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Assessment of Antioxidant, Anti-Lipid Peroxidation, Antiglycation, Anti-Inflammatory and Anti-Tyrosinase Properties of *Dendrobium sulcatum* Lindl

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Abstract: *Dendrobium sulcatum* Lindl or "Ueang Jampa-Nan" (Orchidaceae family) is widely distributed in Thailand and Laos. It is classified in the genus *Dendrobium*, which is used in both traditional Chinese medicine and Ayurvedic medicine for health enhancement and anti-aging. The purpose of this study was to investigate the phytochemical constituents and bioefficacy of stems, leaves and flowers from *D. sulcatum* for cosmetic and cosmeceutical applications. Phenolic and flavonoid contents were tested for the phytochemical evaluation. The antioxidant (DPPH, FRAP and ABTS assays), anti-lipid peroxidation, antiglycation, anti-inflammatory and anti-tyrosinase properties were assessed for their bioefficacy. The results showed that the extracts of stem and leaf had higher total phenolic content than that of the flower, and the leaf extract had the highest flavonoid content. The antioxidant, anti-lipid peroxidation and anti-inflammatory activities of the extracts were greater in those from the stem and leaf compared with that of the flower. The leaf extract exhibited the greatest antiglycation property. The results of anti-tyrosinase analysis of the extracts showed that the leaf and flower exhibited potent activities with a percentage inhibition greater than 70% (at a concentration of 50 μg/mL). In conclusion, these findings suggest that the ethanolic extracts from different parts of *D. sulcatum* are promising sources of natural active ingredients for further cosmetic and cosmeceutical products.

Keywords: antiglycation; anti-inflammation; anti-lipid peroxidation; antioxidation; *Dendrobium sulcatum*

Citation: Rungsang, T.; Srivilai, J.; Rakasawapokin, P.; Rakasawapokin, P.; Mungmai, L.; Saesue, K.; Aoonboontum, P.; Plukham, N.; Siriwipanan, P.; Chaichanathawikit, P.; et al. Assessment of Antioxidant, Anti-Lipid Peroxidation, Antiglycation, Anti-Inflammatory and Anti-Tyrosinase Properties of *Dendrobium sulcatum* Lindl. *Cosmetics* **2023**, *10*, 43. https://doi.org/10.3390/cosmetics10020043

Academic Editor: Adeyemi Oladapo Aremu

Received: 3 February 2023
Revised: 21 February 2023
Accepted: 27 February 2023
Published: 1 March 2023

1. Introduction

Orchidaceae are large flowering plants among the angiosperms. This plant family is important in the decoration industry, and is popularly used as a cosmetic and herbal ingredient due to its potential biological activity. Thailand is one of the largest orchid growing areas in the world [1]. *Dendrobium sulcatum* Lindl (see Figure 1), commonly known as "Ueang Jampa-Nan" in Thailand or "Ju Cao Shi Hu" in China, is widely distributed in Southeast Asia, including in Thailand, Laos and Myanmar. This plant is small to medium in size and reaches 25–40 cm high. The stem has a flat shape (2–3 cm width) with a dark green and a longitudinal groove. The leaves have an oval-lanceolate shape (4–5 cm width and 6–8 cm length) and are green, smooth and shiny on the top. The flowers have a long bouquet shape (9–12 cm length) with 10–15 flowers, and they have a yellow color [2]. In Thailand, this plant is found mainly in the northern part, including in the Chiang Mai and

Nan provinces. *Dendrobium* species are the second largest genus in the Orchidaceae family which have been used in Chinese folk medicine as a tonic, astringent and anti-inflammatory, and in Ayurvedic medicine [3,4]. Recently, cosmetic and cosmeceutical applications of the Orchidaceae family and some *Dendrobium* species such as *D. sonia* and *D. officinale* have been reported to be potentially active in lowering melanin production, antioxidation, anti-aging with MMP2 inhibitory effect and tyrosinase inhibition [5–7]. In addition, various pharmacological properties of *Dendrobium* species have been reported, including anti-hypertension properties, promotion of digestion, reduction of hyperglycemia, immunomodulation, antifatigue properties [8] and antiviral properties against SARS-CoV-2 [9]. It is also used in the treatment of skin diseases and nervous system disorders [10]. The major chemical constituents of *Dendrobium* species are polysaccharides [3], alkaloids [11], aromatic compounds [12], sesquiterpenoids [13], coumarins [14], fluorenones [15], phenanthrenes [16], bibenzyls [17] and flavonoids [18,19]. However, the biological properties of *D. sulcatum* in relation to cosmeceutical applications and its traditional use have not been reported yet. Therefore, the present study aimed to investigate and elucidate the possibility of using *D. sulcatum* for cosmetic and cosmeceutical applications. The phytochemical constituents of *D. sulcatum* extracts were determined. Subsequently, the antioxidant, anti-lipid peroxidation, antiglycation, anti-inflammatory and anti-tyrosinase activities of *D. sulcatum* extracts were also investigated.

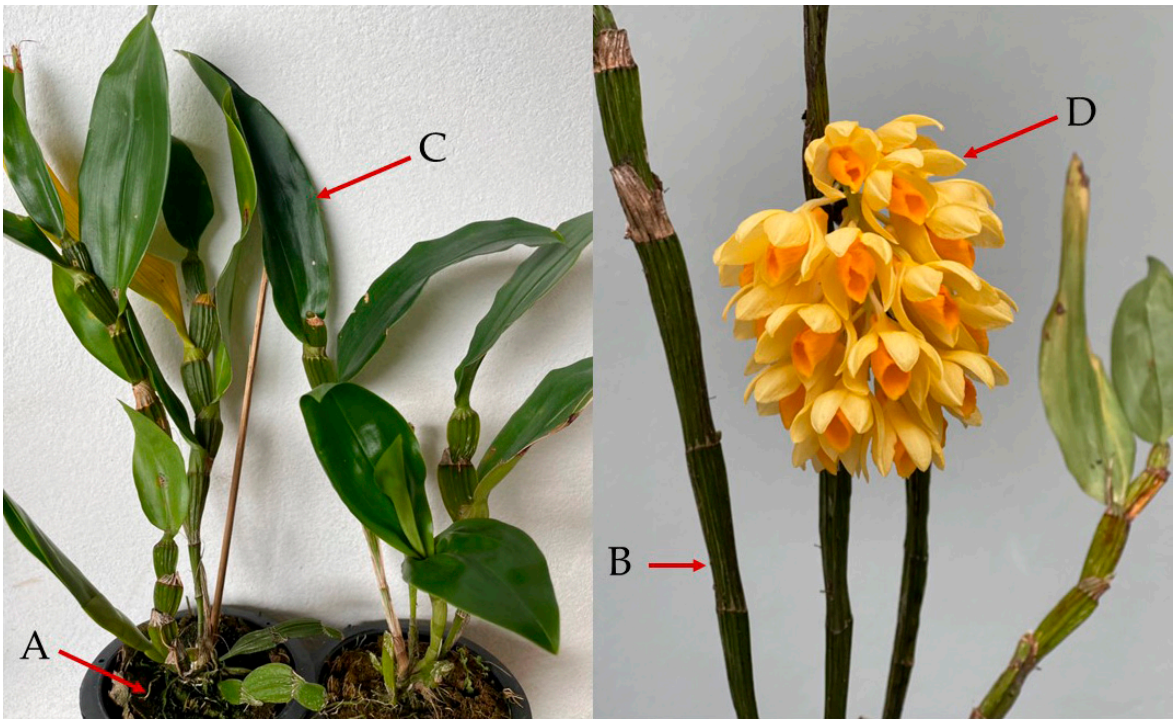

Figure 1. The different parts of *D. sulcatum*: (A) root, (B) stem, (C) leaf and (D) flower.

2. Materials and Methods

2.1. Chemical and Reagents

Analytical grade methanol and ethanol were obtained from RCI Labscan (Bangkok, Thailand). DPPH, phosphoric acid, LPS, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), dimethyl sulfoxide (DMSO), macrophage cells (RAW264.7), ferrous sulfate ($FeSO_4$), ferric chloride ($FeCl_3$), trichloroacetic acid, thiobarbituric acid, Fe_2SO_4, ABTS, BSA, trichloroacetic acid, thiobarbituric acid, mushroom tyrosinase and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), 0.25% trypsin-EDTA, penicillin (10,000 units/mL) and streptomycin (10,000 µg/mL) (P/S) were obtained from GIBCO (Grand Island, NE, USA) and fetal bovine serum (FBS) was

purchased from GIBCO (Paisley, UK). Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) was purchased from LOBA Chemie (Mumbai, India) and sodium nitrite ($NaNO_2$) from Ajax Finechem (Taren Point, Australia). *D*-frustose was purchased from KEMAUS (NSW, Australia), and sulphanilamide and N-(1-Naphthyl) ethylenediamine dihydrochoride (NEDD) from AppliChem GmbH (Darmstadt, Germany). Aluminum chloride (hexahydrate) and *L*-DOPA were obtained from LOBA CHEMIE (Mumbai, India).

