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Technical note

Assessment of antioxidant activities of *Epidendrum secundum* Jacq, a terrestrial orchid from southern Ecuadorian highlands

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ABSTRACT

A comparison of antioxidant and antibacterial activities among *in vitro* cultured seedlings and Orchidarium plant specimens of *Epidendrum secundum* Jacq, a terrestrial orchid from southern Ecuadorian highlands, was performed. The aim of this work was to evaluate the enzymatic and non-enzymatic antioxidant compounds production from *in vitro* cultured seedlings and Orchidarium plants from the orchid species under study. For this purpose, a group of spectrophotometric assays was set up, comparing the free radical scavenging capacity, phenolic and flavonoids content from seedling leaves and plant leaves organic extracts, as non-enzymatic antioxidant indicators. In addition, the antibacterial activity of the organic plant extracts was evaluated versus *Escherichia coli* and *Staphylococcus aureus*. Catalase and ascorbate peroxidase activities were assessed as enzymatic antioxidant systems in aqueous extracts. The results showed higher non-enzymatic antioxidant activity for leaves from *Epidendrum secundum* Orchidarium plants compared with leaves from *in* vitro seedling cultures. The organic extracts from the orchid under study displayed moderate antibacterial activity against *Staphylococcus aureus*. In the enzymatic antioxidant evaluation, catalase activity was significantly higher at *in vitro* conditions.

The results from this work allow us to highlight the potential of *Epidendrum secundum* Jacq as a source of bioactive metabolites with promising antioxidant and antibacterial activities.

1. Introduction

Plant secondary metabolites play important roles in plant growth and development, including signaling, pigmentation, pollinator attraction, structural support, defense against herbivores and pathogens, protection from UV radiation and modulating enzymatic actions (Lin et al., 2016). Some of these compounds are distinctive of a plant genus (Taiz et al., 2015). Plant secondary metabolites are classified in three main groups: phenolic compounds, alkaloids and terpenes (Dias et al.., 2012). The production of secondary metabolites is related to plant environmental conditions and is increased by factors such as air pollution, oxidant-forming herbicides, heavy metals, drought, heat stress, cold stress, wounding and UV radiation (Bray et al., 2000).

Reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals, hydrogen peroxide and singlet oxygen are formed during certain redox reactions, at incomplete reduction of oxygen and oxidation of water by the mitochondrial or chloroplast electron transfer chain (Mittler, 2002; Apel and Hirt, 2004; Gill and Tuteja, 2010). In order to control ROS proliferation, plant metabolism produces non-enzymatic and enzymatic antioxidants. These compounds and enzymes vary among specific subcellular compartments (Quan et al., 2008). Reactive oxygen species production is regulated by phenolic compounds, including α-tocopherols, and enzymes such as ascorbate peroxidase (APX) and catalase (CAT) (Jadhav et al., 1996). Plants display different grades of resistance to environmental stress conditions. The representatives of the Orchidaceae family are able to grow and develop in temperate and tropical areas and are adapted to various growth environments, including epiphytic and lithophytic (Arditti, 1992).

The Orchidaceae family is one of the most diverse plant families with over 28,000 reported species (Christenhusz and Byng, 2016). Orchids are distributed worldwide, except in desert and polar areas (Chase, 2005). Each year, approximately 200 new species are reported, primarily in tropical zones (Chase et al., 2015; Givnish et al., 2015). Orchids are valuable plants because of their ornamental value and medicinal properties (Gutiérrez, 2010). According to the IUCN red list, 38.5% of orchid species are critically endangered or endangered because of their commercial value and habitat loss (Favre et al., 2020). In addition, world orchid populations are under continual threat due to climate change (Schödelbauerová et al., 2009; Kottke et al., 2010; Waud et al., 2016).

Epidendrum is an orchid genus that includes epiphytic and terrestrial species. It is present from southern United States to northern

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Argentina (Dunsterville and Garay, 1979; Rasmussen, 1995; Riofrío et al., 2013; Thwala et al., 2013). *Epidendrum* is the most diverse Orchidaceae genus in South America (Endara et al., 2017). The antioxidant and anti-inflammatory activity of organic extracts from *Epidendrum nocturnum* Jacq and *Epidendrum coryophorum* Kunth have been evaluated, showing that this genus is an important source of bioactive compounds (Gonzalez Mera et al., 2020; Mencias et al., 2021).

