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Biomarkers of oxidative stress in the equine erythrocytes after *in vitro* treatment with extracts derived from different pseudobulbs of *Dendrobium parishii* Rchb.f. (Orchidaceae) plants

Lyudmyla Buyun[1], Oleksandr Gyrenko[1], Lyudmyla Kovalska[1], Maryna Opryshko[1], Myroslava Maryniuk[1], Halina Tkaczenko*[2], Natalia Kurhaluk[2]

[1]M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine, Kyiv, Ukraine
[2]Pomeranian University in Słupsk, Institute of Biology, Poland

Lyudmyla Buyun: https://orcid.org/0000-0002-9158-6451
Oleksandr Gyrenko: https://orcid.org/0000-0003-3296-3787
Lyudmyla Kovalska: https://orcid.org/0000-0001-6410-6603
Maryna Opryshko: https://orcid.org/0000-0001-5048-4961
Myroslava Maryniuk: https://orcid.org/0000-0003-2590-448X
Halina Tkaczenko: https://orcid.org/0000-0003-3951-9005
Natalia Kurhaluk: https://orcid.org/0000-0002-4669-1092

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The current study was conducted to investigate the antioxidant properties of extracts derived from different pseudobulbs of *Dendrobium parishii* Rchb.f. using biomarkers of oxidative stress (2-thiobarbituric acid reactive substances as biomarkers of lipid peroxidation, aldehydic and ketonic derivatives of oxidative modification of proteins (OMP), total antioxidant capacity (TAC)) in the equine erythrocytes after *in vitro* treatment with the extracts. The current study is a continuation of our cooperation with M.M. Gryshko National Botanic Garden, National Academy of Science of Ukraine (Kyiv, Ukraine) concerning investigations of antibacterial and antioxidant properties of extracts derived from leaves and pseudobulbs of some species belonging to the Orchidaceae family. The antioxidant properties of extracts derived from different pseudobulbs of *D. parishii* using biomarkers of oxidative stress in the equine erythrocytes after *in vitro* treatment with the extracts revealed that extracts derived from different pseudobulbs of *D. parishii* exhibited varying activity. Extracts derived from the first, second, and third pseudobulbs of *D. parishii* increased lipid peroxidation after *in vitro* treatment of equine erythrocytes. Extracts derived from the first, sixth, and seventh pseudobulbs of *D. parishii* caused to decrease in the levels of aldehydic derivatives of OMP. On the other hand, ketonic derivatives of OMP in the erythrocyte samples after incubation with extracts derived from all parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) were decreased. Moreover, extracts derived from the first and sixth parts of pseudobulbs of *D. parishii* after incubation with erythrocyte samples caused to increase in the TAC levels. The study of extracts derived from *D. parishii* supports its favorable biological activities and lays a strong foundation for further exploration of its structure-activity relationships and activity development, providing experimental data for the development and utilization of extracts of *D. parishii*.

Keywords: *Dendrobium parishii*, pseudobulb extract, equine erythrocytes, biomarkers, lipid peroxidation, oxidatively modified proteins, total antioxidant capacity

***Corresponding Author:** Halina Tkaczenko, Institute of Biology, Pomeranian University in Słupsk, Arciszewski 22b, 76-200 Słupsk, Poland
halina.tkaczenko@upsl.edu.pl

Introduction

Orchids (Orchidaceae) are a family of monocotyledonous perennial herbs; over 750 genera and 20 thousand species (according to other sources 35 thousand), in both hemispheres; most abundant and diverse in the tropics of America and South Asia. Some orchids are used in industry (vanilla) and medicine (orchid); many are grown in greenhouses. About 100 species, are in the tropics; Several species are cultivated there for their fruits containing vanillin (Zhang et al., 2018; Wang et al., 2019). In traditional Chinese medicine, orchids are still used for medicinal purposes. According to the studies, the uses of dried orchids range from improving vision to treating cancer. Information about the healing properties of orchids and their use as food moves from West to East more than from East to West. Many good discoveries in the East are kept secret from the West even today, with the development of information technology. Additionally, many cultures that use orchids for food do not document it as well as we would like (Bulpitt et al., 2007; Rokaya et al., 2014; Shang et al., 2017; Jiang et al., 2021).

