RESEARCH / INVESTIGACIÓN

Phytochemical screening and antioxidant activity of Epidendrum nocturnum

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Abstract: The objective of this study was to evaluate the antioxidant capacity of *Epidendrum nocturnum* using the DPPH technique to determine the capacity for scavenging free radicals, as well as to identify secondary metabolites in ethanolic extracts of the previously mentioned species by phytochemical screening, with analysis of alkaloids, flavonoids, saponins, tannins, and triterpenes. The results determined in the phytochemical screening that the secondary metabolites were most present were flavonoids, tannins, and saponins; no alkaloids or triterpenes were found. In the analysis of antioxidant activity, *Epidendrum nocturnun* in the three extracts showed that with an average concentration of 3.50 ppm, it could inhibit 50% of the free radicals present in the test solution.

Key words: Antioxidant, *Epidendrum*, DPPH, metabolites, screening.

Introduction

Orchids belong to the Orchidaceae family, one of the most numerous families with approximately 35,000 species and striking for their beautiful flowers[1]. In Ecuador, Endara and Jost[2] mention that there are 1707 endemic orchid species, and because of this, it is said that this family is considered the most diverse of vascular plants.

We will find several secondary metabolites within the orchids such as alkaloids, flavonoids, phenols, terpenoids, and phenanthrenes; these being complex substances used as active principles in the pharmaceutical industry, they have a pharmacological or physiological action on the body[3].

Within the scientific field, interest in verifying the ethnobotanical uses of plants, or obtaining new compounds with pharmacological activity, especially with antioxidant action. Because Ecuador has a great diversity of orchids used in traditional medicine that still do not have a phytochemical screening, the lack of scientific studies that corroborate these applications is evident; with those mentioned above, it is essential since active principles can be obtained to replace synthetic compounds used in the pharmaceutical industry[4].

Materials and methods

Location and collection of the sample

The collection was carried out in the "Orquideario de Sarina," located in the province of Pichincha, in the canton of Quito, in the parish of El Quinche.

The plant material selected for this study was 100g of leaves; the leaves were cut with pruning scissors previously sterilized with 96% alcohol, placed in paper bags, and stored in plastic bags with a seam to avoid deterioration the samples.

Obtaining the ethanolic extract

To obtain the ethanolic extracts, a maceration was carried out according to the methodology described by Moreno and Jaramillo[5], the process consisted of: taking 100 g of young leaves, it was crushed in a porcelain mortar of 80 mm in diameter, 70 mL of 96% ethanol and macerated in an amber flask for 8 days in complete darkness. It was then filtered to remove leaf residues and stored in the dark and kept under ambient conditions; all this was repeated three times, obtaining three similar extracts.

Phytochemical screening

Using the Miranda and Cuellar Manual qualitative technique, preliminary colorimetric tests were used, which are fast and straightforward for determining secondary metabolites. The presence of alkaloids (Draggendorf;), flavonoids (Shinoda), saponins (foam), tannins (Gelatin-Salt), and triterpenes (Liebermann-Burchard) were determined in the ethanolic extracts.

If opalescence (+), turbidity (++), or precipitate (+++) is observed, it is considered that the sample contains the secondary metabolite; in all analyses, 96% alcohol was used as a negative control.

Test for alkaloids

Following the methodology described by Carrera *et al.*[6], using the Draggendorf reagent, 3 mL of sample was placed in a test tube, then 3 drops of the draggendorf reagent were added, vigorously shaken, and waited for 30 minutes. Caffeine was used as a positive control.

Test for flavonoids

Following the methodology described by Ramos *et al.*[7], using the Shinoda reagent; For which 3 mL of sample was placed in a test tube, then several magnesium filings were added, the test tubes were placed in a water bath at 60 ° C, then 3 drops of concentrated HCl were placed. Apple was used as a positive control.

Test of saponins

Following the methodology described by Moreno and Jaramillo[5], using the foam test; for which 3 mL of sample was placed in a test tube, then 5 mL of distilled water was added and vigorously stirred for one minute. Quinoa was used as a positive control.

Tannins test

Following the methodology described by Sánchez and Calle[8], using the Gelatine-Salt reagent, 3mL of the sample was placed in a test tube, then 2 mL of reagent was added. Black tea was used as a positive control.

Triterpenes test

Following the methodology described by Carrera *et al.*[6], using the Liebermann-Burchard reagent; for which 3 mL of sample was placed in a test tube, then 1 mL of acetic anhydri-

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de was slowly added through the wall of the tube, and finally, with caution, 2 drops of concentrated sulfuric acid were added. Calendula was used as a positive control.

