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PHYTOCHEMICAL SCREENING OF A FEW ENDEMIC AND MEDICINALLY IMPORTANT ORCHIDS OF NORTHEASTERN INDIA

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Abstract

The present study investigates the phytochemical activity of a few endemic and medicinally important orchids of NorthEast India, highlighting their potential as valuable sources of bioactive compounds for pharmaceutical, nutraceutical, and industrial applications. The species exhibited substantial variation in total phenol content (TPC), ranging from 15.70 to 83.16 mg GAE g^{-1}, with a mean value (43.70 mg GAE g^{-1}). Total flavonoid content (6.86-29.54 mg QE g^{-1}) and flavanol content (0.09-2.35 mg QE g^{-1}) were similarly diverse, with *Bulbophyllum odoratissimum* consistently demonstrating the highest levels across phenolic and flavonoid categories. Antioxidant activity, measured by FRAP (3.25-16.59 mg $FeSO_4$ g^{-1}), also varied significantly amongst species, with *Liparis odorata* exhibiting the greatest reducing potential. Sugar profiling revealed notable differences in total sugar, reducing sugar, and non-reducing sugars, with sucrose identified as the predominant carbohydrate in orchid leaves. Correlation analysis indicated a strong positive relationship between total sugar and reducing sugars ($r = 0.998$; $p < 0.01$), while other biochemical parameters showed no significant associations. Overall, the present findings underscored the biochemical richness and interspecific variability amongst medicinal orchids, suggesting promising opportunities for further phytochemical characterization, pharmacological evaluation, and potential biotechnological applications.

Introduction

ORCHIDS ARE one of the most important ornamental plants in the world and are recognized for their captivating hues, forms, sizes, and fragrances. These belong to family Orchidaceae, the second largest family of flowering plants in the world which comprises 693 genera with 29,481 species worldwide (POWO, 2025; WFO, 2023). India is a home to roughly 1484 species of orchids (Kumar *et al.,* 2022), which is more than 6% of world's orchid population with high rate of endemism. The NorthEastern India occupies 7.7% of India's total geographical area supporting 50 % of the flora (*ca*. 8000 species), of which 31.58 % (*ca*. 2526 species) are endemic (De *et al.,* 2016). A total of 856 species of orchids are reported in the NorthEastern region and about 150 species are endemic to different states of this region (De and Singh, 2015; De *et al.,* 2016). Studies showed that Arunachal Pradesh has the highest number of orchids, with 612 species, followed by Sikkim with 560 species, Meghalaya [430 species (Singh *et al.,* 2019)], Nagaland [423 species (Deb *et al.,* 2021)], Assam [398 species (Gogoi, 2019)], Manipur [389 species (Nageswara and Vikas, 2018)], Mizoram [253 species (Ninawe and Swapna, 2017)], and Tripura with the least [51 species (Baishnab *et al.,* 2025)]. Orchids are mainly used as cut flowers and as potted plants in the horticulture industry (Murthy *et al.,* 2018; Park *et al.,* 2018). However, these plants have been also highly valued for their use in traditional medicine for centuries. A total of 145 medicinally important orchid species are documented in NorthEast India which are used for treating diseases like cancer, tumours, nervous disorders, and skin ailments (Gupta *et al.,* 2024). There has been increasing interest in using antioxidants from plants as functional foods and nutraceutical products with antioxidant properties (Brewer, 2011). Recent studies have identified various orchid species as rich sources of antioxidants, highlighting their potential health benefits (Chinsamy *et al.,* 2014; Schuster *et al.,* 2017).

A plethora of orchid species have been found to possess an array of bioactive compounds, conferring upon them a wide range of pharmacological properties. Notably, *Aerides odorata* contains 2-Methyl-5-(1,2,2-Trimethy cyclopentyl) phenol, which has been reported to possess anticancerous property (Katta *et al.,* 2019). *Dendrobium candidum* contains *Dendrobine*, which has been reported to exhibit potential in the treatment of diabetes (Shiau *et al.,* 2005), while *D. nobile* has been identified as a rich source of *Denbinobin*, a compound with anti-tumour activity (Mohanty *et al.,* 2012). *Rhynchostylis retusa* has been found to contain gigantol and flavidin, showed positive antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi,* and *Staphylococcus aureus* (Hossain, 2011). Further research is imperative to elucidate the phytochemical composition and potential biological activities of these orchid species, thereby bridging the knowledge gap in this domain. A number of bioactive compounds like alkaloids, phenanthropyrans,

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phenanthrenes, stilbene, and related compounds have already been isolated from orchids (Sut *et al.,* 2017). It is high time to create awareness amongst the public regarding the medicinal benefits of our indigenous orchids, in order to protect our genetic resources from over-exploitation and habitat destruction due to urbanization. As only a few studies have been conducted on the medicinal potential of orchids (Hoque *et al*., 2024; Kumari and Pathak, 2025a,b; Rahman *et al*., 2023; Sharma and Pathak, 2024), the present study was aimed at conducting phytochemical analyses of some important medicinal orchids in the NorthEastern region so as to ensure their importance, conservation and sustainable usage.