Experiments with phytocomponents such as alkaloids, terpenes, stilbenoids, bibenzyls, phenanthrenes, flavonoids, and polysaccharides isolated from Orchidaceae have shown their potential medicinal utility (Sut et al., 2017). To date, several classes of phytocomponents have been isolated from therapeutically used orchids, demonstrating great chemical diversity (Sut et al., 2017). Among them, phenol derivatives have been studied for their biological activity, especially anticancer properties (Wang et al., 2021; Śliwiński et al., 2022), anti-inflammation (Jiang et al., 2019; Zhang et al., 2021), and anti-neurodegeneration properties (Li et al., 2017; Zhang et al., 2022).

Based on traditional folk uses, chemical composition, and pharmacological studies, *Dendrobium* is considered a promising medicinal and edible plant with multiple pharmacological activities (Li et al., 2023). *Dendrobium* was recorded in the Chinese Pharmacopoeia as an astringent, analgesic, tonic, and anti-inflammatory substance as early as around 200 AD (Li et al., 2023). Interestingly, *Dendrobium* stems and leaves have become a major part of research by Chinese and foreign researchers (Moretti et al., 2013; Prasad et al., 2017; Ke et al., 2020; Lou et al., 2020; Wang et al., 2022; Zhong et al., 2022; Li et al., 2023).

Wang (2021) in the review demonstrated that 131 compounds from *Dendrobium* plants have been reported to possess anti-inflammatory, antimicrobial, antioxidant, antiaging, anti-psoriasis, and tyrosinase-inhibitory activities, implying that *Dendrobium* plants are important resources for the discovery of active compounds and the development of new drugs and cosmetics. *Dendrobium crepidatum* Lindl. & Paxton, *Dendrobium denneanum* Kerr, *Dendrobium loddigesii* Rolfe, *Dendrobium nobile* Lindl., and *Dendrobium officinale* Kimura & Migo have been extensively studied. The major active compounds found in *Dendrobium* species are phenanthrenes, alkaloids, flavonoids, phenylpropanoids, and lignans. Several compounds, such as loddigesiinol A, (S)-5-methoxy-2,4,7,9-tetrahydroxy-9,10-dihydrophenanthrene, (S)-4-methoxy-2,5,7,9-tetrahydroxy-9,10-dihydrophenanthrene, 2,5-dihydroxy-4-methoxy-phenanthrene 2-O-β-D-glucopyranoside, (9R)-1,2,5,9-tetrahydroxy-9,10-dihydrophenanthrene 5-O-β-D-glucopyranoside, (+)-homocrepidine A, and vicenin 2, have significant anti-inflammatory activities and inhibit nitric oxide production (Wang, 2021).

The current study was conducted to investigate the antioxidant properties of extracts derived from different pseudobulbs of *Dendrobium parishii* Rchb.f. using biomarkers of oxidative stress in the equine erythrocytes after *in vitro* treatment with the extracts.

Material and methodology

Collection of plant materials and preparation of plant extracts

The pseudobulbs of *D. parishii* plants cultivated under glasshouse conditions were sampled at M.M. Gryshko National Botanic Garden (NBG), Kyiv, Ukraine. Since 1999, the whole collection of tropical and subtropical plants (including orchids) has had the status of a National Heritage Collection of Ukraine and is supported through State Funding. Besides, the NBG collection of tropical orchids was registered at the Administrative Organ of CITES in Ukraine (Ministry of Environment Protection, registration No. 6939/19/1-10 of 23 June 2004). Freshly collected pseudobulbs (seven parts beginning from the base of the growing tip of the rhizome, designated as numbers 1, 2, 3, 4, 5, 6, and 7) were washed, weighed, crushed, and homogenized in 0.1 M phosphate buffer (pH 7.4) (in the ratio of 1 : 9, w/w) at room temperature. The extracts were then filtered and used for analysis. The extract was stored at -25 °C until use.

Horses and collection of blood samples

Eighteen healthy adult horses from the central Pomeranian region in Poland (village Strzelinko,

N 54° 30' 48.0" E 16° 57' 44.9"), aged 8.9 ±1.3 years old, including 6 Hucul ponies, 5 Thoroughbred horses, 2 Anglo-Arabian horses, and 5 horses of unknown breed, were used in this study. All horses participated in recreational horseback riding. Horses were housed in individual boxes, with feeding (hay and oat) provided twice a day, at 08.00 and 18.00 h, and water available *ad libitum*. All horses were thoroughly examined clinically and screened for hematological, biochemical, and vital parameters, which were within reference ranges. The females were non-pregnant.

