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Phytochemical profile and physicochemical characterization of the endemic vine *Tynanthus croatianus (Bignoniaceae)* in Costa Rica

Perfil fitoquímico y caracterización fisicoquímica de la planta trepadora endémica *Tynanthus croatianus (Bignoniaceae)* en Costa Rica

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Written by:
German Madrigal Redondo[1]
https://orcid.org/0000-0002-9856-4044
Jeniffer Sandí Flores[2]
https://orcid.org/0009-0009-4495-9671
Maynor Carranza Varela[3]
https://orcid.org/0009-0001-8861-6322
Gustavo Carazo Berrocal[4]
https://orcid.org/0000-0002-1778-5559

Abstract

Traditional medicine utilizes species of the *Bignoniaceae* family to treat diabetes, malaria, and leishmaniasis. However, *Tynanthus croatianus*, an endemic vine from Costa Rica, lacks comprehensive phytochemical studies validating its potential therapeutic use. **Objective:** This study aimed to characterize the secondary metabolites in *T. croatianus* stems using solvents of varying polarity and to determine the physicochemical properties of the aqueous extract. **Methods:** Stem samples were collected in Puntarenas, Costa Rica. Extracts were prepared via maceration using distilled water, ethanol (96%), methanol, and chloroform. Qualitative phytochemical screening (Dragendorff, Shinoda, etc.) and physicochemical analyses (pH, specific gravity, UV-Vis spectroscopy) were performed. **Results:** Phytochemical screening revealed the presence of flavonoids, terpenes, gallic tannins, catechins, and steroids across all extracts. Notably, alkaloids were detected in ethanolic and methanolic phases but were absent in aqueous extracts. The aqueous solution exhibited an acidic

Resumen

La medicina tradicional utiliza especies de la familia *Bignoniaceae* para tratar la diabetes, la malaria y la leishmaniasis. Sin embargo, *Tynanthus croatianus*, una planta trepadora endémica de Costa Rica, carece de estudios fitoquímicos exhaustivos que respalden su potencial uso terapéutico. **Objetivo**: Caracterizar los metabolitos secundarios en los tallos de *T. croatianus* mediante solventes de polaridad variable y determinar las propiedades fisicoquímicas del extracto acuoso. **Métodos:** Se recolectaron muestras de tallos en Puntarenas, Costa Rica. Los extractos se prepararon mediante maceración con agua destilada, etanol (96%), metanol y cloroformo. Se realizó un cribado fitoquímico cualitativo (Dragendorff, Shinoda, etc.) y análisis fisicoquímicos (pH, gravedad específica, espectroscopía UV-Vis). **Resultados:** El cribado fitoquímico reveló la presencia de flavonoides, terpenos, taninos gálicos, catequinas y esteroides en todos los extractos. Cabe destacar que se detectaron alcaloides en las fases etanólica y metanólica, pero no en los extractos acuosos. La solución acuosa presentó un pH ácido (5,61) y una densidad de

[1] Master's degree in Quality Control of Medicines and Master's degree in Intellectual Property, Pharmaceutical Research Institute (INIFAR), Pharmacy School, University of Costa Rica, San José, Costa Rica. Email: generacionlcr96@gmail.com
[2] Bachelor´s degree in Pharmacy, Pharmaceutical Research Institute (INIFAR), Pharmacy School, University of Costa Rica, San José, Costa Rica. Corresponding author. Email: jeniffer.sandi@ucr.ac.cr
[3] Master's Degree in Natural Resource Management and Production Technologies, Pharmaceutical Research Institute (INIFAR), Pharmacy School, University of Costa Rica, San José, Costa Rica. Email: maynor.carranza.varela@una.cr
[4] Master's degree in Analysis and Quality Control of Medicines, Pharmaceutical Research Institute (INIFAR), Pharmacy School, University of Costa Rica, San José, Costa Rica. Email: gustavo.carazo@ucr.ac.cr

pH (5.61) and a specific gravity of 0.9977. UV-Vis analysis indicated the presence of low-molecular-weight compounds, likely sugars and terpenes. **Conclusion:** *T. croatianus* possesses a diverse metabolic profile rich in antioxidant and antimicrobial candidates. These findings provide a scientific baseline supporting its ethnopharmacological potential and justifying future *in vitro* and *in vivo* bioactivity assays.

