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Antioxidant capacity of *Coelogyne pandurata* extracts at different phenological phases[1]

Capacidade antioxidante dos extratos de *Coelogyne pandurata* em diferentes fases fenológicas

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HIGHLIGHTS:

Ethanol extracts of preflowering bulbs had the highest phenolic and flavonoid contents.
Ethanol extracts of preflowering bulbs had the highest antioxidant activity.
Ethanol showed superior extraction efficiency for Coelogyne pandurata *phenolics and flavonoids.*

ABSTRACT: *Coelogyne pandurata* L., an orchid native to Indonesia, has medicinal properties and potential for lowland cultivation. This study assessed the phenolic and flavonoid contents and antioxidant activity of the leaves, bulbs, and flowers in the vegetative and generative phases of *C. pandurata*. The total phenolic content (TPC) and total flavonoid content (TFC) were measured using colorimetric methods. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing antioxidant power (FRAP) assays. Plant parts (leaves, bulbs, and flowers) were analyzed across two phenological phases (vegetative and generative) with three replicates for each combination. Each experimental unit comprised five biological replicates. The extracts were prepared via ultrasonic extraction using methanol, ethanol, and n-hexane. The range of TPC was 2.51–11.44 mg gallic acid equivalent per g dry weight (DW), that of TFC was 6.05–38.16 mg quercetin equivalents per g DW, that of the DPPH antioxidant activity was 0.21–1.41 µmol Trolox equivalent (TE) per g DW, and that of the FRAP capacity was 15.63–80.70 µmol TE g^{-1} DW. Ethanol extracts, particularly from preflowering bulbs, exhibited the highest TPC, TFC, and antioxidant activity, underscoring its superior extraction efficiency for *C. pandurata*.

Key words: antioxidant capacity, growth phase, phenolic, flavonoid, solvent

RESUMO: *Coelogyne pandurata* L., uma orquídea nativa da Indonésia, possui propriedades medicinais e potencial para cultivo em áreas de baixa altitude. Este estudo avaliou os teores de fenólicos e flavonoides, bem como a atividade antioxidante das folhas, bulbos e flores nas fases vegetativa e generativa de *C. pandurata*. O teor total de fenólicos (TPC) e o teor total de flavonoides (TFC) foram medidos usando métodos colorimétricos. A atividade antioxidante foi avaliada utilizando ensaios de sequestro de radicais 2,2-difenil-1-picrilhidrazila (DPPH) e de poder antioxidante redutor de ferro (FRAP). As partes da planta (folhas, bulbos e flores) foram analisadas em duas fases fenológicas (vegetativa e generativa) com três réplicas para cada combinação. Cada unidade experimental foi composta por cinco réplicas biológicas. Os extratos foram preparados via extração ultrassônica utilizando metanol, etanol e n-hexano. A faixa de TPC variou de 2,51–11,44 mg de equivalente de ácido gálico por g de peso seco (DW), a de TFC foi de 6,05–38,16 mg de equivalentes de quercetina por g DW, a atividade antioxidante pelo método DPPH foi de 0,21–1,41 µmol de equivalente Trolox (TE) por g DW, e a capacidade FRAP variou de 15,63–80,70 µmol TE g^{-1} DW. Os extratos etanólicos, particularmente de bulbos antes da floração, exibiram os maiores valores de TPC, TFC e atividade antioxidante, destacando sua superior eficiência de extração para *C. pandurata*.

Palavras-chave: capacidade antioxidante, fase de crescimento, fenólico, flavonóide, solvente

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Introduction

Coelogyne pandurata L. orchid is a plant endemic to Kalimantan Island, Indonesia. As epiphytes are rich in biodiversity, orchids have considerable tolerance to hot temperatures in lowlands (Hartati & Muliawati, 2020). Orchid advancement relies on their market value, phenolic and flavonoid content, and antioxidant capacity to address shortages in medicinal and cosmetic raw materials (Thitikornpong et al., 2022). Since the onset of the global COVID-19 pandemic, the use of plant-derived natural materials has been crucial. In particular, natural compounds that enhance immunity boost human health benefits (Brahmi et al., 2023). Consequently, the COVID-19 pandemic has highlighted the need for plant polyphenols and antioxidants. In particular, plant-based supplements offer the advantages of the use of natural nutrients, safety, and ecofriendliness. They provide essential vitamins, minerals, antioxidants, and phytonutrients, which are more easily absorbed than synthetic compounds. These supplements support the immune function, reduce inflammation, and promote organ health. Moreover, they contain bioactive compounds that regulate blood sugar and cholesterol levels.

Orchids, with their potential phenolic, flavonoid, and antioxidant contents, require further research for practical applications (Arora et al., 2023). The phenolic, flavonoid, and antioxidant capacity of *Bulbophyllum* orchids have been reported (Bhinija et al., 2022). Kumar et al. (2022) obtained the phytochemical content of *Cymbidium aloifolium* (L.). Bhattacharyya et al. (2022) noted the polyphenol content of *Malaxis acuminata* orchids. Natta et al. (2022) investigated the phenolic and flavonoid content and antioxidant capacity of *Dendrobium nobile*, *Dendrobium moschatum, Dendrobium densiflorum, Acampe papillosa, Coelogyne nitida*, and *Arundina graminifolia*. However, the evaluation of phenolics, flavonoids, and antioxidants extracted using hexane, methanol, and ethanol solvents from *C. pandurata* plants is yet to be reported.

