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From Tradition to Science: Chemical, Nutritional, and Cytotoxic Characterization of *Erythroxylum coca* from Indigenous Colombian Communities

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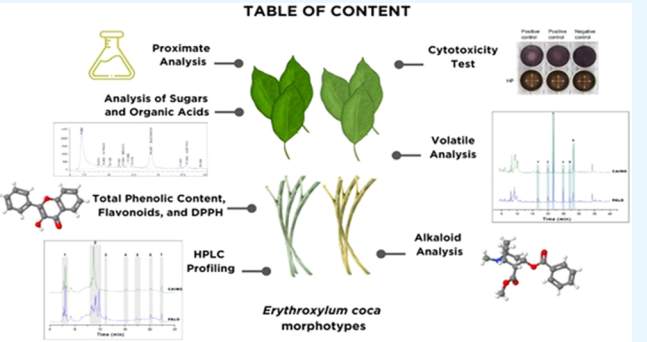
ABSTRACT: *Erythroxylum coca*, commonly known as "coca" is a plant native to the South American Andes, recognized for its high alkaloid content and potential medical and nutritional applications. This study aimed to characterize the chemical, nutritional, and cytotoxic properties of two *E. coca* morphotypes (Palo and Caimo) cultivated by Colombian indigenous communities, with the goal of promoting legal uses and economic opportunities in the region. Comprehensive analyses included the evaluation of sugars, organic acids, total polyphenols, flavonoids, antioxidant capacity, volatile compounds, and cytotoxic activity. Chemical analysis revealed that *E. coca* leaves contain over 50% dietary fiber, while stems surpass 76%, primarily consisting of insoluble fiber. Significant amounts of sucrose, glucose, and fructose were detected, with succinic acid identified as the predominant organic acid. Cytotoxicity evaluation demonstrated that while both morphotypes are safe for consumption, they also exhibit cytotoxic activity against L929 murine fibroblast cell line. Volatile compound analysis highlighted the presence of hexadecanoic and octadecanoic acids, alongside characteristic alkaloids such as cocaine and benzoylecgonine. These findings underscore the nutritional, chemical, and cytotoxic potential of *E. coca* as a sustainable crop. Its cultivation and research can serve as a valuable resource for indigenous communities, contributing to the development of local economies and fostering its legal and beneficial applications.



1. INTRODUCTION

Erythroxylum coca, commonly known as "coca" is a plant native to the Andes of South America, including Colombia, Ecuador, Peru, Bolivia, and Brazil. It is one of the oldest cultivated medicinal species, with use dating back at least 8000 years.[1] The name "coca" derives from the Aymara term "Kkoka" (divine plant) and the Quechua term "mama coca" (mother coca).[2] Traditionally, indigenous communities consume coca leaves by forming a chewable "bolo" in the cheek. The dried leaves are often processed with plant ashes, animal bones, and seashells creating an alkaline mixture known as "mambe".[1] *Erythroxylum* includes about 230 species, with *E. coca* and *E. novogranatense* being the primary natural sources of cocaine.[3,4] While coca has been culturally significant in South America for medicinal and ritual uses, it has also become associated with illicit cocaine production.[1,5−8] Cocaine is a tropane alkaloid known as a potent central nervous system stimulant and anesthetic, which was previously used in medicine for pain relief.[9,10] It was later enhanced and included in alcoholic preparations, beverages, and foods.[10] However, high doses lead to addiction, hyperactivity, increased blood pressure, heart rate, and euphoria.[9] For these reasons, this alkaloid has become a controversial molecule worldwide as the precursor of cocaine hydrochloride, an addictive narcotic that poses a public health problem.[10]

Despite the stigma surrounding coca leaves, the plant synthesizes other important alkaloidal compounds like cinnamylcocaine, benzoylecgonine, and nicotine.[1,11,12] These alkaloids help plants resist pests and diseases.[13,14] Additionally, the coca plant synthesizes flavonoids, disaccharides, and fatty acids, which are of chemical importance, although the proportion of these compounds is low and often masked by the alkaloids.[6]

In Colombia, strict regulations have limited research on the coca plant due to its role in cocaine production. In 2022, coca cultivation increased by 13%,[15] leading to policies restricting

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scientific study and product commercialization.[16,17] The Colombian government introduced a bill aligned with the Nagoya Protocol to regulate coca use for medical, scientific, and industrial purposes,[18] reopening research opportunities.[13,14,19−21] However, a full characterization of coca's alkaloids, flavonoids, and nutrients using modern techniques is still needed, which hinders further research. Hence this study characterized the chemical, nutritional, and cytotoxic properties of two coca plant morphotypes cultivated by indigenous communities in Vaupés, Colombia. The aim was to expand scientific knowledge, identify potential uses, and propose alternatives that could lead to economic and social transformation, ensuring active participation and equitable benefit-sharing among the indigenous communities.

