Article original

Chemical Characterization and Evaluation of the Antioxidant Activity of Bactris Minor Extract

Caracterización química y evaluación de la actividad antioxidante del extracto de *Bactris minor*

Angie Paola Solorzano Padilla[1] https://orcid.org/0000-0002-6119-1221
Andrés Felipe Narvaez Martelo[1] https://orcid.org/0000-0001-5629-4341
Stephanie De La Espriella Angarita[1] https://orcid.org/0000-0003-1879-3005
Clemente Granados Conde[1]* https://orcid.org/0000-0002-3201-4357
Glicerio León Mendez[2] https://orcid.org/0000-0002-9899-5872

[1]Universidad de Cartagena, Facultad de Ingeniería, Grupo de Ingeniería en Investigación, Innovación, Calidad Alimentaria y Salud (INCAS). Cartagena, Bolívar, Colombia.
[2]Fundación Universitaria Tecnológico Comfenalco, Facultad de Ingeniería, Grupo de Investigación CIPTEC. Cartagena, Bolívar, Colombia

.

*Autor para la correspondencia: cgranadosc@unicartagena.edu.co

RESUMEN

Introducción: Los antioxidantes son sustancias químicas complejas que pueden encontrarse de forma natural extraídas de frutas y plantas o sintéticamente. Su importancia es valiosa para la salud, debido a su capacidad para neutralizar los radicales libres que contienen uno o más electrones no apareados responsables de muchas enfermedades degenerativas.

Objetivo: Evaluar la actividad antioxidante del corozo (*Bactris minor*).

Métodos: La pulpa de *Bactris minor* se extrajo del fruto fresco. Se determinaron su pH, grado Brix, acidez titulable, humedad, cenizas, grasas, proteínas, fibra cruda y contenido de carbohidratos. La actividad antioxidante se evaluó mediante los métodos del radical difenil-1-picrilhidrazilo$^{\bullet}$ y del ácido 2,2-azino-bis$^{\bullet+}$.

Resultados: Los sólidos solubles totales resultaron de 4,4 Brix, la acidez del 1,º7 %, el pH de 3,57, el contenido de cenizas de 2,82 %, proteínas del 1,13 %, grasa de 0,120 % y la fibra cruda de 1,103 %. La actividad antioxidante presentó valores de concentración inhibitoria de 80,3 µg/L y 34,8 µg/L mediante los métodos del radical difenil-1-picrilhidrazilo$^{\bullet}$ y del ácido 2,2-azino-bis$^{\bullet+}$ respectivamente.

Conclusiones: La pulpa de corozo (*Bactris minor*) se considera prometedora para diseñar productos nutracéuticos por su alta actividad antioxidante.

Palabras clave: actividad antioxidante; composición química; productos naturales; *Bactris minor.*

ABSTRACT

Introduction: Antioxidants are complex chemicals that can be found naturally extracted from fruits and plants or synthetically. Their importance is valuable for health, due to their ability to neutralize free radicals containing one or more unpaired electrons responsible for many degenerative diseases.

Aim: To evaluate the antioxidant activity of corozo (Bactris minor).

Methods: Bactris minor pulp was extracted from fresh fruit. Its pH, Brix, titratable acidity, moisture, ash, fat, protein, crude fiber and carbohydrate content were determined. Antioxidant activity was evaluated by the diphenyl-1-picrylhydrazyl- and 2,2-azino-bis-+ acid methods.

Results: Total soluble solids were 4.4 Brix, acidity 1.7%, pH 3.57, ash content 2.82%, protein 1.13%, fat 0.120% and crude fiber 1.103%. Antioxidant activity presented inhibitory concentration values of 80.3 µg/L and 34.8 µg/L by the diphenyl-1-picrylhydrazyl- and 2,2-azino-bis-+ acid methods, respectively.

Conclusions: Corozo (Bactris minor) pulp is considered promising for designing nutraceutical products due to its high antioxidant activity.

Keywords: antioxidant activity; chemical composition; natural products; Bactris minor.

