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CHEMICAL COMPOSITION, POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF THE ETHANOL EXTRACT OF *Gentianella dianthoides* (Kunth) Fabris

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Abstract: The chemical composition, total polyphenols and antioxidant activity in the ethanolic extract of *Gentianella dianthoides* (Kunth) Fabris. The study was of an experimental type in which the lyophilized extract of *Gentianella dianthoides* (Kunth) Fabris. The chemical composition was determined by phytochemical screening, it was carried out by color or precipitate formation reactions. The content of total polyphenols was determined colorimetrically using the Folin-Ciocalteu reagent, the gallic acid standard was used at concentrations of 10 to 250 µg/mL. To evaluate the antioxidant activity, it was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method, in which Trolox® (6-hydroxy-2,5,7,8-tetramethylchromic acid) was used as a standard solution. -2-carboxylic) and the lyophilized extract of the sample. Under the experimental conditions it was shown that the lyophilized ethanolic extract of: *Gentianella dianthoides* (Kunth) Fabris has a yield of 11.73%, the phytochemical test showed abundant content of flavonoids and phenolic compounds, moderate content of anthraquinones and slight content of saponins, the determination of total polyphenols by the Folin - Ciocalteu method showed that the amount of polyphenols is 10.0365 mg/g of extract, the determination of antioxidant activity by the DPPH method, the IC 50 of Trolox® (3.8 µg/mL) and of the ethanolic extract was calculated, which in this case was 27.65 µg/mL. It is concluded that the plant *Gentianella dianthoides* (Kunth) Fabris presents polyphenolic composition and antioxidant activity.

Keywords: Chemical composition; antioxidants; lyophilized; Gentianella dianthoides.

INTRODUCTION

The megadiversity of Peru is evidenced in the diversity of species, genetic resources, and ecosystems, which is necessary to highlight and be able to identify those with the greatest potential to prevent or treat diseases, benefiting humanity, in their application in pharmaceutical laboratories, among others (1). For Peru, it is estimated that there are 25,000 existing species, although some scientists calculate that this figure can even be doubled, with a significant percentage of these species being endemic. On the other hand, approximately 4000 species have various uses, in food and health, in cosmetics, in dyeing as aromatizers and flavoring agents, as biocides, in industry, agroforestry, ornamental, among other uses; We must mention that much of this use is rooted in popular knowledge and transmitted from generation to generation, and unfortunately with little scientific basis (2). Numerous studies of medicinal plants have been carried out for decades, due to their potential use as a source of substances with biological properties. One of the main compounds of plants are antioxidants, substances found in certain foods that act by protecting the body from the action of free radicals, which cause aging processes and other diseases. Likewise, oxidative stress has been associated with the pathogenesis of many human diseases, such as: arteriosclerosis, arthritis, dementia, cancer, among others. That is why the use of antioxidants is intensively studied (3).

The Gentianaceae family is recognized in Peru for presenting around 15 genera and approximately 170 species, mostly herbs and shrubs. 103 endemic species in seven genera are recognized. The genera with the highest number of endemic species are: *Gentianella* y *Macrocarpaea*. The endemic species mainly occupy the regions of the Humid and Dry Puna, Paramo and Very Humid Montane

Forest, between 1000 and 5100 m altitude. Thirty-three endemic species are represented within a protected area (4). The species: *Gentianella* (Familia Gentianaceae), they are also found at a height greater than 3000 meters above sea level. This species is known from several collections from Cajamarca, made in the jalca, as well as in the puna vegetation in the south of the country. The area of presence of this species reaches 10,000 km2 (4). As expected from chemotaxonomy, mainly xanthones and to a lesser extent secoiridoids and flavonoids have been isolated. Some of the extracts and isolated xanthones have been tested for hypoglycemic, antimicrobial and antioxidant activity (2).