Blood was drawn from the jugular vein of the animals in the morning, 90 minutes after feeding, while the horses were in the stables (between 8:30 and 10 AM). Blood samples were processed for analysis less than 12 hours after blood withdrawal. Blood was stored in tubes with sodium citrate as the anticoagulant and held on the ice until centrifugation at 3,000 rpm for 5 min to remove plasma. The pellet of erythrocytes was re-suspended in 4 mM phosphate buffer (pH 7.4). A volume of 0.1 ml of the plant extracts was added to 1.9 ml of equine erythrocytes. For positive control, incubation of equine erythrocytes with 4 mM phosphate buffer (pH 7.4) was used. After incubating the mixture at 37 °C for 60 min with continuous stirring, biomarkers of oxidative stress were assessed. Erythrocyte aliquots were used in the current study.

The 2-Thiobarbituric acid reactive substances assay

The level of lipid peroxidation was determined by quantifying the concentration of 2-thiobarbituric acid reacting substances (TBARS) with the Kamyshnikov (2004) method for determining the malonic dialdehyde (MDA) concentration and described in the paper by Tkachenko et al. (2022). This method is based on the reaction of the degradation of the lipid peroxidation product, MDA, with 2-thiobarbituric acid (TBA) under high temperature and acidity to generate a colored adduct that is measured spectrophotometrically. The nmol of MDA per mL was calculated using $1.56 \cdot 10^5$ $mM^{-1}.cm^{-1}$ as the extinction coefficient.

The carbonyl derivatives of oxidative modification of protein assay

To evaluate the protective effects of the extracts derived from pseudobulbs of *D. parishii* against free radical-induced protein damage in equine erythrocytes, a carbonyl derivatives content of protein oxidative modification (OMP) assay based on the spectrophotometric measurement of aldehydic and ketonic derivatives in the erythrocyte suspension and plasma was performed. The rate of protein oxidative destruction was estimated from the reaction of the resultant carbonyl derivatives of amino acid reaction with 2,4-dinitrophenylhydrazine (DNFH) as described by Levine and co-workers (1990) and as modified by Dubinina et al. (1995) and described in the paper by Tkachenko et al. (2022). DNFH was used for determining carbonyl content in soluble and insoluble proteins. The carbonyl content was calculated from the absorbance measurement at 370 nm and 430 nm, and an absorption coefficient of 22,000 $M^{-1} \cdot cm^{-1}$. Carbonyl groups were determined spectrophotometrically from the difference in absorbance at 370 nm (aldehydic derivatives, OMP_{370}) and 430 nm (ketonic derivatives, OMP_{430}).

Measurement of total antioxidant capacity

The total antioxidant capacity (TAC) level in the samples was estimated by measuring the 2-thiobarbituric acid reactive substances (TBARS) level after Tween 80 oxidation. This level was determined spectrophotometrically at 532 nm (Galaktionova et al., 1998) and described in the paper by Tkachenko et al. (2022). The level of TAC in the sample (%) was calculated according to the absorbance of the blank samples.

Statistical analysis

Statistical analysis of the data obtained was performed by employing the mean ± S.E.M. All variables were tested for normal distribution using the Kolmogorov-Smirnov and Lilliefors test ($p > 0.05$). The significance of differences between the OMP level (significance level, $p < 0.05$) was examined using the Kruskal-Wallis one-way analysis of variance (Zar, 1999). The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., USA) (Zar, 1999).

Results and discussion

Levels of TBARS in the equine erythrocytes after *in vitro* incubation with extracts derived from seven parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) were presented in Figure 1.

Our results revealed that extracts derived from first three parts of pseudobulbs of *D. parishii* after incubation with equine erythrocyte samples caused to statistically significant increase in the TBARS levels (50.78 ±7.17 $nmol \cdot mL^{-1}$, 59.23 ±11.45 $nmol \cdot mL^{-1}$, and 52.77 ±9.59 $nmol \cdot mL^{-1}$, respectively) (by 41.5%, 65.1%,

