



## Corozo (*Bactris guineensis*) fruit extract has antiviral activity *in vitro* against SARS-CoV-2

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### ABSTRACT

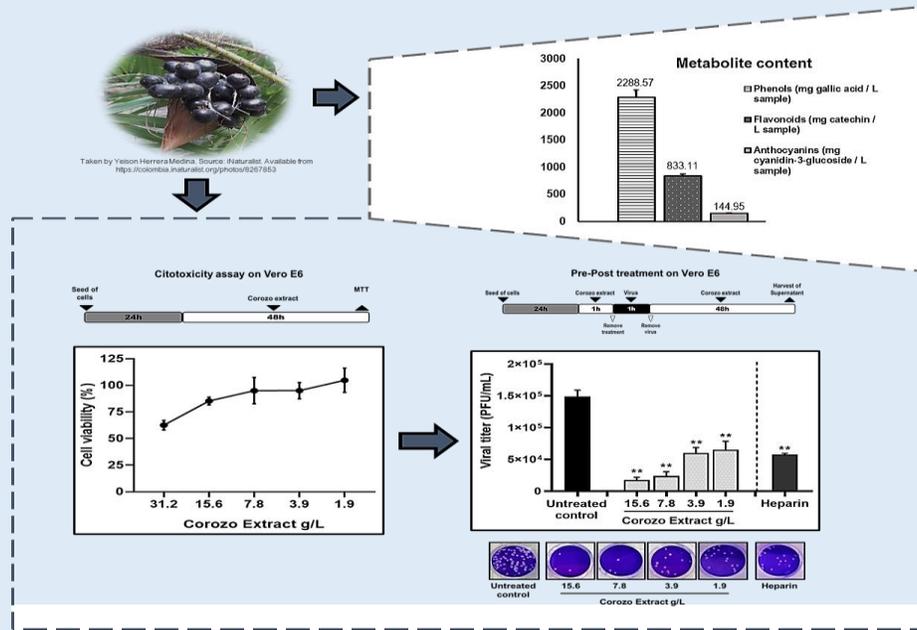
**Background:** Given the current COVID-19 pandemic, numerous drug development studies are being carried out for the treatment and control of this disease. This study aimed to evaluate the *in vitro* antiviral potential of Corozo fruit extract (*Bactris guineensis*) against SARS-CoV-2.

**Methods:** Corozo extract (CE) was prepared from the pulp of mature Corozo fruits. The total content of phenols, flavonoids, and anthocyanins in the extracts was determined using the Folin-Ciocalteu, aluminum chloride, and pH differential methods, respectively. The cytotoxicity on Vero E6 cells was evaluated by MTT assay. Antiviral activity was evaluated by pre-post-treatment using a Colombian isolate of SARS-CoV-2. Viral titer was quantified by plaque assay.

**Results:** Anthocyanin concentration of CE was  $144.95 \pm 10.3$  mg cyanidin-3-glucoside/L. The cytotoxicity of CE on Vero E6 was lower to 20 % at 15.6 g/L. Corozo extract inhibited SARS-CoV-2 at 15.6, 7.8, 3.9 and 1.9 g/L with inhibition percentages of 88.2%, 84%, 59.6% and 56.3%, respectively.

**Conclusion:** This is the first report on the *in vitro* antiviral effect of Corozo fruit extract against SARS-CoV-2. Since this is a natural product, proven safe for consumption, in the future and with further studies, it could be considered an important functional food that can be useful in preventing strategies to fight against COVID-19.

**Keywords:** *Bactris guineensis*, Corozo fruit, antiviral activity, SARS-CoV-2, COVID-19.



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**INTRODUCTION**

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causing agent of COVID-19, has been threatening the health and lives of millions of people worldwide, since it was identified in Wuhan (China) in late 2019 [1]. To date, COVID-19 treatment has been mainly supportive, based on symptoms management, including the use of anti-inflammatory drugs and, in severe cases, oxygen administration and mechanical ventilation, due to the absence of approved drugs or preventing treatment [2]. Many drug and vaccine development studies are currently being conducted to combat SARS-CoV-2; however, conventional approaches are expensive and time-consuming to be approved for widespread use. In this sense, the use of dietary molecules with antiviral activity, which are abundantly

found in functional foods, has acquired great interest worldwide [3].