In Ecuador, *Epidendrum secundum* Jacq grows in southern highlands and is used in traditional medicine to treat fever and nervous ailments (Ríos et al., 2007). Currently, this species has been studied in the frame of its germination and relationship with mycorrhizal fungi (Durán et al., 2019; de Araújo Amatuzzi et al., 2020). However, the antioxidant activity from this plant is still not evaluated.

The aim of the present investigation was to assess the enzymatic and non-enzymatic antioxidant strategies from *in vitro* cultured seedlings and Orchidarium plants from *Epidendrum secundum* Jacq. For this purpose, a set of enzymatic and non-enzymatic assays was set up. The phenolic content, the flavonoid content and the scavenging of the free radical $ABTS^{\bullet+}$ were tested in organic extracts. Moreover, the ascorbate peroxidase and catalase activities were evaluated in aqueous extracts. To our best knowledge, this is the first report of the antioxidant potential of *Epidendrum secundum* Jacq, a terrestrial orchid with traditional uses in Ecuador.

2. Materials and methods

2.1. In vitro *seed culture,* in vitro *seedling culture and plant leaves collection*

Mature closed capsules were obtained from *Epidendrum secundum* Jacq plants growing under environmental conditions at the Orchidarium ascribed to Universidad de Cuenca, Ecuador from October to December, 2021 (Fig. 1). The capsules were the source of seeds for establishing *in vitro* cultures at the Micropropagation Laboratory of the Orchidarium, Universidad de Cuenca. The capsules were washed under tap water, immersed in a 5% sodium hypochlorite solution for 10 min, rinsed in sterile distilled water for 2 min and cut longitudinally by utilizing a sterile surgical blade in order to collect seeds. Seeds viability was evaluated by using an optical microscope (Olympus, Japan). The presence of thick and round embryos indicated a good viability. Approximately 1 g of seeds was inoculated in glass jars that contained a modified Phitamax™ Orchid Maintenance Medium (Sigma Aldrich, San Luis, Mo. USA). The medium was enriched with coconut water and a vitamin solution composed of thiamine, pyridoxine, glycine, folic acid and calcium pantothenate. Afterwards, 1 ml of distilled sterile water was added to each seed culture. *In vitro* seed cultures were maintained at 20–25 °C, 16/8 photoperiod supplied by cool white fluorescent light and 65–75% relative humidity according to the protocol reported by Ali et al. (2005) during 4 months. Subsequently, the seedlings were cultured in glass jars that included a modified Phitamax™ Orchid Maintenance Medium (Sigma Aldrich, San Luis, Mo. USA). The medium was enriched with a blended banana solution (fruit:water 1:1 w/v), the vitamin solution mentioned previously, 1-naphthaleneacetic acid (2.5 mg L^{-1}) and 6-benzylaminopurine (2 mg L^{-1}). *In vitro* seedling cultures were preserved under the same conditions of *in vitro* seed cultures for 8 months (Fig. 2.). On the other hand, leaves from 4 year old *E. secundum* orchid plants growing under environmental conditions at the Universidad de Cuenca Orchidarium, were collected in July 2022.

2.2. *Sample preparation and extraction procedure for total phenolic content, total flavonoids content, antioxidant and antibacterial activity from plant leaves and* in vitro *cultured seedling leaves*

In order to prepare organic and aqueous extracts, leaves from 4-year-old *E. secundum* plants and leaves from one-year-old *in vitro* cultured seedlings were processed for extract preparation according to Yuzuak et al., (2018) with few modifications. Both plant and seedling leaves were lyophilized and ground. The plant material (0.3 g) was placed in separate Falcon® conical centrifuge tubes, mixed with 10 mL of anhydrous methanol, shaken for one minute with a vortex mixer and agitated in an ultrasonic bath for 10 min. The tubes were centrifuged at 6000 rpm for 15 min. Supernatants were recovered and placed in fresh Falcon® conical tubes. Afterwards, 10 mL of methanol were added to the supernatants. Subsequently, the tubes were centrifuged under the conditions mentioned previously. Extracts

Fig. 1. *Epidendrum secundum* Jacq inflorescence, from the Orchidarium, Universidad de Cuenca, Ecuador.

