



Effect of drying temperature on polyphenolic compounds and antioxidant activity of *Piper aduncum* L. leaves

Efecto de la temperatura de secado sobre los compuestos polifenólicos y actividad antioxidante de las hojas de *Piper aduncum* L.

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Abstract

The objective of the research was to evaluate the effect of drying temperature on the polyphenolic compounds and antioxidant activity of the leaves of *Piper aduncum* L. The leaves were collected in the city of Puyo, El Paico sector, located in the Amazon of the province from Pastaza, Ecuador. The ultrasound-assisted extraction technique was used to obtain the aqueous extracts. Total polyphenols were determined using the Folin-Ciocalteu analysis method and antioxidant activity was evaluated using the FRAP and ABTS methods. The effect of three levels of drying temperature (45, 50 and 55 °C) on the polyphenolic compounds and antioxidant activity of matico leaves was evaluated. Design Expert version 10 software was used to evaluate the significance of the study factor on the experimental response ($P < 0.05$). ANOVA analysis was performed to assess the influence that temperature had on the response variables. Drying temperature had a negative effect on polyphenolic compounds and antioxidant activity in *P. aduncum* leaves. The highest values were obtained at 45 °C (79.92 mg eq. of gallic acid·100 g⁻¹ of dry biomass) for total polyphenols (74,898.90 and 508.07 µg eq. of TROLOX·g⁻¹ of dry biomass), for antioxidant activity using the FRAP and ABTS techniques, respectively, so it is advisable to dry at this temperature, prior to any subsequent use or processing in order to preserve its properties as a natural antioxidant.

Keywords: total polyphenols; antioxidant capacity; FRAP; ABTS.

Resumen

El objetivo de la investigación fue evaluar el efecto de la temperatura de secado sobre los compuestos polifenólicos y actividad antioxidante de las hojas de *Piper aduncum* L. Las hojas fueron recolectadas en la ciudad de Puyo, sector El Paico, ubicado en la Amazonía de la provincia de Pastaza, Ecuador. Se utilizó la técnica de extracción asistida por ultrasonido para obtener los extractos acuosos. Los polifenoles totales fueron determinados mediante el método de análisis de Folin-Ciocalteu y la actividad antioxidante fue evaluada utilizando los métodos FRAP y ABTS. Se evaluó el efecto de tres niveles de temperatura de secado (45, 50 y 55 °C) sobre los compuestos polifenólicos y la actividad antioxidante de las hojas de matico. Se usó el software Design Expert versión 10, para evaluar la significancia del factor de estudio sobre la respuesta experimental ($P < 0,05$). Se realizó el análisis ANOVA para valorar la influencia que tuvo la temperatura sobre las variables de respuesta. La temperatura de secado tuvo un efecto negativo sobre los compuestos polifenólicos y la actividad antioxidante en las hojas de *P. aduncum*. Los mayores valores se obtuvieron a 45 °C (79,92 mg eq. de ácido gálico·100 g⁻¹ de biomasa seca) para polifenoles totales, (74.898,90 y 508,07 µg eq. de TROLOX·g⁻¹ de biomasa seca), para actividad antioxidante mediante las técnicas FRAP y ABTS, respectivamente, por lo que es recomendable realizar el secado a esta temperatura, previo a cualquier utilización o procesamiento posterior con vista a preservar sus propiedades como antioxidante natural.

Palabras clave: polifenoles totales; capacidad antioxidante; FRAP; ABTS.



Introduction

The amazonian region of Ecuador is considered to be one of the most biodiverse areas of the planet. The many diverse species found there have been the cause for constant research, in particular for about the production of raw materials for the food, pharmaceutical and cosmetic industries (Luna-Fox et al., 2023a). The vast majority of plant species are unknown to the modern world; nonetheless, the different indigenous cultures have profited from the healing properties of the plants, such as the *Piper aduncum* L., commonly known as matico (Bedón and León, 2022).

Matico, a member of the Piperaceae family, grows to about 4 m in height, its greenish brown color bark is rough and granular, and with alternate individual leaves (Bedón and León, 2022). It grows wild in the coasts of central and southern America, the Amazon rainforest, and in the Andean valleys at 3,000 masl; it is native to Peru, Chile and Argentina (Alvarado, 2019).

