Short Note [Nota Corta]

PRELIMINARY SCREENING OF SECONDARY METABOLITES FROM FIVE MEDICINAL FOOD PLANTS FROM CARIBBEAN DARIEN †

[ANÁLISIS PRELIMINAR DE METABOLITOS SECUNDARIOS DE CINCO PLANTAS MEDICINALES DEL DARIÉN CARIBEÑO]

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SUMMARY

Background: Remote regions of Colombia, particularly in areas such as the Darién region, lack adequate healthcare services for vulnerable populations. In response, these communities have developed strategies to identify and utilize therapeutic plants from their local environment. **Objective:** To document the traditional knowledge of medicinal food plants in San Francisco de Asís (Acandí, Chocó) and identify key secondary metabolites through phytochemical analysis, validating their therapeutic uses and potential applications in healthcare. **Methods:** This research was conducted in the village of San Francisco de Asís (Acandí, Chocó), situated at 7°25' N latitude and 74°26' W longitude. The study involved Black and inland communities who have historically relied on traditional knowledge for health and wellness. Ethnobotanical methods were employed to document the knowledge of local peasants regarding food plants with medicinal properties. Subsequently, phytochemical analyses were performed to identify the secondary metabolites responsible for these therapeutic effects. **Results**: Five food plant species with medicinal properties were identified: pepper (*Capsicum annuum*), flame of the woods (*Ixora coccinea*), jagua (*Genipa americana*), long coriander (*Eryngium foetidum L*), and achiote (*Bixa orellana L*). **Implications:** These findings underscore the importance of traditional knowledge in healthcare practices and highlight the potential of these plants for broader medicinal applications. **Conclusions**: The phytochemical analyses of the five species revealed significant groups of secondary metabolites that correlate with the medicinal uses reported by the inhabitants of Darién.
Key words: Caribbean Darien; ethnobotany; food plants; medicinal plants.

RESUMEN

Antecedentes: Las regiones remotas de Colombia, especialmente en zonas como la región del Darién, carecen de servicios sanitarios adecuados para las poblaciones vulnerables. En respuesta, estas comunidades han desarrollado estrategias para identificar y utilizar plantas terapéuticas de su entorno local. **Objetivo:** Documentar el conocimiento tradicional de plantas medicinales alimenticias en San Francisco de Asís (Acandí, Chocó) e identificar metabolitos secundarios claves mediante análisis fitoquímico, validando sus usos terapéuticos y potenciales aplicaciones en salud. **Métodos:** Esta investigación se realizó en el corregimiento de San Francisco de Asís (Acandí, Chocó), situado a 7°25' de latitud norte y 74°26' de longitud oeste. En el estudio participaron comunidades negras y de tierra adentro que históricamente han confiado en el conocimiento tradicional para la salud y el bienestar. Se emplearon métodos etnobotánicos para documentar el conocimiento de los campesinos locales sobre plantas alimenticias con propiedades medicinales. Posteriormente, se realizaron análisis fitoquímicos para identificar la presencia de metabolitos secundarios responsables de estos efectos terapéuticos. **Resultados:** Se identificaron cinco especies de plantas alimenticias con propiedades medicinales:

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pimienta (*Capsicum annuum*), llama del bosque (*Ixora coccinea*), jagua (*Genipa americana*), cilantro largo (*Eryngium foetidum L.*) y achiote (*Bixa orellana L.*). **Implicaciones:** Estos hallazgos subrayan la importancia del conocimiento tradicional en las prácticas de salud y destacan el potencial de estas plantas para aplicaciones medicinales más amplias. **Conclusiones:** Los análisis fitoquímicos de las cinco especies revelaron grupos significativos de metabolitos secundarios que se correlacionan con los usos medicinales reportados por los habitantes de Darién.
Palabras claves: Darien caribeño; etnobotánica; plantas alimenticias; plantas medicinales.

INTRODUCTION

The rural communities of the Caribbean Darién in Colombia have access to a diverse range of flora from the tropical rainforest's characteristic of the region. These plants are used for medicinal (Otero-Patiño *et al.*, 2000), nutritional (Álvarez-Salas, 2014), decorative, magico-religious (Fonnegra-Gomez and Botero-Restrepo, 2006), and artisanal purposes (Feuillet-Hurtado *et al.*, 2011). The relationship between biodiversity and its cultural uses is a growing concern for both scientific communities and the local populations that rely on these natural resources. Increased awareness of environmental degradation, species loss, and the declining quality of life in rural areas has driven more comprehensive research initiatives and created new opportunities for interaction and alternative approaches to improving socio-ecological environments (Sandifer *et al.*, 2015).

Historically, the population of San Pacho has been closely connected to the extraction of both timber and non-timber natural resources, including the exploitation of vegetable ivory (*Phytelephas pittieri O.F.Cook*.), natural rubber (*Ficus elastica Roxb*.), ipecacuanha (*Cephaelis ipecacuanha (Brot.) A. Rich.),* and fine woods. This region was also the site of early banana plantations in the Urabá area of Chocó in the early 20th century, which led to the migration of agricultural workers from coastal and inland areas, particularly Black people from Bolívar and Chocó (Molano, *et al.*, 1996). Additionally, Black and Indigenous Zenú communities from the savannas of Córdoba and Sucre arrived in the region in the early 20th century to participate in the expansion of the agricultural frontier (Gálve *et al.*, 2009).