According to the Functional Food Center (FFC), the definition of functional foods is: “Natural or processed foods that contain biologically-active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms” [4]. Currently, the benefits of functional food consumption are widely accepted for the prevention and management of several diseases, especially those characterized by an inflammatory component [5, 6]. Several dietary molecules such as savinin, curcumin, and anthocyanins have been identified by molecular docking studies as effective agents against SARS-CoV-2 [7, 8].

Corozo palm (*Bactris guineensis*), belonging to the *Aceraceae* family, is native to warm regions of South and Central America [9]. It has an oval shape, reddish-purple fruit of approximately 3 cm in diameter, which hangs in 50-100 unit clusters [10]. Corozo fruit has a high polyphenol content, especially flavonoids like anthocyanins. It has been reported that anthocyanins have anti-inflammatory and anti-carcinogenic effects, in addition to a high antioxidant and antiviral activity, suggesting the potential therapeutic effect of anthocyanins consumption on human health [11, 12].

*In vitro* anthocyanin fractions and whole extracts have shown high inhibition of the cytopathic effect of different respiratory viruses, particularly influenza A and B viruses [13, 14]. Some natural product extracts, that are rich in these compounds, have also been evaluated in clinical trials, exhibiting a reduction in duration of common cold symptoms and severity [15]. It has been reported also that some flavonoids, including anthocyanins, have the potential to inhibit 3C-like protease (3CLpro), the main protease of SARS-CoV-2 *in silico*, suggesting they can act as potent inhibitors of protease dependent processes of SARS-CoV-2 [8, 16]. Additionally, recent studies have shown an antiviral effect of different herbal extracts developed from natural sources against SARS-CoV-2 [17, 18], which lead to the assumption that corozo extract could also serve as a promising natural source to create antiviral agents as treatment for COVID-19 in future studies.

Herein, we used an anthocyanin-rich extract of corozo (*Bactris guineensis*) fruit to evaluate its antiviral potential against SARS-CoV-2 and to determine the effect of this promising food over SARS-CoV-2 infection in an *in vitro* model.

## METHODS:

**Cells and virus:** Cercopithecus aethiops kidney cell line, Vero E6 was grown in Dulbecco's Modified Eagle Medium

(DMEM, Sigma-Aldrich) supplemented with 2% heat-inactivated fetal bovine serum (FBS) (Gibco), 1% penicillin-streptomycin (Sigma-Aldrich), and 2mM L-glutamine (Sigma-Aldrich) at 37°C with 5% CO<sub>2</sub>. Vero E6 cell line was donated by Instituto Nacional de Salud (INS) (Bogotá, Colombia). Infections were carried out with a viral stock of the D614G ancestral strain of SARS-CoV-2 (lineage B.1, EPI\_ISL\_536399) [19].

### **Corozo (*Bactris guineensis*) fruit extract preparation:**

Corozo extract (CE) was prepared from mature Corozo (*Bactris guineensis*) fruits obtained from CUDESAC (*Corporación unificada para el desarrollo ecológico, económico, social y ambiental de Colombia*) as follows. First, the fruit pulp was obtained from the mature fruits discharging seeds and peels. Next, a mixture of Corozo fruit pulp (45.3 % w/v), extraction solvents, which are polar substances with an affinity for anthocyanins (4.17 % w/v), liquid rosemary (0.09 % w/v), natural preservatives, commonly used as food additives (0.3 % w/v) and distilled water (50.14 % v/v) was heated at 90°C at constant stirring for 4 h. Then, the mixture was filtered through a cloth filter and centrifuged at 1540 RCF for 5 min. After, the supernatant was filtered through a 0.2 µm syringe filter to obtain the sterile CE. The obtained extract was stored at -20 °C until use.

The two main components of CE, pure Corozo pulp (CP) (Corozo Pulp 45.3 % w/v and distilled water 54.7 % v/v) and additives in the absence of Corozo pulp (AD), were prepared separately to be evaluated along with corozo extract. Concentrations of CE, CP and AD are 498.64, 452.96 and 45.6 g/L. concentrations are given as solid per liter.