In Ecuador, the plant grows between 0-2000 masl, mainly in the provinces of Azuay, Cañar, Carchi, Chimborazo, Cotopaxi, Pastaza and Zamora Chinchipe; locally, it is also known as “hierba del soldado” or “cordoncillo” (Portal et al., 2020). Many amazonian cultures have profited from its healing properties, using it to treat scars, stomach pains, ulcers, diarrheas, hepatic ailments and colitis (Alvarado, 2019). It contains important metabolites such as natural polyphenols, like tannins and flavonoids, which are found in a concentration of up to 5.7%. It also contains various glycosides and has antioxidant and cell membrane protective properties (Cai et al., 2016).

The relationship between free radicals and antioxidants has been the subject of research for almost four decades (Taverne et al., 2018). Different plant species have been studied, their active metabolites have been identified and their high antioxidant activity has been demonstrated, which can be used in the manufacture of medicines and also in the food sector (Soro et al., 2021).

The high antioxidant activity, oxidase inhibition, free radical scavenging and iron chelating capacity of polyphenolic compounds has aroused the interest of researchers. These metabolites have a broad and diverse pharmacological impact; Studies have shown that they modify the body's sensitivity to dangerous substances such as allergens (Vidal-Gutiérrez et al., 2020). Polyphenolic compounds have protective mechanisms on vascular and capillary walls, reducing fragility and antimicrobial activity (Muchiutti et al., 2019).

Various techniques have been used for the extraction of polyphenolic compounds in plant species, such as: microwave

extraction, supercritical fluid, ultrasound, among others (Otalora-Rodríguez et al., 2021). The efficiency of the techniques depends on factors such as time and type of solvent; likewise, the treatment of the plant material, the drying temperature and the particle size play an important role in the extraction performance (Cai et al., 2016). With this background, the objective of the research was to evaluate the effect of drying temperature on the polyphenolic compounds and antioxidant activity of the leaves of *Piper aduncum* L.

Materials and methods

The research was carried out in the Bromatology Laboratory of the Amazon State University (UEA), located at km 2½ Tena highway, Pastaza province. The matico leaves were re-collected in the city of Puyo, El Paico sector, located in the amazonian rainforest of the Pastaza province, Ecuador, on an altitude of 940 masl, 00°59'1" and 77°49'0" (Luna-Fox et al., 2023b). Before analyses, the leaves were washed with demineralized water and dried at room temperature. After that, they were placed in ovens at 45, 50 and 55 °C for 48 h. The moisture content was determined by biomass difference, and this result was used to calculate the initial biomass on a dry basis. The leaves were crushed in a KitchenAid brand mill, model BCG1110B and nominal frequency of 60 Hz, then sieved to obtain a particle size less than 0.5 mm (Gayosso-Rodríguez et al., 2018).

Obtaining the extracts

The extracts were obtained by applying ultrasound-assisted extraction, using a Branson 38000 ultrasonic bath equipment, CPXH series, with 5.7 L tank capacity, frequency of 40 Hz and power 110 W. Distilled water was used as solvent, following what was reported by Luna-Fox et al. (2023a), for which maximizing the caffeine and polyphenol content was considered a priority. The best conditions to extract the greatest amount of caffeine and polyphenols were with 15 g of guayusa in 100 mL of distilled water, applying the decoction for 30 min. The extracts were filtered using Whatman No 4 paper and stored at 4 °C in glass balls covered with aluminum foil until further use (Surco-Laos et al., 2020).

Spectrophotometric determination of total polyphenols

From the aqueous extract, 1 mL was taken in a 10 mL volumetric flask and 0.5 mL of the Folin-Ciocalteu reagent diluted in half with distilled water was added and left to rest for 10 min, then 0.5 mL of sodium carbonate at 20% was added and made up to volume with distilled water; it was shaken and protected from light with aluminum foil for 2 h at room temperature. Finally, the absorbance was measured at

765 nm in a Thermo brand UV-vis spectrophotometer, model Genesis 5 and series 3V0H228001 (Luna-Fox et al., 2023a).