The region's development has been hampered by a limited state presence and institutional fragility, making it one of the poorest areas in the country. This is reflected in low education levels, high poverty rates, and inadequate healthcare coverage. Approximately 30% of the population is not enrolled in any health service system, whether subsidized or contributory. The region also suffers from high rates of malnutrition, as well as respiratory, skin, and tropical diseases (Carrillo-Bonilla *et al.*, 2014). In response, the population of Darién has developed a sophisticated system of using medicinal plants as a local strategy to prevent and treat illnesses, a practice well-documented in ethnobotanical literature (Otero-Patiño, *et al.*, 2000).

This paper is one of the outcomes of an ethnobotanical research project that seeks to explore the knowledge, experiences, and practices of social agents regarding food plants with medicinal properties. Similar research has been conducted in Indigenous communities across the Americas (Salomon-Tarquini, 2019), leading to integrative findings that enhance our understanding of traditional knowledge, including cultural values and mythical beliefs (Gómez *et al.*, 2006). This study focuses on the local knowledge of plants with therapeutic uses, which often arises informally through the observation of ecosystems, and is shaped by common sense, social interactions, and the practical experiences of past, present, and future generations. Consequently, this knowledge is both cumulative and dynamic, adapting to technological changes and not confined to Indigenous communities or specific ethnic groups (Hofstde, 2014).

The aim was to identify five food plants with medicinal properties that were promoted, reproduced, and incorporated into the diet by rural populations as part of their local knowledge. These findings are linked to active compounds identified through preliminary phytochemical analysis. This research provides an alternative to the inadequate healthcare system in Darién, where agricultural family production among peasants, including Indigenous and Black communities, remains a key aspect of their cultural identity.

MATERIALS AND METHODS

Study area

This research was conducted in the village of San Francisco de Asís, situated at coordinates 7°25' north and 74°26´ west, within the municipality of Acandí, Colombia. The municipality spans an area of approximately 212,510 acres (Álvarez-Salas and Gálvez-Abadía, 2014). Locally known as San Pacho, the village lies in the northern Chocó region along the western slope of the Gulf of Urabá, on the Caribbean coast. San Pacho is part of the Caribbean Darien region, alongside three other municipalities: Unguía, Juradó, and a portion of Riosucio. It is approximately 229 miles away from Medellin, Colombia, and is inhabited by around 1,200 individuals of Black (Afrocolombian) and Chilapo (Zambo) descent, many of whom migrated from Cordoba, along with other inland residents settled in the rural areas (Salazar Zapata, 2012). Data and information were collected during three field visits conducted over a three-year period (2021-2023). The first visit took place between July 20 and December 11 of the first year, covering both the rainy and dry seasons. The second visit took place during March and April of the following year, a period marked by increased agricultural productivity. The final visit was conducted during the second semester of the third year, with the purpose of verifying the previously gathered information and observations. During this visit, interviews were also conducted with community members to corroborate findings and gather additional insights related to local knowledge and plant use.

Field survey approach

In this research, conducted in the corregimiento of San Francisco de Asís and its surrounding areas, the study extends beyond understanding how local communities appropriate forest foods. It delves into the relationships established with the larger encompassing ecosystem, as well as the sociopolitical and cultural dynamics that influence it directly or indirectly. An ethnobotanical method was employed for species identification, enabling the examination of the biology of forest resources and the local knowledge and beliefs associated with them (Alexiades and Sheldon, 1996). This method also highlighted the uses linked to processes of transformation and conservation. Two field visits were conducted. The first visit, in January 2021, aimed to recognize the area through written and photographic records of initial impressions, incidental conversations with residents, and adjustments to field guides. The main field season took place between July and December 2023. The application of the ethnographic method enabled the observation of participants' actions and statements, following Malinowski's concept of "being there" (Malinowski *et al.*, 1986). Daily activities related to the environment were observed (Guber, 2012), and ethnobotanical knowledge, appropriation forms, and conservation strategies were collected.

Field diary records were maintained, and 36 semi-structured interviews were conducted on the use and appropriation of the forest. Interviewees ranged in age from 25 to 62 years, many of whom were experienced in the forest setting. The interviews were divided into three groups: ten interviews each with Afro-descendants, Chilapos and Paisas. Seventeen interviews were conducted with women and thirteen with men. Informed consents were obtained, ensuring the free and spontaneous participation of interlocutors. The forest and plots were explored with one of the locals, following recommended techniques for socio-economic and environmental studies of tropical forests (Harrison, 2002). Plants were identified in situ using local knowledge. Taxonomic classification was performed with assistance from experts at the Herbarium of the University of Antioquia.