Material and Methods

Experimental Site

The present experiment was conducted to study the phytochemical properties of a few endemic and medicinally important orchids of NorthEast India in the research farm, Division of Floriculture & Landscaping, ICAR Research Complex for NEH Region, Barapani, Meghalaya for a period of three years (2021-23). The endemic medicinal orchid species were collected from different regions of NorthEastern India (Table 1). The plants were grown in pots containing sphagnum moss, charcoal, and coconut husk (1:1:1), placed in a controlled-environment greenhouse under an 8-hrs photoperiod at 28/22 °C day/night temperatures and a relative humidity of 80%. They were uniformly spaced to encourage similar growth and development. Plants were watered once or twice a wk, and an optimal amount of soluble fertilizer solution (N:P:K 19:19:19) was applied once, every 2 wks.

Table 1. Presently studied endemic and medicinally important orchids of NorthEastern India.

Species	Collected from
Aerides odorata Lour.	Khawbung, Mizoram
Bulbophyllum odoratissimum (Sm.) Lindl. ex Wall.	Ruallung, Mizoram
Coelogyne corymbosa Lindl.	Nogsder, Meghalaya
Dendrobium chrysanthum Wall. ex Lindl.	Reiek, Mizoram
Liparis odorata (Willd.) Lindl.	Cherrapunjee, Mizoram
Phaius tankervilleae (Banks) Blume	Mausmai, Meghalaya
Papilionanthe teres (Roxb.) Schltr.	Mausmai, Meghalaya
Rhynchostylis retusa (L.) Blume	Khawbung, Mizoram

Phytochemical Estimation

Fresh leaf samples of uniform sizes from each treatment were taken for the phytochemical analyses in three replicates.

Determination of total phenolic content (TPC)

The estimation of total phenolic content was performed by using the Folin–Ciocalteu method (Singleton and Rossi, 1965) and expressed in mg of gallic acid equivalents (GAE) per 100g of sample as:

$$\text{Total Phenol Content (mg GAE per g)} = \frac{\text{Conc.of GA from standard graph} \times \text{Vol.of extract} \times 100}{\text{Weight of sample}}$$

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by using the aluminium chloride colorimetric method (Chandra *et al.,* 2014). The total flavonoid content of the sample was calculated from a standard curve of quercetin (0.10-3.0 µg, r^2=0.999) and expressed in mg of quercetin equivalent (QE) per gram of extract weight (QE/g ext. wt) as:

$$\text{Flavonoid content (mg QE per g)} = \frac{\text{(Conc.of Q from Standard curve} \times \text{Volume of extract)}}{\text{(Weight of the sample)}}$$

Determination of Total Flavonol Content (TFC)

The total falvonol content was determined as per Miliauskas *et al.* (2004). The total flavonol content was calculated and expressed as:

$$\text{Total flavonol content (mg QE per g)} = \frac{\text{(Conc.of Q from standard curve} \times \text{Vol.of extract)}}{\text{(Weight of the sample)}}$$

Determination of Total Antioxidant Capacity Using FRAP Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was performed using the methodology described by Benzie and Strain (1999) with little modifications and expressed as mg $FeSO_4$ g^{-1}

Determination of Total Sugar

The total sugar content was determined as per Dubois *et al*. (1956) by Phenol-Sulfuric method.

Determination of Reducing Sugar

Reducing sugar was determined based on Miller (1959) by Dinitrosalicylic acid method and expressed as mgg^{-1}.

Determination of Non-Reducing Sugar

The amount of non-reducing sugar was calculated from the formula:

Non-reducing sugar (mg per g)=
Total sugars (mg per gram)- Reducing sugars (mg per gram)

Data Analysis

Data recorded were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test at a 5% level of significance using the SPSS Version 26.