The total polyphenol content was determined using a gallic acid calibration curve and the concentration of total polyphenols was expressed in milligrams equivalent to gallic acid per 100 g of matico leaves with a dry biomass base (mg EAG·100 g⁻¹ of dry biomass), for this, equation 1 was used, obtained from the mathematical model of the gallic acid calibration line, made with five concentrations and three replicates (Luna-Fox et al., 2023b).

$$A = 0.0734C - 0.0028 \quad (1)$$

where:

A: sample absorbance

C: sample concentration (mg·L⁻¹)

Antioxidant activity

The test was carried out by applying two methods, which were characterized as reliable and easy to apply (Suárez-Rebaza et al., 2019).

Antioxidant activity by ABTS

The ABTS radical cation decolorization assay, described by Re et al. (1999) was selected in order to determine the free radical scavenging activity. The ABTS radical was prepared by mixing solutions of 7 mM ABTS and 2.45 mM potassium persulfate, in equal parts. The solution was kept in the dark at room temperature for 16 h to allow the formation of the radical, which was diluted in ethanol to obtain an absorbance of 0.873. The preparation of the potassium persulfate solution was carried out by adding 0.663 g of the salt to distilled water and diluting to make up to 100 mL. The ABTS solution was prepared by dissolving 0.384 g in 10 mL of distilled water (Arteaga-Crespo et al., 2020). The results were expressed in micrograms equivalent of TROLOX·g⁻¹ of dry biomass (µg eq. T·g⁻¹ of dry biomass), calculated from equation 2.

$$A = 0.873 - 0.1304C \quad (2)$$

where:

A: absorbance of the sample read at 730 nm.

C: sample concentration (mg·L⁻¹).

Antioxidant activity by FRAP

Antioxidant activity was calculated by the FRAP assay, according to Benzie and Strain (1996). Eighty µL of each extract was placed in a 10 mL volumetric flask, to which 5 mL of freshly prepared FRAP solution was added. After adding the reagent, distilled water was added to the flask to make 10 mL, and it was left at 37 °C for 30 min, protected from light. The reading was recorded at a wavelength of 593 nm against the control solution. The FRAP reagent was prepared by mixing 2.5 mL of 2,4,6-pyridyl-s-triazine (TPTZ) solution with 2.5 mL of iron III chloride solution and 25 mL of acetate buffer.

To prepare the TPTZ solution, 0.03 g of reagent was weighed and placed in a 10 mL volumetric flask and diluted to the mark with 40 mM hydrochloric acid. Acetate buffer was prepared by

dissolving 0.0061 g of sodium acetate in 200 mL of distilled water, 40 Mm hydrochloric acid was added until the mixture reached a pH of 3.5, then diluted to volume with distilled water to 250 mL. To prepare the iron (III) chloride solution, 0.1352 g was dissolved in 25 mL of distilled water. The results were expressed in equivalent micrograms of Trolox·100 g⁻¹ of dry biomass (µg eq.T·g⁻¹ of dry biomass), and were calculated using equation 3 (Arteaga-Crespo et al., 2020).

$$C = \frac{A}{0,1879} \quad (3)$$

where:

A: absorbance of the samples read at 593 nm.

C: concentration of the samples (mg·L⁻¹)

Experimental design

The effect of three levels of drying temperature (independent variable) of matico leaves (table 1) on the concentration of polyphenolic compounds and the antioxidant activity of the aqueous extracts obtained (response variables) was assessed. The Design Expert version 10 software was used to assess the significance (P<0.05) of the study factor on the experimental response. ANOVA analysis was performed to assess the influence that temperature had on the response variables. In the table 1 shows the factorial design with intermediate point, carried out.

Table 1. Level of the variables selected in the factorial design.

Independent variable	Level of codified variable			
	Symbol	Low	Central	High
Drying temperature (°C)	A	-1 45	0 50	1 55

Results and discussion

Effect of temperature on the total polyphenolic compounds of matico

Figure 1 shows the Pareto Diagram, including the Bonferroni limit which is a statistical cut line; likewise, the study factor is represented by the vertical bar and the color shows the effect it had on the response variable; if the color is blue, then the effect of the factor on the response variable was negative, if the color is orange it was positive. The study factor was statistically significant (P<0.05), if it is above the Bonferroni limit. In this research, temperature significantly affected the polyphenolic compounds of matico, which was expected because these antioxidant compounds underwent an oxidation reaction that was accelerated by the effect of temperature.