Post data collection, the interviews were transcribed, systematically organized, and categorized into 20 analytical groups using ATLAS.ti 6 software. This systematic approach facilitated the extraction of pertinent excerpts from interviews, where participants referenced, explained, or defined elements relevant to these categories.

Plant material collection and preparation.

Thirteen small farms engaged in food production were carefully chosen to gather plant samples. After washing, the samples were air-dried and sealed within zippered bags. Following proper labeling (M-1Pepper (*Capsicum annuum)*; M-2 Achiote Leaves (*Bixa orellana L)*; M-3 Long Coriander Leaves (*Eryngium foetidum L)*; M-4, Flame of the Woods Leaves (*Ixora coccinea)* and M-5; Jagua Fruit (*Genipa americana)*), they underwent pulverization via a grinding machine, resulting in a fine powder. This powder was then securely stored in airtight containers, ready for future testing and analysis.

Phytochemical Analysis

Extraction

A total of 25 grams of dried and pressed samples were utilized for extraction, combined with 250 mL of ethanol, and subjected to ultrasound treatment for one hour. Subsequently, the mixture was stored in darkness for 48 hours to optimize extraction efficacy. Following this, the solution was filtered under cold vacuum, and the residue was washed with 100 mL of ethanol to ensure thorough extraction (Swami-Handa *et al.*, 2008). Sample M-6 (jagua tincture) underwent a modified procedure, as it was not subjected to conventional extraction. Instead, the process commenced with 25 mL of tincture, followed by a liquid–liquid extraction (partitioning) using 15 mL of dichloromethane (DCM). This method was selected to effectively separate compounds based on their differential solubility in immiscible solvents. DCM was chosen due to its ability to dissolve a wide range of polar and nonpolar compounds, making it highly versatile for extraction processes, while also helping to prevent degradation of the extracted constituents. The resulting organic phase, referred to as filtrate B, was then recovered. Additional tests were conducted using the pure tincture, designated as filtrate A, after slight dilution of the dye to minimize potential colorimetric interference. Furthermore, another fraction of the 25 mL tincture was alkalinized with ammonium hydroxide and subsequently extracted with DCM, yielding a separate organic phase, referred to as filtrate C (adapted from Rocha de Albuquerque *et al.*, 1996).

Alkaloids

The solvent from the extract, corresponding to 15 grams of dry plant material (or 15 mL of M-6), was evaporated. The resulting residue was then subjected to extraction with 20 mL of 5% HCl at 50ºC. Subsequently, 0.5 mL of the acid extract was placed into four test tubes, and to each tube, 2 drops of Dragendorff, Mayer, Valser, and ammonium Reineckate reagents for alkaloids were added. Positive results were indicated by color changes or precipitate formation. In case of positive results, the remaining acid extract was alkalinized using 20% NaOH. The alkaline mixture was then sequentially extracted first with $CHCl_3$ and then with $CHCl_3$-EtOH (3:2). The solvents were evaporated, and the resulting residues were subjected to extraction again with 3 mL of 5% HCl. Finally, the four alkaloid precipitation reactions were performed again to confirm the presence of alkaloids (Koçanci *et al.*, 2022).

Steroids and/or triterpenoids

The residue from the ethanolic extract, corresponding to 20 grams of dried plant material (or 20 mL of M-6), underwent extraction with two portions of 20 mL petroleum ether. The resulting solution was filtered and concentrated to 5 ml. In a separatory funnel, it was then vigorously shaken with 10 mL of MeOH-H_2O (9:1). The ether phase was separated and subjected to two-dimensional column chromatography (CC) on silica gel. Elution was first performed with cyclohexane-AcOEt (95:5), followed by petroleum ether-ethyl ether-AcOH (80:20:1). The developed chromatogram was visualized using Liebermann-Burchard reagent, and heating at 105°C revealed the appearance of new spots with hues of red, green, or blue, indicative of the presence of steroids and/or triterpenoids. The residue obtained from the petroleum ether extraction was subsequently extracted with 20 mL and 10 mL of EtOH-H_2O (1:7) at 60°C, filtered, resulting in Solution 1 (Neela *et al.*, 2010).

Flavonoids

Solution 1, obtained from the previous extraction, was utilized for conducting specific tests. Firstly, with 1 mL of Solution 1, the Cyanidin or Shinoda reaction was executed, characterized by the appearance of a red coloration upon addition of powdered Mg and HCl, indicating the presence of γ-benzopyrones. Secondly, leucoanthocyanidins were identified by heating 1 mL of Solution 1 along with 0.5 mL of HCl in a water bath for 15 minutes, resulting in the production of red colorations (Zhu *et al.*, 2013).

Naphtho and/or anthraquinones

Bomtrager-Krauss reaction, heat in boiling water bath 15 minutes 5 mL of Solution 1, 1 mL of 20 volume H_2O_2, 1 mL of 50 % H_2S0_4, cool, extract with 3 mL of benzene and shake with 1 mL of 5 % NaOH containing 2 % NH_4OH; a red coloration in the alkaline layer indicates positive reaction for these substances.