### **Determination of total phenol, flavonoid and anthocyanin content:**

**Determination of total phenol content:** The total phenol content of CE, CP and AD was determined using the Folin-

Ciocalteu colorimetric method with some modifications [20]. Briefly, 15  $\mu\text{L}$  of the sample, 37  $\mu\text{L}$  of Folin reagent and 125  $\mu\text{L}$  type I water were added to 96-well plates and incubated for 5 min protected from light. Then, 120  $\mu\text{L}$  of sodium carbonate (7.1% w/w) were added for 1h to end the reaction. The absorbance was read at 760 nm in a multiskan GO spectrophotometer (Thermo-Scientific). Finally, the results were compared to a calibration curve, previously performed with gallic acid as standard. Data are expressed as mg equivalents of gallic acid/L sample.

**Determination of total flavonoid content:** Total flavonoid concentration in CE, CP and AD was quantified using the aluminum chloride colorimetric method with some modifications [21]. Briefly, 30  $\mu\text{L}$  of the sample, 15  $\mu\text{L}$  of  $\text{NaNO}_2$  (5% w/v) and 114  $\mu\text{L}$  of type I water were added to 96-well plates. The reaction was achieved in the dark at room temperature for 5 minutes. Then, 15  $\mu\text{L}$  of  $\text{AlCl}_3$  (10% w/v), 60  $\mu\text{L}$  of  $\text{NaOH}$  (1 M) and 66  $\mu\text{L}$  of type I water were added. The plates were read at 510 nm in a spectrophotometer (Thermo-Scientific). The results were compared with a calibration curve previously performed with (+) -catechin as standard, to be expressed as mg equivalents of (+) -catechin/L sample.

**Determination of total anthocyanin content:** The differential colorimetric method of pH was used to quantify total anthocyanin content in CE, CP and AD [22]. Briefly, sample (100  $\mu\text{L}$ ) was dissolved in the buffer solutions (900  $\mu\text{L}$ ) of potassium chloride (pH 1.0) and sodium acetate (pH 4.5), separately. Then, the absorbance was measured at 540 nm and 700 nm for both. The cyanidin-3-glucoside with a molar extinction coefficient ( $\epsilon$ ) of 26900 was used as a reference, to express the results as mg equivalents of cyanidin-3-glucoside/L sample, according to equation 1

$$[(A \times PM \times f \times 10^3)/\epsilon].$$

The differences in absorbances were calculated using the equation 2

$$[A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}1.0} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH}4.5}],$$

where  $A$  was absorbance,  $PM$  was molecular weight and  $f$  was the dilution factor.

**Cytotoxicity assay:** The cytotoxicity of CE was evaluated on Vero E6 cells by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Briefly, Vero E6 cells were seeded in 96-well plates at a density of  $1.0 \times 10^4$  cells/well in DMEM supplemented with 2% FBS. The plates were incubated for 24h at 37°C with 5%  $\text{CO}_2$ . After incubation, 150 $\mu\text{L}$ /well of serial double dilutions of Corozo extract from 1.9 to 31.2 g/L were added to each well and incubated for 48 h, at 37°C with 5%  $\text{CO}_2$ . After, the supernatants were removed, and cells washed twice with Phosphate Buffered Saline (PBS) (Lonza) and 30 $\mu\text{L}$ /well of MTT (2mg/mL) were added. After MTT addition, plates were incubated for 2h at 37°C, with 5%  $\text{CO}_2$ , protected from light. Finally, formazan crystals were dissolved by adding 100 $\mu\text{L}$ /well of pure DMSO. The cytotoxicity of CP (1.8-28.2 g/L) and AD (0.2-2.9 g/L) was evaluated as well.

Plates were read at 570 nm using a multiskan GO spectrophotometer (Thermo-Scientific). The average absorbance of untreated cells was used as viability control. Cell viability of each treated well was calculated based on the viability control. Concentrations with cell viability >80% after treatment was considered non-cytotoxic. For the MTT assay, two independent experiments with four replicates each were performed (n=8).