Keywords: *T. croatianus*, phytochemical profile, secondary metabolites, extracts, therapeutic potential.

0,9977. El análisis UV-Vis sugirió la presencia de compuestos de bajo peso molecular, probablemente azúcares y terpenos. **Conclusión:** *T. croatianus* posee un perfil metabólico diverso, con un rico contenido de candidatos antioxidantes y antimicrobianos. Estos hallazgos proporcionan una base científica que respalda su potencial etnofarmacológico y justifica futuros ensayos de bioactividad *in vitro* e *in vivo*.

Palabras clave: *T. croatianus*, perfil fitoquímico, metabolitos secundarios, extractos, potencial terapéutico.

Introduction

Traditional medicine, or ethnobotany, has been used to treat diseases since ancient times and has shown clear benefits for communities that continue to preserve and use medicinal plants (Zambrano Intriago et al., 2015). Among the important medicinal plants is the *Bignoniaceae* family, which includes species with active compounds used to treat diseases such as cancer, diabetes, syphilis, malaria, hepatitis, rabies, and even (Gargiullo et al., 2004). Some examples of these species belong to the *genus Tynanthus miers,* such as *Tynanthus guatemalensis, Tynanthus panurensis, Tynanthus micranthus,* and *Tynanthus croatianus.*

The genus *Tynanthus miers* comprises approximately 15 species (Medeiros & Lohmann, 2015), and is characterized mainly by lianas and some small trees. The small white flowers are a distinctive characteristic of the genus within the family, as is the intense clove aroma present in many of its species, as well as its fruits with wide margins. (Lohmann, 2006; Cortés, 1897).

Tynanthus guatemalensis is one of the most important species, its use in Mayan culture being linked to treating the symptoms of diabetes. According to a study conducted in conjunction with healers from this population, promising results were obtained when using the plant to treat this disease, identifying verbascoside as the main secondary metabolite associated with the plant's significant anti-glycation activity (Ferrier et al., 2018). .On the other hand, in Peru, *Tynanthus panurensis*, known as "clavo huasca," is traditionally used for its aphrodisiac, energizing, and analgesic properties, as well as for the treatment of diabetes and rheumatism. A study identified the presence of phenylpropanoid glycosides, including verbascoside, isoverbascoside, and leucosceptoside, in this plant. These compounds exhibit analgesic, antioxidant, and antimicrobial pharmacological activity (Plaza et al., 2005; Jiménez & Riguera, 1994).

Tynanthus micranthus is used in natural medicine. It is often brewed as a tea to help relieve colds. The clove-flavored species has been studied for its eugenol content a compound used as an analgesic and disinfectant. Researchers were able to extract this compound and evaluate its antimicrobial activity against fungi, bacteria, and yeasts (Custódio et al., 2010).

In Costa Rica, the species *Tynanthus croatianus* (Figure 1) is found in the La Amistad Pacífico Conservation Area, Puntarenas province. It is characterized as a liana whose fruit is capsule-shaped and grows in limestone-derived soils, typically at altitudes below 300 m above sea level. It is worth noting that this species is also found in Panama (Cortés, 1897).

Figure 1. *Tynanthus croatianus* La Amistad Pacifico Conservation Area in the province of Puntarenas.
Source: Original photograph

Tynanthus croatianus is prized for its medicinal and aphrodisiac properties. Its limited habitat puts it at risk of extinction, making it significant for this article. In Costa Rica, it is called the cinnamon vine or clove vine, but the species remains relatively unknown.

Figure 2. Flowers of Tynanthus croatianus, Gallardo, Los Dos Brazos del Rio Tigre, La Palma de Puerto Jimenez, province of Puntarenas.
Source: Original photograph

Given the limited research on this regionally endemic species, the primary objective of this study is to identify the presence of secondary metabolites in Tynanthus croatianus and assess their potential pharmacological effects.

