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Natural Products

Preliminary phytochemical characterization of the ethanolic extracts of leaf, green and ripe fruit of *Terminalia catappa L.* (Almendro) in Panama

Caracterización fitoquímica preliminar de los extractos etanólicos de hoja, fruto verde y maduro de *Terminalia catappa L.* (Almendro) en Panamá

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Abstract

The almond tree (*Terminalia catappa L.*) in Panama is for timber, nutritional, and medicinal use, among others. Little is known about the secondary compounds present in the different parts of this plant species. This work aimed to carry out the phytochemical characterization of the green leaf (GL), green fruit (GF) and ripe fruit (RF) of the almond tree. The sampling was carried out between May to June 2017. The GL, GF and RF of the almond tree were placed separately, in direct extraction treatment with 95% ethanol for one day. The ethanolic extract was obtained by gravity filtration and concentrated in a vacuum rotary evaporator (BUCHI Rotavapor^{TM} R-210). Phytochemical screening was performed to determine the three main groups of secondary compounds (phenolics, nitrogenous and terpenes). An Infrared Spectroscopy (FTIR) and Ultraviolet-Visible (UV) Spectroscopy analysis were performed to find out which functional groups were present. Past 4.02 program was used to generate a graph of relative abundance of the secondary compounds present, and Whittaker index was applied to evaluate the percentage of difference in the phytochemical composition of the plant's parts. With these data, it was able to identify if the semi-quantitative phytochemical characterization is variable for each part, with nitrogen compounds (alkaloids) and phenolic compounds (tannins, flavonoids) presenting greater abundance. No presence of cardiotonic glycosides was found, or gums and mucilage. This information indicates that *T. catappa L.* is a potential resource for health, being of great ethnobotanical, pharmacological value and for the food industry in Panama.

Keywords: Secondary compounds, ethnobotany, bioactive functions, medicinal plant, natural products, *Terminalia catappa*.

Resumen

El almendro (*Terminalia catappa L.*) en Panamá es de uso maderable, nutricional, medicinal, entre otros. Se sabe poco sobre los compuestos secundarios presentes en las diferentes partes de esta especie vegetal. Este trabajo tuvo como objetivo realizar la caracterización fitoquímica de la hoja verde (HN), fruto verde (FV) y maduro (FM) del almendro. El muestreo se llevó a cabo entre los meses de mayo a junio del 2017. Se colocaron las HN, FV y FM del almendro por separado en tratamiento de extracción directa con etanol al 95% durante un día. El extracto etanólico fue obtenido por filtrado de gravedad y concentrado en un rotavapor al vacío (BUCHI Rotavapor^{TM} R-210). Se realizó un tamizaje fitoquímico para determinar los tres principales grupos de compuestos secundarios (compuestos fenólicos, compuestos nitrogenados y terpenos). También se realizó un análisis de Espectroscopía Infrarroja (FTIR) y Espectroscopía Ultravioleta-Visible (UV) para saber cuáles eran los grupos funcionales presentes. Se utilizó Past 4.02 para generar una gráfica de abundancia relativa de los compuestos secundarios presentes, y se aplicó el índice de Whittaker para evaluar el porcentaje de diferencia en la composición fitoquímica de las diferentes partes vegetales. Con estos datos se logró identificar que la caracterización fitoquímica semicuantitativa es variable para cada parte, presentando mayor abundancia de compuestos nitrogenados (alcaloides) y compuestos fenólicos (taninos, flavonoides). No se observó presencia de glicósidos cardiotónicos, ni de gomas y mucilagos. Esta información resalta que *T. catappa L.* es un potencial recurso para la salud, siendo de gran valor etnobotánico, farmacológico y para la industria alimenticia en Panamá.

Palabras clave: Etnobotánica, funciones bioactivas, metabolitos secundarios, planta medicinal, productos naturales, *Terminalia catappa.*

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1 Introduction

Panama is one of the countries with the greatest biological diversity worldwide (Olmedo et al., 2018; López and Mainieri, 2019), with 8 of the 200 recognized eco-regions around the world with 21 times more plant species per km^2 than Brazil (ANAM, 2010). About 10000 species of vascular plants have been described in the country (Correa et al., 2004), but this list has increased in recent years with reports of described species and new distribution ranges in Panama (Flores et al., 2016, 2017, 2018; Ortiz et al., 2019).

The Panamanian flora presents reservoirs of natural products with great value for bioprospecting new therapeutic treatments with high economic and medicinal potential (Gupta, 2004, 2008; Leija et al., 2014). Therefore, it is important and necessary to increase further chemical, biological and toxicological studies of the country's plants.