This study used polar solvents (ethanol and methanol) and nonpolar n-hexane to assess the polarity of the phenolic and flavonoid compounds in *C. pandurata*, marking a pioneering effort in the research field. Previous research on various orchids (salep orchids, *Anacamptis* species, *Neotinea tridentata*, and *Ophrys* species) has identified chloroform as the optimal solvent for analyzing phenolic, flavonoid, and antioxidant contents (Hürkan et al., 2019). Several studies have highlighted the effectiveness of solvents, such as butanol, for the antioxidant activity of *Eria tomentosa* (Akter et al., 2020). Sanjaya et al. (2024) found that 100% acetone was the best solvent for determining the total flavonoid content (TFC) and antioxidant activity of *Phalaenopsis* orchid leaves. Moreover, this study aimed to assess the phenolic and flavonoid content and antioxidant activity of the leaves, bulbs, and flowers of *C. pandurata* during two physiological phases. The differences in the yield and qualitative parameters were hypothesized between the bulbs at different phases, which was consistent with the findings of Zhang et al. (2023), who demonstrated the varying metabolic activities and metabolite production in bulbs at different ages.

Material and Methods

The bulbs, leaves, and flowers of *C. pandurata* were collected from the Orchid House of Leuwikopo Experimental Station of IPB University, Ciampea, Bogor Regency, West Java, Indonesia, at the coordinates of 6° 33' 50.3" S 106° 43' 29.3" E and altitude of 188 m. Leaf, bulb, and flower samples were collected before the flowering phase between September and November 2022 and after the flowering stage between November and December 2022.

This study aims to assess the phenolic and flavonoid content and antioxidant activity in different parts (leaves, bulbs, and flowers) of *C. pandurata* L. during two distinct physiological phases (vegetative and generative). The experiment consisted of three replicates for each plant part and phenological phase combination. Samples were collected from five individual plants, and pooled samples were used for extraction.

Ultrasonication was used to extract the phenolic and flavonoid compounds using three solvents, namely methanol, ethanol, and n-hexane. The total phenolic content (TPC) and TFC were quantified by colorimetric methods. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric-reducing antioxidant power (FRAP) assays.

This study focused on determining the polarity of the phenolic and flavonoid compounds in *C. pandurata* using polar (ethanol and methanol) and non-polar (n-hexane) solvents for extraction. The chemical composition and antioxidant potential of *C. pandurata* were initially investigated.

Figure 1A and B show the sampled plant in its preflowering and postflowering stage, respectively. Figure 1C and 1D show leaf samples before and after flowering, respectively. Figure 1E and 1F display the bulb sample before and after flowering, respectively. Figure 1G shows the flower sample. Figures 1H–L illustrate the ground plant organs to obtain their powder form.

Dried samples of orchid leaves, bulbs, and flowers were ground into a powder using a blender. Subsequently, the powder of each sample was divided into three parts of 4 g each. Three powder samples from each plant part were extracted using methanol, ethanol, or n-hexane. Samples from different phases were sequentially extracted using a microwave extraction device for 3 min at middle to low levels. After cooling to room temperature, the extracts were filtered through

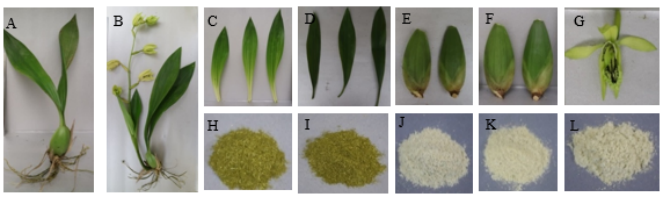

Figure 1. Plant before (A) and after (B) flowering, leaves before (C) and after (D) flowering, bulbs before (E) and after (F) flowering, flower (G), leaf powder before (H) and after (I) flowering, bulb powder before (J) and after (K) flowering, and flower powder (E)

a filter paper (Whatman no. 1, Whatman Limited, Maidstone, UK), dried at room temperature, and stored after labeling.

TPC was determined using a previously reported method (Khumaida et al., 2019) with a modified extraction time. In this study, TPC was determined using a nanospectrophotometer (Spectro^{starNano} BMG Labtech) based on the Folin–Ciocalteu method. A total of 20 µL (0.2 g mL^{-1}) sample extract of *C. pandurata* plant parts was placed into a 96-well microplate (Biologix Europe GmbH), added with 120 µL Folin–Ciocalteu (10%, v/v), homogenized, and incubated for 5 min in the dark. Subsequently, 80 µL Na_2CO_3 (10%, w/v) solution was added and incubated again for 30 min in the dark at room temperature. The absorbance of the sample extracts from each plant part was measured at a wavelength of 750 nm using a nanospectrophotometer (Spectrostar^{Nano} BMG Labtech). TPC was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE g^{-1} DW).

TFC was determined based on a previously described method (Calvindi et al., 2020) using a nanospectrophotometer (Spectro^{starNano} BMG Labtech) based on the colorimetric method with an aluminum chloride ($AlCl_3$) reagent. A total of 10 µL sample (0.2 g mL^{-1} concentration) of leaf, bulb, and flower extract samples was placed in a 96-well microplate (Biologix Europe GmbH), added with 50 µL ethanol pro analysis, 10 µL $AlCl_3$ 10% (w v^{-1}), 10 µL glacial acetic acid (CH_3COOH), and 120 µL distilled water. Samples and reagents were homogenized and incubated for 30 min in the dark at room temperature. The absorbance of the sample extracts from each part of the black orchid plants was measured at a wavelength of 415 nm using a nanospectrophotometer (Spectro^{starNano} BMG Labtech). TFC was expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE g^{-1} DW).