The pharmaceutical and cosmetic industries have deployed great efforts in the scientific research of plant compounds with antioxidant activity. Topically administered antioxidant compounds are effective in skin rejuvenation treatment because they neutralize the damaging effects of free radicals before they can attach to cell membranes and destroy cells. Free radicals contribute to the hardening of collagen and elastin cells (5). Natural antioxidants present in plants have gained great interest in the last two decades since oxidative stress (an imbalance between oxidant and prooxidant substances) is implicated in a large number of health conditions (6).

MATERIAL AND METHODS

COLLECTION AND POST-HARVEST TREATMENT

The plant: *Gentianella dianthoides* (Kunth) Fabris commonly known as "Lirambo", was collected in the month of May 2017, in the province of Hualgayoc, district of Bambamarca and department of Cajamarca at an altitude greater than 3000 meters. The freshly collected material (1 Kg wet weight) and then dried at room temperature in an approximate time of 10 days. For the taxonomic study, the Arthur Cronquist Botanical classification system was used, occupying the following taxonomic categories (Table 1):

Type	**Plant**
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Asteridae
Segment:	Gentianales
Family:	Gentianaceae
Gender:	Gentianella
Species:	*Gentianella dianthoides* (Kunth) Fabris ex J.S. Pringle

Table 1. Taxonomic Classification.

PROCESSING OF PLANT MATERIAL:

Gentianella dianthoides (Kunth) Fabris plants were subjected to a drying process in an oven at a constant temperature of 40 °C for 22 days. After the drying process, the particle size was reduced by passing the dry material through a knife mill.

OBTAINING THE ETHANOLIC EXTRACT

A total of 115 g of dry and ground plant material were subjected to maceration for a period of 14 days, using 96% ethyl alcohol as extraction solvent in an amount of 1000 ml, with constant agitation. Filtration was then carried out through Whatman No. 42 filter paper, in a 500 ml Kitasate flask Brand: Pyrex with a Buchner funnel coupled to a vacuum pump. After completing the filtration process, the sample was concentrated to dryness in a drying oven at 40°C. This concentrate constituted the total extract, which was subjected to a lyophilization process for 12 hours in a Lyophilizer Brand: Labconco Model: 7400040, obtaining a final volume of 2.07 grams (Table 2).

Segment	Ramp (°C/min)	Hold	Time (Hours)
1	0.2	-55°	2
2	0.6	-25°	5
3	0.5	0°	3
4	0.6	10°	2
5	1	24°	-

Table 2. Lyophilization Protocol.

In the Table you can see the 5 segments in which the lyophilization process consisted. Prior to the temperature to be maintained (Hold) for a certain time (Time), the temperature drops to a rate indicated in the Ramp column.

CHEMICAL COMPOSITION

The chemical composition was made by Phytochemical Screening. To carry out the phytochemical screening, the lyophilized extract of: *Gentianella dianthoides* (Kunth) Fabris, was performed by physicochemical test (reactions of color formation or precipitate). The presence of the metabolite was qualitatively cataloged in Intense Presence (++++), Moderate (++), Mild (+) and Negative (-). The presence of the following metabolites was evaluated: Flavonoids (Shinoda), Phenolic Compounds (Ferric Trichloride), Tannins (Gelatin, Potassium Dichromate, Sodium Hypochlorite), Alkaloids (R. Dragendorff, R. Bertrand), Saponins (Foam Test), Steroids (Liebermann-Buchard) and Anthraquinones (Borntrager (Krauss).

DETERMINATION OF TOTAL POLYPHENOLS

To determine the content of total polyphenols, it was determined colorimetrically using the Folin-Ciocalteu reagent (7). A volume of 100 µL of the lyophilized extract of *Gentianella dianthoides* (Kunth) Fabris, was mixed with 500 µL of phosphomolybdic-phosphotungstic acid reagent (known as:Folin-Ciocalteu 0,2 N) y 400 µL of Na_2CO_3 al 7,5% w/v. The mixture was homogenized and left to stand for one hour in the dark. At the end of the time, the absorbance at 765 nm was measured in a UV-Vis spectrophotometer Brand: Thermo Scientific Model: GENESYS 10S UV-Vis. The calibration curve of the gallic acid standard was prepared at concentrations from 10 to 250 µg/mL. The content of total polyphenols (TPC) is expressed as equivalents of gallic acid.