One of the first works on ethnobotany in Panama was conducted by Esposito et al. (1985), including the first phytochemical characterizations of leaves and stem in river almond (*Terminalia catappa L.*), finding the presence of tannins. Muhammad and Mudi (2011); Mena et al. (2015) mentioned the importance in traditional and medicinal uses (e.g., tonic-astringent properties), in countries such as Costa Rica, Colombia and others. In Panama, locals of Los Santos report their use as a medicinal plant (Torres et al., 2017). This species is native to Southeast Asia and was introduced in Panama (Correa et al., 2010; Farnum and Murillo, 2015; Farnum and Yángüez, 2015; Jiménez and Espino, 2020).

Secondary compounds (e.g., triterpenic acids, anti-inflammatory) of ethanolic extracts of *T. catappa* leaf have been reported (Fan et al., 2004). Some flavone glycosides [6-C-(2''-O-galloyl)-b-D-glucopyranoside] have also been isolated from ethanolic extracts of the dry leaf with bioactive antioxidant functions (Lin et al., 2000). Aphrodisiac functions have been reported to the seed, with the presence of alkaloids, oils, amino acids and peptides (Ratnasooriya and Dharmasiri, 2000). Similarly, it has been observed that dry leaf extract inhibits the growth of *Bacillus subtilis*, a soil bacterium that can act as a biofungicide (Lahlali et al., 2013), and *Staphylococcus aureus*, a bacterium in our body that could cause infections if not controlled (Lowy, 1998).

Hepatoprotective bioactive functions against acute liver damage induced by carbon tetrachloride (CCl_4) and D-galactosamine, hepatocyte injury induced by (D-Galn) have also been determined from chloroform extracts of leaves. This active function is related to the presence of triterpenes (Gao et al., 2004). Researchers have established that the study of the different parts of T. catappa L. is pivotal for bioprospecting and creating possible drugs for diseases (Muhammad and Mudi, 2011; Chanda et al., 2013; Jiménez and Rebolledo, 2015; Calderón et al., 2013).

There are few studies carried out in Panama on *T. catappa L.* and upon analyzing the potential of this species for future studies focused on bioprospecting secondary bioactive compounds, the aim of this research is to conduct the first preliminary characterization of the ethanolic extracts of new leaves, green and mature fruits in *T. cattappa L.* in Panama.

2 Materials and Methods

2.1 Collection Area

Vegetable parts of *T. catappa* were collected in the community of Bella Vista, Limones Community, Punta Burica, Panama (8° 2′7.15"N, 82°53′22.38"W) (Figure 1). This community separates the Pacific coast of Panama with Costa Rica. The human population living in this area has fragmented forests for socio-economic activities, such as grasslands and oil palm monoculture (Miranda, 2013). The area has a humid tropical climate, with an annual rainfall of 4247.8 mm and an annual average temperature of 26.4-27°C. According to Holdridge, the original dominant vegetation in the region is the Tropical Wet Forest (Miranda, 2013).

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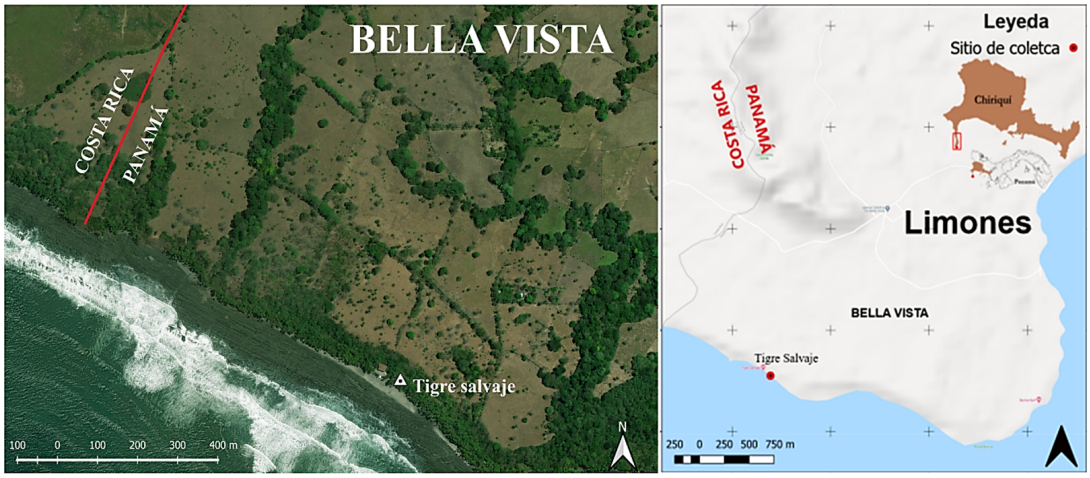

Figure 1. *T. catappa L.* collection area.