Results and Discussion

The present study provided valuable insights into the phytochemical composition of medicinal orchids, highlighting their potential as a rich source of bioactive compounds for pharmaceutical and nutraceutical applications. The Total Phenol Content (TPC) of the medicinally important orchid species examined ranged from 15.70-83.16 mg GAEg^{-1} and revealed a notably elevated total phenol content (TPC), with a mean value of 43.70 mg GAE g^{-1} (Fig. 1), surpassing the average

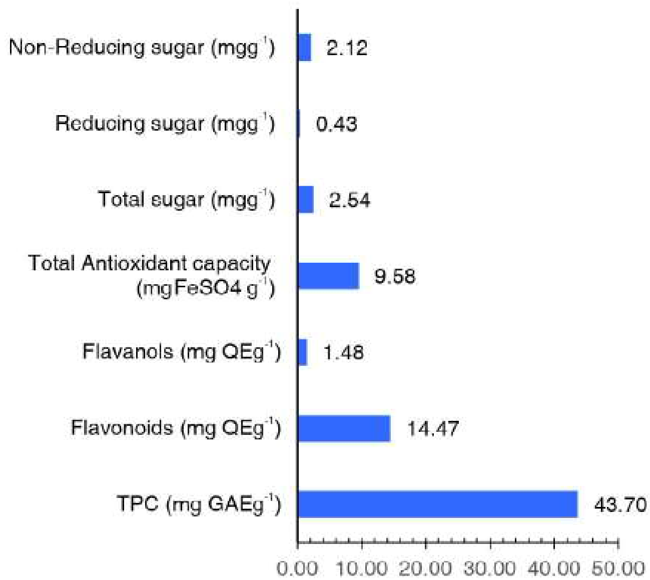

Fig. 1. Phytochemical contents in a few medicinally important orchid species in the present investigation.

TPC of 31.94 mg GAE g^{-1} reported by Hurkan *et al.* (2019) for a range of orchid species. Total phenol content is an important component of the orchids due to their antioxidant activity, and it plays an important role in plant defense against pathogens, insects, and environmental stresses, as well as in its potential medicinal uses. These also play an important role in stabilizing lipid peroxidation (Wei and Shiow, 2001). The present investigation indicates that these orchid species are promising candidates for further phytochemical and pharmacological analyses, with potential implications for drug discovery and development.

Orchids are known to contain a variety of flavonoids, which are a class of plant secondary metabolites with diverse biological activities. Flavonoids are plant secondary metabolites responsible for flower pigmentation, pollinator attraction, and fruit dispersal (Griesbach, 2005). These protect plants against biotic and abiotic stresses, including UV radiation (Takahashi and Ohnishi, 2004), and contribute to tolerance against frost, drought, and heat (Samanta *et al.,* 2011). The quantitative analysis of the study materials revealed an exceptionally high total flavonoid content, spanning a range of 6.86-29.54 mg QE g^{-1} with a mean value of 14.47 mg QE g^{-1}. Notably, the total flavanol content exhibited a comparable range of 0.09-2.35 mg QE g^{-1} with a mean value of 1.48 mg QE g^{-1}. Ferric Reducing Antioxidant Power (FRAP) is a reliable and widely employed assay for evaluating the total antioxidant capacity of plant extracts, including those of orchids. The FRAP method measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), thereby indicating the reducing potential of the sample. The total antioxidant capacity exhibited by the orchid species under investigation demonstrated a notable range of 3.25-16.59 mg FeSO4 g^{-1}, with a mean value of 9.58 mg $FeSO_4$ gl^{-1}. This variation in antioxidant capacity highlighted the diverse biochemical composition among the orchid species under study. A wide range of Fe^{2+}-chelating effect (3.44 mL^{-1}-24.40 mL^{-1}) of leaf extract of different orchid species was also reported by Nguyen *et al.* (2018). Total sugar content plays a crucial role in the physiological and ecological processes of orchids, contributing to their growth, development, and survival. A quantitative analysis of the sugar content in different orchid species was found to be 2.54 mg g^{-1} for total sugar content, 0.43 mg g^{-1} for reducing sugar content, and 2.12 mg g^{-1} for non-reducing sugar content. These findings contribute to our understanding of the carbohydrate composition in orchid species, with implications for their nutritional and physiological significance.

