Artículo original

Chemical Screening and Antioxidant Activity of Couepia subcordata Benth. (Guaijí) Harvested in the Ecuadorian Coast Region

Tamizaje químico y actividad antioxidante de *Couepia subcordata* Benth. (guaijí) cosechada en la Costa Ecuatoriana

Jaime Naranjo-Morán[1*] https://orcid.org/0000-0002-4410-9337

Jairo Jaime-Carvajal[1] https://orcid.org/0000-0001-6206-1181

John Contreras-Eras[1] https://orcid.org/0000-0001-6976-4584

Patricia Manzano-Santana[1] https://orcid.org/0000-0003-3189-6455

Milton Barcos-Arias[1] https://orcid.org/0000-0003-0863-6778

Juan Cevallos-Cevallos[1] https://orcid.org/0000-0003-4609-7998

[1]Escuela Superior Politécnica del Litoral, ESPOL. Centro de Investigaciones Biotecnológicas de Ecuador (CIBE), Guayaquil, Ecuador

[*]Corresponding autor: jaianara@espol.edu.ec

ABSTRACT

Introduction: Couepia subcordata Benth. (Chrysobalanaceae), is a fruit of Amazonian origin, established in the coastal region of Ecuador and consumed fresh or processed in rural areas. Although important compounds such as triterpenes and antioxidants have been studied in wild relatives of the genus Couepia sp., little is known about other metabolites contained in this fruit and their medicinal properties.

Objective: To characterize the volatile compounds, antioxidant capacity and the presence of ascorbic acid, coumarins, alkaloids, saponins, reducing sugars, phenols and flavonoids in Couepia subcordata.

Methods: About 50 mature fruits of C. subcordata were obtained in the city of Milagro and Naranjito, Ecuador. The pulp of each fruit was dried in an oven and subjected to alcoholic extractions and then analyzed for phytochemical profile by HPLC. Antioxidant capacity was determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method and volatile compounds were analyzed by solid-phase microextraction followed by gas chromatography coupled to mass spectrometry.

Results: The results showed the significant presence of alkaloids, coumarins, low molecular weight flavonoids, reducing carbohydrates, and phenolic compounds (pyrocatechol tannins), while the levels of precipitates, saponins, and flavonoids were not detectable. The antioxidant capacity of the dehydrated pulp of C. subcordata (Guaijí) was 7.899 umol TE 100 g-1.

Conclusions: Couepia subcordata fruits provided new data associated with high contents of coumarins and alkaloids linked to antitumor, anti-inflammatory, antiasthmatic and antioxidant activity. In addition, the fruit presented a richness of volatile compounds that can be used in the pharmaceutical and food industry. These results support efforts to characterize local fruits with potential to develop biodiversity valorization processes.

Keywords: Chrysobalanaceae; coumarins; alkaloids; anthocyanins; antioxidant; antitumor; anti-inflammatory; antiasthmatic.

RESUMEN

Introducción: *Couepia subcordata* Benth. (Chrysobalanaceae) es un fruto de origen amazónico establecido en la región costera de Ecuador y consumido fresco o procesado en zonas rurales. Aunque en parientes silvestres del género *Couepia* sp. se han estudiado compuestos importantes como triterpenos y antioxidantes, se conoce muy poco sobre otros metabolitos contenidos en esta fruta y sus propiedades medicinales.

Objetivo: Caracterizar los compuestos volátiles, la capacidad antioxidante y la presencia de ácido ascórbico, cumarinas, alcaloides, saponinas, azúcares reductores, fenoles y flavonoides en la especie *Couepia subcordata*.

Métodos: Alrededor de 50 frutos maduros de *C. subcordata* fueron obtenidos en las ciudades de Milagro y Naranjito, Ecuador. La pulpa de cada fruto fue secada en estufa y sometida a extracciones alcohólicas para luego analizar el perfil fitoquímico mediante HPLC. La capacidad antioxidante se determinó mediante el método de DPPH (1,1-difenil-2-picrilhidrazilo) y los compuestos volátiles se analizaron mediante microextracción de fase sólida seguida de cromatografía de gases acoplada a espectrometría de masas.

Resultados: Los resultados mostraron una presencia significativa de alcaloides, cumarinas, flavonoides de bajo peso molecular, hidratos de carbono reductores y compuestos fenólicos (taninos pirocatecólicos), mientras que los niveles de precipitados, saponinas y flavonoides fueron no detectables. La capacidad antioxidante de la pulpa deshidratada de *C. subcordata* (guaijí) fue de 7,899 umol TE 100 g-1.

Conclusiones: Los frutos de *Couepia subcordata* aportaron nuevos datos asociados con altos contenidos de cumarinas y alcaloides vinculados con la actividad antitumoral, antiinflamatoria, antiasmática y antioxidante. Además, la fruta presentó una riqueza de compuestos volátiles que pueden ser usados en la industria farmacéutica y la alimentaria. Estos resultados apoyan los esfuerzos por caracterizar las frutas locales con potencial para desarrollar procesos de valorización de la biodiversidad.