The antioxidant activity was analyzed using the DPPH method based on a previous study (Nurcholis et al., 2016) with a modified extraction time. A total of 100 µL (0.2 g mL^{-1}) sample extract from each part of the black orchid plant and 100 µL of 125 µM DPPH reagent (in ethanol pro analysis) were added into a 96-well microplate (Biologix Europe GmbH), homogenized, and incubated for 30 min in a dark room at room temperature. The absorbance of the sample extract from each part of the orchid plant was measured at 515 nm using a nanospectrophotometer (Spectro^{starNano} BMG Labtech). The final unit was expressed in µmol Trolox equivalent per g dry weight (µmol TE g^{-1} DW).

Antioxidant activity analysis using the FRAP method was developed based on a previous study (Nurcholis et al., 2022) with a modified extraction time. FRAP reagent was prepared by mixing acetate buffer (pH 3.6), 10 µM tripyridyl-s-triazine (in 40 mM HCl), 20 mM $FeCl_3$ in 10:1:1 (volume basis) ratio, and stored in a bottle in the dark. For the FRAP assay, 20 µL sample extract was placed in 96 well-microplates and 290 µL FRAP solution was added and incubated for 30 min in the dark. The absorbance was measured at 593 nm using a microplate reader (Spectrostar Nano, BMG Labtech). The antioxidant capacity was expressed as µmol TE g^{-1} DW.

The values are reported as the mean ± standard error mean (SEM) for three replicates. The ExpDes package in R was used to perform ANOVA, followed by the Tukey test at $p \leq 0.05$. Correlation analysis was performed using the Corrplot mixed package.

Results and Discussion

The TPC in the ethanol, methanol, and n-hexane extracts of leaves, bulbs, and flowers of *C. pandurata* before and after the flowering phase was obtained. The leaves before entering the flowering stage contained the highest TPC of 11.44 ± 0.87 mg GAE (gallic acid equivalent) g^{-1} DW (Table 1). The leaves before flowering exhibited the highest TPC in the n-hexane extract (11.44 mg GAE g^{-1} DW), followed by ethanol (10.20 mg GAE g^{-1} DW) and methanol (9.06 mg GAE g^{-1} DW) extract. After flowering, leaves achieved the highest TPC in the ethanol extract (8.03 mg GAE g^{-1} DW), followed by the methanol (7.68 mg GAE g^{-1} DW) and n-hexane (3.05 mg GAE g^{-1} DW) extracts. The bulbs before flowering exhibited the highest TPC in the ethanol extract (9.90 mg GAE g^{-1} DW), followed by methanol (7.27 mg GAE g^{-1} DW) and n-hexane (2.66 mg GAE g^{-1} DW) extracts. After flowering, the bulbs showed the highest TPC in the methanol extract (5.70 mg GAE g^{-1} DW), followed by the ethanol (3.51 mg GAE g^{-1} DW) and n-hexane (2.51 mg GAE g^{-1} DW) extracts. The flowers exhibited the highest TPC in the methanol extract (7.96 mg GAE g^{-1} DW), followed by the ethanol (5.00 mg GAE g^{-1} DW) and n-hexane (4.81 mg GAE g^{-1} DW) extracts.

During the flowering phase, plants undergo significant metabolic changes. Before flowering, leaves contain higher levels of phenolics for protective and growth functions owing to the increased defense against pathogens and herbivores. After flowering, the resources shift to flower and fruit production, thereby reducing leaf phenolics. Preflowering leaves exhibit a higher phenolic content in n-hexane extracts owing to the accumulation of lipophilic phenolic compounds that help protect leaves from environmental stress. Preflowering bulbs store phenolics that are used during flowering to support flower structure formation. The decreased phenolic content in bulbs after flowering indicates the use of these reserves. Meanwhile,

Table 1. Total phenolic content in different parts and growth phases in ethanol, methanol, and n-hexane extracts of *C. pandurata*

Plant parts	Solvents	Total phenolic content (mg GAE g^{-1} DW)
Leaf before flowering	Ethanol extract	10.20 ± 0.16 ab
	Methanol extract	9.06 ± 0.29 bc
	n-hexane extract	11.44 ± 0.87 a
Leaf after flowering	Ethanol extract	8.03 ± 0.28 cd
	Methanol extract	7.68 ± 0.48 cd
	n-hexane extract	3.05 ± 0.59 g
Bulb before flowering	Ethanol extract	9.90 ± 0.80 b
	Methanol extract	7.27 ± 0.38 d
	n-hexane extract	2.66 ± 0.21 g
Bulb after flowering	Ethanol extract	3.51 ± 0.29 fg
	Methanol extract	5.70 ± 0.70 e
	n-hexane extract	2.51 ± 0.01 g
Flower	Ethanol extract	5.00 ± 0.28 e
	Methanol extract	7.96 ± 0.64 cd
	n-hexane extract	4.81 ± 0.01 ef

GAE - Gallic acid equivalent; DW - Dry weight. Each value is presented as mean ± standard error mean; The numbers followed by different lowercase letters indicate significant differences obtained by Tukey test ($p \leq 0.05$)

the high phenolic content in the methanol extracts of the flowers is associated with the role of phenolic compounds in coloration, aroma, ultraviolet protection, and defense against pathogens. Phenolic compounds such as flavonoids play a role in attracting pollinators and protecting plant reproductive parts (Longchar & Deb, 2021).

