species are prolific producers of secondary metabolites, which possess antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties. These metabolites include alkaloids, bibenzyl derivatives, flavonoids, phenolics, terpenoids, and polysaccharides [10]. For example, *Dendrobium officinale* extracts have shown significant antioxidant and antimicrobial activities, whereas *Dendrobium nobile* is noted for its neuroprotective effects [11,12]. Such findings highlight the potential of Dendrobium cultivars and hybrids as valuable sources of pharmacologically significant metabolites.

The cultivated orchid hybrid *Dendrobium thongchai* (gold) is predominantly esteemed for its ornamental value. Although it is extensively utilized in horticulture, there is a significant scarcity of scientific data on its pharmacological attributes and phytochemical profile [13]. Our prior study was among the initial efforts to reveal the antimicrobial capabilities of *D. thongchai* extracts and to verify the presence of key secondary metabolites. Nonetheless, its potential as an antioxidant has yet to be explored, and its chemical profile remains inadequately defined. A comprehensive investigation of this orchid is imperative, considering the crucial role of antioxidants in alleviating oxidative stress and the importance of antimicrobial agents in addressing resistance.

One particularly effective analytical method for examining volatile and semi-volatile phytochemicals is gas chromatography-mass spectrometry (GC-MS) [14]. By employing retention indices and spectral matching, this technique enables the tentative identification of compounds, thereby offering valuable insights into the chemical diversity present in plant extracts [15]. Historically, GC-MS-based metabolite profiling has been instrumental in identifying bioactive compounds such as terpenoids, phenolics, and fatty acid derivatives. They are recognized for their antibacterial and antioxidant qualities in orchids and various medicinal plants [16].

The relationship between chemical constituents and their functional activities can be discerned by analyzing GC-MS profiles alongside biological assays. Antioxidant assays serve as practical evidence of a plant's capability to counteract free radicals and reduce oxidative damage, thereby complementing chemical profiling. Evaluations of total phenolic content and total flavonoid content are instrumental in elucidating structure–activity relationships, while *in vitro* assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), and ferric reducing antioxidant power (FRAP) are routinely utilized to measure antioxidant potential [17]. Considering that oxidative stress plays a pivotal role in the development of neurodegenerative diseases, examining these properties in *D. thongchai* is particularly significant for its potential therapeutic application in these conditions.

Moreover, phytochemicals identified through GC-MS may possess antimicrobial properties, addressing the pressing need for new solutions in the fight against antibiotic resistance. This research underscores the dual biomedical importance of *D. thongchai* in combating antibiotic resistance and oxidative stress-related neurodegenerative disorders by linking its chemical profile to antioxidant efficacy.

METHODS

Plant collection and extraction

Fresh *D. thongchai* (gold) roots were sourced from a commercial nursery in Kerala, India. The roots underwent cleaning and were subsequently shade-dried at ambient temperature. Once dried, they were ground into a coarse powder using a mortar and pestle. For the extraction process, maceration was utilized with a solvent-to-sample ratio of 10:1 (w/v) over a period of 72 h at room temperature, with periodic shaking. Methanol and ethanol were used to extract 50 g of the powdered root material separately. Whatman No. 1 filter paper was used to filter the extracts, and a rotary evaporator operating at lower pressure was used to concentrate them [18]. The dried residues were stored in sealed containers at 4°C until further analysis. The weight of the dried extract in relation to the powdered sample was used to calculate the percentage yield (w/w) of the methanolic (MeOH) and ethanolic (EtOH) extracts, which came out to be 7.0% and 8.3%, respectively.

In this study, *D. thongchai* plant material was cultivated and propagated within a controlled greenhouse environment. On the specimen's full flowering and morphological stabilization, authentication is planned. Following the development of the inflorescence, a certified botanist will conduct a taxonomic confirmation of the specimen, and a voucher specimen will be deposited in the departmental herbarium for future reference.

GC-MS analysis

The MeOH extract was analyzed using a Shimadzu GC-MS QP2010 Plus system equipped with a Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium, the carrier gas, moved at a speed of 1.0 mL/min. The sample was injected in split mode with a split ratio of 1:10, while the injector temperature was maintained at 250°C. The oven temperature was increased to 280°C at a rate of 10°C/min after 2 min at 70°C, and it was kept there for 10 min. Under electron ionization (EI), mass spectra were obtained at 70 eV with a scan range of m/z 40–600 [19]. Compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) 2008 library, and relative percentage abundance was calculated using peak area normalization. To evaluate identification confidence, the reverse match factor (R. Match) was noted for every significant peak. The identification of compounds is regarded as tentative and is based only on mass spectral similarity with NIST library spectra because retention index calibration using an n-alkane standard series was not carried out.