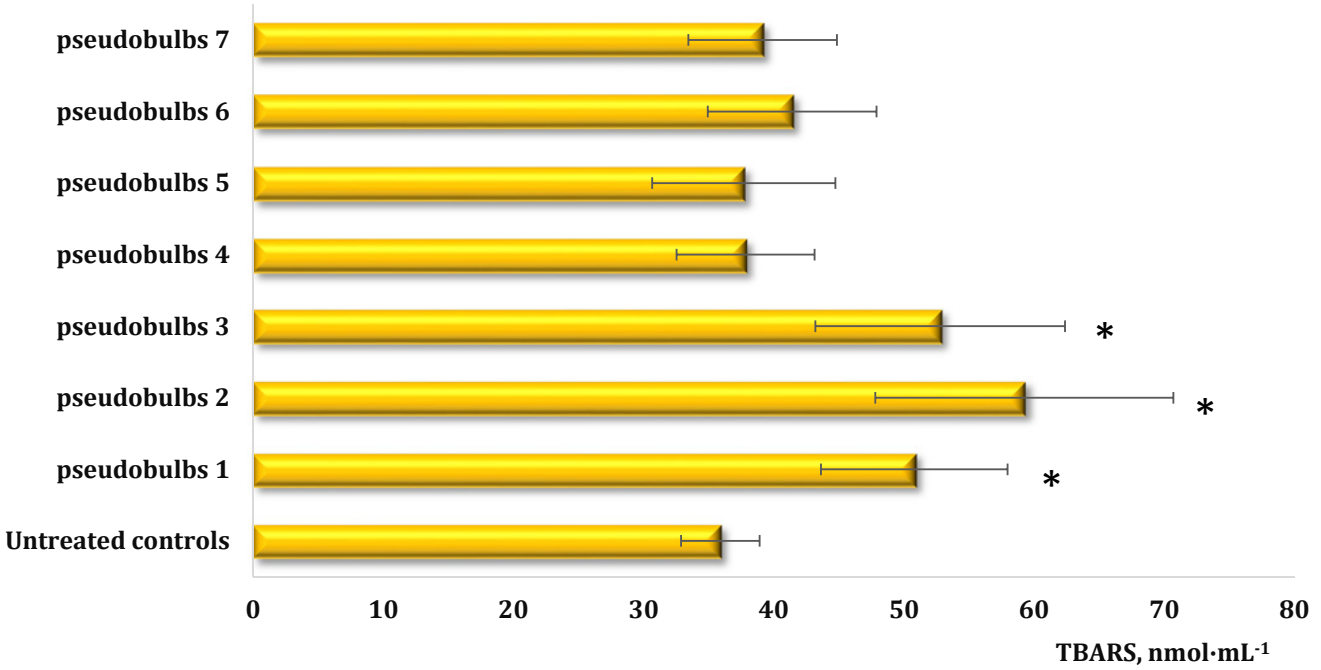

Figure 1 Levels of TBARS in the equine erythrocytes after *in vitro* incubation with extracts derived from seven parts of pseudobulbs of *Dendrobium parishii* Rchb.f. (beginning from the base of the growing tip of the rhizome)
*– changes were statistically significant (p <0.05) compared to untreated control

and 47.1%, p <0.05) compared to untreated samples (35.88 3.02 nmol·mL^{-1}). Extracts derived from last four parts of pseudobulbs of *D. parishii* after incubation with equine erythrocyte samples caused to statistically non-significant increase in the TBARS levels (37.82 ±5.30 nmol·mL^{-1}, 37.68 ±7.04 nmol·mL^{-1}, 41.40 ±6.48 nmol·mL^{-1}, and 39.13 ±5.71 nmol·mL^{-1}, respectively) (by 5.4%, 5%, 15.4%, and 9.1%, p >0.05) compared to untreated samples (35.88 ±3.02 nmol·mL^{-1}) (Figure 1).

Levels of aldehydic and ketonic derivatives of OMP in the equine erythrocytes after *in vitro* incubation with extracts derived from seven parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) were presented in Figure 2.

On the other hand, the contents of aldehydic derivatives of OMP in the erythrocyte samples after incubation with an extract derived from the first part of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) decreased to (28.14 ±1.08 nmol·mL^{-1}) compared to the untreated samples (31.16 ±1.89 nmol·mL^{-1}) (by 9.7%, P <0.05). Extracts derived from the last two parts of pseudobulbs of *D. parishii* after incubation with equine erythrocyte samples caused to statistically non-significant decrease in the levels of aldehydic derivatives of OMP to (29.30 ±1.35 nmol·mL^{-1}, and 29.03 ±1.08 nmol·mL^{-1}, respectively) (by 6%, and 6.8%, p >0.05) compared to untreated samples. Extracts derived from the second to fifth parts of pseudobulbs of *D. parishii* after incubation with equine erythrocyte samples caused to statistically non-significant increase in the levels of aldehydic derivatives of OMP by 3.9%, 2.6%, 5.6%, and 1% (p >0.05) for second, third, fourth, and fifth parts of pseudobulbs of *D. parishii*, respectively (Figure 2A).