The different medicinal orchids studied (Table 1) showed significant variations in their phytochemical contents ($p < 0.05$) and Tukey's post-hoc test was used to compare the means of phytochemical traits amongst the treatments (Table 1). Results have shown a wide range of variation in phenolic content amongst the orchid species with *Liparis odorata* having significantly highest total phenol content (TBC) (83.16 mg GAE g^{-1}) which was at par with that of *Bulbophyllum odoratissimum* (71.13 mg GAE g^{-1}) and *Phaius tankervilleae* (63.65 mg GAE g^{-1}). The lowest TPC was, however, observed in *Aerides odorata* (15.70 mg GAE g^{-1}) and *Coelogyne corymbosa* (16.51 mg GAE g^{-1}). In traditional Indian medicine, the whole plant paste of *Bulbophyllum* *odoratissimum* is used in treatment of tuberculosis, chronic inflammation, and fractured bones (Gutierrez, 2010; Tsering *et al.,* 2017). These results corroborated the findings of other researchers (Chand *et al.,* 2016; Chimsook, 2016; Hurkan *et al.,* 2019; Natta *et al.,* 2022), in a variety of orchid species. In terms of their total flavonoid content (mg QE g^{-1}), the results showed significant variations among the treatments ($p < 0.05$). The highest TFC was recorded in *Bulbophyllum odoratissimum* (29.54 mg QE g^{-1}), followed by *Papilionanthe teres* (18.52 mg QE g^{-1}), and *Liparis odorata* (16.02 mg QE g^{-1}). The lowest TFC was observed in *Rhynchostylis retusa* (6.86 mg QE g^{-1}). The highest flavanol content was recorded in *Bulbophyllum odoratissimum* (2.35 mg QE g^{-1}), followed by *Phaius tankervilleae* (2.20 mg QE g^{-1}), and *Papilionanthe teres* (2.12 mg QE g^{-1}). In contrast, the lowest flavanol content was observed in *Dendrobium chrysanthum* (0.09 mg QE g^{-1}). *Bulbophyllum odoratissimum* has been found to possess a diverse array of bioactive compounds, including phenolic constituents such as syringaldehyde, syringin, and bulbophythrins, as well as flavonoid compounds comprising Batatasin III, Coelonin, Densiflorol B, Gigantol, Moscatin, 7-hydroxy-2,3,4-trimethoxy-9,10-dihydrophenanthrene, and tristin, as has also earlier been reported by Singh (2022).

The highest antioxidant capacity was recorded in *Liparis odorata* (16.59 mg $FeSO_4$ g^{-1}), followed by *Dendrobium chrysanthum* (14.79 mg $FeSO_4$ g^{-1}), and *Coelogyne corymbosa* (10.94 mg $FeSO_4$ g^{-1}). Conversely, *Aerides odorata* showed the lowest total antioxidant capacity (3.25 mg $FeSO_4$ g^{-1}). The highest total sugar content was recorded in *Bulbophyllum odoratissimum* (3.569 mgg^{-1}), followed by *Papilionanthe teres* (3.101 mgg^{-1}), and *Aerides odorata* (3.069 mgg^{-1}). While the lowest content was observed in *Liparis odorata* (0.447 mg g^{-1}). The highest reducing sugar content was recorded in *Coelogyne corymbosa* (0.563 mgg^{-1}), followed by *Papilionanthe teres* (0.493 mgg^{-1}), and *Bulbophyllum odoratissimum* (0.433 mgg^{-1}). The lowest sugar content was observed in *Aerides odorata* (0.363 mgg^{-1}). The non-reducing sugars (primarily sucrose) contribute more to total sugar content than reducing sugars (glucose and fructose), indicating that sucrose is the main sugar component in orchid leaves. The highest non-reducing sugar content was recorded in *Bulbophyllum odoratissimum* (3.136 mgg^{-1}), followed by *Papilionanthe teres* (2.608 mgg^{-1}), and *Aerides odorata* (2.707 mgg^{-1}); the lowest content was observed in *Liparis odorata* (0.085 mgg^{-1}) (Table 2).

Some earlier studies have elucidated the pivotal role of light intensity in modulating sucrose production in orchid leaves. A study on effect of light intensity on *Phalaenopsis* sp. has demonstrated that exposure to low-intensity light (0.6 $molm^{-2}$ per day) for a period of three wks resulted in a significant reduction of sucrose content and delay in flower spike emergence. Conversely, high-intensity light (4.3 $molm^{-2}$ per day) was associated with elevated sucrose levels and an earlier onset of flower emergence (Kataoka *et al.,* 2004). The present data highlighted the diverse biochemical results amongst the studied orchid species, with potential applications in various fields such as medicine, cosmetics, and the food industry. These variations in biochemical content may be attributed to genetic and environmental factors, and further research is needed to elucidate the underlying mechanisms.

A correlation analysis using Pearson's correlation coefficient (r) revealed a high significant positive

Table 2. Phytochemical contents of presently studied endemic and medicinally important orchids of NorthEastern India.