While both MeOH and EtOH extracts were made, only the MeOH extract was subjected to GC-MS analysis because ethanol frequently produces larger amounts of non-volatile or thermally labile phenolics that are difficult to resolve under typical GC-MS conditions, while methanol efficiently solubilizes a wide range of moderately polar and semi-polar metabolites appropriate for GC-MS ionization. Methanol was thus selected as the representative solvent for chemical profiling.

Antioxidant assays

DPPH radical scavenging assay

The extracts' ability to scavenge DPPH radicals was evaluated using a traditional method. In short, 1 mL of 0.1 mM DPPH solution in methanol was combined with 1 mL of extract solution (1–5 mg/mL). The mixture's absorbance was measured at 517 nm following 30 min of dark incubation at room temperature. The positive control was ascorbic acid (Table 1). Percent inhibition was used to represent radical scavenging activity, and linear regression was used to extract IC_{50} values from concentration–response curves [20,21]. By modifying the sample volume within the range of 10–50 μL and maintaining the overall reaction volume at 250 μL, the final concentrations of the extracts in the reaction mixture were systematically adjusted to 0.8, 1.6, 2.4, 3.2, and 4.0 mg/mL.

FRAP assay

The reducing power of the extracts was assessed using the FRAP method. The FRAP reagent was made by mixing 2,4,6-Tri(2-pyridyl)-s-triazine (10 mM in 40 mM HCl), ferric chloride hexahydrate (20 mM), and acetate buffer (300 mM, pH 3.6) in a 10:1:1 ratio. A 100 μL aliquot of extract solution (1–5 mg/mL) was combined with 3 mL of FRAP reagent, and the mixture was incubated for 30 min at 37°C (Table 2). The absorbance at 593 nm was measured using a standard calibration curve, and the results were translated to μM $Fe2^+$ equivalents [22]. By modifying the sample volume within the range of 10–50 μL and maintaining the overall reaction volume at 250 μL, the final concentrations of the extracts in the reaction mixture were systematically adjusted to 0.8, 1.6, 2.4, 3.2, and 4.0 mg/mL.

Statistical analysis

The data are presented as mean±standard deviation (SD), with each experiment performed in triplicate (n=3). A one-way analysis of variance (ANOVA) was utilized to statistically evaluate the MeOH and EtOH extracts. Following this, Tukey's *post hoc* test was conducted to identify any significant differences among the groups. A statistically significant p-value was defined as <0.05. To determine IC_{50} values and verify the reliability of the data, regression equations and correlation coefficients (R^2) were derived for concentration-response curves. The statistical analyses were executed using GraphPad Prism 9.0 and Microsoft Excel.

RESULTS AND DISCUSSION

GC-MS profiling of *D. thongchai* (gold)

The MeOH root extract of *D. thongchai* (gold) was subjected to GC-MS analysis, resulting in a total ion chromatogram that extended from 3.0 to 47.0 min. Seven dominant chromatographic peaks were identified, collectively accounting for more than 99% of the total ion current. These peaks demonstrated peak area contributions ranging from 0.6% to 39.8%, with retention times (RT) spanning from 10.7 to 45.0 min (Table 3 and Figs. 1-5) [23-27].

At a RT of 12.1 min, the main compound, representing 39.8% of the total peak area, was identified and is postulated to be indole, 3-[2-(3-methylphenyl)ethenyl]- (Fig. S1-S5). Indole derivatives,

Table 1: Procedure for evaluation of antioxidant activity by DPPH assay

Components	S_1 (in µL)	S_2 (in µL)	S_3 (in µL)	S_4 (in µL)	S_5 (in µL)	Positive control (in µL)	Negative control (in µL)
DPPH Reagent	200	200	200	200	200	200	200
Sample	10	20	30	40	50	50 (ascorbic acid)	-
Solvent	40	30	20	10	-	-	50

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2: Procedure for evaluation of antioxidant activity by FRAP assay