In ethanol extract, the highest TPC is obtained in the leaves before flowering (10.20 mg GAE g^{-1} DW), whereas the lowest TPC is noted in the bulbs after flowering (3.51 mg GAE g^{-1} DW). For the methanolic extract, the highest and lowest TPC is noted in the flowers (7.96 mg GAE g^{-1} DW) and leaves after flowering (7.68 mg GAE g^{-1} DW), respectively. The n-hexane extract demonstrated the highest TPC in the leaves before flowering (11.44 mg GAE g^{-1} DW) and lowest TPC in the bulbs after flowering (2.51 mg GAE g^{-1} DW).

The differences in the effectiveness of the solvents for extracting phenolics from various plant parts influence these outcomes. As polar solvents, ethanol and methanol are more effective in extracting hydrophilic phenolics, whereas n-hexane, as a nonpolar solvent, is better at extracting lipophilic phenolics. Consequently, the preflowering leaves in n-hexane extracts have a high TPC, which may contain lipophilic phenolics for protection (Minh et al., 2017). Several significant differences were identified, namely the higher TPC of preflowering leaves in n-hexane extracts than post-flowering leaves, higher TPC in preflowering bulbs in ethanol extracts than post-flowering bulbs, and highest TPC in flowers in methanol extracts compared to all other parts.

Medicinal orchids can be used as raw materials for manufacturing drugs and cosmetics owing to various factors that induce the production of secondary metabolites, such as phenolic compounds, which are useful for humans. In this study, the extracts obtained from preflowering leaves have a high TPC because the biosynthetic pathway of phenolic compounds involves carbohydrate compounds that undergo glycolysis. High carbohydrate formation rate is noted in the preflowering leaves because of their high photosynthetic activity. In particular, photosynthesis forms primary metabolites in the leaves before flowering, which undergo glycolysis to form phenolic compounds via the cyclic or malonic acid pathways. The TPC values obtained in this study are lower than those in ethanol extracts of *Phalaenopsis* spp., which was 11.52 ± 0.43 mg GAE g^{-1} DW (Minh et al., 2016). This different is ascribed to the morphological, physiological, and genetic differences among various orchid types. The lowest TPC of 2.51 ± 0.01 mg GAE g^{-1} DW was noted in the bulb after flowering because most carbohydrates do not undergo glycolysis into phenolic compounds but are utilized for energy and plant metabolic needs.

TFC was evaluated using a colorimetric assay with quercetin as the standard flavonoid compound. Among the orchid plant parts, the highest TFC of 38.16 ± 0.86 mg QE g^{-1} DW is noted in *C. pandurata* leaves before flowering, as shown in Table 2. The highest TFC is observed in n-hexane extract (38.16 ± 0.86 mg QE g^{-1} DW), followed by ethanol (31.49 ± 0.14 mg QE g^{-1} DW) and methanol (15.83 ± 0.43 mg QE g^{-1} DW) extracts. This indicates that n-hexane is the most effective solvent for extracting flavonoids from leaves before flowering. After flowering, the TFC of the leaves in n-hexane extract remains high (35.06 ± 0.33 mg QE g^{-1} DW), which is significantly higher than in ethanol (16.02 ± 0.07 mg QE g^{-1} DW) and methanol (16.33 ± 0.47 mg QE g^{-1} DW) extracts. Similar to that observed before flowering, n-hexane is the most effective solvent for the plant leaves. For the bulbs before flowering, the highest TFC is obtained ethanol extract (37.59 ± 0.18 mg QE g^{-1} DW), followed by methanol (25.04 ± 0.48 mg QE g^{-1} DW) and n-hexane (10.47 ± 0.28 mg QE g^{-1} DW) extracts, suggesting that ethanol is the most effective solvent for extracting flavonoids from bulbs before flowering. After flowering, the TFC of the bulbs is the highest in methanol extract (16.16 ± 0.68 mg QE g^{-1} DW), followed by n-hexane (13.09 ± 0.56 mg QE g^{-1} DW) and ethanol (6.05 ± 0.66 mg QE g^{-1} DW) extracts. The effectiveness of methanol was higher than that of n-hexane and ethanol. For the flowers, the highest TFC is obtained in the methanol extract (27.25 ± 0.16 mg QE g^{-1} DW), which is significantly higher than that in n-hexane (13.83 ± 0.10 mg QE g^{-1} DW) and ethanol (9.12 ± 0.81 mg QE g^{-1} DW). Thus, methanol is the most effective solvent for the extracting flavonoids from flowers.

The solvent analysis reveals that the ethanol extract exhibits the highest TFC in bulbs before flowering (37.59 ± 0.18 mg QE g^{-1} DW), which decreased after flowering (6.05 ± 0.66 mg QE g^{-1} DW), denoting its decreased effectiveness in extracting flavonoid. Similarly, ethanol has moderate effectiveness for leaves before flowering and low effectiveness for flowers. The methanol extract is the most effective solvent for the flowers (27.25 ± 0.16 mg QE g^{-1} DW) and exhibits high effectiveness for bulbs before flowering (25.04 ± 0.48 mg QE g^{-1} DW). However, it maintains consistent but lower effectiveness for leaves before and after flowering compared to n-hexane. The n-hexane extract is the most effective solvent for the leaves before (38.16 ± 0.86 mg QE g^{-1} DW) and after (35.06 ± 0.33 mg QE g^{-1} DW) flowering. However, it is least effective for bulbs before flowering (10.47 ± 0.28 mg QE g^{-1} DW) and has moderate effectiveness for flowers (13.83 ± 0.10 mg QE g^{-1} DW). These findings indicate that n-hexane is the most effective solvent for extracting flavonoids from leaves both before and after flowering, ethanol is the most effective solvent for the

Table 2. Total flavonoid content in different parts and phases of *C. pandurata* in ethanol, methanol, and n-hexane extracts