Evaluation of antioxidant activity

For this test, the methodology of Noriega *et al.*[9] was used, for which a solution of 2,2-diphenyl-1-picrylhydracil DPPH was made. For each of the extracts, dilutions were prepared in different concentrations: 10 uL, 50 uL, and 80 uL in amber vials; it was completed with 96% alcohol obtaining a volume of 100 uL; 2.9 mL of DPPH was added to these dilutions until the final volume was 3 mL, it was homogenized and stored in the dark for 30 minutes.

Preparation of the DPPH solution

24 hours before the analysis, 500 mL of DPPH was prepared in 96% ethanol, for which 19.70 mg of DPPH was weighed, it was dissolved in 200 mL of drinking alcohol, then it was added to 500 mL, this The solution was stored in an amber bottle wrapped in aluminum foil refrigerated at 4 ºC, a technique described by Castañeda *et al.*[10].

Vitamin C standard curve

The standard was prepared by dissolving 20 mg of ascorbic acid in 100 mL of ultrapure water as described by Noriega *et al.*[9].

The concentration of vitamin C obtained was 0.2 mg / mL, with which the following dilutions were made:

100 uL of vitamin C + 0 uL of pure water
80 uL of vitamin C + 20 uL of pure water
60 uL of vitamin C + 40 uL of pure water
40 uL of vitamin C + 60 uL of pure water
20 uL of vitamin C + 80 uL of pure water
0 uL of vitamin C + 100 uL of pure water

The prepared solutions were placed in amber vials, and 2.9 mL of previously prepared DPPH was added to these, stirred, and remained in the dark for 30 minutes.

The prepared solutions were placed in 3 mL plastic cells to be analyzed in the spectrophotometer (JASCO V-730) at a wavelength of 517 nm; the samples were analyzed to increase their concentration, each sample was analyzed in triplicate.

The calibration curve obtained in this investigation can be seen in figure 1, which presents an $R^2 = 0.9958$, being acceptable because it is close to 1.

Preparation of samples for analysis in the spectrophotometer

From the extracts of each species under study and the following solutions were made:

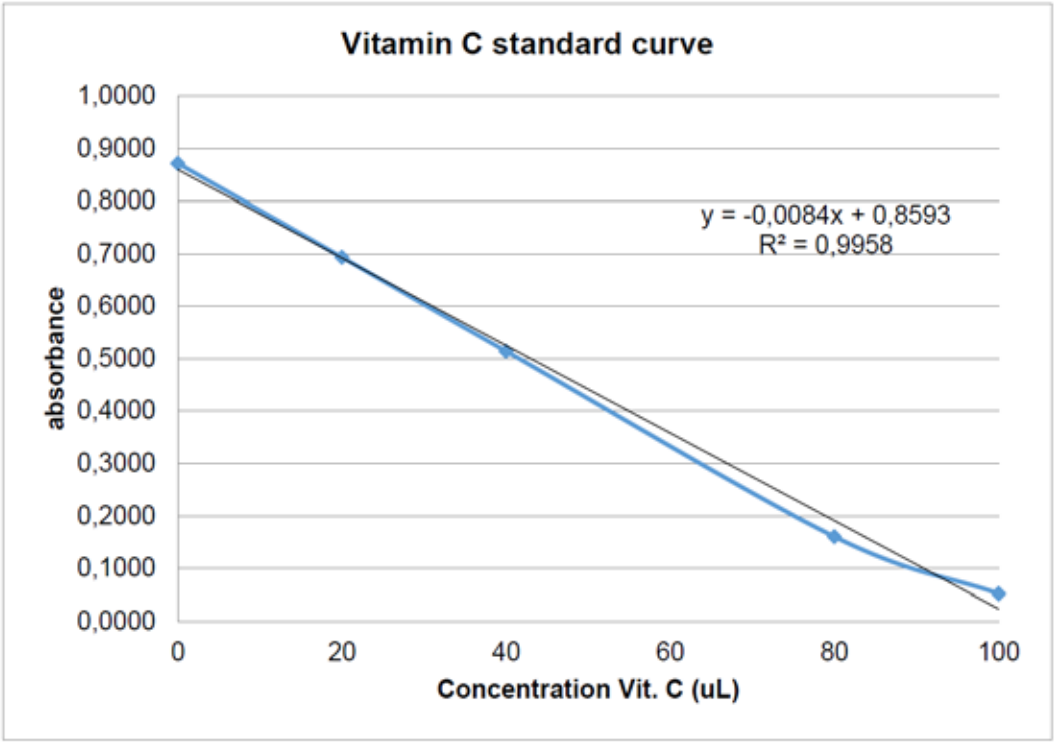
10 uL of extract + 90 uL of 96% ethanol
50 uL of extract + 50 uL of 96% ethanol
80 uL of extract + 20 uL of 96% ethanol
100 uL of alcohol 96% + 2.9 mL DPPH

To analyze the samples in the spectrophotometer, the procedure used for vitamin C was replicated.