Tannins: Begin by adding 1 mL of gelatin-salt reagent (Segelman *et al.*, 1969) to 1 mL of Solution 1. Centrifuge the mixture at 2,000 RPM and then dissolve the resulting residue in 1-2 mL of 10M urea solution. Next, introduce 2-3 drops of 5% $FeCl_3$ solution. The presence of tannins will be indicated by the emergence of intense red, blue, or green colorations (Tanira *et al.*, 1994).

Terpenic lactones, coumarins, and cardiotonics

The procedure began with an ethanolic extract equivalent to 10 grams of dry plant material (or 10 mL of M-6), which was then reduced to half its volume. Chlorophylls were precipitated by adding 4% lead acetate (AcOPb) containing 0.5% acetic acid (AcOH). The mixture was filtered, and the filtrate was concentrated to 2/3 of its volume using a rotary evaporator. Two extractions were performed using 20 mL of chloroform ($CHCl_3$). The resulting extract was dehydrated with sodium sulfate (Na_2SO_4) and further concentrated to 3 mL, resulting in Solution 2.

Solution 2 was applied onto four plates coated with silica gel G, along with standard compounds: steroids (cholesterol, β-sitosterol), coumarins (umbelliferone), terpene lactones (helenalin), and cardenolides (digitoxin). Two plates were developed using a chloroform-acetone ($CHCl_3$-$MeCO_2Me$) mixture at a 90:10 ratio. One plate was treated with vanillin-phosphoric acid to detect terpenoids, while the other was treated with ferric hydroxamate to identify terpenic lactones. The remaining two plates were developed using a dichloromethane-methanol-water (CH_2Cl_2-MeOH-H_2O) mixture at an 87:12:1 ratio. One plate was revealed with vanillin-o-phosphoric acid for coumarins, and the other was treated with Raymond's reagent for cardenolides (Arias *et al.*, 2017).

Coumarins appeared as spots showing green or blue fluorescence under UV light, indicating a positive reaction with ferric hydroxamate but no reaction with vanillin-o-phosphoric acid. Terpenic lactones, both diterpenic and sesquiterpenic, reacted positively with both reagents. Cardenolides were revealed by Raymond's reagent, producing violet spots when treated with 2% m-dinitrobenzene in ethanol, followed by 2% NaOH in 50% ethanol (Morsy, 2017).

Aminoacids

Extraction involved the utilization of a methanol/chloroform/water blend, facilitating subsequent elimination of pigments and lipids within the chloroform phase. Following extraction, without further refinement, constituents of the aqueous phase underwent separation through thin-layer high-voltage electrophoresis. Amino acids were detected via ninhydrin or autoradiography, following the procedure of R.L. Bieleski, N.A. Turner (Bieleski and Turner, 1966).

Data Analysis

The metabolite presence-absence matrix (Table 2) was transformed using Chi-square standardization with the ***decostand*** function from the ***vegan*** package in R, to prepare the data for multivariate analysis. Additionally, a Principal Component Analysis (PCA) was performed using a binary matrix (presence = 1; absence = 0) to explore patterns of metabolite distribution across the different plant extracts.

RESULTS AND DISCUSSION

The rural area of San Francisco is a tropical rainforest, characterized by its isolation from urban centers and low healthcare coverage (Carrillo-Bonilla *et al.*, 2014). Minor accidents in agricultural work or forest use, such as snakebites and stings from vectors of Leishmaniasis and malaria, are initially treated with plants that have medicinal uses (Tariq *et al.*, 2016). The study highlights the therapeutic applications of these plants (Table 1): *Capsicum annuum* (Pepper) and *Ixora coccinea* (Jungle Flame) are known for their antibiotic properties, crucial for wound healing and infection prevention. An interlocutor mentioned, "*pepper and turmeric poultices have helped me heal wounds from the garden. The pepper prevents infection and is much better than antibiotics, while the turmeric helps the wound heal*" (JP: November 2023).

Interlocutors maintain that *Genipa americana* (Jagua) offers a multifaceted approach to treating various ailments, from gastrointestinal problems to skin care. A woman mentioned, "*here, the radiation is very strong, and jagua ink prevents sunstroke. People don't use it much because it darkens the skin for the days you use it, but that's precisely what protects*" (RL: October 2023). *Eryngium foetidum L*. (Long Coriander) stands out for its role in maternal health and respiratory treatments, while *Bixa Orellana L.* (Achiote or Bixa) is valued for its regenerative properties and treatment of tonsillitis.

To establish the existence of secondary metabolites associated with the uses documented, these plants were analyzed through a qualitative phytochemical study of their components (Table 2).