2. MATERIALS AND METHODS

2.1. Plant Material. *E. coca* plants of the Palo (Collector code: JIJ6032) and Caimo (Collector code: JIJ6033) morphotypes were collected from indigenous chagras in Vaupés, Colombia, with specimens deposited in the Colombian Amazon Herbarium (COAH). The Caimo morphotype was collected at 1.239795° N, 70.162814° W, and the Palo morphotype at 1.238087° N, 70.162915° W. The material was dehydrated at 40 °C in a forced convection oven UF1060 (Memmert GmbH + Co. KG, Germany) and separated by organs: leaves and stems. The stem of the Palo morphotype was divided into four sections from base to apex, while the Caimo morphotype's stem was divided into two sections, with the base as section one and the apex as section two. Experiments were conducted according to this classification. Subsequently, the plant material (leaves and stems) was ground in a cutting mill Pulverisette 15 (Fritsch GmbH - Milling and Sizing, Germany), vacuum-packed, and stored at refrigeration temperature (±4 °C). In total, eight samples were analyzed and named as follows: HP: Palo leaves; TP1: Palo stem 1; TP2: Palo stem 2; TP3: Palo stem 3; TP4: Palo stem 4; HC: Caimo leaves; TC1: Caimo stem 1; and TC2: Caimo stem 3.

2.2. Reagents. All chemicals used in this study were of analytical grade. Sodium carbonate, aluminum acetate, and thiazolyl blue tetrazolium bromide (MTT, 98%) were obtained from Sigma-Aldrich. Ethanol, chloroform, and dichloromethane were sourced from Merck (Germany). Aluminum chloride was acquired from Sigma-Aldrich (Burlington), while sulfuric acid and hydrochloric acid were supplied by Honeywell and Fisher Scientific, respectively. Standards used for liquid chromatography, including HPLC-grade raffinose, sucrose, and glucose, were supplied by TCI Chemicals (Japan). Fructose, ribose, glycerol, oxalic acid, malic acid, citric acid, succinic acid, and pyruvic acid were obtained from Sigma-Aldrich, while lactic acid, formic acid, and acetic acid were sourced from Merck (Germany).

2.3. Proximate Analysis. Proximate analysis was conducted following the AOAC-established protocols for the following components:

2.3.1. Carbohydrates.[22] Calculated using the formula

100% − (ether extract + crude fiber + protein (dry basis))

2.3.2. Ash.[22] Approximately 1 g of the sample was weighed into a crucible and dried in an oven at 105 °C for 1 h. The crucible containing the dried sample was weighed, then placed in a muffle furnace at 700 °C for 3 h. Finally, the crucible with the calcined sample was weighed.

2.3.3. Ether Extract.[23] Soxhlet extraction was performed. About 5 g of the sample was placed in a thimble and weighed. A round-bottom flask was also weighed prior to refluxing with benzine at 80 °C for a minimum of 5 h. After reflux, the thimble was dried and weighed again. The solvent in the round-bottom flask was concentrated under reduced pressure, and the flask was weighed to determine the extracted fat content.

2.3.4. Crude Fiber.[24] Approximately 1 g of the sample was processed using a Fiber Analyzer (FIWE Scientific). The sample was sequentially washed with 150 μL of 1.25% H_2SO_4, followed by 150 μL of 1.25% KOH, and finally with 25 mL of acetone. The crucible containing the sample was dried, weighed, and then ashed in a muffle furnace at 700 °C for 5 h. The weight difference was used to calculate the percentage of crude fiber.

2.3.5. Dietary Fiber, Insoluble Fiber, Soluble Fiber.[25] To determine the total dietary fiber, approximately 1 g of defatted and dried sample was incubated in a Velp Scientific enzymatic digester with 50 mL of phosphate buffer (pH 6.0) and 100 μL of α-amylase at 95−100 °C for 30 min. Then, 10 mL of distilled water was added, the pH was adjusted to 7.5 with 0.275 N NaOH, and 100 μL of protease was added, followed by incubation at 60 °C for 30 min. Subsequently, the pH was adjusted to 4.5 with 0.325 N HCl, 200 μL of amyloglucosidase was added, and the mixture was incubated again at 60 °C for 30 min. The sample was precipitated with 280 mL of 95% ethanol at 60 °C for 1 h, filtered using a Velp Scientific CFS6 system, and washed with 78% ethanol (3 × 20 mL), 95% ethanol (2 × 10 mL), and acetone (2 × 10 mL). Finally, it was dried at 105 °C to a constant weight and ashed at 550 °C for 5 h. For insoluble fiber, the same procedure was followed, modifying only the washing step: 30 mL of hot water, 78% ethanol (3 × 20 mL), 95% ethanol (2 × 10 mL), and acetone (2 × 10 mL). The total and insoluble fiber content is determined by subtracting the weight of the ashed sample, the protein content, and the blank weight from the initial sample weight, then dividing the result by the sample weight. Soluble fiber is calculated as the difference between total dietary fiber and insoluble fiber.

2.3.6. Moisture.[26] Approximately 5 g of the sample was weighed into a Petri dish and dried in an oven at 105 °C for 4 h. The sample was weighed, dried again for 1 h, and weighed repeatedly until a constant weight was achieved.

2.3.7. Protein.[27] About 100 mg of the sample was weighed and mixed with 900 μL of phosphate buffer (pH 6.0), followed by shaking for 20 min. The mixture was centrifuged at 10,000 rpm at 4 °C for 15 min. Subsequently, 200 μL of the supernatant was mixed with 800 μL of Bradford reagent in a spectrophotometric cuvette. Absorbance was measured at 600 nm, and the data were interpolated using a calibration curve generated with BSA standards.

2.4. Preparation of Ethanolic Extracts. The powdery plant material was extracted with 96% ethanol in a 1:3 (w/v) ratio through maceration overnight at room temperature (20 °C). The mixture was then filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator Hei-VAP (Heidolph, Germany). The obtained extract was dried, weighed, and stored at 4 °C, protected from sunlight.