Palabras clave: Chrysobalanaceae, cumarinas, alcaloides, antocianinas, antioxidante, antitumoral, antiinflamatoria, antiasmática.

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Introduction

The Chrysobalanaceae family retains a 10.25 % richness in species studied of great importance in the Amazon region.[1] Between these, the members of the

genus *Couepia* are distributed in all the countries that share the Amazon basin.[2] These plant species are vitally relevant to humans and some endemic animals such as several species of monkeys (*Cebus apella* and *Pithecia monachus*).[3]The human being fancy of several species of this family, for example, *Couepia edulis* is consumed by its almond.[4] While, *Couepia grandiflora* is used for its cytotoxic, antioxidant and antibacterial activity.[5] Alternatively, the pulp of *Couepia bracteosa* contains carotenoids of type all-trans-Neocromo (17 %) and all-trans-ß-carotene (16 %).[6] On the other hand, *Couepia subcordata* with food and ornamental feature is very attractive in patios and gardens so genetically has a pattern of human dispersion.[7]

In the Ecuadorian coast, *Couepia subcordata* flourishes in the months of july and august and is harvested in the months of october and november, producing fruits of which are made milk drinks with a regular acceptance.[8,9] The mestizo and indigenous communities have used this species in a long way with timber, medicinal and nutritional purpose, however, few scientific evidences are made of such ethnobotanical uses. On the other hand, nutritional and food information (e. G. Brix, pH, total and soluble pulp solids) of this species is still limited.[10] For this reason, the objective of this work was to carry out a chemical screening and antioxidant activity of the dehydrated pulp of *Couepia subcordata* to improve the characterization of the fruits of this species as a previous step towards the development of process of valorization of this species.

Methods

The fruits of *Couepia subcordata* were obtained from trees in the cantons Naranjito (2 ° 9'41.19 "S and 79 ° 27'39.07" W) and Milagro (2 ° 8'8.22 "S and 79 ° 30'8.94" W) in the province of Guayas - Ecuador, harvested in the month of October 2018, (Fig. 1).

Fig. 1. Fruits of *Couepia subcordata* harvested from trees of the cantons Naranjito and Milagro, Ecuador.

The families of compounds were determined by alcoholic, ethereal and aqueous extracts of dehydrated fruit. The analyses were carried out in the Bioproducts Laboratory of the Center for Biotechnological Research of Ecuador of the Polytechnic High School of the Littoral. The material was dried in stove 60 ° C for 8 hours with air recirculation. Subsequently, the samples were crushed in a domestic blender to obtain a coarse powder with particles of < 2 mm in diameter.[11]

On the other hand, the extracts obtained alcoholic, ethereal and aqueous, were made with solvents that has increased polarity to achieve greater depletion of the dry sample. The determination of secondary metabolites, in accordance with the solubility in each of the used solvents were determined by the methods of; Baljet (metabolite 1), Dragendorf (metabolite 2), resins (metabolite 3), Mayer (metabolite 4), Wagner (metabolite 5), foam (metabolite 6), Fehling (metabolite 7), ferric chloride (metabolite 8) and Shinoda (metabolite 9) according to the protocols of *Lal* et al.[12]

Fifty milligrams of sample were mixed with 1 ml of methanol at 80 %. Subsequently, the mixture was incubated in an ultrasound bath for 30 min, then it was centrifuged at 12000 rpm for 10 min. Then, 200 ul of the supernatant was collected and 32 ml of DPPH was added. After incubating for 30 minutes of reaction, the absorbance was measured at 517 nm. The results were expressed in umol TE 100 g^{-1} sample.[13]

Samples were prepared as previously indicated until coarse powder with < 2 mm diameter particles were obtained. Subsequently, 5 g of samples were placed in flask of 125 ml and was added 50 ml of buffer containing sodium acetate in doubly distilled water at a concentration of 0.05 M and adjusted with acetic acid at a pH of 5.2. The mixture was then mixed for 30 min at 60 ˚ C in a dark bath and stirred every 5 min. The content was filtered through paper Whatman N ˚ 3 to obtain a volume of 50 ml. The sample was filtered once more using a 0.22 µm and 13 mm nylon filter.

The filtered sample was analyzed by liquid chromatography with a 254 nm detector, which had an allast C18 5Micron column, of 250 nm x 4.6. In addition, a 10 ul injection was made with 1 mm/min flow and 254 nm detector; UFS = 0.04. The standard of reference used was 30 mg of ascorbic acid in 30 ml of buffer, later this solution was dissolved 10 ul of the previous solution in 1 ml of the buffer, taking as reference the area obtained with the injection of 10 ul of the standard pattern.[14]

Results

Chemical screening tests demonstrated the presence of secondary metabolites, mainly coumarins, alkaloids, triterpenes, reducing sugars, pyrocatecholic tannins and anthocyanidins observed in table 1.