DETERMINATION OF ANTIOXIDANT ACTIVITY

To evaluate the antioxidant activity, it was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction method. A volume of 800 µL of the 40% DPPH methanolic solution was mixed with 400 µL of the lyophilized extract of *Gentianella dianthoides* (Kunth) Fabris, dissolved in 96% ethyl alcohol, the mixture was homogenized and allowed to stand for 30 minutes at room temperature and in the dark. After the time elapsed, the absorbance at 517 nm was measured in a UV-Vis spectrophotometer Brand: Thermo Scientific Model: GENESYS 10S UV-Vis. The reduction of the reagent is followed by measuring the decrease in absorbance spectrophotometrically at 517 nm. All analyzes were performed in triplicate (n=3). The lyophilized extract was evaluated at different concentrations of 10.4; 20.8; 41.7 µg/mL. Trolox® (6-hydroxy-2,5,7,8-tetramethylchromo-2-carboxylic acid) was used as the standard solution. The percentage of inhibition was calculated according to the following formula:

$$\textbf{\% Inhibition:DPPH=} \frac{\text{(Abs. DPPH - Abs. Sample)}}{\text{Abs. DPPH}} \text{ x } 100$$

The IC50 (minimum concentration necessary to inhibit DPPH by 50%) of the lyophilized extract of *Gentianella dianthoides* (Kunth) Fabris and the Trolox® standard. The results were processed using the MINITAB program. They were statistically analyzed using the t-student test and ANOVA. Results expressed as mean values +/- standard deviation (SD).

RESULTS

EXTRACTION AND YIELD

The yield percentage of the ethanolic extract of lyophilized *Gentianella dianthoides* (Kunth) Fabris, as indicated in Table 3.

	Dry plant weight	Extract Weight	% of Performance
Plant	115 g	13.49 g	11.73%

Table 3. Yield percentage of the Et/OH Extract of *Gentianella dianthoides.*

CARACTERIZACIÓN QUÍMICA

The chemical composition by means of the phytochemical analysis carried out on the lyophilized extract of *Gentianella dianthoides* (Kunth) Fabris made it possible to identify the families of the most abundant chemical compounds in the species, and which could be responsible for their biological activity. These results are shown in Table 4.

Metabolite	Test or reaction	Extraction Et/OH of *Gentianella dianthoides*
Flavonoids	shinoda	++++
Phenolic compounds	Ferric trichloride	++++
Tannins	Jelly	-
	Potassium dichromate	-
	sodium hypochlorite	-
Alkaloids	R. Dragendorff	-
	R. Bertrand	-
Saponins	foam test	+
Steroids	Liebermann	-
Anthraquinones	Borntrager (Krauss)	++

Negative (-), Mild Presence (+), Moderate (++), Intense (++++).

Table 4. Phytochemical march of the metabolic extract.

DETERMINATION OF TOTAL POLYPHENOLS

The absorbance of standard gallic acid solutions was measured (see Table 5). With these data, the respective standard curve was elaborated (Figure 1).

Concentrations (µg/mL)	Average Absorbance
10	0,0551
25	0,2129
50	0,4539
250	2,2203

Table 5. Gallic acid standard absorbances at 765 nm.

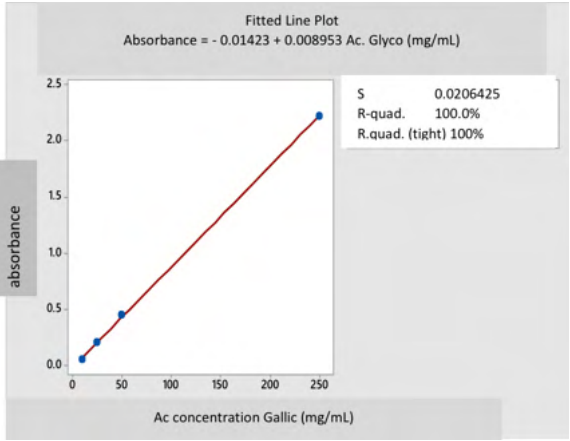

Figure 1. Gallic Acid standard calibration curve.