The standard compounds including Trolox, gallic acid, quercetin, ascorbic acid and aminoguanidine HCl (AMG) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and L-NG-monomethyl arginine citrate (L-NMMA) was from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Plant Collection and Extraction

The whole plant of *D. sulcatum* was collected from Muang, Nan Province, Thailand. The voucher specimen number Wongwad.EAF001 has been deposited in the herbarium of the Queen Sirikit Botanic Garden (QSBG), Chiang Mai, Thailand and complies with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Three different parts of *D. sulcatum*, namely the stems, leaves and flowers were separated, cleaned and dried in a hot-air oven at 60 °C for 2 days. Each part of the plant was then individually ground to a fine powder using a blender. Then, 5 g of the plant powder from each part was extracted with ethanol (100 mL) and sonicated (100 MHz) for 30 min, then filtered. The filtrated solution was evaporated under reduced pressure to obtain a semi-solid crude ethanolic extract of *D. sulcatum* stems, leaves and flowers (0.51, 5.16 and 4.6 % yield, respectively). The crude extracts were kept at −20 °C awaiting further investigations.

2.3. Determination of Total Phenolic Contents

The total phenolic contents of *D. sulcatum* ethanolic extracts of stems, leaves and flowers was determined according to the previous report with some modifications [20]. 1 mg of each sample was weighed and diluted with 1 mL of methanol to obtain a concentration of 1 mg/mL of the extracts. Then, 20 µL of each sample solution was mixed with 100 µL of the 10% Folin-Ciocalteu reagent in 96-well microplates. Subsequently, 80 µL of 10% Na_2CO_3 was added and the samples were incubated for 30 min at room temperature. Absorbance was measured at 765 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). Gallic acid was used to construct the standard curve at the working concentration of 10.0–180.0 µg/mL. The content of phenolic compounds in the extract samples was expressed as gallic acid equivalent (GAE) in mg per gram of extract sample. All samples were performed in triplicates.

2.4. Determination of Total Flavonoid Contents

The total flavonoid contents in three different parts of *D. sulcatum* extracts were investigated according to the previously reported method [21]. Briefly, 100 µL of each sample extract was mixed with 100 µL of 2% aluminum chloride (dissolved in 0.2 M ammonium acetate) in 96-well plates. The mixed solutions were then incubated at room temperature for 60 min and the absorbance was measured at 420 nm using a microplate reader. Quercetin at a working concentration of 1.25–100.0 µg/mL was used as the standard compound to generate the standard calibration curve. Flavonoid content in extract samples was expressed as mg quercetin equivalent (QE)/g extract.

2.5. Antioxidant Activities

The three different parts of *D. sulcatum*, namely stem, leaf and flower extracts were investigated for antioxidant properties using DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP) and ABTS radical scavenging assays.

2.5.1. Sample Preparation for Antioxidant Activities

For the DPPH assay, the stock solution of Trolox standard and sample extracts were separately prepared at concentrations of 1 and 30 mg/mL, respectively, using ethanol as solvent. These stock solutions were serially diluted with ethanol to different concentrations of 0.01–1.0 mg/mL for the Trolox standard solution and 3.0–30.0 mg/mL for the sample extract solution.

For the FRAP and ABTS assays, the stock solution of Trolox standard and sample extracts were separately prepared at 1 mg/mL using ethanol as solvent. Subsequently, each stock concentration of the sample extract and Trolox was diluted with ethanol to obtain the concentrations of 0.5 mg/mL and 0.05 mg/mL, respectively.

2.5.2. DPPH Radical Scavenging Assay

The abilities of the three different parts of *D. sulcatum* extracts in free radical scavenging were tested using a DPPH radical scavenging assay. The DPPH method from a previous report was followed [22]. A solution of 0.2 mM of DPPH was prepared in methanol, and 150 µL of this solution was then mixed with 75 µL of diluted sample solution at different concentrations. The mixed solution was then kept in the dark at room temperature for 30 min. The absorbance of the tested solution was measured at 515 nm using a microplate reader. Trolox was used as the positive control. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample containing all reagents including the test compound.

The IC_{50} value, or the effective concentration of the extract required to scavenge 50% of DPPH radicals, was calculated using the GraphPad Prism 9 program. The dose–response curve was plotted between the percentage DPPH radical scavenging and the various concentrations of sample extracts.

2.5.3. FRAP Assay

The ferrous reducing antioxidant power (FRAP) of sample extracts was investigated according to the method described earlier [22,23]. Briefly, 20 µL of the extract solutions was mixed with 180 µL of FRAP reagent (300 mM acetate buffer pH 3.6: 10 mM TPTZ in 40 mM HCl: 20 mM $FeCl_3$ in ratio of 10:1:1) in 96-well plates. The mixture solution was kept in dark conditions at room temperature for 30 min and the absorbance was then measured at 595 nm using a microplate reader. Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) in a working concentration of 0.01–1.0 mM was used as a standard to construct a calibration curve. The Trolox was used as the positive control and the results were expressed in mmol Fe^{2+}/g extract.

2.5.4. ABTS Radical Scavenging Assay

The scavenging activity of the sample extracts on an ABTS radical cation was performed according to the previous method with some modifications [24,25]. Briefly, ABTS was dissolved in water to a concentration of 7 mM and the solution was added to 2.45 mM potassium persulfate (a final concentration) in a ratio of 1:1 to produce an ABTS radical cation ($ABTS^{\bullet+}$). The mixture solution was kept in the dark at room temperature for 12 h and diluted with ethanol to obtain an absorbance of 0.70 ± 0.20 (250 µL) at 734 nm before further investigation.

For the ABTS assay, 250 µL of the diluted ABTS solution was added to 10 µL of the sample extract solution at the working concentration of 1 mg/mL. Six minutes after initial mixing, the test solution was measured at 734 nm, and the temperature was controlled at 30 °C during the test. The Trolox was used for creating the standard calibration curve of the working concentration in the range of 3.0–1000.0 µM and the results were expressed as mg of Trolox equivalent (TE)/g extract.

2.6. Anti-Lipid Peroxidation of the Extracts Using TBARS Assay

D. sulcatum extracts were evaluated for their lipid peroxidation inhibitory activity using a TBARS assay, which was performed following a previous method with some modifications [26,27]. In this assay, 20 µL of each sample extract (2 mg/mL in ethanol) and 140 µL of homogenized pig brain (1 g) were mixed in a microcentrifuge tube (size 1.5 mL) and incubated at 37 °C for 30 min. After incubation, 20 µL of 2 mM ascorbic acid and 4 mM Fe_2SO_4 were added to the mixed solution and incubated for 60 min at 37 °C. Subsequently, 200 µL of TBARS reagent (40% trichloroacetic acid, 1.4% thiobarbituric acid and 8% HCl) was added to the mixture solution and incubated at 90 °C for 60 min. After incubation, the mixture solution was left at room temperature and centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was then collected and measured at 530 nm using a microplate reader. Trolox (100 µg/mL) was used as the positive control and the lipid peroxidation inhibitory activity was calculated in comparison with the control. The absorbance of the control was in the range of 0.7–0.8.

2.7. Antiglycation of D. sulcatum Extracts Using a BSA-Fructose Assay

An antiglycation assay using the non-enzymatic reaction between fructose and BSA was used to investigate the anti-aging activity of the *D. sulcatum* extracts. The advanced glycation end-products (AGEs) were determined using a BSA-fructose assay with some modifications from previous studies [22,28]. To perform this assay, 1 mg of each *D. sulcatum* extract was dissolved in 1 mL of DMSO to produce the stock solution of 1 mg/mL and 80 µL of this solution was mixed with 80 µL of 30 mg/mL BSA in PBS in a black 96-well plate. The mixture solution was then added to 80 µL of 300 mM *D*-fructose in PBS, covered with aluminum foil and incubated in the dark for 60 h at 37 ± 2 °C. After that, the formation of AGEs was measured for intrinsic fluorescence using a microplate reader at excitation and emission wavelengths of 340 and 435 nm, respectively. The AMG was used as the positive control and the percentage of the inhibition for glycation was calculated following the equation below:

$$\text{Inhibition (\%)} = [(\Delta A_{\text{control}} - \Delta A_{\text{sample}})/\Delta A_{\text{control}}] \times 100$$

where $\Delta A_{\text{control}}$ is the difference between the absorbance value of the control (containing all test reagents except the test sample) and the blank control (containing all reagents the same as the control except the fructose). ΔA_{sample} is a difference in the absorbance value of the sample (containing all test reagents and the sample) and the blank of the sample (containing all reagents including the sample except the fructose).