Table 1. Secondary metabolites the ethereal, alcoholic and aqueous extracts of the dehydrated fruit of *Couepia subcordata*

Metabolites*	Results	Observation
Coumarins	++	Positive (precipitated)
Alkaloids	+	Ethanolic extract
Precipitate	-	Negative
Alkaloids	+++	Ethanolic extract
Alkaloids	++	Ethanolic extract
Saponins	-	Negative
Reducing sugars	+++	Red precipitate

Volatile compounds	+++	Positive (yellow-greenish)
Phenolic compounds	Intense green	Pirocatecólicos tannins
Flavonoids	-	Negative
Flavonoids group sequence C6-C3-C6 structures	+++	Positive
High-mass-index hydrophilic colloid	+++	Positive

* Specifications with a sample amount of 15.0 g and an extraction volume of 30 ml. Qualitative content of secondary metabolites presents in aqueous extracts: high intensity of coloration caused by metabolite = +++, intermediate intensity coloration = ++, slight intensity coloration = +, and absence of metabolite = -.

In the analysis of the antioxidant capacity and vitamin C produced in the pulp of this fruit, there was a concentration of 7.899 µmol TE 100 g^{-1} of antioxidant capacity and 0.13 mg 100 g of vitamin C, these concentrations are not representative in comparison with standard fruits of higher content such as apple, mango, and others. The volatile compounds resulting from the ripe fruit (fifth ripening stage) were aldehydes, acetones, terpenes among other characteristics of fruity and floral aromas, (Table 2).

Table 2. Volatile metabolites and their aroma descriptor observed in alcoholic extracts of *Couepia subcordata* dehydrated fruit

Metabolites volatiles *	**Aroma descriptor**
Ethyl acetate	Pineapple
2 - Hexanal (E)	Lawn
2-Butanona	Fruity
3 - Hexanal	Lawn
2 - Hexanal	Green apple
(2S) -2-methyl-ethyl-ester-butanoic acid	Apple
2 - Hexen -1 -ol	Fruity to wine
2 ethyl-3 vinyloxirane	N/A
Propanic acid 2 methyl ethyl ester	Spicy, rancid
4 methyl, 2 pentanal	Lawn
Ethyl hexanoate	Fruity
High-mass-index hydrophilic colloid	+++
α - Pineno	Pine
Delta -3-carene	Citrus
Ocimens (beta and delta)	Grass

Santolina triene	N/A
Nona -3-5 dien -2 ol	Cucumber

Discussion

The phytochemical screening of the fruit presented secondary metabolites of interest, however, in none of the samples evaluated were resins, saponins or flavonoids. The results previously reported differ from those analyzed in *Couepia paraensis* in which the methanolic extract contained three flavonoids that cause the inhibition of the enzyme glucose-6-phosphatase responsible for controlling blood glucose levels.[15] In particular, the species *Exellodendron coriaceum* belonging to the same family also possesses three flavonoids with activity hypoglycemic.[16] The pulp of *Couepia subcordata* it also contained simple phenolic compounds, like the stems of *Couepia ulei* that induced the quinone reductase activity.[17] It has also been detected triterpenoids inhibitors of the activity lyase DNA polymerase beta *Couepia polyandra*.[18] In addition, the results show the presence of flavonoids compounds low molecular weight (C6-C3-C6) with antioxidant properties that allow multiple patterns of substitution in the ring C, that is to say, anthocyanidins that have joined the group-OH in the position 3 but with double bond of carbon (C3 and C4) in ring C.[19] The presence of a polysaccharide capable of forming hydrophilic colloids that increased the density of the extract was also detected.

The antioxidant capacity and vitamin C content of the pulp of *Couepia subcordata* showed insignificant values in comparison to that reported in *Couepia bracteosa* having greater antioxidant capacity in its shell.[20] Likewise, other families of plants with great antioxidant and antitumor potential have been reported in the Amazon, such as *Copaifera multijuga* and *Virola venosa.*[21,22] On the other hand, the content of ascorbic acid of the pulp of *Couepia subcordata* turned out to be very negligible compared to reference fruits in ascorbic acid such as citrus fruits.[23]

The results showed the presence of secondary metabolites of interest in the pulp of *Couepia subcordata*, as well as a low concentration of antioxidant capacity and vitamin C. However, more studies are required to characterize all the metabolites provided in this species.

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Conflicto de intereses

Los autores declaramos que el presente trabajo no posee conflicto de intereses en relación con la investigación realizada.

Contribución de los autores

Jaime Naranjo Morán: Recolección de frutas y elaboró el manuscrito.

Jairo Jaime Carvajal: Realización del tamizaje químico.

John Contreras Eras: Realización de la actividad antioxidante.

Patricia Manzano Santana: Realización del análisis de vitamina C.

Milton Barcos Arias: Realización de la selección de muestras en estudio.

Juan Cevallos Cevallos: Revisión del contenido científico del manuscrito.