**Evaluation of the antiviral activity against SARS-CoV-2:**

The antiviral activity of CE was evaluated by a pre-post-infection treatment strategy. Briefly, Vero E6 cells were seeded in 96-well plates ( $1.0 \times 10^4$  cells/well) and incubated for 24 h, at 37°C with 5% CO<sub>2</sub>. Then, serial double dilutions (1.9-15.6 g/L) of Corozo extract were prepared and added to the cell monolayers (50µL/well) for 1 h. After pre-treatment, the extract was removed, and the virus inoculum was added at a multiplicity of infection (MOI) of 0.01 in 50µL/well of DMEM supplemented with 2% FBS. After 1 h of virus adsorption at 37 °C, the inoculum was removed and replaced by 150µL/well of the same pre-treatment dilutions. Finally, the plates were incubated for 48 h at 37 °C under a 5% CO<sub>2</sub> atmosphere. After 48h of incubation, the supernatants were harvested and stored at -80 °C for titration by plaque assay. In order to establish if the antiviral effects were due to the corozo components instead of other substances present in the corozo extract, the same dilutions of CP (1.8- 14.1 g/L) and AD (0.2-1.4 g/L) were included as controls. Heparin at 100 µg/mL was included as positive inhibition control. The supernatant of infected cells without treatment was used as control. Two independent experiments with four replicate each were performed (n=8).

**SARS-CoV-2 quantification by Plaque assay:** The antiviral activity of CE was determined by the reduction of the infectious particles of SARS-CoV-2 in supernatants of treated cells by plaque assay. Briefly,  $1.0 \times 10^5$  Vero E6 cells/well were seeded in 24-well plates for 24 h, at 37°C, with 5% CO<sub>2</sub>. Subsequently, tenfold serial dilutions of the supernatants obtained from the antiviral assay (200µL/well) were added to cell monolayers and

incubated for 1h at 37°C with 5% CO<sub>2</sub>. The viral inoculum was then removed and replaced by 1 mL of semi-solid medium (1.5% carboxymethyl-cellulose in DMEM 1X with 2% FBS and 1% Penicillin-Streptomycin). Cells were incubated for 3 days at 37°C, with 5% CO<sub>2</sub>. Then, cells were washed twice with PBS, fixed-stained with 4 % Formaldehyde/ 1% Crystal violet solution, and viral plaques were counted and expressed as Plaque Forming Units per milliliter (PFU/mL).

The difference between the viral titer after treatment and the untreated control was expressed as inhibition percentage. Supernatants of treatment with CP and AD were quantified by plaque assay as well. Two independent experiments with two replicates each were performed (n=4).

**Statistical analysis:** All data were analyzed with GraphPad Prism (La Jolla, CA, USA). Data were presented as mean  $\pm$  SEM. Statistical differences were evaluated by Student's t-test or Mann-Whitney U test based on Shapiro Wilk normality test. A p-value  $\leq 0.05$  was considered significant, with \* p  $\leq 0.05$  and \*\* p  $\leq 0.01$ .

**RESULTS:**

**Determination of the total content of phenols, flavonoids and anthocyanins:** The concentration of total phenols and flavonoids in CE was higher than that of CP and AD. Specifically, CE contained 2288.57 $\pm$ 133.33 mg gallic acid/L sample and 833.11 $\pm$ 37.91 mg catechin/L sample, while CP contained 1264.76 $\pm$ 84.25 mg gallic acid/L sample and 608.67 $\pm$ 15.56 mg catechin/L sample (Tables 1-2). In contrast, the total phenols and flavonoids in AD were of 86.10 $\pm$ 6.90 mg gallic acid/L sample and 109.08 $\pm$ 4.14 mg catechin/L sample, respectively (Tables 1-2).

**Table 1.** Total phenol content

Total phenols (mg gallic acid/L sample)				
Samples	Concentration	Mean	Standard deviation	Coefficient of variation
Corozo Extract (CE)	2288.57	2288.57	133.33	5.83
	2155.24			
	2421.90			
Corozo Pulp (CP)	1188.57	1264.76	84.25	6.66
	1250.48			
	1355.24			
Additives (AD)	93.00	86.10	6.90	8.02
	79.19			
	86.10			

**Table 2.** Total flavonoid content

Total flavonoids (mg catechin/L sample)				
Samples	Concentration	Mean	Standard deviation	Coefficient of variation
Corozo Extract (CE)	790.89	833.11	37.91	4.55
	844.22			
	864.22			
Corozo Pulp (CP)	593.11	608.67	15.56	2.56
	624.22			
	608.67			
Additives (AD)	104.30	109.08	4.14	3.80
	111.63			
	111.30			