2.8. Anti-Inflammatory Activity of D. sulcatum Extracts

The anti-inflammatory activity of the different parts of *D. sulcatum* extracts was evaluated by the inhibition of NO secreted from LPS-induced macrophage cells. The method was performed following the methods from previous studies with some modifications [22,29,30].

2.8.1. Macrophage Cell Culture

Macrophage cell lines (RAW 264.7) were seeded and cultured in a 75 cm^2 cell culture flask containing supplemented DMEM medium (10% FBS and 1% P/S solution). The cell culture flask was incubated in a cell culture incubator at 37 °C under a humidified atmosphere of 95% air and 5% CO_2 [31] until the cells were 80–90% confluent; they remained under these conditions while awaiting further investigation.

2.8.2. MTT Macrophage Cell Viability Assay

The effect of *D. sulcatum* extracts on macrophage cell viability was investigated using the mitochondrial-dependent reduction of MTT to formazan following the method described in previous reports [22,32]. Briefly, macrophage cells were seeded at a density of 5.0×10^4 cells/well in 96-well plates and incubated in a cell culture incubator at 37 °C

under a humidified atmosphere of 95% air and 5% CO_2 for 24 h until the cells were attached to the well plate. Cells were then treated with 100 µL solution of different concentrations of each *D. sulcatum* extract, namely those from stems, leaves and flowers, using an FBS-free DMEM medium as the control. After an incubation period of 20 h, 20 µL of MTT solution (3 mg/mL in PBS buffer) was added to the cells and incubated for 4 h at 37 °C and 5% CO_2. The medium was removed from the 96-well plates and the formazan precipitate was then dissolved in 200 µL of 50% ethanol in DMSO. The absorbance of the solution was measured at 595 nm using a microplate reader and the percentage cell viability was calculated in comparison with the control group.

2.8.3. Induction of Macrophage Cells

Macrophage cells were induced by LPS to secrete the NO production following previous studies [29]. Briefly, the macrophage cells were seeded in 96-well plates at the density of 5.0×10^4 cells/well and cultured in 100 µL of the supplemented DMEM medium. The cell culture plate was incubated in a cell culture incubator at 37 °C under a humidified atmosphere of 95% air and 5% CO_2. After 24 h incubation, the medium was removed and replaced with 80 µL of FBS-free DMEM medium, and 10 µL of LPS solution (at the final concentration of 5 µg/mL) was then added, followed by 10 µL of each sample extract solution (at a final concentration of 10 µg/mL), which was immediately treated. Then, the LPS-induced macrophage cell plates were further incubated for 24 h and 50 µL of the supernatant in each differently treated sample was collected and used for further study in the NO assay.

2.8.4. NO Inhibition Assay

The NO inhibitory activity of ethanolic extract of *D. sulcatum* stems, leaves and flowers was investigated using the colorimetric Griess reaction method according to the previous report with some modifications [22]. Briefly, 50 µL of the supernatant collected from LPS-induced macrophage cells was mixed with 50 µL of 1% sulphanilamide in 5% phosphoric acid and left at room temperature for 5 min. After that, 50 µL of 0.1% NEDD in sterile water was added and the mixed solution was left for 5 min. The absorbance of the tested solution was detected at 510 nm using a microplate reader. $NaNO_2$ (at the working concentration in the range of 3.0–300.0 µM) was used for creating the standard calibration curve and *L*-NMMA (at the final concentration of 10 and 25 µg/mL) was used as the positive control.

2.9. Anti-Tyrosinase Activity of D. sulcatum Extracts

The anti-tyrosinase assay was performed following a previously described method with some modifications [33,34]. Briefly, 1 mg of each *D. sulcatum* extract was dissolved in 1 mL of 15% DMSO in 0.6M phosphate buffer pH 6.8 (P buffer) to obtain the stock concentration of 1 mg/mL solution. Then, 40 µL of the sample extract solution was mixed with 80 µL of P buffer and 40 µL of tyrosinase enzyme (200 U/mL) in 96-well plates. This mixed solution was then incubated at room temperature for 10 min and 40 µL of 2.5 mM *L*-DOPA in P buffer was added. After that, the test well plate was incubated again for 10 min at room temperature and the absorbance was measured at 492 nm using a microplate reader. Ascorbic acid (200 µg/mL) was used as a positive control.

2.10. Statistical Analysis

All performed experiments were undertaken in triplicate ($n = 3$) and the results were expressed as the average ± SD. The percentage inhibitory activity was plotted against log10 [concentration] and the half maximum inhibitory concentration (IC_{50}) was calculated using Graph-Pad Prism Software version 9 (San Diego, CA, USA). SPSS version 24 for Windows (SPSS, Chicago, IL, USA) was used for statistical analysis.

3. Results and Discussion

3.1. Total Phenolic and Total Flavonoid Contents

The flavonoid and phenolic compounds are known as plant secondary metabolites for antioxidation, anti-inflammation, etc., which might be related to the traditional use of this plant as a health tonic and anti-aging treatment. Therefore, the content of flavonoids and phenols was characterized in this study. Phenolic content was investigated using gallic acid equivalent. The linearity of the standard gallic acid calibration curve at working concentrations of 10.0–180.0 µg/mL was obtained with $R^2 = 0.9992$; $y = 0.0052x - 0.004$. Using this standard gallic acid calibration curve equation, the total phenolic contents obtained from the ethanolic extract of *D. sulcatum* from stems, leaves and flowers are shown in Table 1. The stem and leaf extracts had higher content of total phenolic contents (46.41 ± 2.22 and 43.85 ± 3.85 mg gallic acid equivalent (GAE)/g extract, respectively) compared with the flower extract (22.05 ± 2.22 mg GAE/g extract). These results indicated that all ethanolic extracts of *D. sulcatum* contain phenolic compounds, which is consistent with previous reports of phenolic constituents in other *Dendrobium* species. Some of the phenolic compounds, such as coelonin, erianthridin, moscatilin, lusianthridin, gigantol and batatasin III from *D. formosum* [35], and shaftoside, isoschaftoside, dihydroresveratro and tristin from *D. huoshanense*, *D. nobile*, *D. chrysotoxum* and *D. fimbriatum*, have been found previously [36].

Table 1. Total phenolic and flavonoid contents and antioxidant activities (DPPH, FRAP and ABTS assays) of ethanolic extract of *D. sulcatum* stems, leaves and flowers.

Sample	TPC (mg GAE/g Extract)	TFC (mg QE/g Extract)	DPPH (IC_{50}, µg/mL)	FRAP (mmol Fe^{2+}/g Extract)	ABTS (mg TE/g Extract)
Stem extract	46.41 ± 2.22 ^{b}	15.70 ± 1.11 ^{a}	353.43 ± 22.73 ^{c}	0.50 ± 0.04 ^{c}	65.76 ± 3.33 ^{b}
Leaf extract	43.85 ± 3.85 ^{b}	22.45 ± 2.17 ^{c}	252.17 ± 20.61 ^{b}	0.35 ± 0.05 ^{b}	116.99 ± 3.53 ^{c}
Flower extract	22.05 ± 2.22 ^{a}	16.03 ± 0.31 ^{b}	797.57 ± 12.16 ^{d}	0.12 ± 0.02 ^{a}	40.73 ± 2.68 ^{a}
Trolox	-	-	4.44 ± 0.18 ^{a}	10.40 ± 0.30 ^{d}	-

Each value represents the mean ± SD ($n = 3$); the different letters within the column are significantly different ($p < 0.05$) according to Tukey's HSD test.

Total flavonoid was characterized by quercetin equivalent (QE) using the aluminum chloride colorimetric method. For this purpose, the ethanolic extract of the stems, leaves and flowers of *D. sulcatum* was determined. The standard quercetin calibration curve was obtained ($y = 0.0202 + 0.2571$; $R^2 = 0.9996$). The results showed that the highest value of total flavonoid content was obtained from the leaf extract (22.45 ± 2.17 mg QE/g extract), followed by the flower and stem extracts with a value of 16.03 ± 0.31 mg QE/g extract and 15.70 ± 1.11 mg QE/g extract, respectively. The flavonoid constituents previously found in *Dendrobium* species were flavone C-glycosides and flavonols [4,37]. Flavonoid constituents in *Dendrobium* species, such as quercetin from *D. tosaense*, naringenin from *D. loddigesii* and vicenin 2 from *D. officinale*, have also been reported [38].