Plant parts	Solvents	Total flavonoid content (mg QE g^{-1} DW)
Leaf before flowering	Ethanol extract	31.49 ± 0.14 c
	Methanol extract	15.83 ± 0.43 f
	n-hexane extract	38.16 ± 0.86 a
Leaf after flowering	Ethanol extract	16.02 ± 0.07 f
	Methanol extract	16.33 ± 0.47 f
	n-hexane extract	35.06 ± 0,33 b
Bulb before flowering	Ethanol extract	37.59 ± 0.18 a
	Methanol extract	25.04 ± 0.48 e
	n-hexane extract	10.47 ± 0.28 h
Bulb after flowering	Ethanol extract	6.05 ± 0.66 i
	Methanol extract	16.16 ± 0.68 f
	n-hexane extract	13.09 ± 0.56 g
Flower	Ethanol extract	9.12 ± 0.81 h
	Methanol extract	27.25 ± 0.16 d
	n-hexane extract	13.83 ± 0.10 g

QE - Quercetin equivalents; DW - Dry weight. Each value represents the mean ± standard error mean; numbers followed by different lowercase letters indicate significant differences obtained by Tukey's test ($p \leq 0.05$)

bulbs before flowering, whereas methanol is more effective for the bulbs after flowering, and methanol is the most effective solvent for the flowers.

The effectiveness of solvents in extracting flavonoids from different plant parts can be influenced by the chemical properties of the flavonoids, cellular composition of the plant, and interactions between the solvent and plant matrix. Flavonoids exhibit a range of chemical structures, ranging from highly polar to nonpolar, affecting their interactions with solvents. In leaves, n-hexane is the most effective nonpolar solvent before and after flowering because nonpolar flavonoids dominate the leaves and are closely associated with lipid structures in the cell membrane. These flavonoids dissolve in n-hexane, which solubilizes the lipid components and increases the extraction efficiency (Chaves et al., 2020). In bulbs, ethanol is the most effective solvent before flowering because of its polarity, which can extract polar flavonoids that are likely to be more prevalent in bulbs with high water content. After flowering, the chemical composition of the bulbs changes, making methanol, which has a higher polarity than ethanol, more effective in extracting more polarized or different flavonoids from bulbs after this phase change. In flowers, methanol is the most effective solvent because of its excellent ability to dissolve polar compounds, such as flavonoids. Flowers contain flavonoid compounds that dissolve well in methanol, enabling a more efficient extraction. The success of each solvent in extracting flavonoids from specific plant parts depends on the compatibility between the properties of the solvent and chemical properties of the flavonoids, which vary across different plant parts. In particular, different solvents can produce varying profiles of phytochemical constituents, thereby influencing the antioxidant activity and overall bioactivity of plant extracts (Sen et al., 2020).

The formation of flavonoid compounds in orchid plants is influenced by genetic, environmental, and endogenous factors, such as growth and developmental phases. The transition from the vegetative to the generative phase necessitates changes in plant metabolism, which affect the formation of flavonoid compounds (Minh et al., 2016). In this study, the extract obtained from the leaves before flowering has a high TFC owing to the breakdown of primary metabolites that form phenolic compounds, which later form flavonoid compounds. Increased metabolic photosynthetic activity further increases the availability of raw carbohydrates to form flavonoid compounds. The highest TFC is obtained in the ethanol and n-hexane solvent extracts of leaves, which contradicts previously reported findings (Chand et al., 2016), whereby TFCs of 11.89 ± 0.64 and 41.77 ± 2.99 mg QE g^{-1} DW were noted in *Gastrochilus acutifolius* orchid leaves and *Luisis trichinize* orchids, respectively. Such discrepancies is ascribed to the differences in the orchid species, which are expected to have different metabolic responses. In this study, the lowest TFC of 6.05 ± 0.66 mg QE g^{-1} DW was obtained in the bulbs after flowering because the available carbohydrates in the bulb are preferably used to meet the metabolic needs during flowering (Minh et al., 2017).

We analyzed the antioxidant capacities of different plant parts and solvents, focusing on the DPPH radical scavenging capacity and FRAP antioxidant capacity. The data revealed significant variations based on the plant part and the solvent used. The antioxidant activity data of *C. pandurata* plant part extracts obtained using DPPH and FRAP assays are shown in Table 3. Higher antioxidant potential is noted in *C. pandurata* extracts using the FRAP method than using the DPPH method. These results indicate the dominant antioxidant properties with a greater reduction capacity than the free-radical capture activity of *C. pandurata* extracts. Antioxidant analysis using the FRAP method obtained the highest antioxidant activity in the bulb ethanol extracts before flowering (80.70 ± 0.70 µmol TE g^{-1} DW). In contrast, using the DPPH method, the highest antioxidant activity was observed in the bulb extracts before flowering (1.41 ± 0.05 µmol TE g^{-1} DW, ethanol extract) and after flowering (1.12 ± 0.007 µmol TE g^{-1} DW, methanol extract) and flowers (0.94 ± 0.26 µmol TE g^{-1} DW, methanol extract).