Materials and methods

- **Sample Tynanthus croatianus**

The plant *Tynanthus croatianus* A. H. Gentry was collected between February 2021 and March 2022, directly from the canton of Puerto Jimenez, Gallardo, along the Tigre River riverbed, in the district of La Palma, Puntarenas province, Costa Rica (coordinates: N: 08°32.399, W 083°21.865' and N:08°32.397', W: 083°21.866'). A total of 2 kg of plant material was collected on each date. A test specimen was deposited in the herbarium of the National Museum of Costa Rica and the University of Costa Rica under the card numbers 08 36 39 Lat and 83 21 55 Long.

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- **Chemicals and reagents**

The following reagents were used for the phytochemical screening: hydrochloric acid 0.01 M and 20% bismuth nitrate Merck (Germany), potassium iodide Merck (Germany), mercuric chloride Sigma-Aldrich (Germany), sublimate iodine Merck (Germany), 1%, 5%, 10% and 2M sodium hydroxide PCC ROKITA (Poland), acetic anhydrous of VWR Chemicals BDH(EE.UU), concentrated sulphuric acid of PQM Fermont (Mexico), ammonium molybdate Chemicals BDH (EE.UU), perchloric acid Merck (Germany), bromine Merck (Germany), glacial acetic acid Fisher Scientific (EE.UU), potassium bromide Sigma-Aldrich (Germany), sodium hypochlorite Merck (Germany), metallic zinc Sigma-Aldrich (Germany), trichloroacetic acid concentrate Sigma-Aldrich (Germany), gelatin, salt, ferric chloride JT BAKER SIGMA (EE.UU), formaldehyde Merck (Germany), ninhydrin Merck (Germany), vanillin, alpha naphthol or beta naphthol Merck (Germany), sodium carbonate Fisher Scientific (EE.UU) , sodium citrate Merck (Germany), cupric sulfate pentahydrate JT BAKER SIGMA (EE.UU), sodium Merck (Germany), potassium Merck (Germany), potassium tartrate JT BAKER SIGMA (EE.UU), silver nitrate SPECTRUM (EE.UU), ammonium hydroxide concentrate Fisher Scientific (EE.UU), hydroxylamine 2% Merck (Germany), Sudan III Merck (Germany), picric acidand ammonium trichloride Merck (Germany).

The following were used as control reagents for the phytochemical profile: quinine, Sigma-Aldrich (Germany); naphthoquinone, Merck (Germany); cholesterol; orange peel; strawberry or blackberry extract; tannins; aloe; hydrolyzed collagen; glucose; aerial parts of *Montanoa hibiscifolia, oil;* and seeds of *Passiflora denopoa*, annatto.

The equipment used for the physicochemical tests was: Hanna® pH meter (HI5522-01) (Poland), Kimble® pycnometer (Mexico), and Thermo Electron Corporation Model 334610 refractometer (EE.UU.).

- **Phytochemical profile**

For the phytochemical profile, the stem of Thynanthus croatianus was first cut on a wooden board with a sharp knife. It was then placed in a laboratory blade mill to reduce its size and facilitate dispersion. Five to ten grams of this plant material were weighed into a glass container using a macrobalance, and solvent was added in a ratio of 1 part Thynanthus croatianus to 2 parts solvent (1:2). The solvents used were distilled water, 96% ethanol (Merck, Germany), 99.8% methanol, and 99.8% chloroform. The mixture was stirred in the glass container, allowed to macerate for 7 days, and finally filtered through a cloth filter. The total amount of extract obtained was weighed on a macrobalance, the container was sealed and labeled, and the extract was finally stored in a freezer (aqueous at -20 °C and other solvents at 5 °C) during testing.