3.2. DPPH Radical Scavenging Activity

The primary screening of antioxidant activity with the hydrogen atom transfer mechanism of ethanolic extract of the *D. sulcatum* stems, leaves and flowers was investigated using a DPPH free radical scavenging assay. The results were expressed as amount required to scavenge 50% DPPH free radicals (IC_{50}) and are shown in Table 1. Among the test samples, the one exhibiting the highest antioxidant effect was the leaf extract as IC_{50} = 252.17 ± 20.61 µg/mL which was about 1.5 and 3 times higher than stem and flower extracts, respectively. These results suggest that the ethanolic extract of *D. sulcatum* leaves has greater potential for DPPH free radical scavenging than the other parts.

These results are consistent with previous reports. Natta et al. [39] reported that the IC_{50} value of antioxidant activity from the DPPH assay of the methanolic stem extract solutions of *D. nobile*, *D. moschatum* and *D. densiflorum* were 2034.1, 1047.2 and 357.9 µg/mL, re-

spectively, whereas the methanolic leaf extract solutions were 972.1, 526.3 and 625.8 μg/mL, respectively. The strongest DPPH scavenger was the ethanolic extract from the *D. crepidatum* stems (IC_{50} = 74.0 μg/mL) [40]. Some ethanolic extract from flowers of *Dendrobium* species, including *D. sonia*, *D. sonia pink*, *D. snow rabbit* and *D. shavin*, have also been reported for antioxidant activity; the IC_{50} values were >500, 492.8, >500 and 463.1 μg/mL [41]. This suggests that the extract of *D. sulcatum* leaves could be a good source of antioxidants.

The antioxidant properties of the extracts, especially those of the leaf, might come from the phenolic and flavonoid compounds (see Table 1). In a previous study, a strong antioxidant was reported which was derived from flavonoids such as rutin (quercetin-3-rhamnosyl glucoside), and narigin isolated from *D. officinale* also had antioxidant activity [42]. Some of the phenolic compounds obtained from *Dendrobium* species such as phenanthrenes, bibenzyls, coumarins and alkaloids might be the sources of corresponding antioxidant activity.

3.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is known as the antioxidant capacity assay and is related to the electron transfer mechanism. The results showed that the ethanolic extract from the stems of *D. sulcatum* had the highest antioxidant power (0.50 ± 0.04 mmol Fe^{2+}/g extract) compared with the leaf and flower parts of the plant (0.35 ± 0.05 mmol Fe^{2+}/g extract and 0.12 ± 0.02 mmol Fe^{2+}/g extract, respectively). However, the results were slightly different from those of the DPPH assay, which might be due to the different amount and type of active chemical constituents responsible for antioxidation with different mechanisms. The leaf extract was the most effective antioxidant based on the hydrogen atom transfer mechanism observed in the DPPH assay, whereas the stem had highest reducing power based on the electron transfer mechanism observed in the FRAP assay. The reducing power of *D. sulcatum* has not been reported previously. The activity of some *Dendrobium* species has been previously reported (with FRAP assay), including extracts from the flower of *D. sabin* [43] and the stem of *D. officinale* [44]. This information confirmed the reducing powers of *D. sulcatum* and other *Dendrobium* species reported by the FRAP assay. These results confirmed the antioxidant activities of the plant extracts, which could be due to both hydrogen atom transfer and electron transfer.

3.4. ABTS Radical Scavenging Assay

The ABTS assay is a method for determining the relative antioxidant ability of a sample to scavenge $ABTS^+$ radicals based on hydrogen atom transfer and electron transfer, usually reported as the standard Trolox equivalent (TE). The ABTS radical cation is generated by a reaction between ABTS salt and a strong oxidizing agent such as potassium persulfate to form a blue-green ABTS radical solution. Antioxidants donate hydrogen atoms to the blue-green ABTS radical solution, which leads to decolorization and can be measured by the long wavelength absorption spectrum. In this study, the results of the ABTS assay agreed with those of the DPPH and FRAP assays, which showed higher antioxidant activities in the ethanolic extracts of the leaves and stems than in those of the flower extract. The antioxidant ability to scavenge the radical $ABTS^+$ was 116.99 ± 3.53, 65.76 ± 3.33 and 40.73 ± 2.68 mg TE/g extract for leaf, stem and flower extracts, respectively (see Table 1). This indicates that the leaf extract tends to be more active in antioxidation than extracts of other parts. Some *Dendrobium* species extracts, such as the methanolic stem extract solution of the *D. nobile, D. moschatum* and *D. densiflorum*, have been reported for ABTS radical scavenging activity with IC_{50} values of 316.5, 226.9 and 64.4 μg/mL, respectively, whereas the IC_{50} value of the methanolic leaf extract solutions were 134.4, 172.5 and 56.4 μg/mL [39].

3.5. Anti-Lipid Peroxidation of D. sulcatum Extracts Using TBARS Assay

Peroxides are usually formed in the human body; this phenomenon is called lipid peroxidation, which is related to the aging process. In this experiment, the anti-lipid peroxidation activities of ethanolic extracts from different parts of *D. sulcatum*, namely stems, leaves and flowers, were evaluated using the TBARS assay. At a final concentration

of 100 µg/mL of each extract, the inhibition of lipid peroxidation was less than 20% and considered inactive, whereas the percentage inhibitory activity became stronger as the concentrations of the extract samples were increased (low activity at a concentration of 300 µg/mL and moderate activity at a concentration of 500 µg/mL; see Figure 2). These results suggested that the anti-lipid peroxidation effects of *D. sulcatum* extracts were dose dependent. The anti-lipid peroxidation effect of the ethanolic extract of the stems, leaves and flowers of *D. sulcatum* was demonstrated for the first time, and this property may be due to antioxidant phenolic compounds and other active compounds of the plant extracts [45,46]. In addition, anti-lipid peroxidation properties have been demonstrated in some *Dendrobium* species, such as aqueous extract of *D. nobile* stems and flowers which can inhibit the lipid peroxidation in both goat liver homogenate (33.96% and 61.22%) and ghost red blood cells (94.97% and 99.05%, respectively) [47].

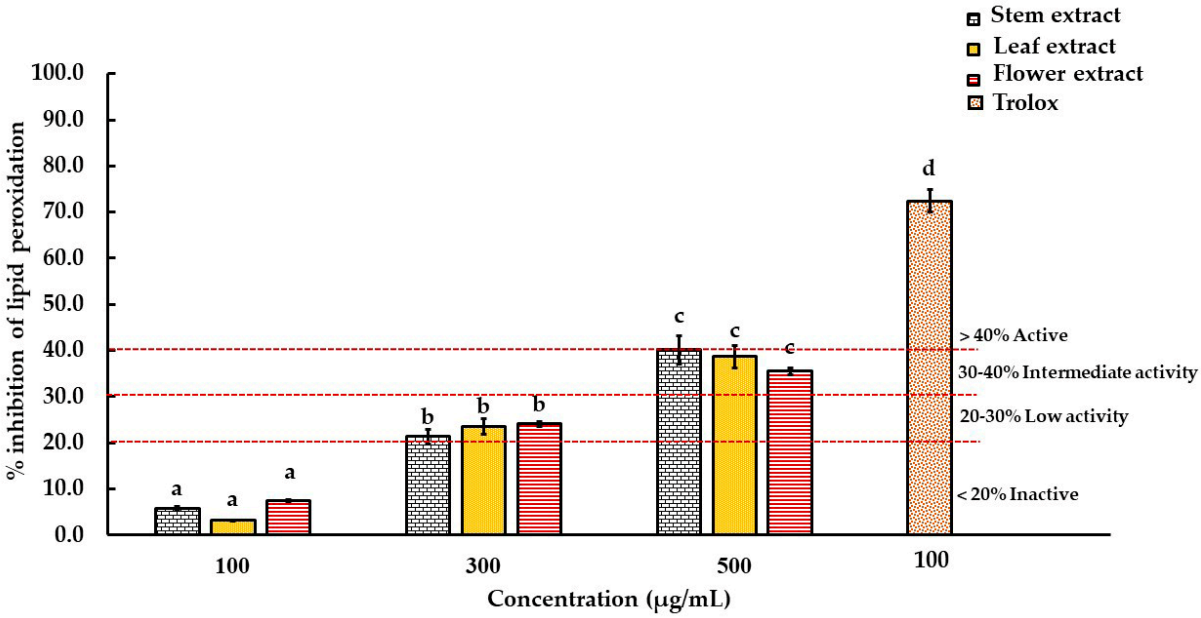

Figure 2. Anti-lipid-peroxidation activities of *D. sulcatum* ethanolic extract from stems, leaves and flowers (at the final concentration of 100, 300 and 500 µg/mL) using thiobarbituric acid reactive substances (TBAR) assay. Each value represents the mean ± SD (n = 3). The different letters are significantly different ($p < 0.05$) according to Tukey's HSD test. The last column depicts Trolox (100 µg/mL) which was used as the positive control. Samples exhibiting <20% inhibition were considered inactive, 20–30% inhibition was low activity, >30–40% inhibition was moderate activity and >40% was considered as active [26].