Figure 2 shows the negative effect of temperature on the response variable, where at higher temperatures the concentration of polyphenols decreased; In this sense, the highest concentration of polyphenols was obtained in matico leaves dried at 45 °C.



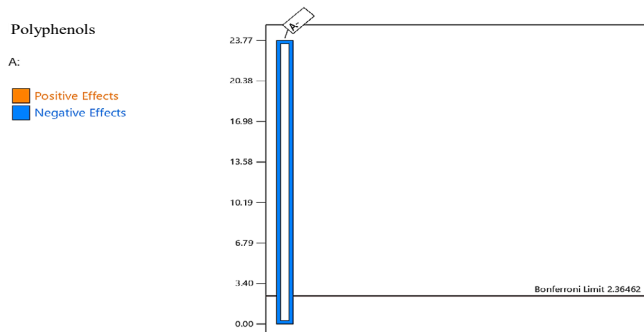


Figure 1. Pareto diagram on the effect of temperature on the total polyphenols of matico..

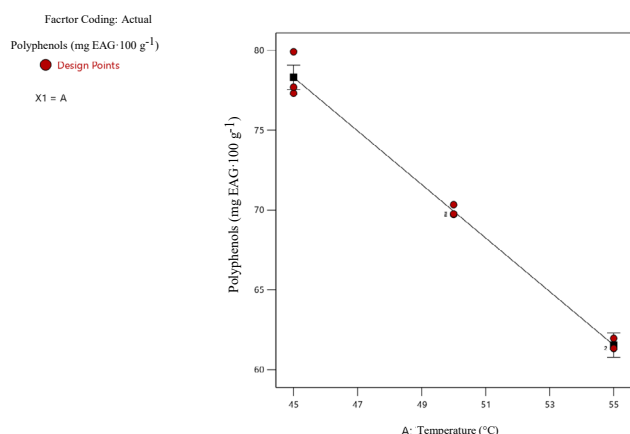


Figure 2. Effect of drying temperature on the total polyphenols of the matico.

Table 2 shows the selected factor model. The model’s F value of 659.19 implied that the model was significant. There was only a 0.01% chance that such a large F value was due to noise.

Table 2. ANOVA for the selected factorial model for total polyphenols.

Source	Sum of squares	gl	Mean square	F-value	P-value	
Model	421.85	1	421.85	659.19	< 0.0001	Significant
Temperature (A)	421.85	1	421.85	659.19	< 0.0001	
Residual	4.48	7	0.6400			
Lack of fit	0.0005	1	0.0005	0.06	0.9812	Not significant
Pure error	4.48	6	0.7465			
Corrected total	426.33	8				

Probability “P” values less than 0.05 indicated that the model terms were significant. In this case, temperature (A) was a significant term in the model. The lack of fit F value of 0.06 implied that the lack of fit was not significant relative to the pure error. There was a 98.12% chance that such a large lack of fit F value was due to noise.

According to Anderson and Whitcomb (2016), the difference between the predicted R² and the adjusted R² must be less than 0.2 for the factor model used in research to be appropriate. The value obtained in this research for the predicted R² of 0.9802 was in reasonable agreement with the adjusted R² of 0.9880; that is, the difference was less than 0.2 (table 3). Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable (Arteaga-Crespo et al., 2020). Its ratio of 44.47 indicated a suitable signal.

Table 3. Adjustment statistics for total polyphenols.

Standard deviation	0.8000	R ²	0.9895
Mean	69.93	Adjusted R ²	0.9880
CV (%)	1.14	Predicted R ²	0.9802
		Adequate accuracy	44.4699

Abbreviation: CV= coefficient of variation.

Design-Expert software generated a linear mathematical model that demonstrated the relationship between drying temperature and total polyphenols (equation 4).

$$\text{Total polyphenols} = 153.79445 - 1.67733A \quad (4)$$

where:

A: temperature (°C).

Equation 4 in terms of real factors can be used to make predictions about the response for given levels of each factor. In this case, the levels must be specified in the original units of each factor.

The experimental values in triplicate and predicted by the mathematical model are presented in table 4. The results obtained showed the suitability of the predictive model to cover the entire range of experimental results, which means that the model can be applied successfully.