Subsequently, the following tests were performed to identify metabolites from the liquid and dry extract stored in the previous phase, according to laboratory procedures: Dragendorff (alkaloids), Mayer (alkaloids), Wagner (alkaloids), Tollens (carbohydrates), Molish (carbohydrates), Fehling (reducing carbohydrates), Benedict (reducing carbohydrates), Shinoda (flavonoids), Pews (flavonoids), Carr-Price (carotenes), Perchloric acid (terpenes), Ammonium molybdate IV (terpenes), Tortelli-Jaffe (terpenes), Sudan (fats), Kedde (cardiotonic glycosides), Guignard (cyanogenic glycosides), Cooling (mucilages), Rosenthaler (saponins), Foam (saponins), Bornträger-Kraus (quinones), Nihydrin (amino acids), Hydrochloric acid-formaldehyde (condensed tannins), Salkowski (terpenes), Gelatin-Salt (tannins), Ferric Chloride III (gallic acid and catechin tannins), Lieberman-Burchard (steroids), Rosenheim (anthocyanins), ferric hydroxamate (sesquiterpene lactones).

Physico-chemical analysis

Specific gravity: The measurement was carried out in triplicate using a Kimble® pycnometer (Mexico) and a RADWAG® Model AS 220 X2 analytical balance (Poland). The mass of the empty pycnometer (clean, dry, and with the lid on) and the mass of the sample (dry and with the lid on) were determined on the same analytical balance. The mass of the sample was then determined by difference, and the same procedure was repeated with distilled water. The data were averaged, and the relative standard deviation (RSD) was calculated. The calculation of the specific gravity was carried out using the following equation:

$DR = \rho\ sustance / \rho\ reference$ **(equation 1)**

pH: The measurement was carried out in triplicate with the aid of a Hanna Instruments pH meter (HI5522-01) (Poland). An aliquot of about 20 mL of the sample was transferred to a 50 mL beaker, and the electrode was properly washed and dried, avoiding contact with the beaker walls. The pH values indicated on the pH meter were recorded after the measurement stabilized. The data were averaged, and the relative standard deviation (RSD) was calculated.

Conductivity: The measurement was carried out in triplicate using a Hanna Instruments pH meter (HI5522-01) (Poland). The pH measurement procedure was repeated, switching the pH meter to conductivity mode. The data were averaged, and the relative standard deviation (RSD) was calculated.

Brix degrees: The measurement was carried out in triplicate with a Thermo Electron Corporation Model 334610 refractometer (EE.UU.). A dropper was loaded with a small amount of sample, the prism cover was removed, and two to four drops of sample were placed in the prism. The sample was checked for distribution, and the prism cover was placed on the prism. The sample was then focused, and the Brix degrees were determined using the refractometer scale. The data were averaged, and the relative standard deviation (RSD) was calculated.

UV-Visible Spectroscopy

Equipment: Thermo Scientific Orion AquaMate 8000 UV-Visible Spectrophotometer (UV-Vis).

An aqueous extract of T. croatianus stems was prepared at 10% w/w in the same manner as described in this section. 5 mL of the filtered extract was placed in a 1 cm x 1 cm quartz cell, and a scan was performed from 200 nm to 700 nm at 5 nm intervals. The data were graphed in Microsoft Excel.

Results

Phytochemical profile

The phytochemical profile of the plant sample in different solvents is presented in **Table 1 below.** In general, the results showed a remarkable difference between the solvents used.

Table 1.
Phytochemical analysis of aqueous, ethanolic, methanolic, and chloroformic extracts using Tynanthus croatianus stem. Nomenclature: (NA) Not applicable, (-) Negative test, (+) Positive test.