Received: 25/06/2023
Accepted: 26/08/2024

Introduction

Antioxidants are compounds used in the food industry for their conservative ability and are considered to prevent disease.[1] These substances have the ability to delay or prevent catalytic oxidations and the processes that drive the formation of free radicals.[2] In addition, they are used to prevent the oxidation of lipid and other constituents to preserve the quality of food.[3]

There is currently a growing interest in natural additives as potential antioxidants, which is why in recent years many sources of plant origin have been studied.[4] *Choksirb*[5] states that products of this origin possess a variety of chemical compounds that act as antioxidant agents, inhibiting the formation and damage produced by free radicals, which not only produce rancidity and food losses in their storage, but are associated with chronic diseases such as cancer, Alzheimer's disease, cardiovascular diseases and are strongly linked to the aging process.

Different methodologies have been used to evaluate the antioxidant capacity in vitro of the fruits, with the results obtained depending on the method used[6] of which frap, méthods radical difenil-1-picrilhidrazilo (DPPH,) acids 2,2-azino-bis$^{\bullet+}$ (ABTS), and oxygen radical absorbance capacity (ORAC) are the most used. It is recommended that at least two (or even all) of these assays be combined to provide a reliable picture of the total antioxidant capacity of a food product.[7]

The consumption of tropical fruits is increasing in both domestic and international markets due to the growing recognition of their value to human health. Colombia has a large number of underexploited native and exotic fruit species that may be of interest to agribusiness and constitute a possible source of income for the local population. These represent an opportunity for local producers to access special markets where consumers appreciate the exotic character of these products and the presence of bioactive compounds capable of preventing degenerative diseases.[8]

The corozo is a fruit that is produced in the northern region of the Colombian Atlantic which has a great cultural value in addition to being an exotic fruit of great acceptance in its flavour, has a high content of anthocyanins and tartaric acid which gives it a great source of antioxidants, it is in the wild in most cases so it appears in a specific season of the year and it is very difficult to acquire.[9]

Considering that there are few studies about this fruit, it was sought to verify its usefulness in the food industry, based on its antioxidant content from its pulp offering a natural alternative typical of the Colombian region, for its application in the preparation of wine aperitif of fruits in addition to determining if they could be exploited as a source of natural antioxidants.[10]

For this reason, the objective of this study was to evaluate the antioxidant activity of corozo (*Bactris minor*).

Methods

The *Bactris minor* fruits were collected in the municipality of Magangué (9°14'48"N 74°45'34"W). The fruits were selected taking into account that they were free of external damage and presented commercial maturity; they were washed and scalded at 90ºC for 5 minutes. The juice obtained was packed in airtight bags and then refrigerated to a temperature of 4°C and freeze-dried with a Labconico brand equipment at -52ºC for 48 hours.[2]

Determination of the physicochemical characteristics of the corozo

The methods used for the determination of physicochemical characteristics are as follows:

- Acidity: Titration - AOAC 940.26.[11]
- pH: Potentiometer - AOAC 10.041.[11]
- Soluble solids: Refraction - AOAC 22.024.[11]
- Density: Gravimetry - AOAC 11.002.[11]
- Moisture: Gravimetry - AOAC 930.15.[11]
- Ash: Gravimetry - AOAC 942.05.[11]
- Protein: Kjeldahl - AOAC 2001.11.[11]
- Carbohydrates: Gravimetry.
- Crude fiber: Polysaccharide analysis - AOAC 45.4.07.[11]
- Fat: Soxhlet.

Determination of the major components of extract by gas chromatography-mass spectrometry (GC / MS)

An Agilent 7890A/5975C chromatograph was used. Each extract sample (50 µL) was dissolved in 450 µL dichloromethane, the injector temperature was 250°C, a HP-5MS 5% Phenyl methyl silox capillary column was used; Helium was used as carrier gas at constant flow rate of 1 mL /min, pressure of 7.6354 psi and linear velocity of 36 cm/sec.