Data Analysis

During data analysis (Figure 1), it was found that the first two dimensions of the PCA accounted for 56.4% of the total variance, with Dim1 explaining 30.9% and Dim2 explaining 25.5%. The biplot revealed a clear separation among samples based on their phytochemical composition. Extracts M6 (jagua tincture) and M4 (Flame of the Woods leaves) were positioned on the far right of Dim1, showing strong associations with the presence of alkaloids and terpenoids, two metabolite groups known for their pharmacological relevance. In contrast, sample M5 (jagua fruit) was located on the opposite side of Dim1, indicating a markedly different chemical profile. Samples M1, M2, and M3 clustered more closely toward the center, suggesting more moderate or overlapping phytochemical traits. Variables such as flavonoids, tannins, and amino acids contributed mainly to variation along Dim2, further influencing the vertical distribution of the samples. This ordination analysis supports the chemical differentiation among plant extracts and offers a valuable basis for connecting metabolite presence with traditional therapeutic uses.

Table 1. Ethnobotanical results of five food plants with medicinal properties.

Common Name	Scientific Name	Family	Part Used	Preparation	Medicinal Use
Pepper	*Capsicum annuum*	Solanaceae	Fruit	The fresh or dried fruit is directly applied to the wound	Antibiotic
Flame of the woods	*Ixora coccinea*	Rubiaceae	Leaves	Poultice	Antibiotic and powerful healing agent
Jagua	*Genipa americana*	Rubiaceae	Ripe fruit	The fruit is either eaten or turned into juice	Diuretic and emetic properties; used to treat scurvy and venereal sores, as well as fluid retention and gastrointestinal problems
			Tincture of unripe fruit	Tincture is obtained	Used as sunscreen, skin patch remover and mosquito repellent
Long coriander	*Eryngium foetidum L.*	Apiaceae	The whole plant	In an infusion	Used to make childbirth easier and for respiratory disease
Achiote or bixa	*Bixa Orellana L.*	Bixaceae	Shoots	Poultice	Used to regenerate tissue after burns or wounds, and to heal tonsillitis

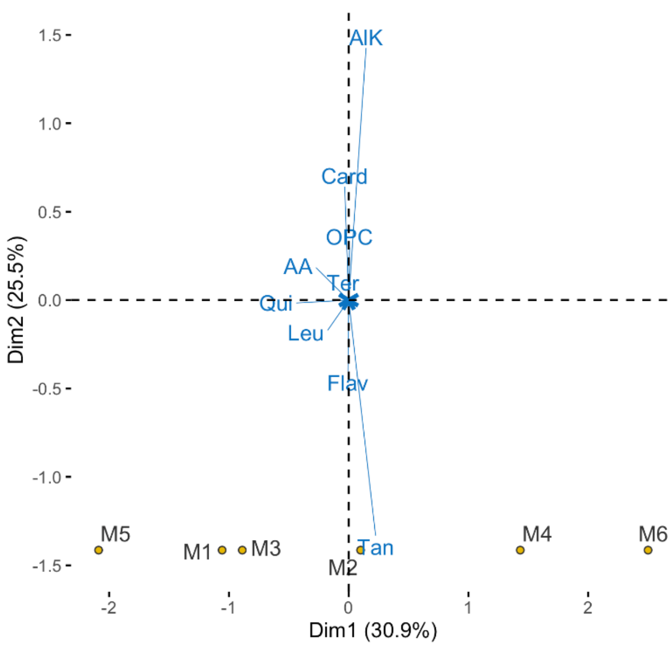

Figure 1. PCA of Plant Extracts Based on Metabolite Presence. Note: AA: Amino Acids; Flav: Flavonoids; Ter: Terpenoids; Alk: Alkaloids; Card: Cardiotonics; Tan: Tannins; OPC: Other Phenolic Compounds; Leuc: Leucoanthocyanidins; Quin: Quinones

Analysis of Peppers (*Capsicum annuum*)

Hot peppers (*Capsicum annuum*) belong to the Solanaceae family and are widely cultivated in various parts of the world, especially in temperate regions. The phytochemical analysis of this samples, denoted as M-1, reveals a diverse profile of bioactive compounds with significant medicinal potential (Jang, Choi and Jang, 2024).

The M-1 samples exhibited a high presence of amino acids (++), highlighting their importance in the plant's medicinal properties. Amino acids play crucial roles in various biological processes and contribute significantly to the therapeutic potential of peppers (Fattori *et al.*, 2016). The analysis indicates the presence of other phenolic compounds (+), while leucoanthocyanidins, tannins, flavonoids, cardiotonics, and quinones are absent (-). Similarly, the absence of alkaloids (-) in the samples suggests that the antibacterial and medicinal properties of Capsicum annuum are not attributed to these compounds. Phenolic compounds are renowned for their potent antioxidant properties, which help mitigate oxidative stress and prevent related diseases (Rahman *et al.*, 2021). Specifically, these compounds act as bactericides, effective against *Helicobacter pylori* (Carrascosa *et al.*, 2011), reinforcing the antimicrobial properties of peppers (Periferakis *et al.*, 2023).

The presence of terpenoids (+) in M-1 samples, although not abundant, is noteworthy. Terpenoids are integral to the plant's antibacterial capabilities, particularly against traditional tuberculosis pathogens (Mahizan *et al.*, 2019; Abdallah *et al.*, 2023). Their mechanism involves disrupting microbial cell membranes, thereby inhibiting pathogen growth. Furthermore, terpenoids have shown promise in anti-cancer therapies by inducing apoptosis in cancer cells (Siddiqui *et al.*, 2024).