Tests	Metabolite	Aqueous phase	Ethanolic phase	Methanolic phase	Chlorophormic phase
Dragendorff	Alkaloids	-	+	+	-
Wagner	Alkaloids	-	-	+	-
Tollens	Carbohydrates	-	+	-	+
Molish	Carbohydrates	-	+	-	+
Fehling	Reducing carbohydrates	+	+	+	NA
Benedict	Reducing carbohydrates	-	+	+	-
Shinoda	Flavonoids	+	+	+	+
Liebermann-Burchard	Terpenes	+	+	+	+
Ammonium Molybdate IV	Terpenes	+	+	+	+
Salkowski	Terpenes	+	+	+	+
Guignard	Cyanogenic glycosides	-	-	-	+
Ferricyanide	Cyanide	NA	NA	NA	NA
Cooling	Mucilages	-	-	-	-
Rosenthaler	Saponins	-	+	+	-
Foaming	Saponins	+	-	-	-
Bornträger- Kraus	Quinones	-	+	+	-
FeCl3	Gallic and catechinic tannins	+	+	+	+
Lieberman- Burchard	Steroids	+	+	+	+
Rosenheim	Anthocyanins	+	+	+	-
Ferric hydroxamate	Sesquiterpene lactones	+	+	+	-

Physico-chemical analysis

Table 2.
Physico-chemical analysis of aqueous extracts of plant material of Tynanthus croatianus.

Sample	Specific gravity	pH	Conductivity (mV)	Brix degrees
1	0.9992	5.61	60.9	15
2	0.9957	5.61	61.4	17
3	0.9982	5.61	61.4	17
Average ± Standard Deviation	0.9977 ± 0.0018	5.61 ± 0	61.2 ± 0.29	16.3 ± 1.15

3,3 UV-Visible Spectroscopy

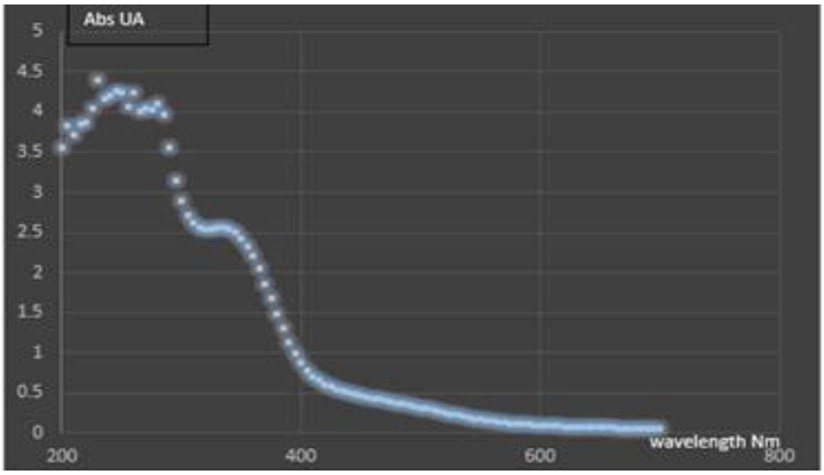

Figure 3. UV-visible spectrum from 200 nm to 700 nm, 10% m/m aqueous extract of the stem of *Tynanthus croatianus.*
Source: Original photograph.

Discussion

Plants synthesize primary metabolites for essential functions and also generate a large number of secondary metabolites for survival under adverse environmental conditions, including enzymatic activity, signaling, and defense (Yadav et al., 2021), so it is important to analyze the presence of these through qualitative phytochemical screening tests, which, although qualitative tests, function to provide a first glimpse of a possible overview of the potential pharmacological properties of the plant under study. In this way, preliminary phytochemical studies are very useful for estimating plant constituents and potential biological activities and, therefore, are used as a preliminary assessment of bioactive compounds in plants (Davoodi et al., 2021).

In the present study, the plant material tested negative for Mayer, Pews, Carr-Prince, Perchloric Acid, Tortelli-Jaffe, Sudan, Kedde, Cooling, Nihydrin, Formaldehyde-HCl, Gelatin, and Salt, so the positive results obtained in at least one test are shown in Table 1.

The positive results in the 4 phases analyzed denoted the presence of flavonoids, terpenes, gallic tannins, catechins, and steroids. This is of great value, as flavonoids have extensive and varied pharmacological actions, being well known for their properties against capillary fragility and coronary dilatation, as well as their spasmolytic, anti-hepatotoxic, estrogenic, and diuretic properties, among others. It also has antimicrobial and fungitoxic properties (de Melo et al., 2023).