3.6. Antiglycation of D. sulcatum Extracts Using a BSA-Fructose Assay

Glycation or the Maillard reaction is a nonenzymatic binding initiated between the free amino group of proteins, lipids or nucleic acids and the carbonyl group of reducing sugars such as glucose and fructose. This process spontaneously and naturally generates advanced glycation end-products (AGEs), which can directly lead to cellular damage to both extracellular and intracellular proteins, resulting in skin exhibiting signs of aging [48]. Therefore, preventing the formation of AGEs in the early stages of aging is an important consideration. In general, the use of AGE inhibitors such as AMG has been observed [49]. Based on our results, the antiglycation activities of the ethanolic extract of the stems, leaves and flowers of *D. sulcatum* are shown in Figure 3. At the tested concentration of 1000 µg/mL, samples showed the highest glycation inhibitory activity (>80% inhibition) for all extracts. Among the different parts of *D. sulcatum*, leaf extract showed the highest antiglycation activity at the test concentrations. AMG, the positive control, showed the percentage inhibition of 90.41 ± 3.70 and 100.00 ± 1.30% for 50 µg/mL and 100 µg/mL, respectively.

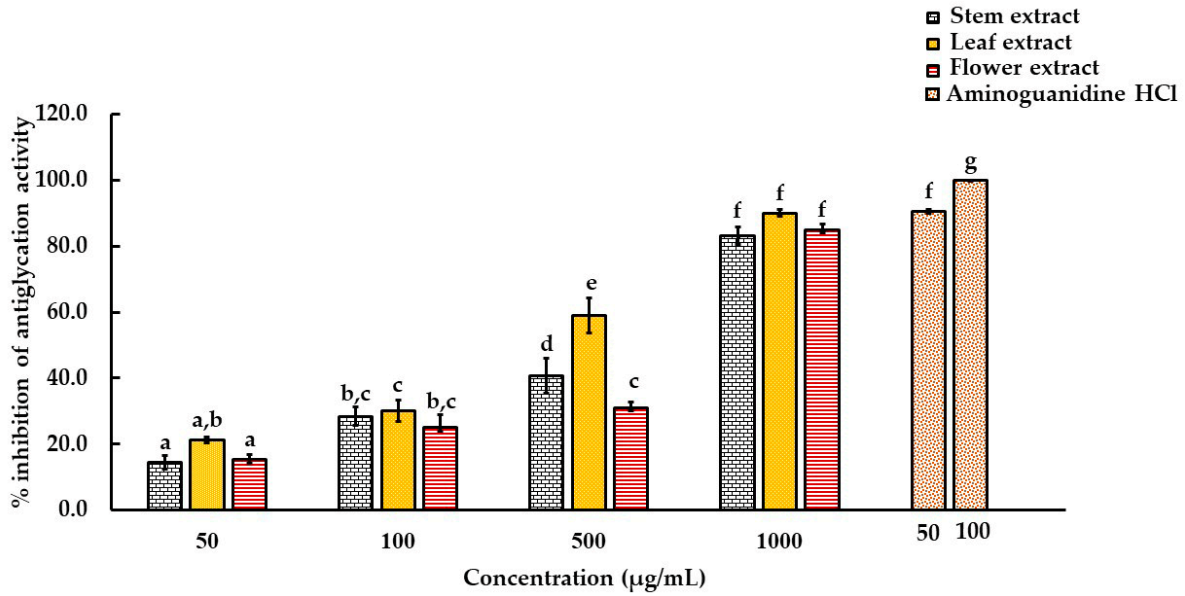

Figure 3. Effect of ethanolic extract of *D. sulcatum* stems, leaves and flowers on antiglycation activity using a BSA-fructose assay. Each value represents the mean ± SD (n = 3). The different letters are significantly different ($p < 0.05$) according to Tukey's HSD test.

These results indicated that various parts of *D. sulcatum*, namely stems, leaves and flowers, expressed moderate glycation inhibitory activity. This is the first time that the antiglycation activity of *D. sulcatum* extracts has been observed. The antiglycation activity of these extracts may mainly involve the early stage of AGE formation by the inhibition of reactive oxygen species (ROS). According to our results regarding the antioxidant activities of various parts of *D. sulcatum*, extracts showed moderate antioxidant activities which were related to moderate inhibition of ROS resulting in the moderate antiglycation property. The active compounds in *D. sulcatum* extracts on the antiglycation property might be from their phenolic compounds such as phenolic acids, luteolin, quercetin and rutin, which have also been reported for antiglycation inhibitors [50].

3.7. Anti-Inflammatory Activity of D. sulcatum Extracts

NO is a product of not only nitric oxide synthase (iNOS) but also other NOS enzymes and plays a role in inflammation (albeit at lower concentrations). It is one of the signaling molecules involved in the inflammatory process through the cyclooxygenase-2 pathway (COX-2). Many agents, such as LPS, cytokines and other substances, cause iNOS to form NO. Excessive formation of NO product can cause the pathophysiology of inflammatory diseases and cell death. Therefore, inhibition of NO products can prevent the process of inflammation and cell damage. For this experiment, macrophage cells were tested for cell viability with *D. sulcatum* extracts and induced to form NO products by LPS. The colorimetric Griess reaction method was used to determine the inhibition of NO level by *D. sulcatum* extracts. The results of the macrophage cell viability assay performed on samples treated with *D. sulcatum* extracts are shown in Figure 4. The results showed that at the concentrations of *D. sulcatum* extracts in the range of 1.0–25.0 µg/mL the cell viability was not significantly different compared with the control group ($p < 0.05$), except for in the case of the flower extract. However, at a concentration of 50–500 µg/mL for the stem and leaf extract and 25–500 µg/mL for the flower extract, the cells were significantly ($p < 0.05$) decreased compared with the control group. The results indicated that these concentration ranges are harmful and toxic to cells. Therefore, appropriate concentrations of each extract were selected for further investigation with the NO inhibition assay: less than 25 µg/mL for the flower extract and less than 50 µg/mL for the stem and leaf extracts.

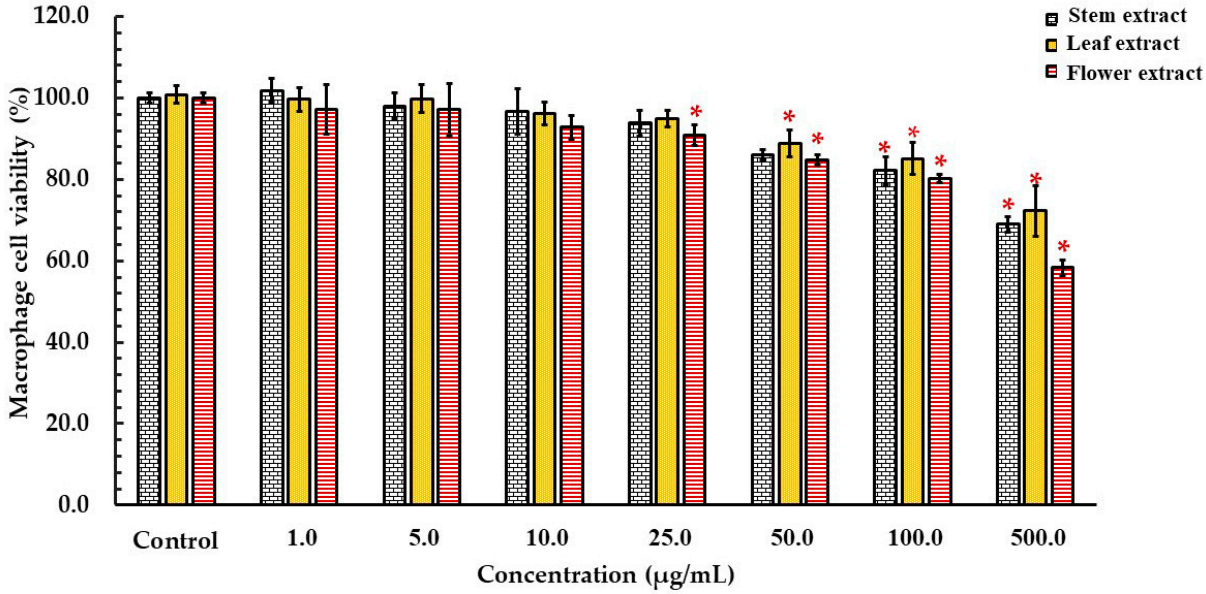

Figure 4. Effect of ethanolic extract of *D. sulcatum* stems, leaves and flowers on macrophage cell viability using MTT assay. Each value represents the mean ± SD, (n = 3). * Significantly different versus the control ($p < 0.05$, Student's t-test).