The drying temperature had a negative effect on the polyphenols of the matico, the highest and lowest amount of total polyphenols was reported in the extract obtained with the samples dried at 45 and 55 °C, respectively. Rojas (2019) tested the effect of four drying temperatures (35, 40, 50 and 60 °C) on total polyphenols in leaves of *P. aduncum* and *Brunfelsia grandiflora*, demonstrating that the concentration of polyphenols decreased with increasing temperature; these results agreed with those obtained by Luisetti et al. (2020) whose application in the formulation of potential

functional foods, due to its beneficial effects on health, makes its selective extraction interesting. It is proposed to optimize the parameters of the extraction process of phenolic compounds, based on an experimental design of three variables at three levels. The parameters were drying temperature, Liquid/Solid ratio (L/S), demonstrating that the drying temperature was inversely proportional to the extraction yield of secondary metabolites.

Tabla 4. Experimental and predicted results for polyphenols.

Temperature (°C)	Polyphenols (experimental) mg EAG·100 g ⁻¹ DB	Predicted values mg EAG·100 g ⁻¹ DB
45	77.32	78.31
	79.92	
	77.69	
	69.71	
50	69.77	69.93
	70.33	
	61.32	
55	61.33	61.54
	61.96	

Ríos-Aguirre and Gil-Garzón (2021) mentioned that drying temperatures above 50 °C degrade some bioactive compounds in plants; In this sense, Nova et al. (2023) indicated that drying temperatures that were between 35-45 °C were appropriate to maintain the secondary metabolites of the plants, with antioxidant activity.

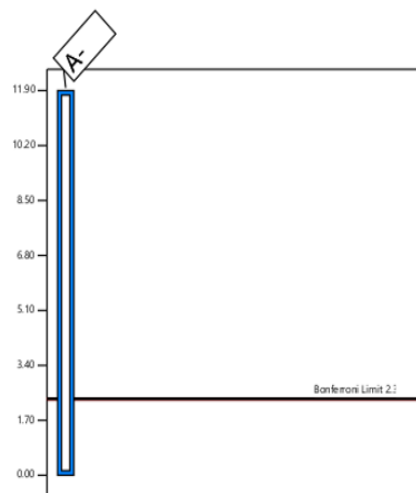
The polyphenol results achieved in this research varied from 61.32 to 79.92 mg EAG·100 g⁻¹ db, these results were higher than those obtained by Załuski et al. (2018). Different investigations such as those by Herrera-Calderon et al. (2019), Uribe et al. (2021) and Aldair et al. (2022) have reported a wide variety of total polyphenols in different species of matico, this variability may be due to factors such as plant age, climatic and soil conditions; In this sense, Alvarado (2019) pointed out that the altitude at which matico leaves were re-collected significantly influenced the polyphenol content of this plant.

On the other hand, the extraction techniques, particle size and the solvent used in the extraction directly influence the yield of the polyphenols. Mamoori and Janabi (2018) evaluated the effect of two extraction methods to quantify polyphenols spectrophotometrically, demonstrating that the results obtained by ultrasound were superior to those obtained by microwaves (figure 3).

FRAP

A:

Positive Effects
Negative Effects



Abts

A:

Positive Effects
Negative Effects

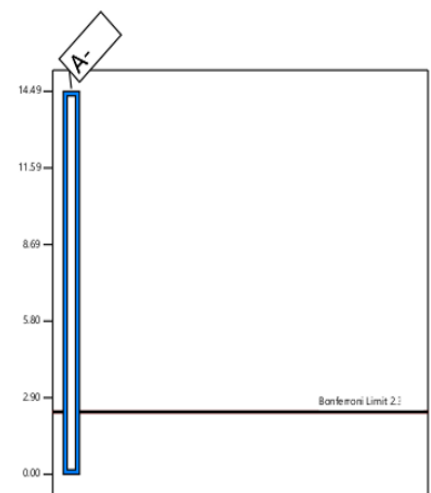


Figure 3. Pareto diagram of the effect of temperature on the antioxidant activity of matico.

Effect of temperature on the antioxidant activity of matico

The influence of temperature on the antioxidant activity of matico evaluated by the FRAP and ABTS techniques is shown in figure 3. The Pareto Diagram shows that the temperature was located above the Bonferroni limit, which indicated that this factor was statistically significant ($P < 0.05$).