Fig 2. *In vitro* seedling culture from *Epidendrum secundum* Jacq, grown in Phytamax™ modified medium, from the Micropropagation Laboratory, Orchidarium, Universidad de Cuenca, Ecuador.

obtained from plant and seedling leaves were concentrated "*in vacuo*". In order to remove chlorophyll, the dry extracts were re-suspended in 1 mL of chloroform, and centrifuged at 6000 rpm for 5 min. After that, the organic phase of each extract was eliminated. The entire procedure to remove chlorophyll was performed three times. Finally, the extracts were evaporated to dryness, re-suspended in 1 mL of methanol, centrifuged at 10,000 rpm for five minutes and stored at 4 °C for further analysis.

2.3. Total phenolic determination

Total phenolic content of the extracts was determined by following Stankovic et al. (2011) protocol with few modifications. Concisely, 25 μL of each extract were mixed with 25 μL of Folin Ciocalteau reagent, in a 96-well microtiter plate. Each mixture was allowed to stand for three minutes. Subsequently, 500 μL of sodium carbonate solution (7.5%) were added to the reaction mixtures. Next, 350 μL of distilled water were added to the reaction mixtures. Then, the reaction mixtures were stored in darkness at room temperature for one hour. Absorbance measurements were taken at 750 nm in an EPOCH® microplate spectrophotometer (Agilent, Santa Clara, CA, United States). A methanol aliquot of 300 μL was utilized as a blank. A calibration curve was prepared using gallic acid as a standard compound. Total phenolic content of the extracts was expressed as milligrams of gallic acid equivalent per gram of dry weight.

2.4. Total flavonoids content

Total flavonoids content from the extracts under study was quantified based on Chand et al. (2016) protocol with slight modifications. Reaction mixtures formulated with 100 μL of the extracts and 100 μL of $AlCl_3$ (2%, in methanol), were placed in a 96 well microtiter plate. The reaction mixtures were stored at room temperature for one hour. Absorbance measurements were taken at 415 nm, in a microplate spectrophotometer. Quercetin was used as a standard compound to generate a calibration curve. Total flavonoids content of the extracts was expressed as milligrams of quercetin equivalent per gram of dry weight.

2.5. $ABTS^{\bullet+}$ scavenging antioxidant capacity assessment

The stable free radical $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) was used to assess the antioxidant capacity from the extracts, according to Mingle and Newsome (2020) protocol with few modifications. For this purpose, a 0.7 mM $ABTS^{\bullet+}$ solution was made by dissolving a ten milligrams $ABTS^{\bullet+}$ tablet (Sigma Aldrich, San Luis, Mo. USA) in 14 mL of phosphate buffered saline, pH 7. Additionally, a 2.45 mM potassium persulfate solution was prepared in distilled water. Afterwards, that, a working reagent was prepared, mixing seven milliliters of the $ABTS^{\bullet+}$ solution with seven milliliters of the potassium persulfate solution.

A 96-well microtiter plate was prepared to assess the antioxidant activity from orchid organic extracts. An ascorbic acid calibration curve, ranging from 1000 to 15.62 $\mu g\ mL^{-1}$ was developed by triplicate. An aliquot of 50 μL of the organic extracts was placed in the microtiter plate and diluted with 50 μL of PBS buffer, reacting with 100 μL of working reagent at dark conditions for 15 minutes. The absorbance was read at 734 nm, in a microplate spectrophotometer. The calibration curve wells were treated under the same conditions as the extracts.

2.6. Antibacterial activity

The organic extracts from fresh plant and seedling leaves and *in vitro* cultures from the orchid under study were submitted to an antibacterial assay, according to Cazar et al. (2005) and Requena et al. (2019). A microdilution assay was performed towards against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). The bacterial strains were isolated from clinical samples. One colony was resuspended in LB Broth medium (Sigma) and incubated for 24 h at 37 °C. The inoculums were adjusted to the Mc Farland scale 1 (3×10^8 CFU mL^{-1}).