The phytochemical composition of *Capsicum annuum* reveals a complex interplay of amino acids, phenolic compounds, and terpenoids, each contributing uniquely to its medicinal properties. The high presence of amino acids and phenolic compounds underscores their critical roles in antimicrobial, antioxidant, and therapeutic applications. Terpenoids, though less abundant, contribute to the plant's antibacterial and potential anticancer properties (Hamed *et al.*, 2019). This analysis validates the traditional uses of peppers in medicine and suggests further exploration into their phytochemical constituents for potential applications in modern healthcare and agriculture.

Analysis of Achiote Leaves *(Bixa Orellana L.)*

The phytochemical screening of *Bixa Orellana L.* (achiote) leaves, denoted as M-2, reveals a diverse profile of bioactive compounds, including amino acids (+++), leucoanthocyanidins (+), phenolic compounds (+++), tannins (+++), and terpenoids (+++). The absence of flavonoids, cardiotonics, quinones, and alkaloids indicates that the identified medicinal properties and traditional uses of achiote are primarily attributed to the present compounds (Da Silva *et al.*, 2023).

The M-2 samples exhibited a high presence of amino acids (+++), essential for various biological processes, including protein synthesis and tissue repair. However, their direct correlation with the traditional uses of achiote, such as skin softening and tonsillitis treatment, is not well-documented in existing literature (Valério *et al.*, 2015). The role of amino acids may be more related to general health and cellular functions rather than specific therapeutic applications.

The strong presence of phenolic compounds (+++) is significant, particularly concerning skin-related treatments. While traditional reports suggest achiote's use for skin softening, phenolic compounds are more accurately linked to anti-wrinkle treatments due to their antioxidant properties. Additionally, achiote is widely used in cosmetic products, capitalizing on the skin's ability to absorb these compounds effectively (Shahid-ul-Islam *et al.*, 2016).

Table 2. Results of the phytochemical screening of five food plants with medicinal properties.

Samples Metabolites	M-1 Pepper	M-2 Achiote Leaves	M-3 Long Coriander Leaves	M-4 Flame of the Woods Leaves	M-5 Jagua Fruit	M-6 Jagua Tincture
Amino Acids	++	+++	+++	+	++	+
Leucoanthocyanidins	-	+	-	-	-	-
Other phenolic compounds	+	+++	++	+	-	+
Tannins	-	+++	-	+	-	+
Flavonoids	-	-	-	-	-	+
Cardiotonics	-	-	+	+	-	-
Quinones	-	-	-	-	-	-
Terpenoids	+	+++	+++	+++	++	+
Alkaloids	-	-	-	+++	-	+++

Positive testing (+), (content: + low; ++ moderate; +++ high)
Negative testing (-), absence of metabolites or presence below the test’s level of detection.

A high positive response for tannins (+++) suggests their pivotal role in the medicinal uses of achiote, especially in treating tonsillitis. Tannins are known for their antibacterial and analgesic effects, which can provide relief in tonsillitis cases. Moreover, tannins have been documented for their neuropharmacological, anticonvulsive, analgesic, antibacterial, and antidiarrheal effects (Shilpi *et al.*, 2006; Coelho-Dos Santos *et al.*, 2022; Kováč *et al.*, 2022), supporting their broader therapeutic applications.

The presence of terpenoids (+++) in the M-2 samples aligns with several traditional uses of achiote. Terpenoids have well-documented antibacterial, anti-inflammatory, and skin regenerative properties, which can contribute to treating burnt tissue and tonsillitis. However, no direct evidence links terpenoids to the specific use of achiote for skin softening, suggesting that their role might be more related to tissue regeneration and anti-inflammatory effects (Proshkina *et al.*, 2020; Trepa *et al.*, 2024).

Although present in smaller quantities (+), leucoanthocyanidins contribute to the antioxidant properties of achiote, supporting overall health and potentially aiding in skin treatments and tissue regeneration (Khoo *et al.*, 2017). On the other hand, achiote seeds are industrially significant for extracting colorants, primarily due to the carotenoid bixin, responsible for the yellow-orange color (Raddatz-Mota *et al.*, 2017). The phytochemical screening did not specifically evaluate bixin, focusing instead on other bioactive compounds. This distinction is crucial as it highlights the dual utility of achiote in both medicinal and industrial contexts.

Analysis of Long Coriander *(Eryngium foetidum L.)*

The phytochemical screening of Eryngium foetidum L. (long coriander) leaves, denoted as M-3, reveals the presence of several bioactive compounds with notable medicinal properties. The identified metabolites include amino acids (+++), phenolic compounds (++), cardiotonics (+), and terpenoids (+++), while leucoanthocyanidins, tannins, flavonoids, quinones, and alkaloids were absent. However, the absence of alkaloids challenges the reported use of the plant as a contraceptive, suggesting that other unidentified compounds or synergistic effects might be responsible.