The results of NO inhibitory activity of *D. sulcatum* extracts are shown in Figure 5. After macrophage cells were induced to secrete NO by LPS, the generated amount of NO was 63.75 ± 0.62 µM, whereas the non-LPS-induced cells had 34.33 ± 0.62 µM. The NO level from the LPS-induced macrophage cells was significantly ($p < 0.05$) decreased to 49.13 ± 1.35 and 50.38 ± 0.89 µM when the cells were treated with (10 µg/mL) ethanolic extract from the stems and leaves of *D. sulcatum*. The level of NO was also significantly decreased when the LPS-induced cells were treated with *L*-NMMA, a positive control with a final concentration of 10 or 25 µg/mL (47.44 ± 0.72 and 39.31 ± 0.87 µM of NO, respectively). These results indicated that the NO inhibitory activity of the ethanolic extracts from stems and leaves of *D. sulcatum* was in the same range as *L*-NMMA, which was considered to be a strong activity. However, the ethanolic extract of *D. sulcatum* flowers showed a slightly NO inhibitory activity with a NO level of 59.66 ± 0.41 µM. The NO inhibitory activity of *D. sulcatum* was demonstrated for the first time. In addition, anti-inflammatory activity via the NO inhibitory activity of the same genus, *Dendrobium*, was detected [51]. The NO inhibitory activity of *D. sulcatum* extracts might be due to the phenolic compounds, phenanthrene, alkaloids and other active compounds of the plants which have previously been described for the *Dendrobium* genus [3]. Several recent studies indicated that flavonoids (such as apigenin, quercetin, isovitexin and luteolin) and phenanthrene (such as erianthridin, ephemeranthol A and 5,7-dimethoxyphenanthrene2,6-diol) have a NO inhibitory activity effect, mainly via inhibition of NFκB, leading to down-regulation of the iNOS process [52–54]. In addition, alkaloids from the *Dendrobium* genus, including Dendrocrepidamine A and Dendrocrepidamine B, have also been reported for the inhibition of LPS-induced NO in macrophage cells [11].

3.8. Anti-Tyrosinase Activity of D. sulcatum Extracts

Tyrosinase is an enzyme that plays an important role in the process of skin pigmentation by oxidation of *L*-DOPA, leading to melanin production. Therefore, inhibition of tyrosinase could lead to a decrease in melanin and a lighter skin tone. The results from our study on the effect of ethanolic extract from the stems, leaves and flowers of *D. sulcatum* on anti-tyrosinase activity are shown in Figure 6. The extracts from the leaves and flowers at 50 µg/mL showed higher tyrosinase inhibitory activity (73.55 ± 1.89% and 75.80 ± 0.70%, respectively) than the extract from the stem (39.28 ± 4.25%) at a fi-

nal concentration of 50 µg/mL. These tyrosinase inhibition patterns of each extract were similar to those for the higher concentrations of each extract (200 µg/mL). Tyrosinase inhibition was 78.29 ± 1.12%, 77.64 ± 1.4% and 44. 65 ± 3.55% for leaf, flower and stem extracts, respectively, whereas ascorbic acid (200 µg/mL), the positive control, reached 98.62 ± 0.91%. These results demonstrate, for the first time, the anti-tyrosinase property of *D. sulcatum*. The anti-tyrosinase activity was also found in some *Dendrobium* species such as *D. Sonia* (IC_{50} = 816.8 µg/mL), *D. sonia pink* (IC_{50} > 2000 µg/mL), *D. snow rabbit* (IC_{50} = 1524.2 µg/mL) and *D. shavin* (IC_{50} = 907.2 µg/mL) [41]. This information indicates that the *D. sulcatum* extracts exhibit higher tyrosinase inhibitory activity than those of these previously studied species.

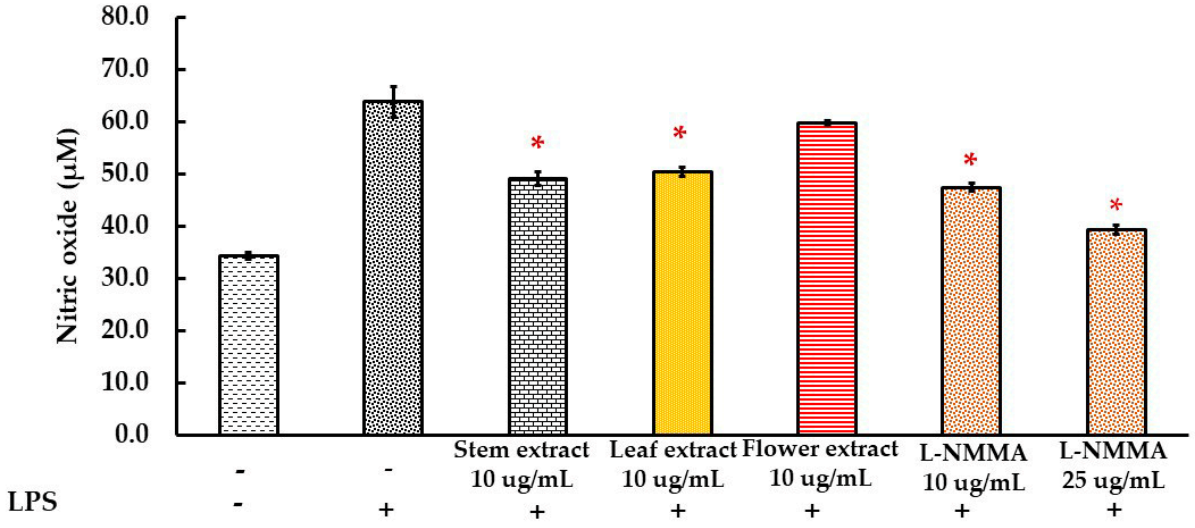

Figure 5. Effect of ethanolic extract of *D. sulcatum* stems, leaves and flowers (10 µg/mL) and *L*-NMMA (10 µg/mL and 25 µg/mL) on NO inhibition. Each value represents the mean ± SD, (n = 3). * Significantly different versus the LPS-induced control at $p < 0.05$ Student's t-test.

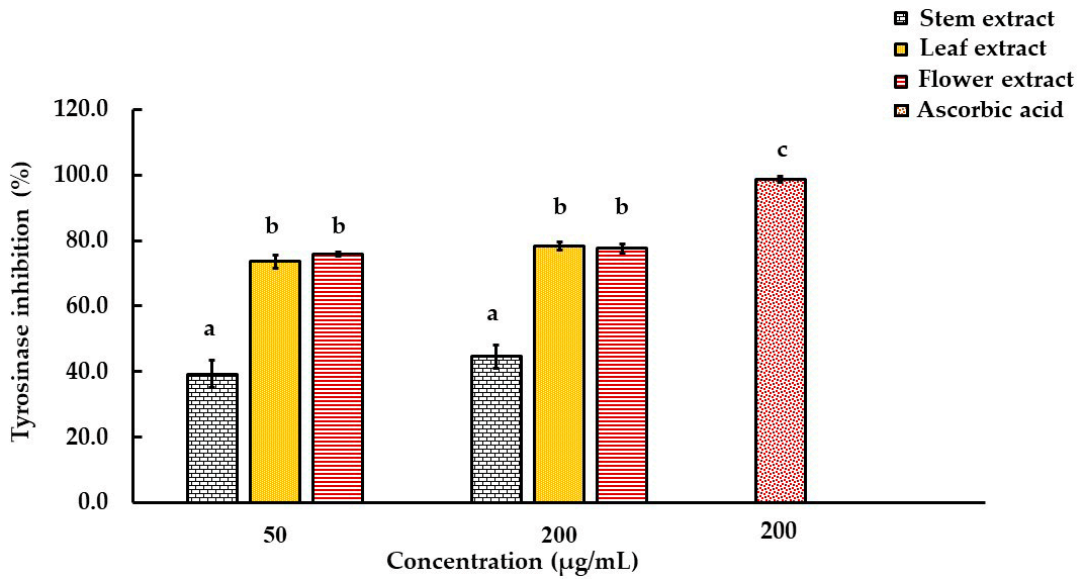

Figure 6. Effect of ethanolic extract of *D. sulcatum* stems, leaves and flowers (50 and 200 µg/mL) on tyrosinase inhibition; the last column depicts ascorbic acid (a positive control, 200 µg/mL). Each value represents the mean ± SD, (n = 3). The different letters are significantly different ($p < 0.05$) according to Tukey's HSD test.