Initial temperature 45°C and transfer line temperature 280°C. Mass spectra were obtained by electron ionization (70 eV), with automatic scanning at a range of m/z 30-400 u.m., at 3.85 scan/s. The components identities were assigned by comparison of each spectrum with the database standards reported in the literature.[12]

DPPH$^{\bullet}$ radical method

Free radical scavenging activity DPPH was determined using the method described by *Silva* et al[13] (with some modifications 75 µL of sample were added to 150 µL of a methanol solution of DPPH (100 ppm) and they were incubated at room temperature for 30 min, after the disappearance of the DPPH radical was determined spectrophotometrically at 405 mm in microplate reader Multiskan Ex (Thermo Scientific®, USA).

The percentage of inhibition (% Inh) was calculated using (equation 1).

$$\%\ inhibition\ = \frac{(A_0 - A_f)}{A_0} * 100 \quad (1)$$

Where A0 and Af are the target absorbance values (DPPH solution in alcohol) and the sample (DPPH solution plus antioxidant dissolved in ethanol), respectively.

ABTS$^{\bullet+}$ radical method

The free radical scavenging activity ABTS was determined using the method described by *Re* et al.[14] with some modifications.

The ABTS radical was formed following the reaction of 3.5 mM ABTS with 1.25 mM of potassium persulfate (final concentration).

The samples were incubated at 5°C and in darkness for 16 h. Once the ABTS radical was formed, it was diluted with ethanol until having an absorbance of 0.7 ± 0.05 at 734 nm.

To a volume of 190 µL of the ABTS radical dilution was added 10 µL of the sample under study and incubated at room temperature for 5 minutes.

After this time, the disappearance of the ABTS radical at 734 nm was determined spectrophotometrically in the microplate reader Multiskan Ex (Thermo Scientific®, USA).[15,16]

Statistical Analysis

All trials were performed by sextupled. The results were expressed as the mean ± SD (standard deviation). Significant differences were determined by ANOVA analysis followed by Dunnett's or Tukey's test or as deemed appropriate.

Results

The *Bactris minor* extract presented a yield of 0.1%, this possibly because the *Bactris minor* is a fruit that has little mesocarp, in addition it has a percentage of water of approximately 83%.

Table 1 shows the physicochemical characteristics of the *Bactris minor* and the the results from the microbiological tests that were performed on the pulp.

Table 1- Results of the physicochemical characteristics and Microbiological tests of the corozo

Characteristics	Results	Other research
Ash	2.82% ± 0.064	1.1%[15]
Brix Grades	4.4	4.5[16]
Moisture	82.2% ± 0.265	79.6%[15]
Protein	1.13% ± 0.029	1.02%[16]
Acidity	1.7%	2.13%[16]
Carbohydrates	13.72% ± 0.280	-
pH	3.57	3.04[16]
Crude fiber	1.103% ± 0.100	-
Compound	0.120% ± 0.017	-
Majority components	-	-
Method	-	-
Antioxidant capacity of corozo extract	-	
Fat	-	-
Analysis	Result	Norm

		M	M
Mesophilic aerobes	123 CFU /g	$5x10^3$	$2x10^4$
Fungi	142 CFU /g	$1x10^3$	$3x10^3$

Where: m: Maximum permissible index to identify the level of good quality. M: Maximum permissible index to identify the acceptable level of quality. *CFU: colony-forming unit

According to NTC 404[17] which establishes the requirements and test methods that fruit juices and pulps must meet, the worked pulp is within the parameters of good quality, which indicates that there were good hygiene practices during the obtaining of the pulp.

Table 2 shows the majority components found in the corozo extract.