2.5. Total Phenolic and Flavonoid Content. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method with a 5 mg/mL dilution following AOAC guidelines.[28] The extract was mixed with Folin-Ciocalteu

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Table 1. Bromatological Parameters Were Identified in Leaves and Stems of the Coca Morphotypes Palo and Caimo, Expressed as Grams Per 100 Grams of Sample (g/100 g)[a,b]

ID	CHO	ash	ether extract	crude fiber	dietary fiber	insoluble fiber	soluble fiber	moisture	protein
HP	70.67 b	4.83 ± 0.09 b	3.42 ± 0.11 a	21.05 ± 0.41 d	54.71 ± 0.15 d	53.39 ± 0.18 e	1.316 e	10.83 ± 0.75 d	6.59 ± 0.51 b
TP1	52.93 c	2.48 ± 0.009 e	0.62 ± 0.06 d	42.55 ± 0.95 c	78.12 ± 0.20 b	77.16 ± 0.11 a	0.959 f	7.81 ± 0.76 f	1.40 ± 0.09 c
TP2	48.56 d	2.07 ± 0.08 g	0.14 ± 0.01 f	47.78 ± 0.87 a	81.42 ± 0.03 a	77.42 ± 0.04 a	4.293 c	20.61 ± 0.56 a	1.43 ± 0.12 c
TP3	47.68 d	2.17 ± 0.07 f	0.18 ± 0.01 f	48.45 ± 0.002 a	83.57 ± 0.16 a	77.84 ± 0.04 a	5.726 b	11.80 ± 0.53 c	1.50 ± 0.09 c
TP4	46.10 d	3.69 ± 0.23 c	0.41 ± 0.02 e	47.88 ± 0.87 a	76.54 ± 1.43 b	72.32 ± 0.13 b	4.216 c	16.16 ± 1.00 b	1.90 ± 0.03 c
HC	77.91 a	3.19 ± 0.08 d	2.15 ± 0.08 b	18.48 ± 0.09 e	49.05 ± 0.34 e	44.45 ± 3.40 f	4.594 c	13.36 ± 0.79 c	9.26 ± 1.91 a
TC1	38.08 f	5.40 ± 0.30 a	0.99 ± 0.02 c	45.54 ± 0.95 b	67.01 ± 0.49 c	60.79 ± 0.40 d	6.226 a	8.84 ± 0.30 f	9.97 ± 0.42 a
TC2	43.90 e	1.77 ± 0.09 h	1.05 ± 0.08 c	43.51 ± 0.42 c	67.6 ± 0.30 c	64.19 ± 0.55 c	3.450 d	9.07 ± 0.89 e	9.75 ± 0.89 a

[a]HP: Palo leaves; TP1: Palo stem 1; TP2: Palo stem 2; TP3: Palo stem 3; TP4: Palo stem 4; HC: Caimo leaves; TC1: Caimo stem 1; and TC2: Caimo stem 3. CHO: carbohydrates. [b]Letters (a−h) indicate significant differences according to Tukey's classification test.

reagent Sigma-Aldrich and sodium carbonate, incubated in the dark for 2 h, and the absorbance was measured at 735 nm using a UV−vis spectrophotometer (UV-3600i Plus, Shimadzu Corporation, Kyoto, Japan). The results were expressed as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g dry weight).

For flavonoid quantification, the sample was mixed with aluminum chloride, aluminum acetate, and ethanol.[29] The mixture was shaken and incubated for 40 min before measuring the absorbance at 424 nm using a UV−vis spectrophotometer Genesys 10S (Thermo Fisher Scientific). The results were expressed as milligrams of quercetin equivalents per gram of dry sample (mg QE/g dry weight).

2.6. DPPH• Radical Scavenging Activity. The antioxidant capacity was evaluated using the DPPH• radical scavenging assay. A mixture of 50 μL of the extract and 800 μL of DPPH• solution was prepared and incubated in the dark for 1 h. After incubation, the absorbance was measured at 515 nm using a UV−vis spectrophotometer Genesys 10S.[30,31]

2.7. HPLC Metabolic Profiling and Analysis of Sugars and Organic Acids. The profiling of the extracts was performed using an HPLC 1200 system (Agilent Technologies) equipped with a variable wavelength detector (VWD) at 280 nm. The mobile phase was 1% formic acid in water and ACN at a flow rate of 1.0 mL/min in a gradient mode of 0% B (0−3 min), 50% B (12−14 min), 100% B (20−22 min), 0% B (23−25 min). The separation was achieved using a Luna C18 column (5 μm; 150 mm × 4.6 mm) (Phenomenex). Organic acid quantification was carried out using an HPLC system 1200 (Agilent Technologies) equipped with a refractive index detector (RID) and variable wavelength detector (VWD) set at 214 nm. The separation was achieved on an Aminex HPX-87H column (9.0 μm; 300 mm × 7.8 mm) (Biorad Laboratories) using a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 mL/min in isocratic mode. Quantification was performed by interpolating the peak areas against calibration curves constructed for individual standards. Calibration curves were prepared using eight different concentrations ranging from 10 to 1000 mg/L for raffinose, sucrose, glucose, fructose, ribose, glycerol, and the organic acids oxalic, malic, citric, succinic, pyruvic, lactic, formic, and acetic acid. Each standard was analyzed six times to determine the area under the curve (AUC) at each concentration. The calibration curves were generated by plotting the AUCs as a function of concentration, ensuring precise quantification of each analyte.