The leaves before flowering in ethanol extract exhibited a DPPH radical scavenging capacity of 0.11 ± 0.04 µmol TE g^{-1} DW and FRAP antioxidant capacity of 35.65 ± 0.90 µmol TE g^{-1} DW. In comparison, the methanol extract had lower capacities (0.05 ± 0.003 µmol TE g^{-1} DW for DPPH and 29.28

Table 3. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) antioxidant scavenging capacity of ethanol, methanol, and n-hexane extracts of different parts and flowering phases of *C. pandurata*

Plant parts	Solvents	DPPH radical scavenging capacity (µmol TE g^{-1} DW)	FRAP antioxidant capacity (µmol TE g^{-1} DW)
Leaf before flowering	Ethanol extract	0.11 ± 0.04 hi	35.65 ± 0.90 f
	Methanol extract	0.05 ± 0.003 i	29.28 ± 0.92 g
	n-hexane extract	0.03 ± 0.01 i	29.21 ± 0.29 g
Leaf after flowering	Ethanol extract	0.60 ± 0.15 ef	58.63 ± 0.23 b
	Methanol extract	0.61 ± 0.004 f	29.74 ± 0.62 g
	n-hexane extract	0.82 ± 0.02 cd	15.63 ± 0.35 j
Bulb before flowering	Ethanol extract	1.41 ± 0.05 a	80.70 ± 0.70 a
	Methanol extract	0.44 ± 0.004 ef	52.65 ± 0.19 c
	n-hexane extract	0.35 ± 0.04 fgh	24.03 ± 0.55 i
Bulb after flowering	Ethanol extract	0.21 ± 0.01 ghi	25.82 ± 0.67 h
	Methanol extract	1.12 ± 0.007 b	37.36 ± 0.36 e
	n-hexane extract	0.96 ± 0.04 bc	16.57 ± 0.45 j
Flower	Ethanol extract	0.14 ± 0.003 hi	25.74 ± 1.12 h
	Methanol extract	0.94 ± 0.26 bc	45.66 ± 0.29 d
	n-hexane extract	0.87 ± 0.01 bc	36.81 ± 0.48 ef

TE - Trolox equivalent; DW - Dry weight. Each value represents the mean ± standard error mean. The numbers followed by different lowercase letters indicate significant differences in the same column, as obtained by Tukey's test ($p \leq 0.05$).

± 0.92 µmol TE g^{-1} DW for FRAP). The n-hexane extract showed the lowest DPPH capacity (0.03 ± 0.01 µmol TE g^{-1} DW), whereas the FRAP capacity (29.21 ± 0.29 µmol TE g^{-1} DW) is maintained. After flowering, the antioxidant capacities of the leaves in ethanol extract significantly increased, showing a DPPH capacity of 0.60 ± 0.15 µmol TE g^{-1} DW and FRAP capacity of 58.63 ± 0.23 µmol TE g^{-1} DW. Meanwhile, the methanol extract of the leaves maintained its moderate DPPH capacity (0.61 ± 0.004 µmol TE g^{-1} DW) but achieved a lower FRAP capacity (29.74 ± 0.62 µmol TE g^{-1} DW). Notably, the n-hexane extract had a higher DPPH capacity (0.82 ± 0.02 µmol TE g^{-1} DW) and a lower FRAP capacity (15.63 ± 0.35 µmol TE g^{-1} DW).

In the bulbs before flowering, the ethanol extract exhibits the highest antioxidant capacities among all samples, with a DPPH capacity of 1.41 ± 0.05 µmol TE g^{-1} DW and FRAP capacity of 80.70 ± 0.70 µmol TE g^{-1} DW. Meanwhile, the methanol extract had a DPPH capacity of 0.44 ± 0.004 µmol TE g^{-1} DW and FRAP capacity of 52.65 ± 0.19 µmol TE g^{-1} DW. The n-hexane extract achieves moderate capacities with 0.35 ± 0.04 µmol TE g^{-1} DW for DPPH and 24.03 ± 0.55 µmol TE g^{-1} DW for FRAP. After flowering, the antioxidant capacities of the bulbs ethanol extract significantly decreased to 0.21 ± 0.01 µmol TE g^{-1} DW for DPPH and 25.82 ± 0.67 µmol TE g^{-1} DW for FRAP. However, the methanol extract exhibits a high DPPH capacity of 1.12 ± 0.007 µmol TE g^{-1} DW and moderate FRAP capacity of 37.36 ± 0.36 µmol TE g^{-1} DW. The n-hexane extract maintains a high DPPH capacity (0.96 ± 0.04 µmol TE g^{-1} DW) but a low FRAP capacity (16.57 ± 0.45 µmol TE g^{-1} DW).

In the flowers, the ethanol extract demonstrates low antioxidant capacities with 0.14 ± 0.003 and 25.74 ± 1.12 µmol TE g^{-1} DW for DPPH and FRAP, respectively. The methanol extract shows higher capacities, with 0.94 ± 0.26 and 45.66 ± 0.29 µmol TE g^{-1} DW for DPPH and FRAP, respectively. The n-hexane extract of flowers displays moderate antioxidant capacities of 0.87 ± 0.01 and 36.81 ± 0.48 µmol TE g^{-1} DW for DPPH and FRAP, respectively.

The antioxidant capacity of plant extracts is highly dependent on the presence of various bioactive compounds, particularly phenolics, flavonoids, and secondary metabolites. These compounds scavenge free radicals and mitigate oxidative stress, which is critical for preventing cellular damage and chronic diseases. The significant variations in the antioxidant capacities among different plant parts and solvents used in this study can be attributed to the differential distribution and solubility of these bioactive compounds.

The leaves before and after flowering demonstrated notable differences in their antioxidant capacities. Before flowering, the lower antioxidant capacity of the leaves is likely ascribed to the limited accumulation of phenolic compounds and flavonoids, which typically increase as plants mature. In contrast, after flowering, the antioxidant activity of the leaves substantially increases, particularly with the ethanol extracts. This increase may be linked to the enhanced production of antioxidants that protect the reproductive tissues and support the higher metabolic demands associated with flowering. Bulbs have the highest antioxidant capacities before flowering, especially in the ethanol extracts, because of the high concentration of storage compounds, including phenolics and flavonoids, which accumulate during the early growth stages. After flowering, the antioxidant capacity of the bulbs decreases, which can be ascribed to the mobilization of these bioactive compounds to support flower and seed development. The flowers exhibit moderate antioxidant activity, particularly in methanol extracts, suggesting their significant amounts of soluble phenolics and flavonoids, which are effectively extracted by methanol.