Table 2 - Majority components of corozo extract and antioxidant capacity, analyzed by DPPH and ABTS methods

Compound		**(rt, min)***	**Relative area (%)**
Majority components	Cyanidin-3-glucoside	10.3	6.23
	Peonidine-3-glucoside	6.8	4.36
	Cyanidin-3-rutinoside	7.8	5.81
	3-hydroxy-2-butanone	4.40	2.20
	Benzyl alcohol	30.5	0.26
Method		**Results**	
Antioxidant capacity of corozo extract	DPPH	80.3±0.74 µg/L	
	ABTS	34.8±0.62 µg/L	

*Retention time (rt)

The antioxidant activity of corozo extract was evaluated by these two different methods: DPPH• and $ABTS^{\bullet+}$. The results are expressed as anti-radical activity or IC_{50}, which is defined as the concentration of the antioxidant that decreases the absorption of the radical to 50% of the initial amount, (table 2).

Discussion

Table 1 shows that it has a high amount of humidity 82.2% this value exceeded up to 20% that obtained by *Arrazola*[16] and also exceeds that exposed by *Acuña*[15] which was 79.6%. In addition, there is a significant difference in the percentage of ash between the result of this research and that demonstrated by *Acuña*[15] 2.82% and 1.1% respectively. It is also observed that it has a low-fat content which is normal characteristic of fruits except avocado and coconut.

The discrepancy between these results is due to the fact that the physicochemical characteristics of the fruit can be affected by the origin and state of the plant, the influence of the environment,[18,19] or variables such as density of plant planted, amount of water used in irrigation and amount of light in the cultivation of the plant, the geographical, botanical and agricultural conditions of its cultivation, and the method of its extraction[20] because we know that not all departments have the same characteristics.

The antioxidant capacities of the fruits vary depending on their content in vitamin C, vitamin E, carotenoids, flavonoids and other polyphenols.

A previous study carried out on this fruit both in the shell and in its edible part, reported the presence of Cyanidin-3-rutinoside and cyanidin-3-glucoside were identified as the main constituents (72.2 and 15.7%, respectively), followed by peonidin-3-rutinoside (5.1%), cyanidin - 3- (6 - O - malonyl) - glycoside (4.1%), cyanidin - 3 - sambubioside (1.8%) and peonidin - 3 - glycoside (1.1%).[21]

The total phenolic values in the peels were higher than in the pulps. Phenolic compounds tend to accumulate in the dermal tissues of the plant body due to their potential role in protecting against ultraviolet radiation, acting as attractants in fruit dispersal and as defense chemicals against pathogens and predators.

Table 2 presents the results obtained in the determination of the antioxidant capacity of corozo extract. These results differ from those found by *Contreras*[3] where he determined the antioxidant capacity of the corozo pulp and the epidermis by means of the ABTS and FRAP methods whose results were for the pulp 16.4 and 7.84 ppm and for the epidermis 48.1 and 24ppm, respectively.

A similar study was carried out by *Barrera*[22] where he measured the antioxidant activity of this fruit, but its value was not very high, they explain that this is because the fruit they used was not in an optimal stage of ripening so it was pale in color indicating a reduced amount of polyphenols.[23,24]

Evaluations based on the use of radicals such as DPPH• and ABTS•+ are considered by the scientific community as the most common spectrophotometric methods used for the determination of the antioxidant capacity of essential oils and plant extracts, because the procedures required in these methodologies are simple, fast, sensitive and reproducible.[18]

Therefore, the pulp of Corozo (*Bactris minor*), is considered promising to design nutraceutical products for its high antioxidant activity.

Acknowledgments

The authors thank the University of Cartagena and Fundación Universitaria Technologic Comfenalco for providing space, resources, and time for researchers

References bibliographic

1. Fernández M, Soto Z, Castro P, Casasa L, Mantella C, Martinez E. High-Pressure Fractionation of Tropical Fruits with Potential Antibacterial Activity: M. Indica L. and B. Guineensis. Chemical engineering transactions. 2019;75:55-60 DOI: 10.3303/CET1975010

2. Torrenegra M, Villalobos O, Castellar E, León G, Granados C, Pajaro N, Caro M. Evaluation of the antioxidant activity of pulps from Rubus glaucus B., Vaccinium floribundum K. and Beta vulgaris L. Rev Cubana Plant Med. 2016 [access 25/04/2023];21:1-8. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-47962016000400009