Components	S_1 (in µL)	S_2 (in µL)	S_3 (in µL)	S_4 (in µL)	S_5 (in µL)	Positive control (in µL)	Negative control (in µL)
FRAP reagent	200	200	200	200	200	200	200
Sample	10	20	30	40	50	50 (1% FAS solution)	-
Distilled water	40	30	20	10	-	-	50

Incubate the tubes for 5 min at 25°C, FRAP: Ferric reducing antioxidant power

Table 3: Predominant compounds identified from GC-MS profiling of *Dendrobium thongchai* methanolic extracts

Peak	RT (min)	Major compound	Formula	Mol. Wt.	Area %	R-Match	Confidence level	Reported bioactivity	References
1	12.58	Indole, 3-[2-(3-methylphenyl) ethenyl]- (Fig. 1)	$C_{17}H_{15}N$	233	39.8	632	Tentative	Antioxidant, neuroprotective	23
3	38.27	Oleic acid, eicosyl ester (Fig. 2)	$C_{38}H_{74}O_2$	562	22.9	536	Weak	Antimicrobial, membrane-active	24
5	45.05	(+)-α-Tocopherol, O-tert-butyldimethylsilyl (Fig. 3)	$C_{35}H_{64}O_2Si$	544	15.9	517	Weak	Potent antioxidant, vitamin E derivative	25
4	44.25	D-Streptamine, glucopyranosyl derivative (Fig. 4)	$C_{18}H_37N_5O_{10}$	482	12.6	613	Tentative	Glycoside, antimicrobial	26
2	35.03	^{1}H-Indol-4-ol, 3-methyl- (Fig. 5)	C_9H_9NO	147	7.4	786	Fair Match	Radical scavenger, neuroprotective	27

Note: Compounds were identified based on NIST library spectral matches from GC–MS analysis of methanolic root extract of *Dendrobium thongchai*. For each major chromatographic peak, the compound with the highest match score (R.Match) was selected to represent the peak. *R.Match: Reverse match factor Values >900=Excellent match, 800–899=Good, 700–799=Fair, and <700=Weak. All identifications in this study are therefore tentative and based solely on spectral matching without retention index calibration.* Relative percentages of the entire chromatogram are used to represent peak regions. The complete list of all tentative compounds (~50 hits) is provided in Supplementary Table S1. GC-MS: Gas chromatography-mass spectrometry

Table 4: DPPH radical scavenging assay of *Dendrobium thongchai* methanolic and ethanolic extracts

Extract	Concentration range (mg/mL)	% Inhibition (mean±SD, n=3)	Regression equation	R^2	IC_{50} (mg/mL)	Significance (p<0.05)
Methanolic	0.8–4.0	18.5±0.9→57.22±1.4	y=8.246x+19.176	0.9547	3.74±0.09	–
Ethanolic	0.8–4.0	21.2±1.0→59.77±1.6	y=7.668x+23.658	0.9243	3.43±0.12	Significant

Values are shown as mean±SD, and all experiments were run in triplicate (n=3). p<0.05 (Tukey's *post-hoc* test) was used after a one-way ANOVA for statistical analysis

Table 5: Ferric reducing antioxidant power (FRAP) of *Dendrobium thongchai* methanolic and ethanolic extracts

Extract	Concentration range (mg/mL)	% Reducing power (mean±SD, n=3)	Regression equation	R^2	Maximum activity (%)	Significance (p<0.05)
Methanolic	0.8–4.0	22.4±1.0→65.4±1.3	y=9.218x+28.674	0.9412	65.4±1.3	–
Ethanolic	0.8–4.0	20.3±1.2→63.5±1.6	y=8.987x+30.214	0.9324	63.5±1.6	n.s. (not significant)

Values represent mean±SD (n=3). One-way ANOVA was used to perform Statistical analysis, followed by Tukey's *post hoc* test (p<0.05)

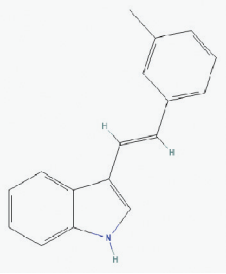

Fig. 1: Indole, 3-[2-(3-methylphenyl)ethenyl]-[$C_{17}H_{15}N$]
Note: Only the major compounds contributing to ~99% of the chromatogram are shown. The complete NIST match list is available in Supplementary Table S1