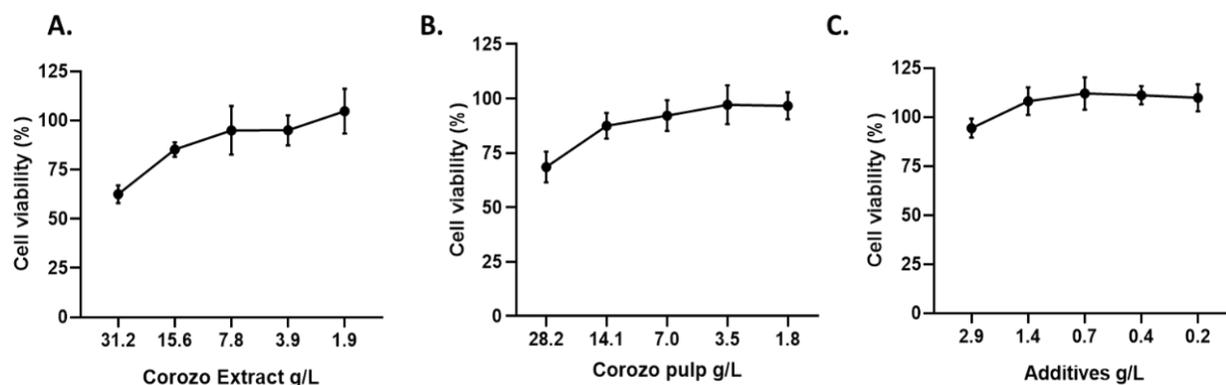
It was confirmed that anthocyanins were the main flavonoids present in CP and CE ( $169.49 \pm 9.86$  and  $144.95 \pm 10.3$  mg cyanidin-3-glucoside/L sample, respectively) while there were no detectable concentrations of flavonoid in AD ( $0 \pm 0.79$  mg cyanidin-3-glucoside/L sample) (Table 3).

**Table 3.** Total anthocyanin content

Total anthocyanins (mg cyanidin-3-glucoside/L sample)				
Samples	Concentration	Mean	Standard deviation	Coefficient of variation
Corozo Extract (CE)	133.09	144.95	10.30	7.10
	150.12			
	151.63			
	151.63			
Corozo Pulp (CP)	158.14	169.49	9.86	5.82
	175.84			
	174.50			
	177.01			
Additive (AD)	-3.34	0	0.79	-18.65
	-4.84			
	-4.51			
	-6.01			

**Corozo extract toxicity on Vero E6 cells:** The viability of Vero E6 monolayers after 48h treatment with the higher concentrations of CE and CP (31.2 and 28.2 g/L) was around 72.9% and 65.3%, respectively, indicating the

cytotoxic potential of these compounds at these concentrations. The successive dilutions of CE and CP exhibited viability higher than 80%. Additives were non-cytotoxic at all evaluated dilutions (Figure 1).