2.8. Alkaloid Extraction. Alkaloid extraction involves two phases: an initial acidic extraction and a subsequent liquid−liquid extraction with chloroform. Ten grams of plant material were mixed with hydrochloric acid, agitated, and filtered. Chloroform was then used to separate the organic phase containing alkaloids, with excess chloroform removed under reduced pressure.

2.9. Volatile Compound Extraction. Volatile compounds were extracted from 400 g of fresh leaves using hydrodistillation with a Clevenger apparatus. The leaves were soaked in 3 L of water, and the volatile compounds were subsequently isolated through liquid−liquid extraction with dichloromethane. Additionally, solid-phase microextraction (SPME) was employed for comparison of volatile compounds, where freshly ground coca leaves from Caimo and Palo genotypes were placed in sealed, independent vials with a divinylbenzene fiber (SPME Fiber Assembly 50/30 μm DVB/CAR/PDMS, Stableflex 24Ga, Manual xHolder (Supelco)). Extracts from both genotypes were incubated in a water bath at 70 °C for 1 h to allow the capture of volatile compounds.

2.10. Gas Chromatography−Mass Spectrometry (GC-MS) Analysis. The dichloromethane fractions and SPME fibers were analyzed using a gas chromatograph 7890D coupled to a mass spectrometer 5877A (Agilent Technologies). Separation was performed on an HP5-MS column (30 m × 0.25 μm × 250 μm) with hydrogen as the carrier gas. The injector temperature was set to 270 °C in split mode, and the oven temperature was programmed with multiple ramps, starting at 50 °C and increasing to 250 °C. The transfer line and ion source were maintained at 250 °C. An electron ionization system (EI) was used with an ionization energy of 70 eV for GC−MS detection. Compound identification was achieved by comparing the spectra with the NIST 14 library (National Institute of Standards and Technology).

The alkaloid extract analysis was conducted using the same equipment and column, with hydrogen as the carrier gas. The injector was set at 280 °C in split mode, and the oven temperature was programmed with two ramps, starting at 80 °C and increasing to 280 °C. The transfer line was maintained at 280 °C, the ion source at 250 °C (EI, 70 eV). Compound identification was performed by comparison with the NIST 14 library.

2.11. Cytotoxicity Evaluation. Cytotoxicity was evaluated using an indirect contact assay with mouse areolar fibroblasts (CCL-1, NTCN clone 929) on coca leaf samples. Cells were seeded at a density of 350,000 cells/mL (700,000 cells/well) and incubated with 25 μL of each sample for 24 h. After incubation, thiazolyl Blue Tetrazolium Bromide 98% (MTT) was added, and the color change was observed and measured. Data were classified according to the reactivity grades specified in ISO 10993-5.[32]

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Table 2. Sugar Content Identified in Leaves and Stems of the Coca Morphotypes Palo and Caimo, Measured by HPLC, Expressed in Milligrams Per 100 g of Sample (mg/100 g)[a,b]

ID	raffinose	sucrose	glucose	fructose	ribose	glycerol
HP	0	187.46 ± 33.84 d	1274.49 ± 164.05 e	1110.24 ± 136.17 e	0	154.03 ± 19.66 c
TP1	0	142.97 ± 8.56 e	3427.62 ± 94.97 a	3294.56 ± 95.66 b	11.21 ± 1.08 b	43.21 ± 1.38 d
TP2	0	17.54 ± 1.99 h	119.88 ± 17.97 f	124.79 ± 15.86 f	65.12 ± 5.55 a	365.43 ± 12.28 a
TP3	54.66 ± 4.06 b	90.66 ± 6.42 f	2183.68 ± 151.97 c	2166.08 ± 165.00 d	8.33 ± 1.28 c	22.45 ± 1.73 e
TP4	64.80 ± 8.35 a	76.35 ± 3.45 g	1575.50 ± 64.15 d	1574.50 ± 50.51 e	2.31 ± 0.39 d	43.34 ± 4.26 d
HC	0	670.38 ± 44.28 b	3651.59 ± 779.53 a	3374.27 ± 170.72 b	0	264.31 ± 20.22 b
TC1	0	2318.65 ± 14.44 a	2315.26 ± 67.34 c	3140.90 ± 100.90 c	0	16.63 ± 1.56 f
TC2	0	319.35 ± 45.91 c	3157.46 ± 162.27 b	4352.50 ± 259.48 a	0	37.34 ± 7.03 d

[a]HP: Palo leaves; TP1: Palo stem 1; TP2: Palo stem 2; TP3: Palo stem 3; TP4: Palo stem 4; HC: Caimo leaves; TC1: Caimo stem 1; and TC2: Caimo stem 3. [b]Letters (a−h) indicate significant differences according to Tukey's classification test.