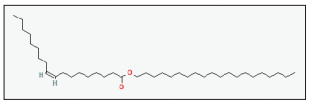

Fig. 2: Oleic acid, eicosyl ester [$C_{38}H_{74}O_2$]
Note: Only the major compounds contributing to ~99% of the chromatogram are shown. The complete NIST match list is available in Supplementary Table S1

recognized for their antioxidant, neuroprotective, and antimicrobial properties, are physiologically significant metabolites which are particularly relevant to the dual biomedical focus of this study. The presence of indole-related structures in the extract was further substantiated by another indolic compound, ^{1}H-Indol-4-ol, 3-methyl-,

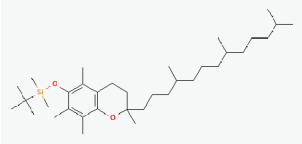

Fig. 3: (+)-α-Tocopherol, O-tert-butyldimethylsilyl [$C_{35}H_{64}O_2Si$]
Note: Only the major compounds contributing to ~99% of the chromatogram are shown. The complete NIST match list is available in Supplementary Table S1

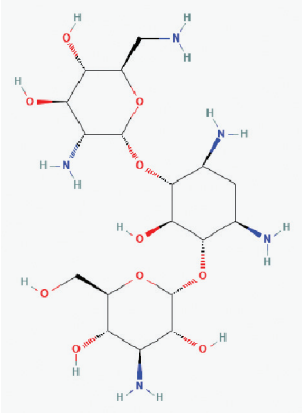

Fig. 4: D-Streptamine, glucopyranosyl derivative [$C_{18}H_{37}N_5O_{10}$]
Note: Only the major compounds contributing to ~99% of the chromatogram are shown. The complete NIST match list is available in Supplementary Table S1

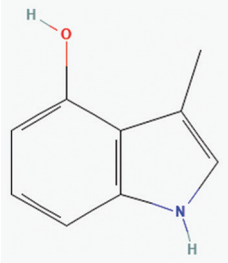

Fig. 5: ^{1}H-Indol-4-ol,3-methyl-[C_9H_9NO]
Note: Only the major compounds contributing to ~99% of the chromatogram are shown. The complete NIST match list is available in Supplementary Table S1

which accounted for 7.4% at RT 35.0 min (Fig. S2). Indolic phenolics, due to their electron-rich aromatic system, are potent radical scavengers and may play a crucial role in the extract's observed antioxidant activity.

At a RT of 38.3 min, a notable peak, accounting for 22.9% of the area, was attributed to oleic acid, eicosyl ester (Fig. S3). This fatty acid ester is recognized for its antibacterial, inflammation-reducing, and antioxidant properties. The ability of these lipid esters to stabilize cellular membranes and reduce oxidative stress suggests their potential utility in treating neurodegenerative disorders and bacterial infections.

A compound detected at RT 44.25 min, representing 15.9% of the area, was identified as a derivative of D-streptamine glycoside, which is structurally akin to aminoglycoside antibiotics (Fig. S4). The presence of such metabolites indicates a potential for natural antibiotic-like activity, which may account for the antimicrobial effects observed in prior studies. Based on its mass spectral fragmentation pattern and NIST library similarity index (R.Match=517), the peak detected at RT 45.05 min was determined to be (+)-α-Tocopherol, O-tert-butyldimethylsilyl derivative. The presence of α-tocopherol indicates that the root extract of *D. thongchai* contains inherent antioxidant components that can stabilize free radicals by donating hydrogen (Fig. S5). However, this assignment remains tentative due to moderate library match scores.

The remaining minor peaks (RT 10.7, 34.8, and 42.3 min, 0.6–7.3% each) were associated with heteroaromatic amines, other lipid esters, and indole-related compounds. Overall, the chemical profile suggests that phenolic derivatives, fatty acid esters, indolic alkaloids, and glycoside-like structures are predominant in the MeOH extract of *D. thongchai* (gold). Supplementary Table S1 provides an extended list of approximately 50 additional candidate compounds identified through NIST library searches across the main peaks. These include structural classes such as tocopherol esters, pyrimidines, benzofuranamines, carbazole derivatives, and quinolines. Among the ~50 tentative compounds identified (Supplementary Table S1), six major metabolites accounted for ~99% of the chromatogram area: Indole derivatives, oleic acid eicosyl ester, octadecenoic acid ester, α-tocopherol derivative, D-streptamine glycoside, and 3-methylindol-4-ol. While their preliminary identifications require further confirmation, the inclusion of these chemical families ensures transparency and serves as a valuable reference for future targeted research, given their well-established radical scavenging, antimicrobial, or neuroactive properties.