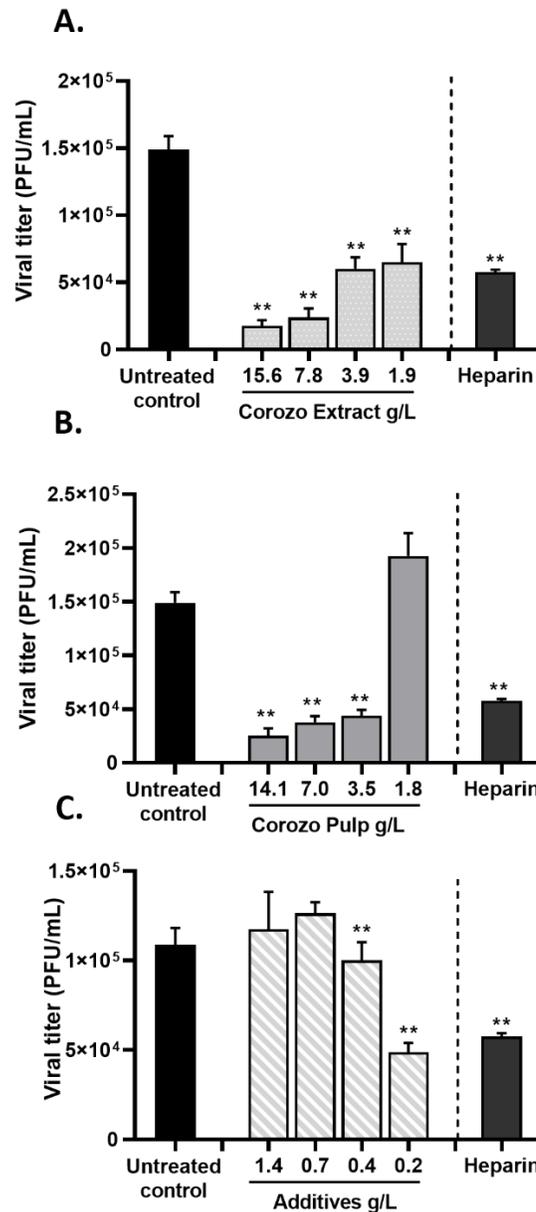
**Figure 1. Cytotoxicity of Corozo extract on Vero E6.** A-C The figures represent the viability percentage of Vero E6 cells after 48h of treatment with double serial dilutions of Corozo extract together with Corozo pulp and -Additives, respectively. Two independent experiments with four replicates each were performed (n=8). A regression curve was done based in cytotoxicity data to establish the 50% cytotoxicity concentration (CC50) of CE. The CC50 of CE was 42.03 g/L. Based on its cytotoxicity CE was evaluated from 15.6 g/L for antiviral assays. CP was evaluated from 14.1 g/L and AD from 1.4 g/L for homogenizing conditions.

**Corozo extract had antiviral activity against SARS-CoV-2 through pre-post treatment strategy:** After a pre-post treatment of SARS-CoV-2 infected cells with the CE, a

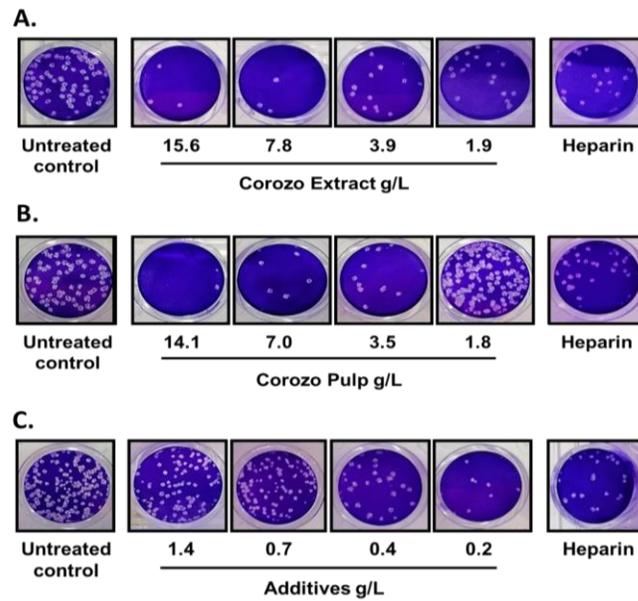
significant reduction in the number of infectious viral particles in the supernatant was observed. It inhibited SARS-CoV-2 in a dose-dependent manner, exhibiting the

higher inhibition capacity at 15.6 g/L with an 88.2% (p=0.004) of viral inhibition compared to untreated control. This antiviral effect was also observed at 7.8, 3.9 and 1.9 g/L concentrations, with 84% (p=0.004), 59.6% (p=0.004), and 56.3% (p=0.004) of viral inhibition, respectively. However, the antiviral effect exhibited by the CP and AD allowed to establish that the antiviral