Figure 4 shows that temperature was inversely proportional to antioxidant capacity; That is, to the extent that the levels of this factor increased, the response variable decreased. In this sense, for the FRAP (A) and ABTS (B) technique, the superior results were achieved at 45 °C with values of 74,898.90 and 508.07 $\mu\text{g ET}\cdot\text{g}^{-1}$ db, respectively.

Table 5 presents the ANOVA with the factorial model selected for antioxidant activity. The model's F value of 113.11 implied that this was significant and that there was only a 0.01% chance that such a large F value would occur due to noise.

P values less than 0.05 proved that the model terms were significant. In this case, temperature (A) was a significant factor in the model. Values found above 0.10 indicated that the model terms were not significant. The lack of fit F value of 2.76 implied that the lack of fit was not significant relative to the pure error. There was a 14.76% probability that such a large lack of fit F value would occur due to noise.

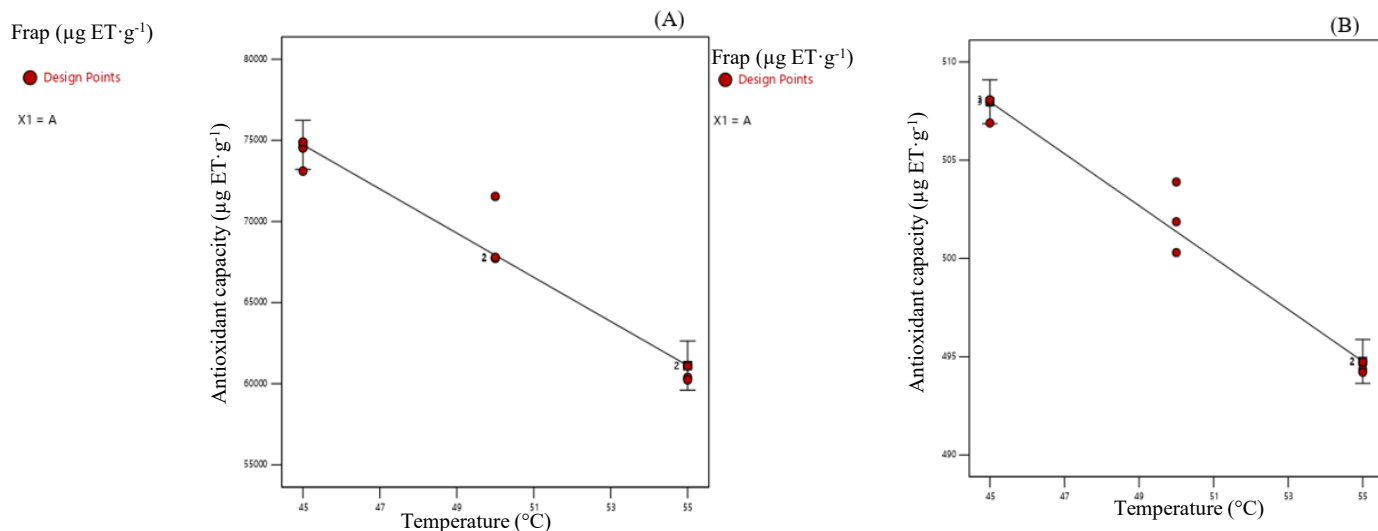


Figura 4. (A) Effect of temperature on antioxidant activity by FRAP. (B) Effect of temperature on antioxidant activity by ABTS.

Tabla 5. Experimental and predicted results of antioxidant activity.

Temperature (°C)	Antioxidant activity (FRAP) µg ET·g ⁻¹ db	Predicted values (FRAP) µg ET·g ⁻¹ db	Antioxidant activity (ABTS) µg ET·g ⁻¹ db	Predicted values (ABTS) µg ET·g ⁻¹ db
	74.528,60		507,97	
	73.105,60	74728,95	508,07	507,97
45	74.898,90		506,89	
	67.727,00		501,87	
	67.787,20	67921,50	503,89	501,37
50	71.544,30		500,30	
	60.383,90		494,37	
	60.234,50	61117,05	494,73	494,76
55	61.088,00		494,21	

Again, considering what was proposed by Anderson and Whitcomb (2016), the difference between the predicted R² and the adjusted R² must be less than 0.2 for the factor model used in research to be appropriate. In the present research the predicted R² of 0.9174 was in reasonable agreement with the adjusted R² of 0.9334; that is, the difference was less than 0.2 (table 6). Adequate precision measured signal-to-noise ratio. A ratio greater than 4 is desirable. Its ratio of 18.421 indicates a suitable signal.