Fair to weak spectral similarity was indicated by the five major peaks' NIST library reverse match (R.Match) values, which varied from 517 to 786. These values demonstrate the complexity of orchid root extracts, where moderate library matches are frequently obtained through co-elution and novel compounds. As a result, all identifications are given as provisional and solely based on spectral similarity. The compound assignments are considered tentative because they are solely based on spectral matching, as retention indices were not established.

Our GC-MS findings support the increasing evidence that Dendrobium species are rich in indoles, alkaloids, and fatty acid derivatives. For example, GC-MS profiling [28] showed that MeOH extracts of *D. jenkinsii* contained bioactive phytoconstituents with antimicrobial and antioxidant properties. Similarly, research on the terrestrial orchid *Himantoglossum affine* identified significant compounds such as monoacetine and 4-pyrone derivatives, which are recognized for their free radical scavenging abilities [29]. The detection of indole derivatives in *D. thongchai* is particularly significant, as indoles have been linked to neuroprotective effects by influencing amyloid aggregation and oxidative stress, suggesting potential applications in addressing neurodegenerative diseases.

Similar observations have been made in other orchids using advanced MS-based profiling techniques. A comprehensive metabolome analysis of *Vanilla planifolia* using LC-MS and GC×GC-MS identified 127 metabolites, including terpenoids, sugars, and flavonoids [30]. The diversity observed in our study is mirrored in this array of phytochemicals, highlighting the effectiveness of MS-based methods in capturing the metabolic complexity of orchids. In a similar context, GC-MS analysis of *Rhynchostylis retusa* roots revealed a variety of alkaloids and phenolics [31]. More recently, it was demonstrated that extracts from *Dendrobium anosmum* produced glycosidic compounds, fatty acid esters, and indole derivatives with documented antimicrobial and anticancer properties [32]. These compounds overlap with those identified in this study, indicating that *D. thongchai* possesses distinct chemical markers while also sharing conserved biosynthetic traits with other Dendrobium species.

Antioxidants activity

DPPH radical scavenging assay

The MeOH and EtOH extracts of *D. thongchai* both showed a notable concentration-dependent development in radical scavenging activity, as determined by the DPPH assay (Figs. 6 and 7 and Table 4). The percentage inhibition rose steadily from 0.8 mg/mL to 4.0 mg/mL, peaking at 59.77±1.6% for the EtOH extract and 57.22±1.4% for the MeOH extract at the highest concentration tested. Apart from

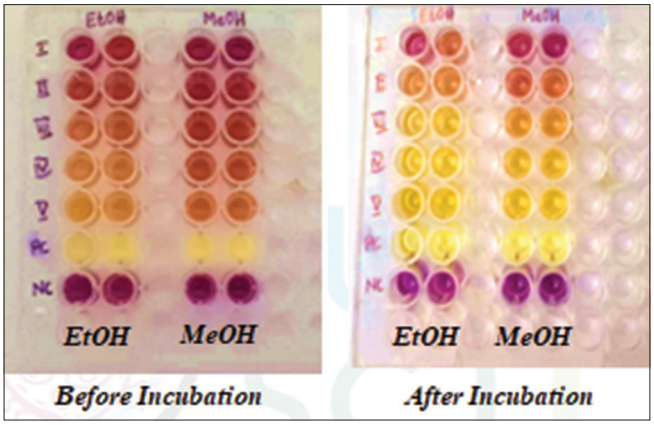

Fig. 6: Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl assay

the 2.4 mg/mL concentration, where the MeOH extract exhibited a slightly higher response, the EtOH extract generally displayed superior activity across most concentrations. Linear regression analysis of the inhibition curves yielded equations y=7.668x+23.658 (R^2=0.9243) for the EtOH extract and y=8.246x+19.176 (R^2=0.9547) for the MeOH extract, indicating strong linearity and reliable fitting. The effective concentration (IC_{50}) was calculated to be 3.43±0.12 mg/mL for the EtOH extract and 3.74±0.09 mg/mL for the MeOH extract. One-way ANOVA statistical analysis ($p<0.05$) verified a notable variations between the extracts, highlighting the higher DPPH radical scavenging efficiency of the EtOH extract. These findings suggest that ethanol's superior phenolic solubility and polarity range likely enhance the extraction of potent antioxidant components from the orchid matrix.