Table 3. Organic Acids Content Identified in Leaves and Stems of the Coca Morphotypes Palo and Caimo, Measured by HPLC, Expressed in Milligrams Per 100 g of Sample (mg/100 g)[a,b]

ID	citric	malic	succinic	lactic	formic	acetic
HP	437.20 ± 79.19 e	0	11,666.01 ± 1683.28 b	0	0	1981.37 ± 172.18 b
TP1	816.80 ± 40.10 c	547.99 ± 27.07 d	1062.04 ± 57.22 d	0	0	0
TP2	283.39 ± 35.64 f	63.44 ± 7.05 f	291.32 ± 74.55 f	0	0	229.72 ± 30.56 e
TP3	464.74 ± 21.94 d	477.50 ± 36.31 de	587.58 ± 91.25 e	0	0	357.86 ± 28.71 d
TP4	1052.94 ± 87.26 b	927.02 ± 74.53 c	370.55 ± 138.47 f	0	0	191.17 ± 6.61 f
HC	0	523.44 ± 200.98 d	20,424.88 ± 627.98 a	595.10 ± 921.94	0	6931.57 ± 2086.75 a
TC1	0	2374.19 ± 107.75 b	1077.90 ± 61.55 d	0	12.17 ± 29.81	1043.89 ± 98.32 c
TC2	1618.75 ± 523.58 a	3721.16 ± 511.70 a	2058.97 ± 86.54 c	0	0	1478.05 ± 41.55 c

[a]HP: Palo leaves; TP1: Palo stem 1; TP2: Palo stem 2; TP3: Palo stem 3; TP4: Palo stem 4; HC: Caimo leaves; TC1: Caimo stem 1; and TC2: Caimo stem 3. [b]Letters (a−f) indicate significant differences according to Tukey's classification test.

2.12. Statistical Analysis. Data were subjected to the Shapiro-Wilk test to determine the normality of the values. An analysis of variance (ANOVA) and Tukey's test were performed to determine significant differences between data at a significance level of 5%. This was conducted using the open-source software R Studio 2021.09.0.

3. RESULTS AND DISCUSSION

3.1. Proximate Analysis. The bromatological analysis in Table 1 shows that leaves of both morphotypes of *E. coca* have high carbohydrate content (HP = 70.67/100 g and HC = 77.91/100 g). Leaves are photosynthetically active organs, which synthesize and store large amounts of carbohydrates and act as energy sinks.[33,34] A study has suggested that people who get carbohydrates from coca leaves by chewing have a hyperglycemic response that could provide a quick energy source during physical activity.[35] The sugars identified with the highest content were glucose, fructose, and sucrose, which are the mono and disaccharides most used by plants in their metabolic processes.[36,37] Additionally, the Caimo morphotype had higher protein content (9.26−9.97/100 g) than the Palo morphotype (1.40−6.59/100 g). However, these values were lower than reported in other studies that used leaves from Peru (6.12/100 g dry weight) and Bolivia (5.0/100 g dry weight).[12,38]

On the other hand, the fiber parameters evidenced that the highest amount of crude fiber was in stems (42.55−48.45/100 g) compared to the leaves (21.05−18.48/100 g) in both morphotypes. Other studies have reported lower content in coca leaves from Peru and Bolivia with 15.46/100 g dry weight and 14.4/100 g dry weight, respectively as well as insoluble fiber content had a similar trend (38.02/100 g dry weight).[12,38] Besides, the Palo morphotype exhibited higher total dietary fiber content (54.71−83.57/100 g) than the Caimo morphotype (49.05−67.6/100 g), with stems containing more fiber than leaves in both cases. Dietary fiber (divided into soluble and insoluble) supports cardiovascular and gastrointestinal health by improving gut microbiota composition, which can positively affect overall colon health.[39] Compounds such as cellulose, hemicellulose, and lignin (insoluble fiber) contribute to increased fecal bulk and stimulate intestinal transit.[40−42] Conversely, the identified ether extract content was low in both morphotypes (0.14−3.42/100 g).

3.2. Analysis of Sugars and Organic Acids. The sugar analysis of the two coca morphotypes showed that sucrose, glucose, fructose, and glycerol were present in all samples (Table 2). The Caimo morphotype exhibited a higher sucrose content, with TC1 showing the highest content (2318.65 mg/100 g dry weight), followed by the leaves, and TC2. In the Palo morphotype, sucrose content was lower, with leaves having the highest content and stems the lowest (187.46 mg/100 g dry weight and 17.54 mg/100 g dry weight, respectively). Glucose and fructose showed similar trends across both morphotypes. Raffinose and ribose were only detected in the stems of the Palo morphotype, with raffinose found in TP3 and TP4, and ribose present in all stem samples, particularly in TP2. Glycerol concentration varied between leaves and stems in both morphotypes without a consistent pattern.

The analysis of organic acids from coca samples showed that succinic, malic, citric, and acetic acids were the most recurrent, while lactic and formic acids were present in smaller amounts Table 3. Succinic acid had the highest levels (HP = 11,666.01 mg/100 g, HC = 20,424.88 mg/100 g dry weight), whereas citric acid levels were higher in the apical stems of Palo and Caimo (TP4 = 1052.94 mg/100 g dry weight and TC2 =

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1618.75 mg/100 g dry weight). Malic acid was absent in Palo leaves but was found in higher quantities in the stems of Caimo (TC2 = 3721.16 mg/100 g dry weight and TC1 = 2374.19 mg/100 g dry weight) and lactic acid was present only in Caimo leaves, whereas formic acid was found only in the basal stems of Caimo. Finally, acetic acid was mainly found in the leaves of both morphotypes (HP = 1981.37 mg/100 g dry weight and HC = 6931.5 mg/100 g dry weight). Organic acids are relevant in plants because they are involved in metabolism and physiological processes.[7] Additionally, these acids are important for the sensory quality of foods, serving as acidity regulators, antioxidants, preservatives, and more.[43] In particular, succinic acid is valued for its ability to produce polybutylene succinate (PBS), as well as for manufacturing surfactants and detergents, flavors and fragrances, herbicides and fungicides, and food additives.[44]