Using the following formula, the concentration of gallic acid was related to the absorbance [9].

$$\text{(Gallic acid)} = \frac{(\text{Absorbancia} + 0.0099)}{0.0089}$$

For the analysis, the lyophilized sample of *Gentianella dianthoides* (Kunth) fabris. Table 6 shows the average absorbance of the ethanolic extract of *Gentianella dianthoides* (Kunth) Fabris, which is substituted in the equation of the calibration line (value of the ordinate, "y") and clear "x" that corresponds to the concentration of gallic acid in the extract. To express the results in mg of gallic acid per g of extract, the amount of solvent used in the extraction and the amount of sample used in the analysis have been taken into account.

Ethanolic Extract	Average Absorbance	CPT (mg of gallic acid equivalents/g of extract)
Gentianella dianthoides (Kunth) Fabris	0,4796 ± 0,037	10,0365

Table 6. Content of Total Polyphenols (TPC) of the Et/OH Extract of: *Gentianella dianthoides.*

DETERMINATION OF ANTIOXIDANT ACTIVITY

Table 7 shows the percentages of antioxidant activity at different concentrations of Trolox® (6-hydroxy-2, 5, 7, 8-tetramethylchrome-2-carboxylic acid)[6]. With these data, the Trolox® calibration curve was elaborated (Figure 2).

Concentrations of Trolox® (µg/mL)	Average Absorbance	% DPPH inhibition	IC 50 (µg/mL)
0	0,701	0,0	3,8
1,2	0,603	14,03	
3,6	0,322	54	
7,2	0,071	89,9	

Table 7. DPPH inhibition percentage of the Standard: Trolox®.

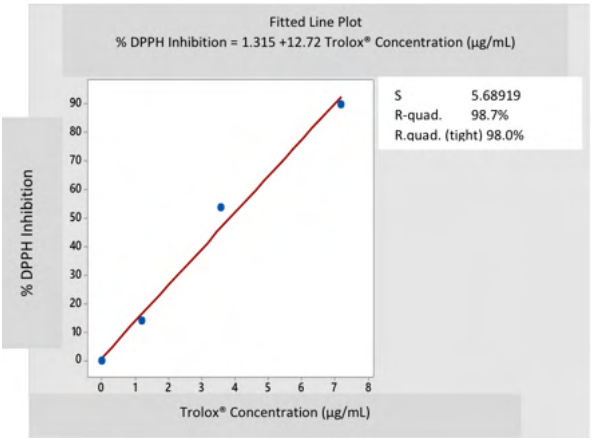

Figure 2. Standard calibration curve: Trolox®.

Pearson's correlation coefficient between the concentration of Trolox® and its percentage of antioxidant activity in the calibration curve was 0.993, indicating good linearity at the concentrations tested and reliability of the data (Figure 2).

Concentrations (µg/mL)	Average Absorbance	% DPPH inhibition	IC 50 (µg/mL)
0	0,4542 ± 0,009	0,0	27,64
10,41	0,3703 ± 0,014	18,48	
20,83	0,2884 ± 0,002	36,51	
41,66	0,1083 ± 0,005	76,16	

Table 8. Percentage of DPPH inhibition of the Et/OH Extract of *Gentianella dianthoides* (Kunth) Fabris.