On the other hand, the presence of terpenes is considered of utmost importance, as they have been reported to possess antioxidant, antimicrobial, antifungal, antiviral, and nutritional properties (de Melo et al., 2023).

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Figure 4. Aqueous extract of the stem of *Tynanthus croatianus.*
Source: Original photograph.

An essential characteristic of tannins is their astringent capacity, as well as their ROS (reactive oxygen species)-reducing, antimutagenic, antiviral, and antimicrobial properties; however, most plants that synthesize tannins produce a mixture of procyanidins and prodelphinidins, which makes their isolation difficult (de Melo et al., 2023).

In addition, steroid compounds have been linked to anti-inflammatory properties due to their inhibitory effects on phospholipase A2, which is critical to their production. Moreover, the literature indicates that steroids can affect gene expression, translation, and enzyme activity, thereby altering physiological processes via multiple biochemical pathways (Ericson-Neilsen & Kaye, 2014).

As for the differences shown in the phytochemical profile of the different phases, after showing a positive result, the following stand out: firstly, in the aqueous phase, a positive response was obtained for reducing carbohydrates, saponins, anthocyanins, sesquiterpene lactones, while for the ethanolic phase it was for alkaloids, reducing carbohydrates, saponins, quinones, catechins, anthocyanins, sesquiterpene lactones.

Besides that, the methanolic phase showed positive results for alkaloids, reducing carbohydrates, saponins, quinones, catechins, anthocyanins, and sesquiterpene lactones; the chloroformic phase indicated the presence of gallic tannins and catechins.

Because this is a preliminary investigation of the plant and recognizing that the tests performed are qualitative, the positive result for alkaloids, not being a specific test, can produce false positives with compounds that present the presence of nitrogenous compounds in the extract, which is a complex matrix (Barrera et al., 2014), so in subsequent studies it is recommended to carry out confirmatory tests such as resolution or thin layer chromatography.

It can also be noted that the polar extracts (aqueous, ethanolic, and methanolic) have the highest number of positive results for metabolite extraction in general, compared to the less polar extracts (chloroformic). Of the four extracts tested, the ethanolic extract has the highest yield based on these positive results; however, it cannot be concluded that this is the best extract from this study alone, as qualitative, and not quantitative, tests cannot determine in quantity which extract has the highest presence of metabolites of interest.

It should also be noted that this analysis of extracts in different media is necessary to verify the affinity of the metabolites, which may function as future active metabolites of interest in studies; however, to continue this analysis in *in vivo* or *ex vivo* models, it is important to consider that chloroform is capable of generating toxicity, thus causing carryover errors in the results of this type of test.

On the other hand, it was necessary to document the physicochemical properties of the plant in question, as this was the first characterization, which facilitates future reproduction and the determination of compatibility with other excipients for use in dermo-cosmetic formulations. **Table 2** shows the physicochemical analysis of the aqueous extract, with a pH of 5.61, indicating its acidic character in solution. As for the specific gravity, a value of 0.9977 ± 0.0018 is reported, which is very similar to that of water at 25°C. Conductivity is 61.2 ± 0.29 mV.

Metabolites found in other Thynanthus species have been associated with aphrodisiac and sensory-stimulation effects, including increased heart rate. However, these compounds have also been associated with a completely different effect in traditional use, such as relaxation and treatment of stomach problems, including stomach pain and diarrhea, according to user reports.

This difference lies primarily in the preparation method. The stimulating action is reported in hydroalcoholic extracts, while the relaxing action is seen in hot aqueous extraction. This difference may be due to the fact that no alkaloids appear in the aqueous phase, while they do in the ethanolic phase. It is known that alkaloids, in some cases, are also usually inactivated when heated, as is the case, for example, with Phaseolus species (Barrera et al., 2014; Maia Ladeira et al., 2025; Suruí, 2025).

Additionally, Table 2 shows how the amount of solids in the aqueous extract is relatively low due to the low conductivity (61.2 ± 0.29 mV) and a specific gravity (0.9977 ± 0.0018), close to that of water. Furthermore, the extract's acidic pH, close to 5.6, is unfavorable for the alkaloids, as they are predominantly present in their acid salt form (Maia Ladeira et al., 2025; Suruí, 2025).