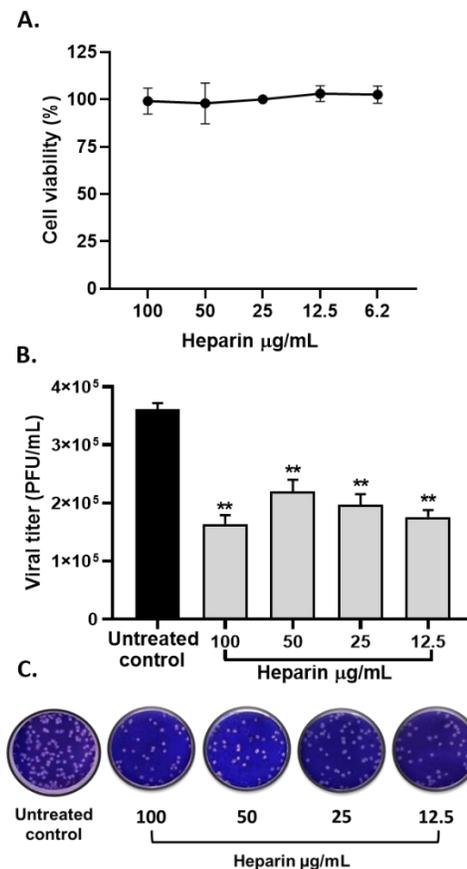
activity observed at 1.9 g/L of CE was not attributable to corozo compounds but to additives (Figure 2-3). Based on the inhibition percentages observed for CE an EC50 (half maximal effective concentration) of 1.67 was calculated for a selectivity index (SI) of 25.32, indicating this extract has a very high SI [23].



**Figure 2. Corozo extract inhibited SARS-CoV-2 in a dose-dependent manner. A-C SARS-CoV-2 viral titer (PFU/mL) of supernatants harvested after Corozo pulp, Corozo extract and additives treatment, respectively. Heparin at 100ug/mL is included as inhibition control. Data are presented as Mean ± SEM. Two independent experiments with two replicates each were performed (n=4). The asterisks represent the statistically significant differences (\*p ≤ 0.05, \*\*p ≤ 0.01; Mann–Whitney U test).**



**Figure 3. Corozo extract treatment reduces the number of viral infectious particles of SARS-CoV-2. A-C.** Representative images of the SARS-CoV-2 plaque assay on Vero E6. On the other hand, Heparin (positive inhibition control) showed a viral inhibition of 54.6% ( $p=0.002$ ), 39.0% ( $p=0.002$ ), 45.3% ( $p=0.002$ ), and 51.2% ( $p=0.002$ ), at 100, 50, 25 and 12.5  $\mu\text{g}/\text{mL}$  respectively (Figure 4).



**Figure 4. Antiviral activity of Heparin against SARS-CoV-2. A.** Cytotoxicity of Heparin on Vero E6 cells. Data are presented as mean  $\pm$  SEM. **B.** SARS-CoV-2 viral titer (PFU/mL) of supernatants harvested after Heparin treatment. The asterisks represent the statistically significant differences ( $*p \leq 0.05$ ; Mann–Whitney U test) **C.** Representative image of plaque assay on Vero E6.

**DISCUSSION:**

This study demonstrated the *in vitro* antiviral activity of Corozo (*Bactris guineensis*) fruit extract against SARS-CoV-2 through a pre-post infection treatment. Corozo extract is rich in polyphenols, especially anthocyanins, which are the major flavonoid compounds in fruits and vegetables, with cyanidin-3-rutinoside and cyanidin-3-glucoside as the main anthocyanin component [9]. Previous studies have shown remarkable stability of anthocyanins, proper of corozo fruit, to high temperatures [9]. Therefore, the results for active compound content showed that the extraction protocol with high temperature obtained a rich-anthocyanin extract as expected. These compounds could be related to the antiviral activity of Corozo, due to their proven biological properties, including antiviral activity against different respiratory viruses [14].

According to the strategy used, the results suggest that the CE inhibits early and/or late steps of SARS-CoV-2 replication cycle [24]. Regarding the initial steps, it has been reported that anthocyanins, from strawberry, alter adsorption and viral entry into host cells [25]. Willing *et al.* proposed three possible mechanisms: (i) anthocyanins could act as antioxidants and scavenge reactive oxygen species (ROS) to prevent them from harming cell membrane (ii) anthocyanins could block virus receptors in the host cell, and (iii) anthocyanins could bind to the viral envelope, avoiding further infection [25]. The two main anthocyanins in strawberries are pelargonidin-3-glucoside and cyanidin-3-glucoside, the second being one of the main anthocyanins in corozo fruit, suggesting that the corozo extract may have similar mechanisms of action as the strawberry extract.

The results for antiviral activity of CP were also promising, but not as much as the results shown for CE and much more than AD results. The comparison between these samples allows thinking that the main compounds responsible for the antiviral activity of the

extract are in the corozo pulp since the additives alone did not show antiviral activity. Additionally, some interactions between the additives and the fruit pulp may enhance antiviral activity since the CE showed more antiviral activity than CP.