A spectrophotometric assay was performed, using a 96-well sterile microtiter plate. Stock solutions from the organic extracts (12,500 $\mu g\ mL^{-1}$) were dissolved in methanol. The stock solutions were diluted with sterile LB broth medium, to a concentration of 500 $\mu g\ mL^{-1}$. A serial dilution was developed in the microplates, in a concentration rank from 250 to 1.95 $\mu g\ mL^{-1}$. The microwells were seeded with the bacterial inoculums. The plates were incubated at 37 °C for 24 h. The bacterial growth was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The absorbance of the microwells were measured in a microtiter spectrophotometer EPOCH (λ = 515 nm).

2.7. Extraction procedure for antioxidant enzyme assays

Extraction process was implemented according to Elavarthi and Martin (2010) protocol with slight modifications. Approximately 1.6 g of plant leaves from *E. secundum* were ground. The same amount of *in vitro* seedling leaves, previously washed in order to remove maintenance medium, were weighed and ground. Subsequently, the plant material was homogenized in 7.2 mL of 0.2 M potassium phosphate buffer with a pH of 7.8 plus 0.1 mM EDTA respectively. Next, the samples were centrifuged at 4000 rpm for 20 min at 4 °C. Afterwards, the supernatant of each sample was removed and kept on ice, the pellet resuspended in 0.8 mL of the potassium phosphate buffer, and the suspension centrifuged under the same conditions previously described. Supernatants were combined and kept on ice to determine ascorbate peroxidase activity and catalase activity.

2.8. Ascorbate peroxidase activity determination

Ascorbate peroxidase (APX) activity was determined based on Nakano and Asada (1981) and Flocco and Giuletti (2007) protocols with minor modifications. A 3 mL assay mixture was prepared by adding 800 μL of 0.5 mM sodium ascorbate, 1.5 mL of 50 mM potassium phosphate buffer (pH=7.0), 100 μL of the plant leaves extract, 100 μL of 0.1 mM H_2O_2 and distilled water until reach the final volume. H_2O_2 was added at last to trigger the reaction. The same process was performed with the *in vitro* seedling leaves extract. The decline of absorbance in the extracts was measured every 30 seconds during three minutes at a wavelength of 290 nm, in a spectrophotometer (Ciba Corning®). An extinction coefficient of 2.8 mM^{-1} cm^{-1} was employed to determine the APX activity. The APX activity was expressed in units per gram of plant leaves weight and *in vitro* seedlings (U g^{-1} fw). One enzymatic unit of APX was arbitrarily established as the quantity of enzyme that causes a modification in absorbance of 0.003 min^{-1} at 290 nm at 25 °C. The APX activity was calculated by utilizing the following formula:

$$\frac{U}{mL} = \left(\frac{V_f}{V_o} x\ a\ x\ l\right)\frac{\Delta Abs}{min} \quad (1)$$

Where: V_f is the final volume in each assay mixture, V_o is the volume of every extract in its assay mixture, a is the extinction coefficient of sodium ascorbate at 290 nm, l is the light path in cm, and ΔAbs min^{-1} is the decrease in absorbance per minute. The results were normalized to U g^{-1} of fresh weight by employing this formula:

$$\frac{U}{g} = \frac{U}{mL}\left(\frac{V_e}{fw_e}\right) \quad (2)$$

Where V_e is the volume of buffer utilized to prepare each extract, and fw_e is the fresh weight of plant leaves and *in vitro* cultured seedlings used to make every extract.

2.9. Catalase activity determination

Catalase (CAT) activity was established based on Aebi (1984) and Flocco and Giuletti (2007) protocols with slight modifications. A 3 mL assay mixture was made by adding 50 μL of the fresh extract, 500 μL of 7.5 mM H_2O_2, 1.5 mL of 50 mM potassium phosphate buffer (pH=7.0), and distilled water until reach the final volume. The H_2O_2 was added at last to trigger the reaction. The same procedure was carried out with the *in vitro* extract. The decline of absorbance in the extracts was measured every 30 s during three minutes at a wavelength of 240 nm. An extinction coefficient of 40 mM^{-1} cm^{-1} was utilized to determine the CAT activity. The CAT activity was expressed in units per gram of fresh weight of plant leaves and *in vitro* cultured seedlings (U g^{-1} fw). One enzymatic unit of CAT was arbitrarily designated as the quantity of enzyme that induces a variation in absorbance of 0.005 min^{-1} at 240 nm at 25 °C. The CAT activity was calculated by using the Eq. (1), where: V_f is the final volume in every assay mixture, V_o is the volume of each extract in its assay mixture, a is the extinction coefficient of H_2O_2 at 240 nm, l is the light path in cm, and ΔAbs min^{-1} is the decrease in plant leaves and *in vitro* cultured seedlings absorbance per minute. The results were normalized to U g^{-1} fw by employing the Eq. (2).