The limitation of this study is that the major chemical constituents associated with the biological activities of *D. sulcatum* extracts are not known. Further studies to identify the biomarkers, evaluate the cytotoxicity of human cells, and perform quality control and pre-formulation study of the plant extracts would be essential for further application of the extracts in cosmetic and cosmeceutical products.

4. Conclusions

The biological activities, including antioxidation, anti-lipid peroxidation, antiglycation, anti-inflammatory and anti-tyrosinase properties of ethanolic extract of *D. sulcatum* stems, leaves and flowers were successfully elucidated and revealed for the first time. The ethanolic extract of *D. sulcatum* stems and leaves exhibited higher antioxidant, anti-lipid peroxidation and NO inhibitory activities compared with the flower extract. As for the antiglycation activity, the results showed that the leaf extract of the *D. sulcatum* exhibited the highest activity, whereas the potential to inhibit tyrosinase was found in the leaf and flower extracts. These results suggest that the stems, leaves and flowers of *D. sulcatum* are a promising source of natural active ingredients for anti-skin-aging and whitening agents in cosmetic and cosmeceutical products.

Author Contributions: Conceptualization, T.R., J.S., L.M., N.K. and E.W.; formal analysis, J.S., L.M., N.K. and E.W.; investigation, T.R. and E.W.; methodology, T.R., K.S., P.A., N.P., P.S., P.C. and E.W.; resources, P.R.(Pawarit Rakasawapokin) and P.R. (Patpitcha Rakasawapokin); supervision, E.W.; visualization, J.S. and E.W.; writing—original draft, T.R.; writing—review & editing, J.S., L.M., N.K. and E.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: The authors would like to acknowledge the Research and Innovation Center in Cosmetic Sciences and Natural products (RCCN), Department of Cosmetic Sciences, School of Pharmaceutical Sciences, University of Phayao, for proving instruments, facilities and space.

Conflicts of Interest: The authors declare that they hold no competing interests.

References

1. Thammasiri, K. Current status of orchid production in Thailand. *Acta Hortic.* **2015**, *1708*, 25–33. [CrossRef]
2. The Botanical Garden Organization. Queen Sirikit Botanic Garden. In *Ueang Jampa-Nan*; O.S. Printing House: Bangkok, Thailand, 2000; Volume 6, pp. 162–163.
3. Xu, J.; Guan, J.; Chen, X.J.; Zhao, J.; Li, S.P. Comparison of polysaccharides from different *Dendrobium* using saccharide mapping. *J. Pharm. Biomed. Anal.* **2011**, *55*, 977–983. [CrossRef]
4. Lam, Y.; Ng, T.B.; Yao, R.M.; Shi, J.; Xu, K.; Sze, S.C.W.; Zhang, K.Y. Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* plants. *Evid.-Based Complement. Altern. Med.* **2015**, *2015*, 841752. [CrossRef]
5. Hadi, H.; Razali, S.N.; Awadh, A.I. A comprehensive review of the cosmeceutical benefits of *Vanda* species (Orchidaceae). *Nat. Prod. Commun.* **2015**, *10*, 1483–1488. [PubMed]
6. Kanlayavattanakul, M.; Lourith, N.; Chaikul, P. Biological activity and phytochemical profiles of *Dendrobium*: A new source for specialty cosmetic materials. *Ind. Crops Prod.* **2018**, *120*, 61–70. [CrossRef]
7. Lin, Y.; Yang, T.; Shen, L.; Zhang, J.; Liu, L. Study on the properties of *Dendrobium officinale* fermentation broth as functional raw material of cosmetics. *J. Cosmet. Dermatol.* **2022**, *21*, 1216–1223. [CrossRef] [PubMed]
8. Lv, G.Y.; Yan, M.Q.; Chen, S.H. Review of pharmacological activities of *Dendrobium officinale* based on traditional functions. *Zhongguo Zhong Yao Za Zhi* **2013**, *38*, 489–493. [PubMed]
9. Jiaranaikulwanitch, J.; Yooin, W.; Chutiwitoonchai, N.; Thitikornpong, W.; Sritularak, B.; Rojsitthisak, P.; Vajragupta, O. Discovery of natural lead compound from *Dendrobium* sp. against SARS-CoV-2 infection. *Pharmaceuticals* **2022**, *15*, 620. [CrossRef] [PubMed]
10. Wang, Y.H. Traditional uses, chemical constituents, pharmacological activities, and toxicological effects of *Dendrobium* leaves: A review. *J. Ethnopharmacol.* **2021**, *270*, 113851. [CrossRef]
11. Hu, Y.; Yang, H.; Ding, X.; Liu, J.; Wang, X.; Hu, L.; Liu, M.; Zhang, C. Anti-inflammatory octahydroindolizine alkaloid enantiomers from *Dendrobium crepidatum*. *Bioorg. Chem.* **2020**, *100*, 103809. [CrossRef]