The Design-Expert software generated two mathematical models, one for FRAP (equation 5) and another for ABTS

(equation 6), which demonstrated the relationship between drying temperature and antioxidant activity b.

Tabla 6. Fit statistics for antioxidant activity.

Standard Deviation	1,567.17	R ²	0.9417
Mean	67,922.00	Adjusted R ²	0.9334
CV (%)	2.31	Predicted R ²	0.9174
		Adequate precision	18.4210

Abbreviation: CV = coefficient of variation.

$$\text{Antioxidant activity (FRAP)} = 1.35966 \times 10^5 - 1360.89A \quad (5)$$

$$\text{Antioxidant activity (ABTS)} = 567.40000 - 1.3207A \quad (6)$$

where:

A: temperature (°C)

Equations 5 and 6 can be used to make estimates about the response variable at different temperature levels. In this case, the levels must be specified in the original units of the study factor.

The experimental responses in triplicates and the values predicted by the mathematical models are presented in table 7. The predicted results showed the suitability of the predictive models to cover the entire range of experimental results, which meant that the model can be successfully applied.

The temperature had a behavior inversely proportional to the antioxidant activity of the matico subjected to drying, this agreed with what was indicated by Soto-Celis and Jáuregui,

(2018) who evaluated the effect of drying temperature on the antioxidant activity of *Psidium guajava* L. and it was shown that this decreased with increasing temperature; Likewise, Rosa-Hernández et al. (2018) demonstrated that drying temperature had a negative effect on the antioxidant activity of *Citrus paradisi*.

Tabla 7. ANOVA for the selected factorial model in antioxidant capacity.

Source	Sum of squares	gl	Mean square	F-value	P-value	
Model	2,778E+08	11	2,778E+08	113,11	< 0,0001	Significant
Temperature (A)	2,778E+08	11	2,778E+08	113,11	< 0,0001	
Residual	1,719E+07	77	2,456E+06			
Lack of fit	5,420E+06	11	5,420E+06	2,76	0,1476	Not significant
Pure error	1,177E+07	66	1,962E+06			
Corrected total	2,950E+08	88				

Zaluski et al. (2018) mentioned that matico is a plant whose antioxidant activity is determined mainly by the phenolic compounds it possesses. According to Indirayati et al. (2020) flavonoids, catechins, flavones, and hydroxycinnamic acids were found to be the polyphenols present in matico with the highest antioxidant activity. The amount of these compounds depended on the species, soil conditions, light hours and climatic conditions (Arroyo et al., 2022). The antioxidant activity results obtained in this study were higher than those reported by Pacheco et al. (2022) where it reached values of 1,770 and 86.4 $\mu\text{g ET}\cdot\text{g}^{-1}$ of dry biomass for the FRAP and ABTS techniques, respectively. The discrepancy in values could be attributed to differences in the samples analyzed (Chizzola et al., 2018), genetic variability (Alipio-Rodríguez et al., 2020) and environmental conditions (Średnicka-Tober et al., 2019).

It is worth emphasizing that the results of this research have transcendental importance when processing *P. aduncum* leaves. as non-food raw material for the manufacture of medicinal products, nutraceuticals, cosmetics, among others, for which it is convenient to dry at a temperature that does not exceed 45 °C.

Conclusion

The drying temperature had a negative effect on the polyphenolic compounds and antioxidant activity of dried matico leaves. The best results were obtained at 45 °C with values of 79.92 mg EAG \cdot 100 g $^{-1}$ of dry biomass for total polyphenols; 74,898.90 and 508.07 $\mu\text{g ET}\cdot\text{g}^{-1}$ of dry biomass, for antioxidant activity determined through the FRAP and ABTS spectrophotometric techniques, respectively. This contribution is crucial in the context of optimizing drying processes for the conservation of bioactive properties in natural products, offering practical guidelines for obtaining products with greater antioxidant benefits.

Conflict of interests

The authors declare that they have no conflicts of interest in this publication in any of its phases.

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