Many anthocyanins from different sources have demonstrated significant antiviral activity in late stages of virus cycle. Anthocyanins from red potatoes, strawberries, red beans, and wild berries have been reported to affect the maturation of viral particles and their further release from infected cells in viral models such as Influenza, Human Herpes, Coxsackievirus, and Poliovirus [13][14, 26]. A similar scenario has been reported for SARS-CoV-2; in which the phacelianin, gentiodelphine, cyanodelphine and tecophilin had a high affinity with the 3CL protease, the main protease of SARS-CoV-2 by molecular docking, suggesting that the treatment with an anthocyanins-enriched plant, such as Corozo, could be affecting the proteolytic maturation of this virus [8, 16].

Most of the evidence presented here corresponds to anthocyanins obtained mainly from berries, representing one of the most studied fruits rich in anthocyanins [27, 28]. Concentrations of anthocyanins for high blush berries range from 0.25 to 4.9mg/g [29], while concentrations of 0.8mg/g have been reported for the corozo fruit [10]; indicating that corozo fruit, despite being a poorly studied food, has comparable concentrations and composition than other widely studied foods with reported antiviral activity including anti-SARS-CoV-2 activity as shown by our results together with a really good selectivity index .

Corozo is usually consumed as fresh fruit in the Colombian Caribbean, and it is also prepared as infusions, beverages, wines, and desserts [9]. Since it is a natural product, proven to be safe for consumption, it can be considered as a nutritional supplement that can become part of the strategies to be implemented as a therapeutic

intervention to fight COVID-19. In fact, it may have a direct antiviral effect or it could potentiate the immune system due to its richness in bioactive compounds and antioxidants [25, 30].

The molecular mechanisms associated with the antiviral effect of Corozo against SARS-CoV-2 remain to be elucidated. The results obtained in this study demonstrate the relevance of further studies that allow the isolation and characterization of anthocyanins present in the Corozo extract and the evaluation of its antiviral effect against SARS-CoV-2 using *in vitro* and *in vivo* models.

### CONCLUSION :

This is the first report on the *in vitro* antiviral effect of the *Bactris guineensis* fruit extracts against SARS-CoV-2. Given the reported safety of this product for human consumption, it is suggested that it may be considered an option for further clinical tests that could classify it as a functional food to modulate the susceptibility to COVID-19. Furthermore, this study demonstrated the importance of continuing with ethnobotanical studies to develop and discover new drugs that can provide benefits in the treatment of infectious diseases.

**List of Abbreviation:** INS: Instituto Nacional de Salud, 3CLpro: 3C-like protease, AD: Additives, MOI: Multiplicity of infection, CC50: 50% cytotoxicity concentrations, MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide, CUDESAC: Corporación unificada para el desarrollo ecológico, económico, social y ambiental de Colombia, CE: Corozo extract, COVID-19: Coronavirus disease-2019, nm: Nanometers, CP: Corozo pulp, PBS: Phosphate Buffered Saline, PFU: Plaque forming units, DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl sulfoxide, RCF: Relative centrifugal force, EC50: half maximal effective concentration, SI: selectivity index, ROS: Reactive oxygen species, SARS-CoV-2: Severe acute

respiratory syndrome coronavirus 2, SEM: standard error of mean, FBS: Fetal bovine serum, FFC: Functional Food Center.

**Author contribution:** Tania Jaimes-Gualdrón-TJG, Lizdany Flórez-Álvarez-LFA, María I. Zapata-Cardona-MZC, Benjamín Alberto Rojano-BAR, María T. Rugeles-MTR, Wildeman Zapata-WZ Conceptualization, MTR and WZ.; Methodology, MTR, WZ, LFA, MZC.; Formal Analysis, TJG, LFA, and MZC; Investigation, TJG, LFA, and MZC.; Resources, BAR, MTR, and WZ; Data Curation, BAR, and WZ; Writing – Original Draft Preparation, TJG, LFA, and MZC.; Writing – Review and Editing, BAR, MTR, and WZ. Project Administration, BAR, MTR, and WZ; Funding Acquisition, BAR, MTR, and WZ”

**Conflicts of Interest:** The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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