Results and Discussion

Secondary metabolites identification

The percentage of presence of secondary metabolites in the analyzed extracts of *Epidendrum nocturnum* were: flavonoids 100% in high concentration, tannins 100% in medium concentration, saponins 66.66% in low concentration, alkaloids and triterpenes 0%, the concerning result the alkaloids contrasts with the study done by Sut *et al.*[11], who found phenanthrenic alkaloids in species of the genus Epidendrum; This would indicate that the species collected has not had an external threat that induces the production of alkaloids, as explained by Farrán *et al.*[12], see table 1 and table 2.



Note: Calibration curves obtained from Microsoft Excel 2013.

Figure 1. Vitamin C calibration curve.

Epidendrum nocturnum					
Metabolites	ALKALOIDS	FLAVONOIDS	SAPONINS	TANNINS	TRITERPENES
Secondary	-	+++	+	+	-
1st extract	-	+++	+	++	-
2nd extract	-	+++	-	++	-
3rd extract	0	100	66.66	100	0

Note:Negative (-), little presence (+), moderate presence (++), high presence (+++).

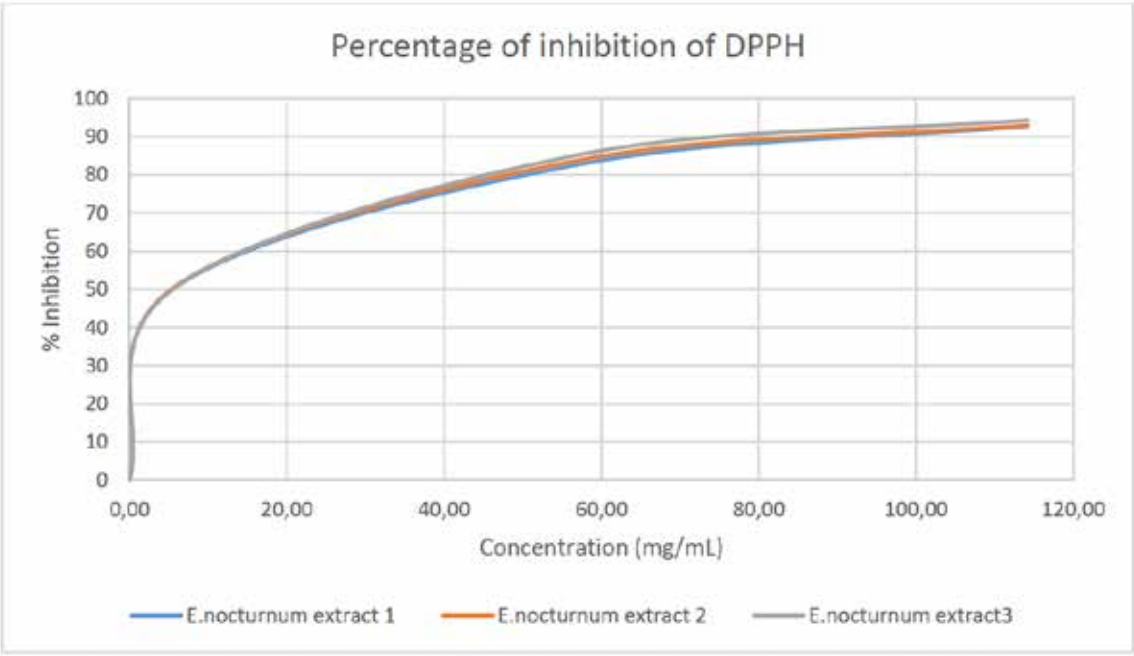
Table 1. Phytochemical screening of Epidendrum nocturnum.

Table 2. Secondary metabolites analysis.
Note: Photographs of the tests carried out in the CIVABI UPS.

Evaluation of antioxidant activity

The extracts of *E. nocturnum* showed antioxidant capacity. The three extracts had similar results that, on average with a concentration of 3.50 ppm they inhibited 50% of the DPPH free radicals. See figure 2.

It was observed that the *Epidendrum nocturnum* species exceeded the antioxidant capacity of *Prosthechea michuacana* reported by González *et al.*[13], which required 13.22 ppm to inhibit the% IC50 of DPPH.



Note: Percentage of inhibition of DPPH of *Epidendrum nocturnum* obtained from Microsoft Excel 2013.

Figure 2. Percentage of inhibition of DPPH of Epidendrum nocturnum.

Conclusions

The secondary metabolites present in *Epidendrum nocturnum* in 100% were flavonoids and tannins; in saponins, it was 66.66%.

The antioxidant activity analysis showed in the 3 extracts *Epidendrum nocturnum* with only 3.5ppm inhibited 50% of DPPH free radicals.

Epidendrum nocturnum presented a significant antioxidant capacity and a high presence of flavonoids in its composition, confirming that this secondary metabolite contributes to these plants' antioxidant capacity.

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