As shown in Figure 4, the extracts tend to darken upon exposure to air, indicating an oxidation mechanism. It is postulated that the oxidized compounds are terpenes, which are abundant across all extraction phases (see Table 1). These metabolites have very diverse biological functions, but one of the most interesting is that they probably contain terpene derivatives: eugenol, cinnamaldehyde, cinnamic acid, and vanillin. These compounds would explain the common name popularly given to cinnamon vine (bejuco de canela) and clove (clavo), which are associated with relaxing, anxiolytic, and aphrodisiac actions. They would also explain the color change of the extracts from pale yellow to dark brown due to oxidation of the double bonds, greater conjugation, and the displacement of the visible spectrum by the pi of the double bonds (Suruí, 2025).

Figure 3 shows the UV - visible spectrum of the 10% m/m aqueous extract of T. croatianus stems, it can be concluded from the results that there is a great absorption in the ultraviolet range, discarding the UV-C spectrum range in which water can also absorb part of the radiation, the UV-B and UV-A ranges have a much higher absorbance than in the visible range beyond 400 Nm, this implies that most substances do not have extensive conjugation systems, that is, they should not be large molecules, nor with broad conjugation systems that allow greater absorption of the electromagnetic spectrum between 400 Nm to 700 Nm, this leads to believe that there must be sugars, and low molecular weight terpenes as the main metabolites, which coincides with the results in Table 1 where the presence of this type of compounds is determined, these types of compounds are usually photosensitive and react in the presence of ultraviolet radiation (Suruí, 2025). It is important to clarify that, for more structural information on the molecules present in the plant, a chromatographic analysis should be carried out in a second stage.

Finally, as shown in table 2, the aqueous extract contains a high content of degrees Brix, approximately 16.3 ± 1.15, which is easily correlated with what the table shows, since it shows the presence of sugars, despite the low concentration of solids, this implies a high content of sugars and, or derived compounds such as aldehydes and ketones, which, in addition to coinciding with the presence of terpenoid metabolites, allows them to be associated with the descriptions given by the informants who consume this plant, who refer to a sweet taste in both the aqueous and ethanolic extract.scribes the dissolved solids, reports a value of 16.3° ± 1.15° (Maia Ladeira et al., 2025; Suruí, 2025).

Conclusion

The study of the medicinal use of different plants was initially approached through the development of phytochemical profiles, which provided an overview of the presence of compounds of interest described in the literature with potential antioxidant and anti-inflammatory activity or with a possible adjuvant effect in pathologies such as diabetes. The results obtained showed the presence of flavonoids and terpenes in the evaluated phases. However, further studies are required to confirm the structural identity of the compounds preliminarily detected and to experimentally evaluate their biological activity in appropriate models.

Studies on *Tynanthus croatianus* are limited, limiting the availability of information and direct comparison with previous research.

The collaboration of a professional in biology, botany, or forestry is essential during fieldwork because it helps avoid confusion with other species and facilitates the efficient location of the plant.

This research constitutes the first study of T*ynanthus croatianus and provides* useful preliminary information on its phytochemical profile and physicochemical properties. These findings provide a foundation for future research focused on the biological and pharmacological characterization of the species.

Along with similar studies supporting the presence of secondary metabolites in species of the *Bignoniaceae* family, the development of in vitro and in vivo assays is recommended to systematically evaluate the plant's efficacy and safety.

Therefore, it is important to continue research on this species, given reports of other species in the genus, and to pursue a progressive approach to the scientific validation of its pharmacological value.

Declaration of competing interests

The authors have no material financial or non-financial interests to disclose.

Expression of Gratitude

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Author's statement

Jeniffer Sandí, German Madrigal, Brayan Murillo, and Gustavo Carazo participated in the study design, discussion of the results, and final writing of the manuscript. Alexander Altukhov carried out the experimental part and participated in the discussion. Jorge Rojas and Noelia Arce participated in the experimental phase and the discussion of the results.

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