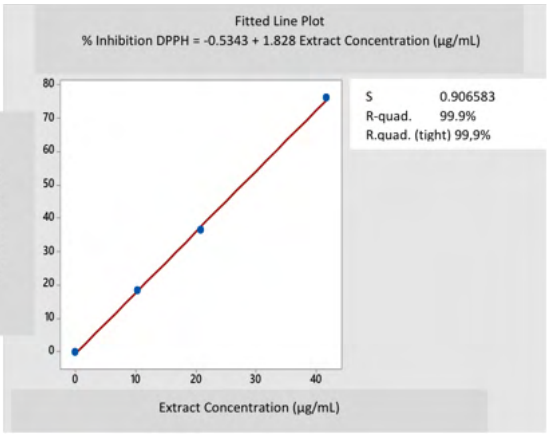

Figure 3. Calibration curve of the ethanolic extract of: *Gentianella dianthoides*.

DISCUSSION

The phytochemical test showed an abundant content of flavonoids and phenolic compounds, to which antioxidant activity is attributed; in addition, moderate content of anthraquinones and slight content of saponins; while no tannins, alkaloids, or steroids were found.

The results obtained in the determination of total polyphenols by the Folin - Ciocalteu method showed that the amount of polyphenols in de *Gentianella dianthoides* (Kunth) Fabris is 10.0365 mg/g of extract. Through statistical analysis, a Pearson correlation coefficient (r) of 1.000 and a determination coefficient (r2) of 0.9997 were obtained. The value of $t_{regression}$ =84,17 and a p-value = 0.000. It was verified that there is linear and significant correlation. With reference to the polyphenol content of the aqueous extract of the *Gentianella* nítida of 65.8 mg/g (1) was higher than the ethanolic extract of *Gentianella dianthoides* showing a value of 10.0365 mg/g. This higher value could be explained by the difference in the polyphenolic secondary metabolite extraction method used or by the protocols followed that vary in the proportions of reagents, concentrations and measurement times, which differs from our study.

On the other hand, it must be considered that the plant of *Gentianella* nítida was collected in the District of Ulcumayo, Province of Junín and Department of Junín (1), while the plant: *Gentianella dianthoides* (Kunth) Fabris was collected in the District of Bambamarca, Province of Hualgayoc and Department of Cajamarca, which could explain the difference in the concentration of polyphenols.

For the results obtained in the determination of antioxidant activity by the DPPH method, the IC 50 of Trolox® (3.8 µg/mL) and of the ethanolic extract (Table 8) was calculated from the regression analysis equation. A low IC 50 value indicates high antioxidant power. Trolox®, as a standard, will always exhibit a lower IC 50 value compared to extract (10), which in this case was 27.65 µg/mL.

The results were statistically analyzed, evaluating the concentrations of the extract vs the percentage of DPPH inhibition found. Figure 3 shows the equation of the straight line, obtaining:

$y = -0.5343 + 1.828x$

The regression statistics, as expressed in Table 9.

Regression Statistics	
Pearson correlation	1,000
R-cuad.	0,9995
R-cuad. (tight)	0,9959
S	0,906583

Table 9. Regression Analysis.

In the statistical analysis, a Pearson correlation coefficient (r) of 1,000 and a coefficient of determination (r^2) of 0.9995. The coefficient of determination indicates that there is good linearity and provides greater statistical significance (Table 9). Using the t-student test and ANOVA, the "t" statistic was found together with a variance test. The value of $t_{regression}$ =62,13 showed that the correlation is linear and significant. The value of p = 0.000 showed that the analysis of variance shows us that there is a high correlation, so the method is linear.

Comparing the reported data of antioxidant capacity IC 50= 145 µg/mL for the *Gentianella* nítida with the data of antioxidant capacity IC 50= 27.64 µg/mL for the *Gentianella dianthoides*, it can be proposed that the latter would have greater antioxidant capacity than the *Gentianella* nítida, even when the polyphenol content is lower. It may be suggested that these

oxygenated metabolites show antioxidant capacity or perhaps the extract may contain other different secondary metabolites that contribute to antioxidant activity (1).

CONCLUSION

The ethanolic extract of *Gentianella dianthoides* (Kunth) Fabris contains flavonoids and phenolic compounds. The determination of antioxidant activity by the DPPH method showed antioxidant activity, with an IC 50 = 27.64 µg/mL, and is directly related to the presence of total polyphenols and flavonoids.

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