3.3. Total Phenolic Content (TPC), Flavonoids (TFC), and DPPH•. Leaves of the Caimo morphotype had the highest total phenolic content (10.534 mg GAE/g dry weight), flavonoid content (0.213 mg Eq Q/g) antioxidant capacity (0.696 mg Eq Trolox/g) Table 4. In contrast, the Palo morphotype showed lower phenolic content, with TP1 having the highest (3.357 mg GAE/g dry weight) and TP3 the lowest (0.942 mg GAE/g dry weight) Table 4. The measures of TPC and TFC are relevant because these large groups of heterogeneous molecules have been used in the pharmaceutical, agricultural, and food industries.[45−48] Flavonoids have been extensively studied in the genus *Erythroxylum* supporting the chemotaxonomic of gender.[6,49,50] Kaempferol, quercetin, and eriodictyol are relevant compounds in the genus *Erythroxylum*, which can be present conjugated with glycosides or in free form.[19,51]

Table 4. Total Phenolic Content, Flavonoid Content, and Antioxidant Capacity of Leaves and Stems of Palo and Caimo Morphotypes[a,b]

ID	total phenolics (mg eq AG/g)	flavonoids (mg eq Q/g)	DPPH (mg eq Trolox/g)
HP	1.176 ± 0.055 d	0.074 ± 0.048 b	0.095 ± 0.032 c
TP1	3.357 ± 0.332 b	0.028 ± 0.004 d	0.161 ± 0.070 b
TP2	1.146 ± 0.027 d	0.060 ± 0.077 c	0.102 ± 0.054 b
TP3	0.942 ± 0.029 g	0.006 ± 0.001 f	0.088 ± 0.055 c
TP4	1.040 ± 0.037 f	0.015 ± 0.004 e	0.107 ± 0.077 b
HC	10.534 ± 0.448 a	0.213 ± 0.026 a	0.696 ± 0.563 a
TC1	1.105 ± 0.123 e	0.010 ± 0 e	0.057 ± 0.051 c
TC2	1.45 ± 0.019 c	0.030 ± 0.004 d	0.147 ± 0.143 b

[a]HP: Palo leaves; TP1: Palo stem 1; TP2: Palo stem 2; TP3: Palo stem 3; TP4: Palo stem 4; HC: Caimo leaves; TC1: Caimo stem 1; and TC2: Caimo stem 3. [b]Letters (a−g) indicate significant differences according to Tukey's classification test.

Our TPC outcomes contrast with values reported in coca leaves, which had values between 3.8934 and 3.780 mg GAE/100 g dry weight.[12] The leaves of both morphotypes had the highest flavonoid content, while the stems showed very low levels, with TP3 of the Palo morphotype nearly devoid of flavonoids. The DPPH• test revealed that the HC sample had the highest antioxidant capacity (0.696 mg Trolox Equivalents/g dry weight), though samples from *E. suberosum* have shown antioxidant capacity 4.5−6 times that of ascorbic acid in aqueous and ethanolic fractions.[52]

3.4. HPLC Profiling of Coca. The ethanolic extracts of coca samples were analyzed using reversed-phase liquid chromatography (RPLC) on a system equipped with a VWD detector and monitored at a wavelength of 280 nm. Eight distinct compound signals were detected in the chromatographic profiles (Figure 1). Both morphotypes, Palo and Caimo, exhibited similar chromatographic signals in leaf extracts. However, notable differences were observed in the stem extracts, particularly in the retention times and signal intensities of certain compounds.

In the Palo morphotype, the chromatographic profiles of the four stem sections showed consistent patterns, with differences identified in Zones 5 and 6 (Figure 1b). Specifically, Zone 5 showed signals in stems 3 and 4 but not in stems 1 and 2, while Zone 6 presented signals in stems 3 and 1 but not in stems 4 and 2. On the other hand, in the Caimo morphotype, differences were more pronounced. Zone 2 exhibited a signal in stem 1 but not in stem 2, and in Zone 5, two signals were detected in stem 1 that were absent in stem 2.

These findings highlight the variability in chemical composition between morphotypes and stem sections. These differences in chromatographic profiles could be related to genetic factors influencing the synthesis and accumulation of specific secondary metabolites in each morphotype. In the case of the Palo morphotype, the more defined and well-resolved signals suggest greater uniformity in the distribution of compounds within the stems. The variations observed in specific zones (e.g., signals in Zones 5 and 6) may reflect differences in the accumulation of compounds along the stem segments. In the Caimo morphotype, the presence of unique signals in certain segments (e.g., signals in Zone 5 of stem 1)