12. Li, J.T.; Yin, B.L.; Liu, Y.; Wang, L.Q.; Chen, Y.G. Mono-aromatic constituents of *Dendrobium longicornu*. *Chem. Nat. Compd.* **2009**, *45*, 234–236. [CrossRef]
13. Zhang, X.; Liu, H.W.; Gao, H.; Han, H.Y.; Wang, N.L.; Wu, H.M.; Yao, X.S.; Wang, Z. Nine new sesquiterpenes from *Dendrobium nobile*. *Helv. Chim. Acta* **2007**, *90*, 2386–2394. [CrossRef]
14. Zheng, W.P.; Tang, Y.P.; Zhi, F.; Lou, F.C. Dihydroayapin, a new coumarin compound from *Dendrobium densiflorum*. *J. Asian Nat. Prod. Res.* **2000**, *2*, 301–304. [CrossRef] [PubMed]
15. Zhang, X.; Xu, J.K.; Wang, J.; Wang, N.L.; Kurihara, H.; Kitanaka, S.; Yao, X.S. Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*. *J. Nat. Prod.* **2007**, *70*, 24–28. [CrossRef]
16. Chen, Y.; Liu, Y.; Jiang, J.; Zhang, Y.; Yin, B. Dendronone, a new phenanthrenequinone from *Dendrobium cariniferum*. *Food Chem.* **2008**, *111*, 11–12. [CrossRef]
17. Majumder, P.L.; Guha, S.; Sen, S. Bibenzyl derivatives from the orchid *Dendrobium amoenum*. *Phytochemistry* **1999**, *52*, 1365–1369. [CrossRef]
18. Lu, H.; Yang, K.; Zhan, L.; Lu, T.; Chen, X.; Cai, X.; Zhou, C.; Li, H.; Qian, L.; Lv, G.; et al. Optimization of flavonoid extraction in *Dendrobium officinale* leaves and their inhibitory effects on tyrosinase activity. *Int. J. Anal. Chem.* **2019**, *2019*, 7849198. [CrossRef]
19. Chang, C.C.; Ku, A.F.; Tseng, Y.Y.; Yang, W.B.; Fang, J.M.; Wong, C.H. 6,8-Di-C-glycosyl flavonoids from *Dendrobium huoshanense*. *J. Nat. Prod.* **2010**, *73*, 229–232. [CrossRef] [PubMed]
20. Margraf, T.; Karnopp, A.R.; Rosso, N.D.; Granato, D. Comparison between Folin-Ciocalteu and prussian blue assays to estimate the total phenolic content of juices and teas using 96-well microplates. *J. Food Sci.* **2015**, *80*, 2397–2403. [CrossRef]
21. Chandra, S.; Khan, S.; Avula, B.; Lata, H.; Yang, M.H.; Elsohly, M.A.; Khan, I.A. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. *Evid.-Based Complement. Altern. Med.* **2014**, *2014*, 253875. [CrossRef]
22. Wongwad, E.; Pingyod, C.; Saesong, T.; Waranuch, N.; Wisuitiprot, W.; Sritularak, B.; Temkitthawon, P.; Ingkaninan, K. Assessment of the bioactive components, antioxidant, antiglycation and anti-inflammatory properties of *Aquilaria crassna* Pierre ex Lecomte leaves. *Ind. Crops Prod.* **2019**, *138*, 111448. [CrossRef]
23. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
24. Mansour, R.B.; Ksouri, W.M.; Cluzet, S.; Krisa, S.; Richard, T.; Ksouri, R. Assessment of antioxidant activity and neuroprotective capacity on PC12 cell line of *Frankenia thymifolia* and related phenolic LC-MS/MS identification. *Evid.-Based Complement. Altern. Med.* **2016**, *2016*, 2843463. [CrossRef]
25. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
26. Saesong, T.; Allard, P.M.; Queiroz, E.F.; Marcourt, L.; Nuengchamnong, N.; Temkitthawon, P.; Khorana, N.; Wolfender, J.L.; Ingkaninan, K. Discovery of lipid peroxidation inhibitors from *Bacopa* species prioritized through multivariate data analysis and multi-informative molecular networking. *Molecules* **2019**, *24*, 2989. [CrossRef] [PubMed]
27. Limpeanchob, N.; Jaipan, S.; Rattanakaruna, S.; Phrompittayarat, W.; Ingkaninan, K. Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. *J. Ethnopharmacol.* **2008**, *120*, 112–117. [CrossRef]
28. Sun, J.; Liu, W.; Ma, H.; Marais, J.P.J.; Khoo, C.; Dain, J.A.; Rowley, D.C.; Seeram, N.P. Effect of cranberry (*Vaccinium macrocarpon*) oligosaccharides on the formation of advanced glycation end-products. *J. Berry Res.* **2016**, *6*, 149–158. [CrossRef]
29. Cheenpracha, S.; Park, E.J.; Rostama, B.; Pezzuto, J.M.; Chang, L.C. Inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 cells by the norsesterterpene peroxide, Epimuqubilin A. *Mar. Drugs* **2010**, *8*, 429–437. [CrossRef]
30. Wisuitiprot, V.; Ingkaninan, K.; Chakkavittumrong, P.; Wisuitiprot, W.; Neungchamnong, N.; Chantakul, R.; Waranuch, N. Effects of *Acanthus ebracteatus* Vahl. extract and verbascoside on human dermal papilla and murine macrophage. *Sci. Rep.* **2022**, *12*, 1491. [CrossRef]
31. Rasmussen, C.; Thomas-Virnig, C.; Allen-Hoffmann, B.L. Classical human epidermal keratinocyte cell culture. *Methods Mol. Biol.* **2013**, *945*, 161–175. [CrossRef]
32. Stockert, J.C.; Blázquez-Castro, A.; Cañete, M.; Horobin, R.W.; Villanueva, Á. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. *Acta Histochem.* **2012**, *114*, 785–796. [CrossRef] [PubMed]
33. Tengamnuay, P.; Pengrungruangwong, K.; Pheansri, I.; Likhitwitayawuid, K. *Artocarpus lakoocha* heartwood extract as a novel cosmetic ingredient: Evaluation of the in vitro anti-tyrosinase and in vivo skin whitening activities. *Int. J. Cosmet. Sci.* **2006**, *284*, 269–276. [CrossRef]
34. Srisuksomwong, P.; Kaenhin, L.; Mungmai, L. Collagenase and tyrosinase inhibitory activities and stability of facial cream formulation containing cashew leaf extract. *Cosmetics* **2023**, *10*, 17. [CrossRef]
35. Pengdee, C.; Sritularak, B.; Putalun, W. Optimization of microwave-assisted extraction of phenolic compounds in *Dendrobium formosum* Roxb. ex Lindl. and glucose uptake activity. *S. Afr. J. Bot.* **2020**, *132*, 423–431. [CrossRef]
36. Zhu, A.L.; Hao, J.W.; Liu, L.; Wang, Q.; Chen, N.D.; Wang, G.L.; Liu, X.Q.; Li, Q.; Xu, H.M.; Yang, W.H. Simultaneous quantification of 11 phenolic compounds and consistency evaluation in four *Dendrobium* species used as ingredients of the traditional Chinese medicine shihu. *Front. Nutr.* **2021**, *8*, 771078. [CrossRef] [PubMed]
37. Williams, C.A. The leaf flavonoids of the orchidaceae. *Phytochemistry* **1979**, *18*, 803–813. [CrossRef]

38. Wang, Y.H.; Wang, Y.H. Traditional uses and pharmacologically active constituents of *Dendrobium* plants for dermatological disorders: A review. *Nat. Prod. Bioprospect.* **2021**, *11*, 465–487. [CrossRef] [PubMed]
39. Natta, S.; Mondol, M.S.A.; Pal, K.; Mandal, S.; Sahana, N.; Pal, R.; Pandit, G.K.; Alam, B.K.; Das, S.S.; Biswas, S.S.; et al. Chemical composition, antioxidant activity and bioactive constituents of six native endangered medicinal orchid species from north-eastern Himalayan region of India. *S. Afr. J. Bot.* **2020**, *73*, 10414. [CrossRef]
40. Paudel, M.R.; Chand, M.B.; Pant, B.; Pant, B. Assessment of antioxidant and cytotoxic activities of extracts of *Dendrobium crepidatum*. *Biomolecules* **2019**, *9*, 478. [CrossRef]
41. Athipornchai, A.; Jullapo, N. Tyrosinase inhibitory and antioxidant activities of Orchid (*Dendrobium* spp.). *S. Afr. J. Bot.* **2018**, *119*, 188–192. [CrossRef]
42. Yang, J.; Guo, J.; Yuan, J. In vitro antioxidant properties of rutin. *LWT-Food Sci. Technol.* **2008**, *41*, 1060–1066. [CrossRef]
43. Abu, F.; Mat Taib, C.N.; Mohd Moklas, M.A.; Mohd Akhir, S. Antioxidant properties of crude extract, partition extract, and fermented medium of *Dendrobium sabin* flower. *Evid.-Based Complement. Altern. Med.* **2017**, *2017*, 2907219. [CrossRef] [PubMed]
44. Kim, S.; Jo, K.; Byun, B.S.; Han, S.H.; Yu, K.W.; Suh, H.J.; Hong, K.B. Chemical and biological properties of puffed *Dendrobium officinale* extracts: Evaluation of antioxidant and anti-fatigue activities. *J. Funct. Foods* **2020**, *73*, 104144. [CrossRef]
45. Yüksel, A.K.; Dikici, E.; Yüksel, M.; Işik, M.; Tozoğlu, F.; Köksal, E. Phytochemicals analysis and some bioactive properties of *Erica manipuliflora* Salisb. (EMS); antibacterial, antiradical and anti-lipid peroxidation. *Iran. J. Pharm. Res.* **2021**, *20*, 422–434. [CrossRef] [PubMed]
46. Shabbir, M.; Khan, M.R.; Saeed, N. Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves. *BMC Complement. Altern. Med.* **2013**, *13*, 143. [CrossRef] [PubMed]
47. Devi, P.U.; Selvi, S.; Devipriya, D.; Murugan, S.; Suja, S. Antitumor and antimicrobial activities and inhibition of in-vitro lipid peroxidation by *Dendrobium nobile*. *Afr. J. Biotechnol.* **2009**, *8*, 2289–2293.
48. Chaudhuri, J.; Bains, Y.; Guha, S.; Kahn, A.; Hall, D.; Bose, N.; Gugliucci, A.; Kapahi, P. The role of advanced glycation end products in aging and metabolic diseases: Bridging association and causality. *Cell Metab.* **2018**, *28*, 337–352. [CrossRef]
49. Friedman, E.A. Evolving pandemic diabetic nephropathy. *Rambam Maimonides Med. J.* **2010**, *1*, e0005. [CrossRef]
50. Yeh, W.J.; Hsia, S.M.; Lee, W.H.; Wu, C.H. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *J. Food Drug Anal.* **2017**, *25*, 84–92. [CrossRef]
51. Mou, Z.; Zhao, Y.; Ye, F.; Shi, Y.; Kennelly, E.J.; Chen, S.; Zhao, D. Identification, biological activities and biosynthetic pathway of *Dendrobium* alkaloids. *Front. Pharmacol.* **2021**, *12*, 605994. [CrossRef]
52. Hwang, J.S.; Lee, S.A.; Hong, S.S.; Han, X.H.; Lee, C.; Kang, S.J.; Lee, D.; Kim, Y.; Hong, J.T.; Lee, M.K.; et al. Phenanthrenes from *Dendrobium nobile* and their inhibition of the LPS-induced production of nitric oxide in macrophage RAW 264.7 cells. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3785–3787. [CrossRef] [PubMed]
53. Duarte, J.; Francisco, V.; Perez-Vizcaino, F. Modulation of nitric oxide by flavonoids. *Food Funct.* **2014**, *5*, 1653–1668. [CrossRef] [PubMed]
54. Chen, L.Q.; Shen, X.F.; Hu, B.Y.; Lin, Y.; Igbe, I.; Zhang, C.G.; Zhang, G.L.; Yuan, X.H.; Wang, F. Nitric oxide production inhibition and mechanism of phenanthrene analogs in lipopolysaccharide-stimulated RAW264.7 macrophages. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 2521–2525. [CrossRef] [PubMed]