The antioxidant assays of *D. thongchai* revealed a significant radical scavenging and reducing capacity, with ethanol extracts consistently surpassing methanol extracts in performance. This observation aligns with previous research on orchids, which indicated that ethanol preferentially extracts the primary contributors to antioxidant activity, namely phenolics and glycosides. For example, chitosan-treated *Pholidota articulata* regenerants accumulated higher levels of phenolics, which were directly correlated with enhanced antioxidant responses [33]. Similarly, ethanol extracts rich in ferulic and p-coumaric acids demonstrated that Phalaenopsis hybrids exhibited stronger radical scavenging activity in their roots compared to their leaves [34].

FRAP assay

The MeOH and ethanolic (EtOH) extracts of *D. thongchai* exhibited a concentration-dependent enhancement in reducing capacity, as concluded by the FRAP assay (Figs. 8 and 9 and Table 5). The antioxidant potential increased progressively from 0.8 mg/mL to 4.0 mg/mL, achieving a peak of 65.4±1.3% for the MeOH extract and 63.5±1.6% for the EtOH extract. The marginally superior reducing potential of the MeOH extract at higher concentrations implies the presence of more effective electron-donating compounds soluble in methanol. Linear regression analysis of the FRAP response curves revealed a robust correlation between concentration and reducing power: y=9.218x + 28.674 (R2=0.9412) for MeOH and y=8.987x + 30.214 (R^2=0.9324) for EtOH. Statistical evaluation (one-way ANOVA, $p<0.05$) indicated that while differences between the solvent extracts were not significant at lower concentrations, they became pronounced at 4.0 mg/mL. These results demonstrate that both extracts have a substantial capacity to reduce ferric ions, highlighting their potent antioxidant properties and supporting their potential role in mitigating oxidative stress.

The improved extraction of polar phenolic and glycosidic compounds, which are less volatile and thus underrepresented in the MeOH extract's GC–MS profiling, may be the cause of the EtOH extract's higher antioxidant performance. Our results corroborate findings from studies on *Serapias vomeracea*, where phenolic enrichment led to EtOH extracts displaying notable DPPH and FRAP activity [35]. The IC_{50} values

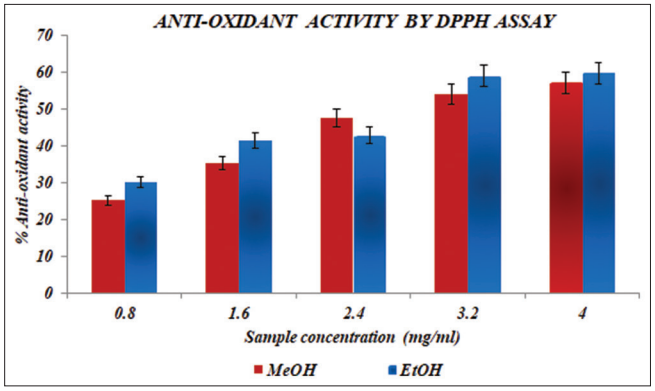

Fig. 7: *Dendrobium thongchai* methanolic and ethanolic extracts' capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals at various concentrations. The standard deviation (SD) of the triplicate readings is shown by the error bars, and the data characterize the mean±SD (n=3)