Solvent choice plays a crucial role in the efficiency of antioxidant extraction. Ethanol has been proven to be the most effective solvent for all plant parts because of its intermediate polarity, facilitating the dissolution of a broad range of phenolic compounds. Moreover, methanol has substantial efficacy in extracting phenolic and flavonoid compounds owing to its polarity. In contrast, n-hexane, which is a nonpolar solvent, was less effective for phenolic extraction, but showed some efficacy in extracting nonpolar antioxidant compounds, such as certain flavonoids and terpenoids. These findings are consistent with those in the existing literature. For instance, Dai and Mumper (2010) reported the higher phenolic content and antioxidant capacity in mature leaves than in young leaves, confirming our observation of increased antioxidant activity in leaves after flowering. Sultana et al. (2009) demonstrated ethanol and methanol as highly effective solvents for extracting phenolic compounds from various plant parts, corroborating our results of higher antioxidant capacities with these solvents (Cosme et al., 2020).

The antioxidant activities of medicinal orchids are used to select useful plant species and their parts. The antioxidant activity of plants is influenced by various endogenous and exogenous factors, such as plant metabolism, growth phase, and environmental stress (Natta et al., 2022). The antioxidant content of these plants can be extracted from the plant parts and consumed by humans, either in tablet or solution forms.

The highest antioxidant activity was found in the bulb extracts before flowering using the FRAP method. This result differs from that of a previous study (Bhattacharyya & Van Staden, 2016), in which the highest FRAP method-based antioxidant activity was exhibited by the leaves. The differences observed can be ascribed to the differences in the orchid species. Furthermore, the antioxidant activity analysis using the DPPH method revealed that the methanol extracts of preflowering bulbs exhibited the highest antioxidant activity. These results differ from those of a previous study (Minh et al., 2017), which noted the highest antioxidant content of 1.663 ± 0.083 mg mL^{-1} for IC_{50} in the root organ using the DPPH method with a hexane solvent. This difference is attributed to the differences in the order of the orchids analyzed.

A simple linear correlation analysis between TPC, TFC, and antioxidant capacities was conducted using Pearson's coefficient to identify the possible phenolic and flavonoid compounds contributing to the antioxidant activity of the ethanolic, methanolic, and n-hexane extracts of *C. pandurata* orchids (Figure 2). This correlation coefficient analysis noted a significant positive correlation between the TPC and FRAP antioxidant activities of the ethanol extract (r = 0.68), and

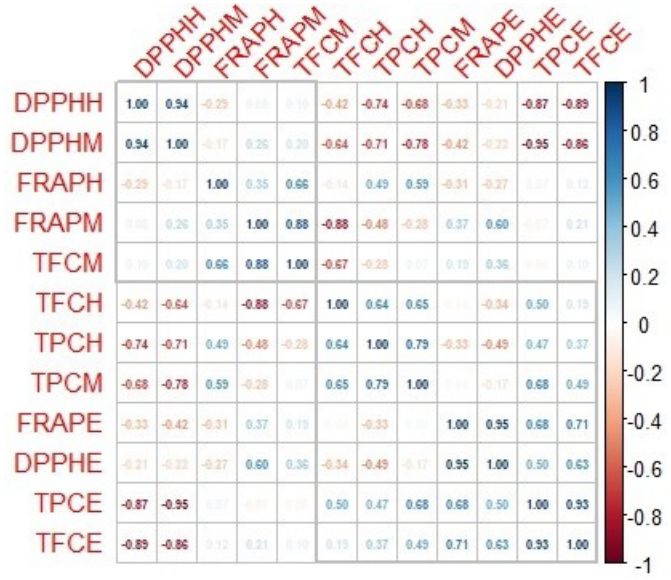

Figure 2. Simple linear correlation of TPC using ethanol (TPCE), methanol (TPCM), and n-hexane (TPCH); TFC using ethanol (TFCE), methanol (TFCM), and n-hexane (TFCH); DPPH results using ethanol (DPPHE), methanol (DPPHM), and n-hexane (DPPHH); and FRAP results using ethanol (FRAPE), methanol (FRAPM), and n-hexane (FRAPH)

n-hexane extract (r = 0.49). In contrast, a negative correlation is noted between the TPC and FRAP antioxidant activities of the methanol extract (r = −0.21). The Pearson correlation coefficient analysis showed a significant positive correlation between the TFC and FRAP antioxidant activity of the ethanol extract (r = 0.71) and methanol extract (r = 0.88), whereas a negative correlation was noted between TFC and FRAP antioxidant activities of the n-hexane extract (r = −0.14).