Moreover, the contents of ketonic derivatives of OMP in the erythrocyte samples after incubation with extracts derived from all parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) decreased to (35.79 ±1.70 nmol·mL^{-1}, 37.61 ±2.01 nmol·mL^{-1}, 33.78 ±1.31 nmol·mL^{-1}, 33.53 ±1.49 mol·mL^{-1}, 36.56 ±2.03 nmol·mL^{-1}, 32.36 1.57 nmol·mL^{-1}, and 35.21 ±1.66 nmol·mL^{-1}) compared to the untreated samples (39.47 ±2.20 nmol·mL^{-1}) (by 9.3%, p >0.05 for extract from first pseudobulbs; by 4.7%, p >0.05 for extract from second pseudobulbs; by 14.4%, p <0.05 for extract from third pseudobulbs; by 15%, p <0.05 for extract from fourth pseudobulbs; by 7.4%, p >0.05 for extract from fifth pseudobulbs; by 18%, p <0.05 for extract from sixth pseudobulbs; by 10.8%, p >0.05 for extract from seventh pseudobulbs) (Figure 2B).

Levels of total antioxidant capacity (TAC) in the equine erythrocytes after *in vitro* incubation with extracts derived from pseudobulbs of *Coelogyne pandurate* were presented in Figure 3.