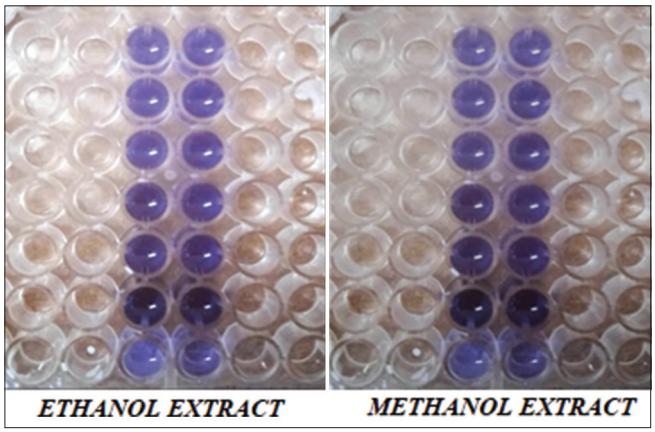

Fig. 8: Antioxidant activity by ferric reducing antioxidant power assay

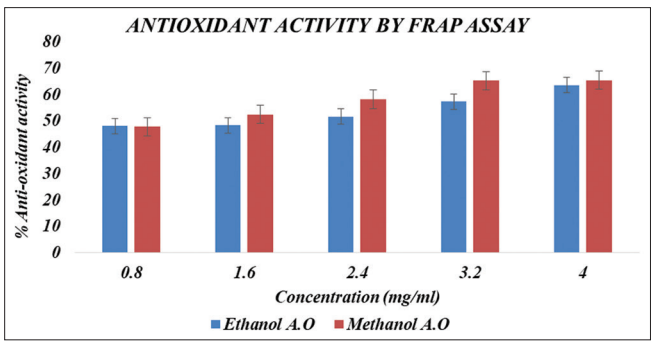

Fig. 9: Ferric reducing antioxidant power reducing power of ethanolic and methanolic extracts of *Dendrobium thongchai* at varying concentrations. Error bars show standard deviation, and values show mean±SD (n=3)

observed in *Rhynchostele rossii* extracts were similar to those of our ethanol extracts, indicating a dose-dependent antioxidant activity and emphasizing the influence of solvent choice on activity [36]. Ethanol is advocated as the preferred solvent for maximizing phenolic recovery and radical scavenging potential, as evidenced by an extensive review of orchid antioxidant research [37].

The antioxidant capabilities of the *D. thongchai* extract are likely due to its polyphenolic and flavonoid content, as demonstrated by the

DPPH and FRAP assays. Previous qualitative screenings of the same batch of extract confirmed the availability of flavonoids and phenolic hydroxyl functional groups, although the current study did not include quantitative measurements of these compounds. These substances are well-known for their availability to contribute hydrogen and chelate metals, which are significant for ferric-reducing and radical scavenging activities. Thus, the enhanced solubility of these polyphenolic compounds in the EtOH extract may explain its superior antioxidant performance.

The comparative analysis reveals that *D. thongchai* follows a consistent antioxidant pattern typical of orchids, where the activity is modulated by the polarity of the solvent and the concentration of phenolic compounds. This study not only sheds light on a previously unexamined orchid species but also highlights its potential therapeutic value, particularly in managing conditions related to oxidative stress, where natural antioxidants are of significant pharmacological interest.

CONCLUSION

This study offers an in-depth analysis of the phytochemical diversity and antioxidant capabilities of *D. thongchai* (gold), a commercially cultivated orchid with untapped medicinal potential. Through GC-MS analysis of MeOH extract, 45–47 compounds were identified, including indole derivatives, fatty acid esters, alkaloids, and glycosides. While these metabolites have been individually identified in other orchid species, their simultaneous presence in *D. thongchai* underscores the plant's chemical diversity and enriches the known phytochemical profile of the genus. The prominence of indole derivatives is particularly noteworthy due to their recognized roles in neuroprotection, specifically in modulating oxidative stress and inhibiting amyloid aggregation.

The antioxidant assays further validated the bioactivity of these extracts. Ethanol extracts consistently exhibited higher activity, although both MeOH and EtOH fractions demonstrated radical scavenging and reducing capabilities. This finding aligns with previous research on orchids, which identified ethanol as more effective in extracting glycosidic and phenolic compounds. The results state that *D. thongchai* is a significant source of natural antioxidants and highlight the significance of solvent polarity in capturing bioactive components.

Significantly, this study identifies two distinct biomedical applications for *D. thongchai*. Its indole-rich profile supports its potential use in treating oxidative stress-mediated neurodegenerative diseases like Parkinson's and Alzheimer's. In addition, the availability of alkyl esters of fatty acid and glycosidic compounds, which correspond with known antimicrobial agents, presents opportunities for addressing antibiotic resistance. This dual focus represents a novel contribution to orchid pharmacology and has not been previously documented for this orchid.

D. thongchai is identified as an inception of multifunctional phytochemicals compounds through the integration of GC-MS profiling and antioxidant assays. Future research should prioritize isolating active fractions, testing in microbial and neuronal models, and conducting structural validation using LC-MS/MS and NMR. These efforts will be crucial in translating *D. thongchai*'s phytochemical potential into medicinal applications.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception, experimentation, data analysis, and preparation of the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this work.

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