The M-3 samples exhibited a high presence of amino acids (+++), which are essential for various biological processes and therapeutic applications. Amino acids play a crucial role in muscle relaxation, which may explain the use of long coriander to ease childbirth and relieve colic. Their involvement in synthesizing proteins and other critical molecules contributes to overall health and wellbeing (Kamei *et al.*, 2020).

The positive presence of phenolic compounds (++) is significant, as these compounds are known for their anti-inflammatory properties. The Apiaceae family, to which long coriander belongs, is particularly noted for its anti-inflammatory effects (Thiviya *et al.*, 2021; Scandar *et al.*, 2023). This property is likely responsible for the plant's use in relieving colic and possibly treating influenza through its analgesic effects. The literature indicates that a dose of 250 mg/kg of dry leaf is required to achieve an analgesic effect (Almeida, 2001), though the specific compounds responsible were not identified in this screening.

The detection of cardiotonic compounds (+) suggests a potential use of long coriander in promoting cardiovascular health. These compounds are known to influence heart muscle contractions and could be linked to the plant's traditional use in treating conditions related to heart health (Patel *et al.*, 2012; Mahleyuddin *et al.*, 2021).

Terpenoids (+++) were abundantly present in the M-3 samples. These compounds have various medicinal applications, including antibacterial, anti-inflammatory, and potential antihypertensive effects (Powder-George *et al.*, 2024). The muscle relaxant properties of terpenoids might contribute to the use of long coriander in facilitating childbirth. Additionally, terpenoids are noted for their role in treating respiratory conditions such as asthma, although other compounds typically associated with this effect, such as flavonoids and tannins, were not present in the analyzed sample.

Despite the absence of flavonoids, tannins, and saponins in the M-3 samples, long coriander is reported to treat burns, earache, hypertension, constipation, convulsions, stomachache, parasites, snakebites, diarrhea, and malaria (Hemachandra *et al.*, 2021). These traditional uses highlight the need for further research to identify and understand the secondary metabolites responsible for these effects.

Analysis of Flame of the Woods Leaves *(Ixora coccinea)*

The phytochemical screening of *Ixora coccinea* (Flame of the Woods) leaves, denoted as M-4, reveals a rich profile of bioactive compounds with significant medicinal potential. The identified metabolites include amino acids (+), phenolic compounds (+), tannins (+), cardiotonics (+), terpenoids (+++), and alkaloids (+++). The absence of leucoanthocyanidins, flavonoids, and quinones narrows the focus to the compounds detected and their contributions to the plant's medicinal properties.

The M-4 samples exhibited a positive presence of amino acids (+), indicating a potential role in the therapeutic properties of Ixora coccinea, particularly its antibiotic and healing effects. Amino acids are fundamental building blocks for protein synthesis, playing a critical role in cellular repair, tissue regeneration, and overall metabolic functions. Their presence in *Ixora coccinea* suggests that they may enhance the plant's ability to promote wound healing and recovery from infections by supporting the synthesis of proteins essential for immune responses and cellular restoration (Rajayan *et al.*, 2024). This finding underscores the importance of amino acids as a key component in the plant's pharmacological profile, providing a biochemical basis for its use in traditional medicine and highlighting its potential for further therapeutic applications.

Phenolic compounds were positively identified (+) in the M-4 samples, known for their potent bactericidal effects. These compounds inhibit microbial growth, particularly bacteria, making them valuable in treating infections (Sun and Shahrajabian, 2023). A high presence of terpenoids (+++) was observed, aligning with the plant's noted antibacterial properties. Terpenoids are recognized for their efficacy in treating traditional tuberculosis and other bacterial infections (Jagatap *et al.*, 2022). Their antioxidant, antibacterial, gastroprotective, hepatoprotective, antidiarrheal, antimutagenic, and chemoprotective effects further underscore the medicinal value of *Ixora coccinea* (Baliga and Kurian, 2012). Additionally, terpenoids have antitumor potential, contributing to the plant's use in cancer treatment (Kamran *et al.*, 2022).

The presence of tannins (+) in *Ixora coccinea* enhances its antibacterial efficacy. Tannins are known for their ability to inhibit bacterial growth and their use in various medicinal applications, including wound healing (Amarowicz *et al.*, 20). While specific wound-healing compounds like hydroxyproline and glycosaminoglycan were not detected in this screening, tannins likely play a significant role in the plant's healing properties (Nayak *et al.*, 1999).

The detection of cardiotonic compounds (+) supports the traditional use of *Ixora coccinea* in treating heart failure. Cardiotonics enhance heart muscle function, making them valuable in managing cardiovascular conditions (Mijatovic *et al.*, 2007; Momin *et al.*, 2012).

A high presence of alkaloids (+++) was also noted. Alkaloids are associated with various therapeutic effects, including the treatment of hypertension (Rajput *et al.*, 2022). While the specific types of alkaloids present in *Ixora coccinea* were not identified, their high presence indicates a potential role in managing blood pressure and cardiovascular health.