2.9. Statistical analysis

The results are reported as the average and standard deviation for 3 replicates. Statistical analysis of results was performed through a t-test. The graphics were generated with GraphPad Prism software (version 8.0, 2019, USA).

3. Results

3.1. Non enzymatic antioxidant and antibacterial activity

The spectrophotometric *in vitro* assays performed on organic extracts from E. *secundum* plant leaves and *in vitro* cultured seedlings allowed us to estimate the total phenolic and flavonoids content. The fresh leaves from *E. secundum*. produced higher phenolic and flavonoids content in comparison with *in vitro* leaves. The free radical scavenging capacity from the extracts was assessed towards the $ABTS^{\bullet+}$ radical. In this assay, the fresh leaves extracts display higher antioxidant capacity than the *in vitro* extract. In the antibacterial assay, the organic extracts from *E. secundum* presented moderate activity towards *S. aureus* and were inactive towards *E. coli*. The results from the non-enzymatic antioxidant and antibacterial assessments are presented in Table 1.

In order to evaluate the enzymatic antioxidant activity from fresh and *in vitro* leaf aqueous extracts of the orchid under study, we quantified ascorbate peroxidase and catalase activity according to the methodology described in Section 2.7 and Section 2.8. Ascorbate peroxidase activity was significantly high in plant leaves, compared to seedling leaves (257, 39 $U.g^{-1}$, vs 178,63 $U.g^{-1}$). The results from the catalase activity assay displayed a higher activity for the seedling leaf extract (16,44 $U.g^{-1}$), compared to fresh leaf extract (6,78 $U.g^{-1}$). Results are shown in Fig. 3.

4. Discussion

In the present study, the antioxidant activity displayed by *Epidendrum secundum* fresh and *in vitro* seedling leaves was evaluated in a pool of enzymatic and non-enzymatic spectrophotometric assays. The results from the non-enzymatic assays allowed us to assess the phenolic compounds and flavonoids production, and relate them with the quenching of the stable free radical $ABTS^{\bullet+}$.

Table 1
Total phenolic content, total flavonoids, antioxidant and antibacterial capacity from organic extracts obtained from *in vitro* seedling leaves and plant leaves from *Epidendrum secundum*. (*significant differences assessed between fresh and *in vitro* leaves via t-test. NA: Not active)

	Organic extracts		
Bioassays	*E. secundum* plant leaves	*E. secundum in vitro* seedling leaves	*p* value*
Total phenolic content (mg gallic acid equivalent per g of dry weight)	4.03 ± 0.1	1.21 ± 0.14	$p < .001$
Total flavonoids (mg quercetin equivalent per g of dry weight)	1.62 ± 0.07	0.06 ± 0.03	$p < .001$
ABTS scavenging assay (μmol of ascorbic acid per g of dry weight)	18.73 ± 1.20	4.9 ± 1.11	$p < .001$
Antimicrobial activity towards *S. aureus* (IC50 $\mu g\ mL^{-1}$)	182,32 ± 2,31	189,27 ± 1,26	$p > .001$
Antimicrobial activity towards *E. coli* (IC_{50} $\mu g\ mL^{-1}$)	NA	NA	-