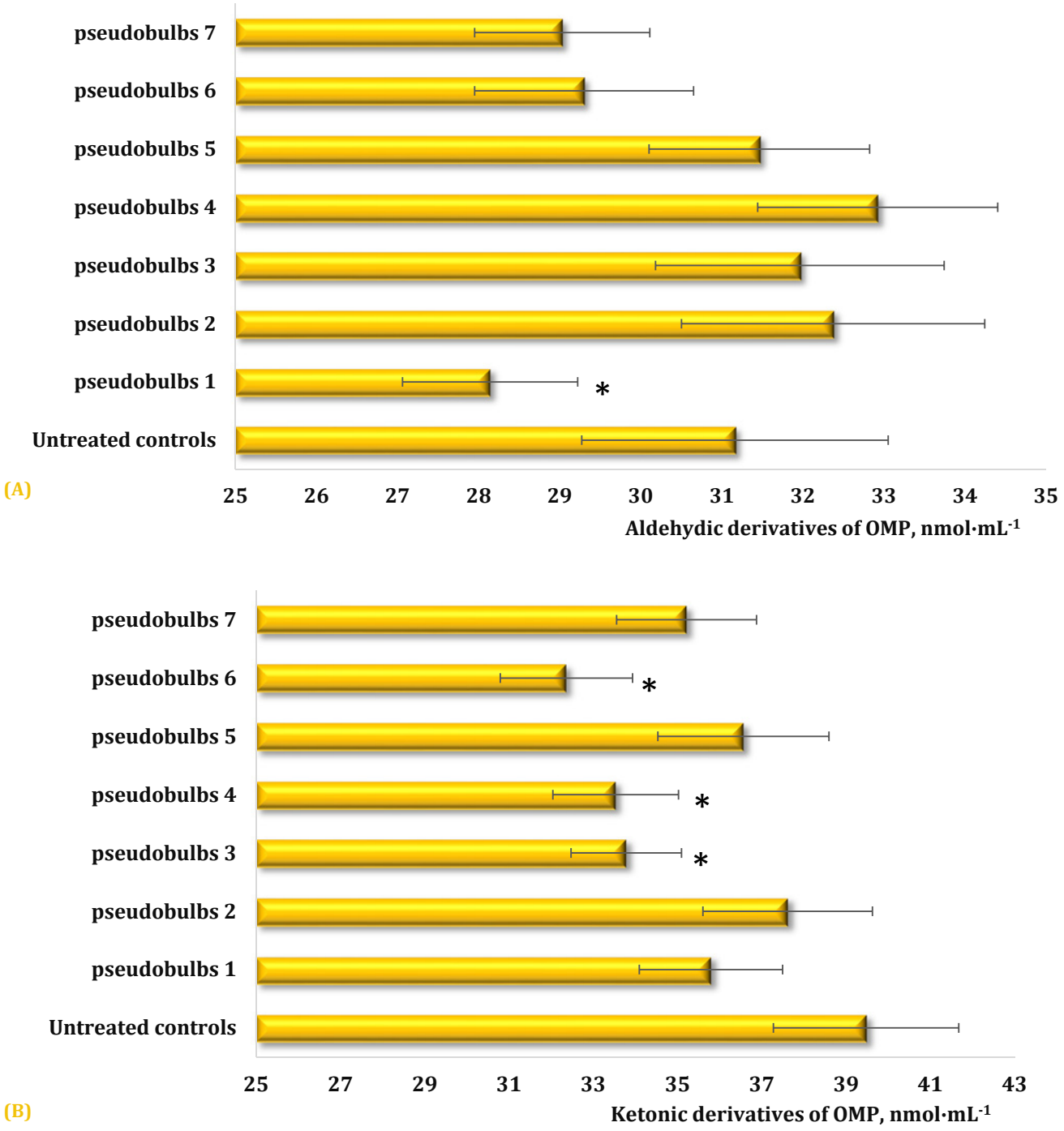

Figure 2 Levels of aldehydic and ketonic derivatives of OMP in the equine erythrocytes after *in vitro* incubation with extracts derived from seven parts of pseudobulbs of *Dendrobium parishii* Rchb.f. (beginning from the base of the growing tip of the rhizome)
*– changes were statistically significant ($p < 0.05$) compared to untreated control

Our results revealed that extracts derived from the first and sixth parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) after incubation with erythrocyte samples caused to increase in the TAC level (by 2.5% and 2.9%, p >0.05). On the other hand, extracts derived from the second, third, fourth, fifths, and seventh parts of pseudobulbs of *D. parishii* after incubation with erythrocyte samples caused to statistically non-significant decrease in the TAC level (by 1.9%, 8.4%, 13.4%, 8.1%, and 1.9%, p >0.05, respectively) (Figure 3).

Similarly, *in vitro* and *in vivo* studies reveal the antioxidant properties of *Dendrobium* plants. Polysaccharides of *Dendrobium* exhibit a variety of biological effects, including immunomodulatory, anti-tumor, gastro-protective, hypoglycemic, anti-inflammatory, hepatoprotective, and vasodilating effects (Chen et al., 2021). For example, *Dendrobium* potential effects with future perspectives for needed future research to maximize the use of bioactive compounds from *Dendrobium* for digestive tract disease treatment (Wu et al., 2023). Recent studies have shown that polysaccharide is one of the main biologically active components in *D. officinale* (He et al., 2022). Polysaccharides of *D. officinale* (DOP) can be considered an effective healthcare product for the treatment of precancerous lesions of gastric cancer and perhaps someday play a critical role in combatting gastric cancer. The research group of Zhao and co-workers has found that *D. officinale* extraction can prevent gastric carcinogenesis in rats through upregulating Bax and downregulating such factors as antiapoptotic B cell lymphoma 2 (Bcl-2), epidermal growth factor (EGF), epidermal growth factor receptor (EGFR), and sphingosine-1-phosphate (S1P) (Zhao et al., 2015, 2017). Further analysis revealed that *D. officinale* extracts could regulate the levels of 8-hydroxy-deoxyguanosine (8-OHdG), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-PX) in plasma and cytokines related to carcinogenesis (Zhao et al., 2016). Polysaccharides, the main effective part of *D. officinale*, have been reported to harbor anticancer effects on gastric cancer cells and protective effects on experimental gastric ulcers in mice (Zeng et al., 2017; Zhang et al., 2018). *D. officinale* polysaccharides (DOP) prevent 1-methyl-3-nitro-1-nitrosoguanidine (MNNG)-induced precancerous lesions of gastric cancer (PLGC) along with subsequent liver and kidney damage. The protective effects of DOP are associated with the reduction of 8-OHdG levels as well as the activation of the NRF2 pathway and its related antioxidant enzymes, heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase-1 (NQO-1) (Zhao et al., 2019).

The *Dendrobium nobile* Lindl polysaccharides as promising therapeutic candidates for UVB-induced