Analysis of Jagua *(Genipa americana)*

The phytochemical screening of *Genipa americana* (jagua) reveals a diverse array of bioactive compounds in both the fruit (M-5) and tincture (M-6). The identified metabolites include amino acids, phenolic compounds, tannins, flavonoids, terpenoids, and alkaloids, each contributing to the plant's extensive medicinal properties. The absence of leucoanthocyanidins, cardiotonics, and quinones refines our focus on the detected compounds and their therapeutic roles.

Amino acids were positively identified in both the fruit (++) and tincture (+) samples, highlighting their importance in jagua's medicinal applications. Amino acids play vital roles in protein synthesis, cellular repair, and overall metabolic functions. Their presence supports jagua's use as an anti-inflammatory agent and in the treatment of chronic enteritis and fluid retention (Bentes *et al.*, 2014; Brauch *et al.*, 2016; Náthia-Neves *et al.*, 2017).

Phenolic compounds were detected in the tincture (+), known for their antioxidant and antimicrobial properties. These compounds are crucial in treating skin conditions and gastrointestinal problems such as diarrhea and constipation due to their spasmolytic (antispasmodic) effects (Cimanga *et al.*, 2010). Phenolic compounds also contribute to jagua's use in treating hemorrhages (Jain *et al.*, 2005) and providing antimalarial effects when used for mosquito bites (Ono *et al.*, 2005).

Tannins were present in the tincture (+) but absent in the fruit. Known for their astringent properties, tannins play a role in treating gastrointestinal issues, such as diarrhea, and enhancing wound healing (Fraga-Corral *et al.*, 2021; Claro *et al.*, 2023). They also contribute to the antimicrobial effects of jagua, supporting its traditional use in treating venereal sores caused by *Neisseria gonorrhoeae*.

Flavonoids were found in the tincture (+) but not in the fruit. These compounds are associated with jagua's spasmolytic properties, aiding in the treatment of gastrointestinal issues like diarrhea and constipation. This compound also contributes to the plant's antimalarial effects and its use in treating hemorrhages (Silva *et al.*, 2018).

Terpenoids were abundantly present in both the fruit (++) and tincture (+), underscoring their significance in jagua's medicinal applications. Terpenoids exhibit strong antibacterial, anti-inflammatory, and antioxidant properties, supporting the plant's use in treating chronic enteritis, skin conditions, and as an antitumor agent (Assis *et al.*, 2023). The presence of terpenoids also aligns with jagua's use as a diuretic, laxative, and in the treatment of fluid retention and scurvy (Dickson *et al.*, 2018).

A high presence of alkaloids was detected in the tincture (+++), though absent in the fruit. Alkaloids from the Rubiaceae family, such as rhynchophylline and iso-rhynchophylline, are known nervous system stimulants (Heitzman *et al.*, 2005). These compounds contribute to jagua's use in treating diabetes, periodontitis, liver failure, cataracts, and in neuroregeneration and wound healing. The pharmacological effects of alkaloids also include stimulation of the nervous system and potential therapeutic uses in managing hypertension (Assis *et al.*, 2023).

Other uses can include the extraction of essential oils, which provide a refreshing effect and are employed as a natural dye for clothes, tools, and skin. These applications highlight the versatility of jagua and its significant cultural and practical value.

CONCLUSIONS

The results of the phytochemical screening offer qualitative evidence that identifies a range of secondary metabolites in the analyzed species. The magnitude of the response, represented by the number of pluses (“+”), correlates with the color intensity observed during testing, compared to a standard molecule from the metabolite group under analysis. This variation in color intensity among the filtrates suggests differences in the concentration of secondary metabolites across the species examined.

Notably, the presence of amino acids and terpenoid compounds, along with the general absence of quinones (both anthraquinones and naphthoquinones), is consistent across all samples. The significant abundance of nitrogenous compounds, particularly alkaloids, in the leaves of flame of the woods (*Ixora coccinea*) and the jagua tincture (*Genipa americana*), stands out. In contrast, the absence of alkaloids in the dry sample of hot peppers (*Capsicum annuum*) is confirmed. Regarding phenolic compounds, the tests indicate a general absence of flavonoids and leucoanthocyanidins, suggesting that the positive response for total phenolic compounds is primarily due to the presence of tannins and other phenolic compounds distinct from flavonoids and anthocyanidins.

These findings underscore the phytochemical diversity among the studied species, highlighting the importance of specific secondary metabolites in their traditional medicinal uses. The consistent presence of amino acids and terpenoids suggests their central role in the therapeutic properties attributed to these plants, while the variability in alkaloid and phenolic compound concentrations indicates the unique medicinal potential of each species.

This research contributes valuable insights into the traditional medicinal practices of the Darién region, providing a potential alternative to the inadequate healthcare system that persists in these remote areas. In a region where agricultural family production remains integral to the cultural identity of Indigenous and Black communities, understanding the medicinal value of local plant species can enhance self-sufficiency and improve health outcomes. Furthermore, these findings reinforce the significance of preserving traditional knowledge and biodiversity, offering a foundation for future studies aimed at integrating ethnobotanical wisdom with modern healthcare practices.

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