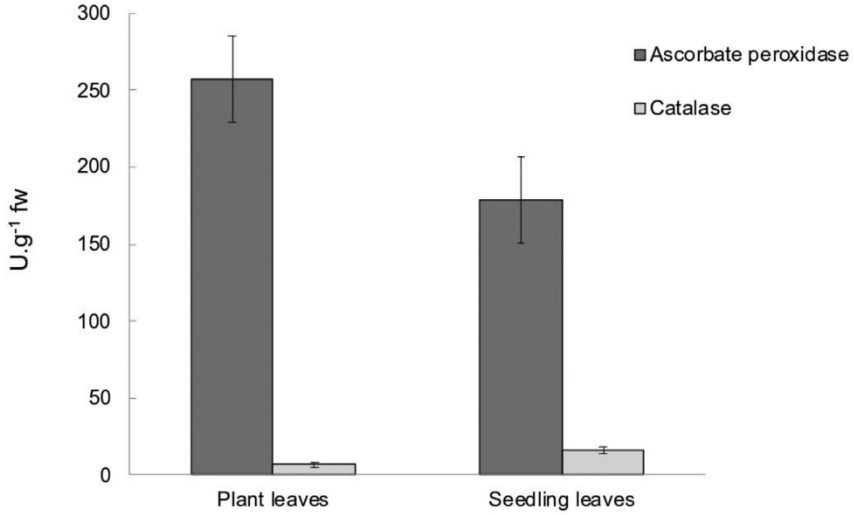

Fig. 3. Enzymatic antioxidant activity of organic extracts from *Epidendrum secundum* plant leaves and *in vitro* seedling culture.

Phenolic compounds are plant secondary metabolites that include around 9,000 compounds, different in structure, molecular weight, physicochemical and biological properties. The biosynthesis of these compounds is endogenously controlled during developmental differentiation (Crozier et al., 2006). In the plant metabolism, phenolic compounds may play a role of signaling molecules, protecting against UV light and pathogens, attracting pollinators, stimulating disease resistance and/or protecting against reactive oxygen species generated when aerobic or photosynthetic metabolism is impaired by environmental stresses (Waskiewicz et al., 2013).

The flavonoids comprise an enormous group of more than 4,500 compounds. Among their subclasses are the anthocyanins (pigments), proanthocyanidins and isoflavonoids. Many of these compounds play defensive roles and act in signaling molecules in plant-microbe interactions (Croteau et al., 2000). Flavonoids promote symbiotic relationships between soil and microorganisms. The effects on the pre-symbiotic growth of arbuscular mycorrhizae fungi has been assessed, showing a specific genus effect, promoting growth and differentiation at fungal sporulation and initial growth (Scervino et al., 2005).

The obtained results showed us that *E. secundum* leaves from plants growing at environmental conditions produce a higher content of phenolic compounds and flavonoids in comparison with *in vitro* seedlings. The $ABTS^{\bullet+}$ assay is an electron-transfer based method, which assesses the ability of a potential antioxidant to transfer one electron to reduce oxidants (Pegg et al., 2007). In the present work, *Epidendrum secundum* plant leaves exhibit higher antioxidant activity in comparison with *in vitro* seedlings. This finding is related with the abundance of phenolic compounds and flavonoids content reported previously (Table 1). Our results are consistent with the study from Minh et al. (2016), focused on phenolic and flavonoids content in *Phalaenopsis* orchid hybrids. Chand et al. (2016), reported the antioxidant activity from nine wild orchids from Nepal. In this work, total phenolic, flavonoids content and antioxidant activity against the free stable radical DPPH was assessed in the orchids under study. Leaves from *Rhynchostylis retusa* (L). Blume were reported as a source of antioxidant compounds, highlighting the potential of orchids as a source of bioactive compounds.

Exposure of plants to stresses can give rise to excess accumulation of reactive oxygen species (ROS) at the cellular level. Cell ROS such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) are mainly produced in chloroplasts, mitochondria and peroxisomes, as well as by the plasma membrane-bound NADPH oxidases and the cell wall-bound NAD(P)H oxidase-peroxidase. ROS plays a role as secondary messengers involved in the stress-response signal transduction pathways. For this reason, their levels must be kept under tight control by ROS-scavenging mechanisms, including enzymatic and non-enzymatic antioxidants (Latowski et al., 2010).

The enzymatic antioxidant defense system in plants mainly includes the antioxidant enzymes, including catalase (CAT) and ascorbate peroxidase (APX). APX is an enzyme that detoxifies H_2O_2, using ascorbic acid as a substrate. The reaction catalyzes the transfer of electrons from ascorbic acid to H_2O_2, producing dehydroascorbate and water (Raven, 2000).