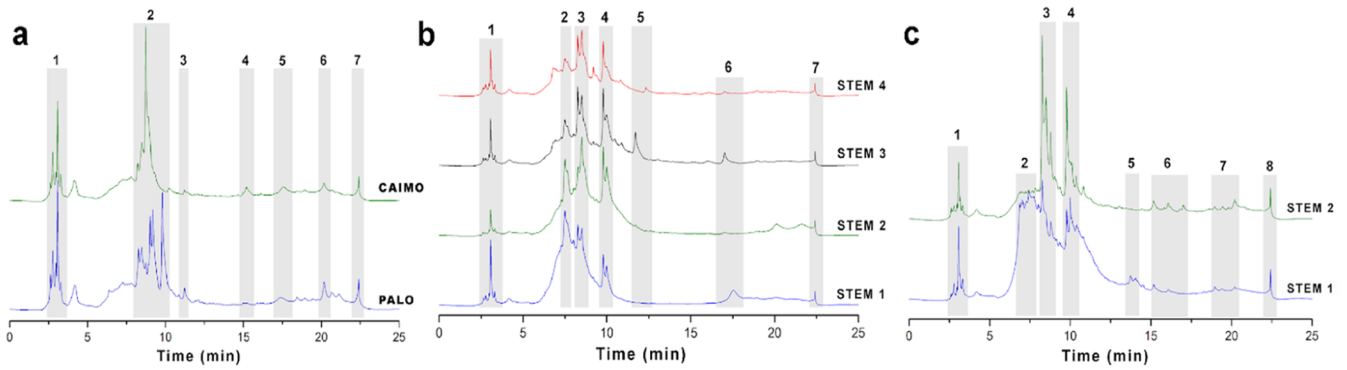

Figure 1. HPLC chromatograms from different plant parts of coca genotypes Caimo and Palo (a) leaves of Palo and Caimo coca; (b) stems of Palo morphotype; (c) stems of Caimo morphotype.

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and their absence in others indicate a more heterogeneous chemical distribution. This heterogeneity could be attributed to genetic differences affecting the biosynthesis of secondary metabolites such as alkaloids, flavonoids, or other phenolic compounds, as well as factors such as tissue age, the physiological state of the stem, or environmental conditions (e.g., soil, light, humidity).[11,20]

3.5. Alkaloid Analysis. The coca samples were analyzed by GC-MS, and the most interesting compounds were observed between the 15th and 30th minute, as shown in Figure S1. Six compounds were identified, with cocaine being the most prominent alkaloid, accounting for 26.948% in the Caimo morphotype and 35.128% in the Palo morphotype (Table 5). In the Caimo morphotype, benzoylecgonine showed a relative percentage comparable to cocaine (26.649%), whereas its percentage was significantly lower in the Palo morphotype (9.988%). Other alkaloids commonly found in coca plants, such as trans-cinnamylcocaine and cinnamylcocaine, were also identified but in lower relative percentages.[6,53−55] Moreover, hexadecanoic and octadecanoic acid, two types of fatty acids, were identified with notable relative percentages in the alkaloid profiles. However, the alkaloid profile varies related to factors such as artificial selection or the spatial location of the plants, as shown by studies of different coca cultivars in Colombia and Bolivia.[11,56] Differences in the relative percentage of alkaloids were found between the Palo and Caimo morphotypes supporting the idea that alkaloidal profile can be a factor chemotaxonomic of genus *Erythroxylum*.[11,20]

Table 5. Compounds Identified in Alkaloid Extracts from Leaves of Palo and Caimo Morphotype, Expressed as Relative Percentages with Calculated and Reported Kovats Index Values (NIST 14)[a]

compound	IK cal	IK ref	% relative Caimo (%)	% relative Palo (%)
ecgonidine, methyl ester	1566	NR	7.7419	5.6527
methyl ecgonine	1500	1504	8.5552	10.790
hexadecanoic acid	2109	2108	1.537 c	2.651 d
octadecanoic acid	2160	2170	2.870 b	3.927 c
cocaine	2192	2163	26.958 a	35.128 a
trans-cinnamylcocaine	2451	2480	2.102 b	6.022 b
cinnamylcocaine	2490	2480	1.281 c	2.619 d
benzoylecgonine	2519	2582	26.649 a	9.988 b

[a]Letters (a, b, c, d) indicate significant differences according to Tukey's classification test.

Cocaine is a controversial alkaloid due to its addictive potential and public health impact as a recreational drug.[10,53] However, it has traditionally been used for its antifatigue benefits and appetite suppression.[12,53] Other alternative uses have been demonstrated, such as agronomic (antimicrobial) and medical (anesthetic, anticoagulant) applications.[1,8,34,57,58] Coca has been widely studied for its biotechnological potential, primarily due to its high alkaloid content and other biologically active compounds,[1,13] which are essential for plant physiology and environmental interactions.[59,60] As a result, coca extracts are seen as a promising alternative for inhibiting the growth of clinically or agro-industrially relevant organisms.

3.6. Volatile Analysis. Ten compounds were identified in the volatile analysis, though the yield could not be determined for the Clevenger extraction Table 6. More compounds were captured using SPME than liquid−liquid extraction using dichloromethane Figure S2. Nonetheless, hexadecanoic acid and octadecatrienoic acid were identified using both methods. In the Caimo morphotype, compounds extracted with dichloromethane showed more intense and defined signals. In contrast, in the Palo morphotype, the signals were faint and of low intensity, indicating a lower quantity of volatiles (Figure S2a). In the microfiber extraction, similar intensities were observed, and the identified compounds were present in both morphotypes. Caryophyllene and humulene were identified, but the humuleno is an analog of caryophyllene. These compounds have been characterized as anesthetics, reinforcing the coca plant's capacity to contribute to medicinal uses.[1,61]