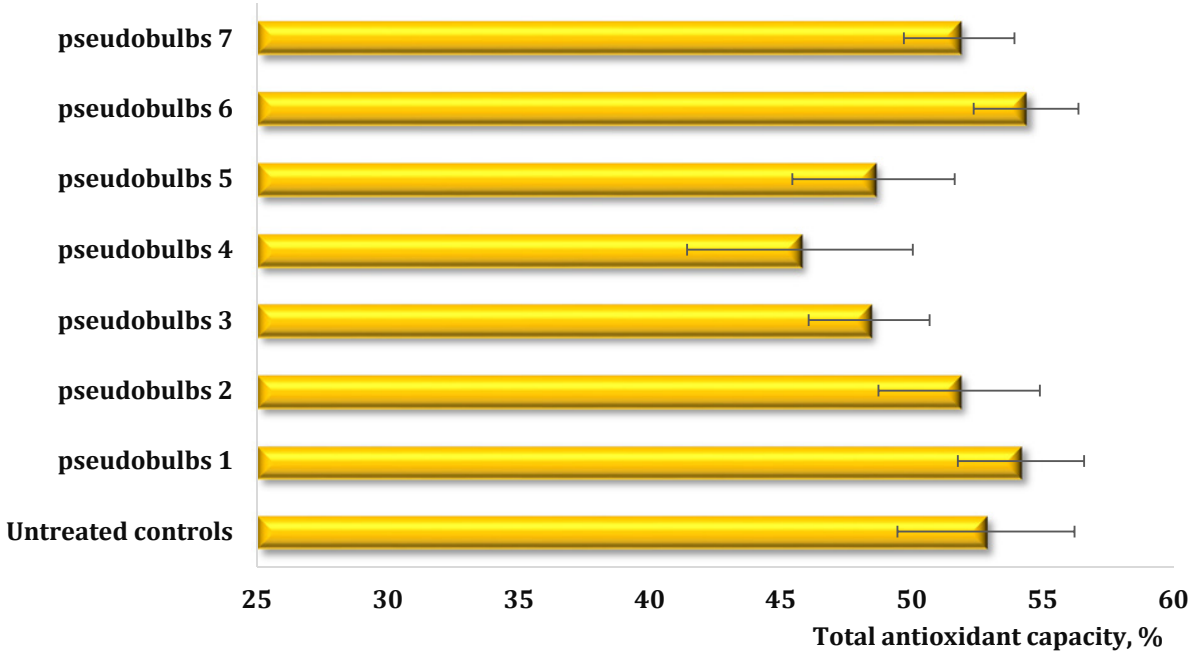

Figure 3 Levels of total antioxidant capacity (TAC) in the equine erythrocytes after *in vitro* incubation with extracts derived from seven parts of pseudobulbs of *Dendrobium parishii* Rchb.f. (beginning from the base of the growing tip of the rhizome)
*– changes were statistically significant (p <0.05) compared to untreated control

photodamage. Li et al. (2022) investigated the antagonistic effect of *Dendrobium nobile* Lindl. polysaccharides (DNLP) on UVA-induced photoaging of Human foreskin fibroblasts (HFF-1) and explore its possible anti-aging mechanisms. Results of these authors revealed that UVA irradiation reduced the viability, lifespan, and proliferation of HFF-1 cells, increased ROS and lipid peroxidation, and decreased the activities of free radical scavenging enzyme systems SOD, CAT, and GSH-Px. DNLP treatment can reverse UVA damage, reduce SA-β-Gal expression, reduce phosphorylation activation of the JNK/c-Fos/c-Jun pathway, and inhibit MMP-1, MMP-2 MMP-3, and MMP-9 protein expression (Li et al., 2022). DOP ameliorated UVB-induced oxidative damage and apoptosis in HaCaT cells via the regulation of MAPKs (Long et al., 2023).

The antioxidant activities of the polysaccharide *in vitro* assay indicate that DOP has a good scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, higher scavenging activity of hydroxyl radical and metal chelating activities (Luo et al., 2016). Zhang et al. (2022) compared the antioxidant activities and polysaccharide characterization of fresh and dry *D. officinale*. This study compared their antioxidant properties both *in vitro* and *in vivo*, and the molecular weight arrangement and monosaccharide composition of the fresh *D. officinale* polysaccharides (FDOPs) and the dried *D. officinale* polysaccharides (DDOPs). The results showed that the FDO and its polysaccharides had more significant effects on scavenging DPPH, ABTS, and hydroxyl radicals than the DDO. In addition, both the FDO and DDO significantly reduced lipid peroxidation levels and increased the SOD, T-AOC, CAT, and GSH levels in mice with acute liver damage caused by CCl_4, while the FDO and its polysaccharides were more effective. Histopathological analysis further verified the protective effect of the *Dendrobium* polysaccharides on CCl_4-induced liver injury (Zhang et al., 2022). The study of Lin et al. (2018) also revealed that DOP treatment exerted potentially hepatoprotective effects against APAP-induced liver injury. The decrease in alanine transaminase (ALT) and aspartate transaminase (AST) levels in the serum and reactive oxygen species (ROS), malondialdehyde (MDA), and myeloperoxidase (MPO) contents in the liver, as well as the increases in glutathione (GSH), catalase (CAT), and total antioxidant capacity (T-AOC) in the liver, were observed after DOP treatment. DOP treatment significantly induced the dissociation of Nrf2 from the Nrf2-Keap1 complex and promoted the Nrf2 nuclear translocation. Subsequently, DOP-mediated Nrf2 activation triggered the transcription and expressions of the glutamate-cysteine ligase catalytic (GCLC) subunit, glutamate-cysteine ligase regulatory subunit (GCLM), heme oxygenase-1 (HO-1), and NAD(P)H dehydrogenase quinone 1 (NQO1) in APAP-treated mice (Lin et al., 2018).