3. Erşan S, Berning J, Esquivel P, Jiménez V, Carle R, May B, *et al*. Composición fitoquímica y mineral de frutos y semillas de palmas silvestres de Bactris

guineensis (L.) HE Moore de Costa Rica. Revista de composición y análisis de alimentos. 2020 [access 25/04/2023];94. Disponible en: https://catedrahumboldt.ucr.ac.cr/ recursos/ publicaciones

4. León G, Osorio M, Ortega R, Pajaro N, Torrenegra M, Herrera A. Design of an Emulgel-Type Cosmetic with Antioxidant Activity Using Active Essential Oil Microcapsules of Thyme (Thymus vulgaris L.), Cinnamon (Cinnamomum verum J.), and Clove (Eugenia caryophyllata T.). International Journal of Polymer Science. 2018;1:1-16. DOI: 10.1155/2018/2874391

5. Choksi R, Boylston W, Rabek J, Widger W, Papaconstantinou J. Oxidatively damaged proteins of heart mitochondrial electron transport complexes. Biochim Biophys Acta. 2004;1688(2):95-101. DOI: 10.1016/j.bbadis.2003.11.007

6. Georgiana I, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021;22(7):3380. DOI: 10.3390/ijms22073380

7. Gulcin I. Antioxidants and antioxidant methods: an updated overview. Arch Toxicol. 2020 [access 15/04/2023];94(3):651-715. Disponible en: https://link.springer.com/ article/10.1007/s00204-020-02689-3

8. Osorio C, Acevedo B, Hillebrand S, Carriazo J, Winterhalter P, Morales AL. Microencapsulation by spray-drying of anthocyanin pigments from Corozo (Bactris guineensis) fruit. J Agric Food Chem. 2010;58(11):6977-85. DOI: 10.1021/jf100536g.

9. Rojano B, Zapata I, Cortes F. Anthocyanin stability and the oxygen radical absorbance capacity (ORAC) values of Corozo aqueous extracts (Bactris guineensis). Revista Cubana de Plantas Medicinales. 2012 [access 15/04/2023];17(3):244-55. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-479620120003000 05& lng=es.

10. León D, De la Espriella S, Leon G, Pacheco S, González M, Tapia Y, *et al*. Development of an antioxidant cosmetic using Bactris guineensis pulp as an active. Pharmacologyonline. 2021 [access 15/04/2023];3:1626-37. Disponible en: https://pharmacologyonline.silae.it/files/archives/2021/vol3/PhOL_2021_3_A177_ LeonMendez.pdf

11. AOAC. Oficial methods of analysis William Horwitz. EEUU, Washington D.C: Association of Analytical Chemists. AOAC.1990. [access 08/05/2023] Disponible en: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf

12. León D, De la Espriella S, Leon G, Granados C, Perez T. Development of an antioxidant cosmetic using as an active essential oil of Rosa spp. Pharmacologyonline. 2021 [acceso 08/05/2023];1:49-59. Disponible en: https://pharmacologyonline.silae.it/files/newsletter/2021/vol3/PhOL_2021_3_N005 _Leon-Mendez.pdf

13. Silva B, Andrade P, Valentao P, Ferreres F, Seabra R, Ferreira M. Quince (Cydonia oblonga Miller) Fruit (Pulp, Peel, and Seed) and Jam: Antioxidant Activity. J. Agric. Food Chem. 2004;52:4705-12. DOI: 10.1021/jf040057v

14. Re R, Pellegrini A, Proteggente A, Pannala A. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 1999;26:1231-37. DOI: 10.1016/s0891-5849(98)00315-3.