Table 6. Compounds Identified in the Volatile Analysis of Leaves from Palo and Caimo Morphotypes with Calculated and Reported Kovats Index Values (NIST 14)

compound number	RT	compound	IK cal	IK ref	% error
1	32.51	hexadecanoic acid	2086	1984	5.16%
2	33.62	octadecatrienoic acid	2242	2108	6.35%
3	33.65	phytol	2244	2135	5.09%
4	34.22	linoleic acid	2173	2152	0.99%
5	34.51	ethyl octadecatrienoate	2299	2173	5.82%
6	10.15	benzoic acid	1215	1171	3.72%
7	16.7	tetradecane	1408	1399	0.66%
8	17.3	caryophyllene	1418	1428	0.69%
9	18.6	humulene	1440	1478	2.59%
10	20.5	pentadecane	1471	1500	1.92%

3.7. Cytotoxicity Test. The cytotoxicity test of extracts from coca leaves of the Palo and Caimo morphotypes revealed cytotoxic activity in both morphotypes against the L929 murine fibroblast cell line (Figure 2). The Palo morphotype exhibited a cell line inhibition percentage of 60.95%, whereas the Caimo morphotype showed an inhibition percentage of 48.57%. The activity was classified as moderate based on the reactivity grades for the indirect contact agar diffusion test.[32] This indicates that the extracts could affect various cell types, including malignant cells. Further evaluation of the ethanolic extracts in different preparations is necessary, as the cytotoxicity depends on the metabolites' concentration.

Cytotoxic activity has been reported in extracts from various species of the Erythroxylum genus.[62,63] The presence of quantified flavonoids and polyphenols, derived from cinnamic acid and shikimic acid precursors, respectively, suggests activation of the shikimate metabolic pathway. This pathway is responsible for the biosynthesis of compounds with cytotoxic properties, including the trimethoxycinnamate group at the C-6 position, which has been associated with malignant cell growth inhibition and enzymatic suppression in humans.[64,65] Additionally, benzoic acid, phytol, and humulene were identified, all of which have been studied for their cytotoxic effects. Phytol and humulene, in particular, have shown significant activity through apoptosis induction, mitochondrial membrane disruption, and reactive oxygen species (ROS) generation.[1,63] Tropane alkaloids (TA), including catuabine (*E. caatingae*) and cocaine, have also been reported to induce early apoptosis (with rates between 53.0 and 74.8%) and cause cellular damage in PC12 cells.[1,66] These compounds are likely contributing to the cytotoxic activity observed in this study,

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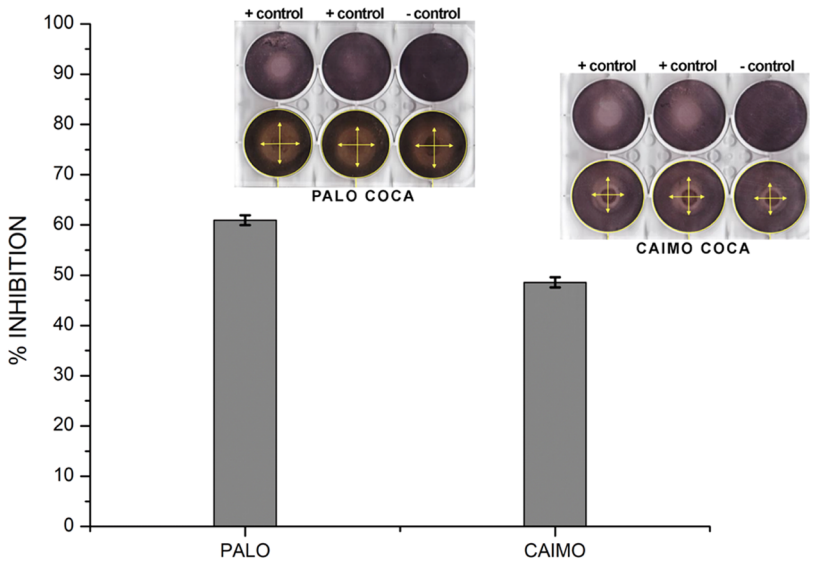

Figure 2. Cytotoxic activity of ethanolic extracts from coca leaves against L929 murine fibroblast cell line.

resulting in the inhibition of L929 murine fibroblast cell line growth.

4. CONCLUSIONS

Coca is an important plant in Colombia that contains a wide variety of chemical compounds, conferring diverse biological properties. Although the evaluated morphotypes are phenotypically similar, significant chemical differences were observed between their leaves and stems. This chemical characterization highlights the importance of distinguishing the various morphotypes present in Colombia. The bromatological, chemical, and cytotoxic characterization revealed a high content of important compounds (insoluble dietary fiber, phenols, flavonoids, volatiles), with high nutritional value and biological activity, which can serve as a basis for exploring potential uses, especially in the food sector, enabling the design of a commercial model for local cultivators.

The findings of this study provide a scientific basis for the potential economic and social transformation of indigenous communities in Vaupés, which could be replicated in other communities across the country or even in the Andean region. By identifying the diverse applications of coca plants, this research supports the development of sustainable industries that could enhance economic opportunities and improve living standards.

Future perspectives regarding *E. coca* should include regulatory frameworks supporting the medical, scientific, and industrial use of coca plants, aligning with sustainable development goals and empowering indigenous communities. It is also essential to explore research alternatives further to concretely define their utility and the levels of technological maturity that their development can achieve.

ASSOCIATED CONTENT

Supporting Information

The Supporting information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c10491.

Results of GC-MS analysis of coca samples (Figure S1); and (a) compounds extracted using L-L extraction with dichloromethane and (b) compounds captured using SPME (Figure S2) (PDF)

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Notes

The authors declare no competing financial interest.

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