The purified polysaccharide from *D. officinale* presented significant immune-modulating activities involving ERK1/2 and NF-κB (He et al., 2016). *D. officinale* and its polysaccharides can significantly enhance cellular immunity and nonspecific immunity in mice (Liu et al., 2011). Humoral immunity was also enhanced after oral administration of *D. officinale*, but the polysaccharides had no influence. Both *D. officinale* and its polysaccharides markedly increased IFN-γ production by murine splenocytes (Liu et al., 2011). Also, *Dendrobium tosaense* Makino (syn. *D. officinale* Kimura & Migo) substantially boosted the population of splenic natural killer (NK) cells, NK cytotoxicity, macrophage phagocytosis, and cytokine induction in splenocytes (Yang et al., 2014). Bioassay using mouse macrophage cell line RAW264.7 indicated that DOP and its two subfractions enhance cell proliferation, TNF-α secretion, and phagocytosis in a dose-dependent manner. They also induced the proliferation of lymphocytes alone and with mitogens (Wei et al., 2016). DOP upregulated messenger RNA (mRNA) expression of anti-inflammatory/antioxidant proteins such as Nrf2 (nuclear factor erythroid 2-related factor), heme oxygenase-1 (HO-1), and NAD(P)H: quinone oxidoreductase (NQO1) in the liver (Chu et al., 2022).

We also investigated the changes in the oxidative stress biomarkers using the model of equine erythrocytes and plasma to evaluate the antioxidant activities of the aqueous extract derived from leaves of *Coelogyne brachyptera* Rchb.f. (Buyun et al., 2022). Results of our study revealed that erythrocytes were more sensitive to the action of an extract derived from leaves of *C. brachyptera*. The levels of aldehydic and ketonic derivatives of oxidatively modified proteins in the treated erythrocytes were significantly decreased, while these parameters were unchanged in the equine plasma. The treatment of equine erythrocytes by extract derived from leaves of *C. brachyptera* increased lipid peroxidation. On the other hand, plasma TBARS level after treatment by extract derived from leaves of *C. brachyptera* was at the same level as in untreated controls. The level of total antioxidant capacity was not-significantly changed after treatment both in equine plasma and erythrocytes (Buyun et al., 2022). Assessment of oxidative stress biomarkers

in the equine blood and the muscle tissue of rainbow trout (*Oncorhynchus mykiss* Walbaum) after *in vitro* incubation with leaf extract obtained from *D. parishii* was conducted in our previous study (Buyun et al., 2019, 2020).

Conclusions

The current study was conducted to investigate the antioxidant properties of extracts derived from different pseudobulbs of *Dendrobium parishii* Rchb.f. using biomarkers of oxidative stress in the equine erythrocytes after *in vitro* treatment with the extracts. The antioxidant properties of extracts derived from different pseudobulbs of *D. parishii* using biomarkers of oxidative stress in the equine erythrocytes after *in vitro* treatment with the extracts revealed that extracts derived from different pseudobulbs of *D. parishii* exhibited varying activity. Extracts derived from the first, second, and third pseudobulbs of *D. parishii* increased lipid peroxidation after *in vitro* treatment of equine erythrocytes. Extracts derived from the first, sixth, and seventh pseudobulbs of *D. parishii* caused to decrease in the levels of aldehydic derivatives of OMP. On the other hand, ketonic derivatives of OMP in the erythrocyte samples after incubation with extracts derived from all parts of pseudobulbs of *D. parishii* (beginning from the base of the growing tip of the rhizome) were decreased. Moreover, extracts derived from the first and sixth parts of pseudobulbs of *D. parishii* after incubation with erythrocyte samples caused to increase in the TAC levels. The study of extracts derived from *D. parishii* supports its favorable biological activities and lays a strong foundation for further exploration of its structure-activity relationships and activity development, providing experimental data for the development and utilization of extracts of *D. parishii*.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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