15. Acuña H. Evaluación del comportamiento de tres enzimas comerciales en la fermentación y clarificación de mosto del fruto de corozo Bactris minor para obtener vino de fruta [tesis en opción del titulo Ingeniero en Alimentos]. [Colombia, Bogotá]: Universidad de la Salle. Facultad de ingeniería. Bogotá, D.C., Colombia, 2009, [access 09/04/2023]:633 Disponible en: https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?params=/context/ing_alimentos/article/1035/&path_info=T43.09_A93e.pdf

16. Arrazola P. Agroindustrialización del Corozo (Bactris Minor) obtención de vino a partir del fruto de corozo. Revista temas agrarios. 1997 [access 09/04/2023];2:58. Disponible en: https://repositorio.fedepalma.org/handle/123456789/84736

17. NTC 404, Instituto Colombiano de Normas Técnicas y Certificación – Colombia. Requisitos y métodos de ensayo que deben cumplir los jugos y pulpas de frutas. Quinta Actualización, Colombia, Bogotá: ICNT; 2007 [access 09/04/2023]. Disponible en: https://n9.cl/ss3oi

18. De Oliveira S, de Souza GA, Rodríguez C. Evaluation of antiradical assays used in determining the antioxidant capacity of pure compounds and plant extracts. Quim. Nova. 2014 [access 15/04/2023];37(3):497-503. Disponible en: https://www.scielo.br/j/qn/a/hmXGgvvdR9MWWw3sYxBQqKs/?lang=en

19. Chlodwig F, Novak J. Essential oil compounds in a historical sample of marjoram (Origanum majorana L., Lamiaceae Breeding of Origanum species. J. Agric. Food Chem. 2001 [access 15/04/2023];48(9):4156–61. Disponible en: https://n9.cl/blz4nb

20. Combariza M, Blanco C, Stashenko E, Shibamoto T. Limonene concentartion in lemon (Citrus volcameriana) peel oil a function of ripeness. J. High Resol. Chromatogr. 1994 [access 13/05/2023];17(9):643-6. Disponible en: https://n9.cl/9iw72

21. Osorio C, Carriazo J, Almanza O. Antioxidant activity of corozo (Bactris guineensis) fruit by electron paragmagnetic resonance (EPR) spectroscopy. Eur Food Res Technol. 2011;233:103–8. DOI:10.1007/s00217-011-1499-4

22. Barrera A. Evaluación de la actividad antioxidante de extractos de cuatro frutos de interés comercial en Colombia y actividad citotoxica In vitro en la línea celular de fibrosarcoma HT1080 [tesis de Liceniado en Biología] [Colombia, Bogotá]: Universidad Javeriana, Facultad Ciencias/Biologia, Colombia. 201 [acceso 07/04/2023] Disponible en: https://repository.javeriana.edu.co/handle/10554/8850

23. Garro A, Cardona W, Rojano B, Robledo SM, Alzate F. Actividad antioxidante y citotóxica de extractos de Pilea dauciodora Wedd (Urticaceae). Rev Cubana Plant Med. 2015 [access 15/10/2024];20(1):88-97. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1028-479620150001000 08 &lng=es

24. Gualdron J. Corozo (Bactris guineensis) fruit extract has antiviral activity in vitro against SARS-CoV-2. Functional Foods in Health and Disease. 2022;12 (9). DOI: 10.31989/ffhd.v12i9.918

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Conceptualization: Clemente Granados Conde, Stephanie De La Espriella Angarita, Glicerio Leon Mendez.

Data curation: Glicerio Leon Mendez.

Formal analysis: Clemente Granados Conde, Stephanie De La Espriella Angarita.

Acquisition of funds: Clemente Granados Conde.

Research: Clemente Granados Conde, Glicerio Leon Mendez, Stephanie De La Espriella Angarita, Angie Paola Solorzano Padilla, Andrés Felipe Narvaez Martelo.

Project management: Clemente Granados Conde, Glicerio Leon Mendez.

Software: Glicerio Leon Mendez, Stephanie De La Espriella Angarita.

Validation: Stephanie De La Espriella Angarita.

Visualization: Glicerio Leon Mendez.

Writing - original draft: Glicerio Leon Mendez, Angie Paola Solorzano Padilla, Andrés Felipe Narvaez Martelo.

Writing – revision and editing: Glicerio Leon Mendez.