The high TPCs in leaves and bulbs before flowering are ascribed to the vegetative phase of plants that experience increased photosynthetic rates, resulting in a high net assimilation rate. Carbohydrates produce glucose, a primary metabolite that undergoes glycolysis to form phosphoenolpyruvate and erythrose-4-phosphate via glycolytic and pentose phosphate pathways. These compounds combine to form 3-deoxy-D-arabinoheptulosonate-7-phosphate, which is then converted to shikimic acid through a series of enzymatic reactions. Subsequently, shikimic acid is converted into phenylalanine, which undergoes deamination by phenylalanine ammonia-lyase to produce transcinnamic acid. Trans-cinnamic acid is hydroxylated and methylated to form phenolic acids, such as p-coumaric acid, which then enter the flavonoid biosynthesis pathway, whereby p-coumaric acid is converted into naringenin through the action of enzymes chalcone synthase and chalcone isomerase. Consequently, naringenin is transformed into various flavonoids, such as flavonols, flavones, and anthocyanins, through enzymatic reactions involving enzymes, including flavanone 3-hydroxylase, flavonoid 3'-hydroxylase, and dihydroflavonol 4-reductase, facilitating the production of important flavonoid compounds in plants, which are essential for defense, pigmentation, and other biological activities (Gantait et al., 2021).

The high production of primary metabolites during the vegetative phase stimulates increased production of phenolic compounds and flavonoids in the leaves and bulbs, which are closely related to the antioxidant content in plants. These compounds are recognized for their strong antioxidant properties, which play a crucial role in protecting plant cells from damage caused by free radicals, which are unstable molecules that can damage cells by stealing electrons from other molecules in the body, thereby leading to oxidative stress. Phenolic compounds and flavonoids act as antioxidants because of their chemical structures, which can donate electrons or hydrogen atoms to free radicals, thereby halting chain reactions that harm cells. Flavonoids such as flavonols, flavones, and anthocyanins, possess phenolic groups that can scavenge and neutralize free radicals. Additionally, phenolic compounds, such as caffeic, p-coumaric, and ferulic acids, are known for their significant antioxidant activities (Cazar et al., 2023).

The Pearson's correlation coefficient analysis noted a significant positive correlation between the TPC and DPPH antioxidant activity in the ethanol extracts (r = 0.50). In contrast, the TPC and DPPH antioxidant activity were negatively correlated for the n-hexane (r = −0.71) and methanol (r = −0.78) extracts. A significant positive correlation was noted between the TFC and DPPH antioxidant activity of the ethanol extract (r = 0.63) and methanol extract (r = 0.20), whereas a negative correlation was noted between the TFC and DPPH antioxidant activity of the n-hexane extract (r = −0.42, Figure 2). Therefore, this study highlight significant correlations between the TPC, TFC, and antioxidant activities, as measured by DPPH and FRAP assays using different solvents (ethanol, methanol, and n-hexane) (Figure 2).

In ethanol extraction, a positive correlation between TPC and DPPH antioxidant activity was observed, indicating a higher TPC was correlated with increased antioxidant potential. Ethanol, not only extracted flavonoids, but also other compounds with antioxidant activity. Similar trends were observed for methanol, where TPC showed a significant positive association with DPPH activity. Conversely, n-hexane exhibited a weaker correlation, which can be attributed to its limited ability to extract polar phenolic compounds. Significant positive correlations were observed between the TFC and DPPH antioxidant activity of ethanol extracts, underscoring the efficacy of ethanol in extracting flavonoids that contribute to the antioxidant capacity. Similarly, methanol showed a strong positive correlation between DPPH activity and TFC, whereas n-hexane showed a weak correlation, reflecting its inefficiency in extracting polar flavonoids.

TPC extracted with ethanol showed a positive relationship with antioxidant activity measured by the FRAP assay, indicating that phenolic compounds extracted with ethanol enhanced antioxidant activity. Comparable results were observed with methanol, whereas n-hexane exhibited a lower correlation, suggesting the less effective extraction of phenolic compounds. Similarly, the TFC extracted with ethanol exhibited a significant positive correlation with the antioxidant activity in the FRAP assay. Methanol also showed a strong positive correlation, whereas n-hexane showed a

weaker correlation, which is consistent with its lower flavonoid extraction efficiency.

These results emphasize the effectiveness of polar solvents, such as ethanol and methanol, in extracting phenolic and flavonoid compounds that contribute substantially to antioxidant activity. These insights are critical for optimizing extraction protocols, particularly in studies focusing on the antioxidant properties of different plant components.

The relationship between antioxidant activity, TPC, and TFC suggests the role of these compounds in the antioxidant activity of the plant. Thus, plant compounds can be selectively targeted for specific antioxidant activity. The positive correlation among antioxidant activity, TPC, and TFC supports the selection of plants and compounds for utilization (Rahim et al., 2022). The correlation of TPC and FRAP antioxidants and are consistent with that of the TFC and FRAP antioxidants, as reported by a previous study (Bhattacharyya et al., 2015), whereby a consistent significant positive correlation (r = 0.18) was noted. Moreover, a significant positive correlation (r = 0.74) was observed between the TPC and FRAP antioxidants in the methanol extracts. This variation can be ascribed to the differences in the plant types and parts used. These results align with those of Longchar and Deb (2021), whereby a negative correlation between TPC, TFC, and FRAP was obtained.

Conclusions

1. Ethanol, methanol, and n-hexane extracts of *C. pandurata* contained phenolics and flavonoids and exhibited antioxidant activities.

2. The correlation of total phenolic content (TPC) and total flavonoid content (TFC) with ferric-reducing antioxidant power (FRAP) power in ethanol extract was higher correlation than that in the methanol and hexane extracts.

3. The ethanol extract had stronger reducing power and antioxidant activity than the methanol and hexane extracts.

4. Before flowering, the bulb exhibited higher TPC and TFC and stronger antioxidant capacity compared to the other plant parts analyzed.

Authors' contributions: Pebra Heriansyah performed the experiments and collected the data. Pebra Heriansyah, Sandra Arifin Aziz, Dewi Sukma, and Waras Nurcholis performed the data analysis, prepared the first version of the manuscript, conducted a literature review, and corrected the manuscript.

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