Species	TPC (mg $GAEg^{-1}$)	Flavonoids (mg QEg^{-1})	Flavanols (mg QEg^{-1})	Total Antioxidant capacity (mg $FeSO_4g^{-1}$)	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non-Reducing sugar (mgg^{-1})
Aerides odorata Lour.	15.7^{d}	8.98^{de}	1.48^{bc}	3.25^{d}	3.069^{a}	0.363^{b}	2.707^{ab}
Bulbophyllum odoratissimum (Sm.) Lindl. ex Wall.	71.13^{ab}	29.54^{a}	2.35^{a}	11.57^{b}	3.569^{a}	0.433^{ab}	3.136^{a}
Coelogyne corymbosa Lindl.	16.51^{d}	13.38^{bcd}	1.80^{ab}	10.94^{b}	3.270^{a}	0.563^{a}	2.707^{ab}
Dendrobium chrysanthum Wall. ex Lindl.	29.68^{d}	11.06^{cde}	0.09^{e}	14.79^{a}	1.620^{b}	0.393^{ab}	1.228^{c}
Liparis odorata (Willd.) Lindl.	83.16^{a}	16.02^{bc}	1.04^{cd}	16.59^{a}	0.447^{c}	0.363^{b}	0.085^{d}
Phaius tankervilleae (Banks) Blume	63.65^{b}	11.38^{cde}	2.20^{a}	4.58^{d}	2.488^{ab}	0.383^{ab}	2.106^{bc}
Papilionanthe teres (Roxb.) Schltr.	48.70^{c}	18.52^{b}	2.12^{ab}	8.67^{bc}	3.101^{a}	0.493^{ab}	2.608^{ab}
Rhynchostylis retusa (L.) Blume	21.07^{d}	6.86^{e}	0.77^{d}	6.23^{cd}	2.787^{a}	0.423^{ab}	2.364^{ab}
Range	15.70-83.16	6.86-29.54	0.09-2.35	3.25-16.59	0.45-3.57	0.363-0.563	0.085-3.136

Means within a column with different letters significantly differ by Tukey's Post Hoc test at $p < 0.05$.

Table 3. Pearson correlation among phytochemicals of presently studied endemic and medicinally important orchids of NorthEastern India.

Phytochemicals	TPC (mg GAE/ 100g)	Flavonoids (mg QEg^{-1})	Flavanols (mg QEg^{-1})	Total antioxidant capacity (mg $FeSO_4$ g^{-1})	Total sugar (mgg^{-1})	Reducing sugar (mgg^{-1})	Non-reducing sugar (mgg^{-1})
TPC (mg GAE/100g^{-1})	1	0.573	0.265	0.472	-0.509	-0.353	-0.503
Flavonoids (mg QE g^{-1})		1	0.571	0.393	0.237	0.213	0.231
Flavanols (mg QE g^{-1})			1	-0.374	0.606	0.357	0.603
Total Antioxidant Capacity (mg $FeSO_4$ g^{-1})				1	-0.605	0.064	-0.631
Total sugar (mgg^{-1})					1	0.544	0.998**
Reducing sugar (mgg^{-1})						1	0.494
Non-Reducing sugar (mgg^{-1})							1

correlation between total sugars and reducing sugars (Table 3, $r = 0.998$, $p < 0.01$). The strong positive correlation suggests that reducing sugars are a significant component of total sugars in the orchid species. The correlation also implied that factors influencing total sugar content, such as photosynthesis, nutrient availability, and environmental conditions, may also impact reducing sugar content. This highlights the importance of considering both total and reducing sugar content when evaluating the biochemical properties of orchid species. The analysis also revealed a lack of statistically significant relationships between various pairs of parameters, including total phenol content with total sugar content, flavanols with total antioxidant capacity, total antioxidant capacity with total sugar content, and non-reducing sugar content. This suggests that these parameters may not be directly related or may be influenced by other factors, masking potential relationships, therefore the present findings have important implications for understanding the complex interactions between phenolic compounds, sugars, and antioxidant capacity in different orchid species.

Conclusion

Orchids have gained popularity not only for their stunning flower colours and diverse forms but also for their medicinal value. It is clear from the study that beyond their ornamental appeal, they also possess a wealth of bioactive compounds with therapeutic potential, making them a valuable resource for the pharmaceutical industry. This dual significance highlights the importance of orchids in both horticulture and medicine, presenting opportunities for interdisciplinary research and conservation efforts. The study revealed significant variations in biochemical parameters; *Bulbophyllum odoratissimum* demonstrated significantly higher concentrations of various phytochemicals as compared to the other species. The present data obtained in eight important endemic and medicinally important species of NorthEastern Himalayas presents a promising opportunity for scientific exploration and exploitation, with potential applications in phytochemistry, pharmacology, agriculture, and conservation.

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