Ascorbate is synthesized in the mitochondria and is transported to the other cell components through a proton-electrochemical gradient, which facilitates diffusion. Ascorbic acid also has been implicated in regulation of cell elongation. Ascorbate occurs in all plant tissues, usually being higher in photosynthetic cells and meristems. Ascorbic acid has effects on many physiological processes, including the regulation of growth, differentiation and metabolism of plants. This key compound influences many enzyme activities, and minimizes the damage caused by oxidative process through synergic function with other antioxidants (Mahmood et al., 2010).

Catalase is an oxidoreductase which regulates the dissociation of H_2O_2 into H_2O and O_2, generated in peroxisomes during oxidative stress. Proliferation of peroxisomes during stress aid in scavenging H_2O_2 diffusing from cytosol. One molecule of catalase converts 6 million molecules of H_2O_2 to H_2O and O_2, having the highest turnover rates for all enzymes (Gill and Tuteja, 2010).

Rahman et al (2015) evaluated the effect of cryopreservation of *Brassidium* Shooting Star orchid protocorms. In this study, catalase levels were significantly high as a result of thermal treatments. The increase in catalase activity may be related to a defense strategy from the plant cells against stress, which triggers the production of superoxide. (Habib et al., 2014). In our study, leaves of *Epidendrum secundum* plants displayed high APX activity, compared with *in vitro* seedling leaves.

One of the key aspects to consider orchids as a source of bioactive compounds is the necessity to establish *in vitro* cultures to ensure biomass production without threatening wild populations. The increased catalase activity in *E. secundum in vitro* seedling leaves compared with plant leaves growing at environmental conditions, remarks the importance of developing *in vitro* propagation methods directed towards the enhancement of antioxidant compounds production.

Epidendrum secundum is traditionally used in Ecuador to alleviate nervous disorders. Orchid extracts with significant antioxidant

activity may be potential sources of bioactive compounds for the treatment of neurodegenerative diseases (Chinsamy et al., 2014). New studies that include inhibition of acetylcolinesterase and monoaminooxidase will validate the use of *E. secundum* in Ecuadorian traditional medicine and must be part of drug discovery research in a similar way than *Ansellia africana* Lindl and other orchids with significant potential in the treatment of central nervous system disorders (Bhattacharyya and Van Staden, 2016).

Conclusions

In the present work, the enzymatic and non-enzymatic antioxidant activity from plant leaves and *in vitro* cultured seedlings from *Epidendrum secundum* was assessed. Applying a set of spectrophotometric assays, the phenolic compounds and flavonoids concentrations were obtained, evaluating organic extracts from the orchid under study. The capture of the free radical $ABTS^{\bullet+}$ was tested to demonstrate the relationship between the production of phenolic compounds and flavonoids and the antioxidant capacity from plant leaves and *in vitro* seedling leaves. *In vitro* cultured seedlings displayed lower phenolic, flavonoids and antioxidant activity, due to the maturity of the developmental stage and the controlled growth conditions. The enzymatic antioxidant activity was assessed via APX and CAT enzymes activity. In this case, catalase activity was significantly higher at *in vitro* seedling leaves from the orchid under study. The CAT activity at *in vitro* conditions might be enhanced as a response to the media composition, which is enriched with growth factors, such as 1-naphtalenacetic acid and 6-bencylaminopurine. For this reason, the importance of research focused on media improvement for *in vitro* cultivation of orchids is highlighted, in order to obtain biomass with higher yield on bioactive compounds.

The present study undertakes the evaluation of *Epidendrum secundum* Jacq, growing at environmental and *in vitro* conditions, as a source of antioxidant compounds. The antioxidant activity is related to other bioactivities, such as the inhibition of bacterial growth. In our report, organic extracts from *E. secundum* plant leaves display moderate antibacterial effect against *S. aureus*. These findings reveal the potential of this orchid species as a source of bioactive compounds, motivating further research on the production of secondary metabolites from orchids, as candidates for drug development. The scope of this research needs to include the ability of *in vitro* cultures to produce bioactive compounds, in order to prevent the decreasing of wild orchid populations.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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