2.2 Plant Material Collection and Preparation

Samples were collected during dry to rainy transition (May-June, 2017). New leaves (GL) were collected, which were closest to the base of the bud and are green, and differ from dried ones because they change from red-orange to brown. Samples of green fruits (GF) and ripened fruits (RP) were also collected. GF is green and RF is yellow, and are qualitatively differentiated (Figure 2). The different plant parts were properly pressed and taken to the Herbarium of the University of Autonomous Chiriquí (UNACHI), where they were subsequently identified by botanical specialists.

Direct treatment of almond GL, GF and RF was performed, which consisted of placing each part separately in 95% of ethanol for one day. The ethanolic extract of these parts was obtained by gravity filtering. A filter paper was placed in a funnel to separate the solid part of the liquid. This extract was then concentrated in a vacuum rotavapor (BUCHI Rotavapor^{TM} R-210) for further analysis (Hostettmann et al., 2008).

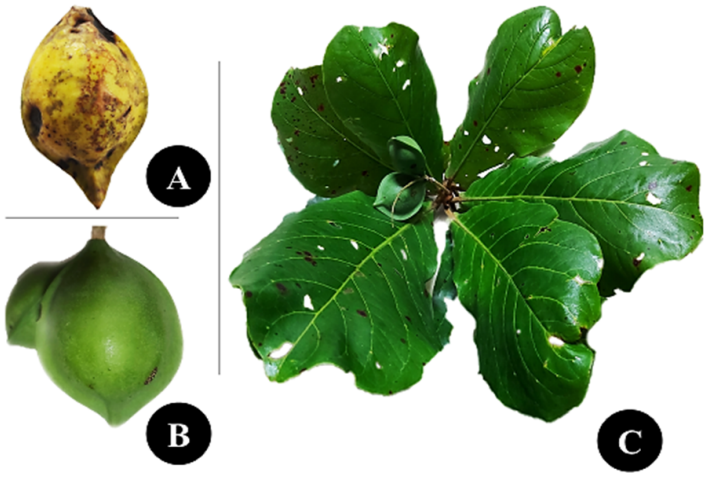

Figure 2. Photographic illustration of the vegetable parts of the almond tree (*T. catappa L.*) A. Ripened fruit, B. Green fruit, C. New leaf and green fruit.

2.3 Phytochemical characterization

Different qualitative and semi-quantitative tests were performed to determine the three large groups of secondary compounds (phenolic compounds, nitrogenous compounds and terpenes). Qualitative signals were identified by precipitates, color change, and foam formation, when the reagent corresponding to the type of metabolite intended to be characterized was added. The semiquantitative ones were evaluated as follows: Absence (–), mild presence (+), mean presence (++), abundant presence (+++), according to the intensity of the qualitative signals mentioned above. Triplicates were performed for each test with reagent and extract control samples, avoiding related false positives.

Nitrogen compounds (alkaloids) were characterized with Draguendorff, Mayer, Wagner and Hager. Each subgroup of terpenes was characterized with the corresponding reagent, terpenoids with Rosenthaler, phytosterols with Liebermann-Buchard and triterpenes with Salkowski. Ferric chloride at 5% ($FeCl_3$) was used in the identification of tannins; flavonoids were characterized with concentrated H_2SO_4 and 5% NaOH. For the identification of saponins, an aqueous solution of the extracts was prepared and agitated, and the formation of foam indicates their presence. The identification of cardiotonic glycosides was done with the Legal test. For the identification of gums and mucilage, 2 mL of the extract was dissolved in 2.5 mL of distilled water, and 5 mL of 95% alcohol was added with constant agitation (Rocha de Albuquerque, 2000; Hostettmann et al., 2008).

Infrared Spectroscopy (FTIR) and UV-Visible Spectroscopy (UV) analysis were performed on the raw extract of each part to determine which functional groups were present and are consistent with the base structures of the screening-detected compounds (Hostettmann et al., 2008).

2.4 Data analysis

The Past 4.02 program was used to graph the relative abundance of the secondary compounds present in each of the extracts of the studied plant parts. The Whittaker index was applied to assess the percentage difference in the phytochemical composition in different plant parts. This analysis was assessed for the presence (1) and absence (0) of the secondary compound groups (Whittaker, 1960).

3 Results and Discussion

This study extends the preliminary phytochemical information of *T. catappa L.* in Panama, based on the qualitative phytochemical characterization of the different parts of *T. catappa L.* It was possible to identify the three large groups of secondary compounds in the plant, presenting similarities in the composition of secondary compounds. The semiquantitative characterization or abundance of these compounds changes in each part, with greater abundance of nitrogen compounds (alkaloids) and phenolic compounds (tannins, flavonoids).

The presence of terpenoids was detected, but in very low abundance, and absence of phytosterols was observed. There was also no presence of cardiotonic glycosides, or gums and mucilage (Table 1, Figure 2). This preliminary characterization is similar to other reports. The abundant presence of phenolic compounds or polyphenolic acids is a characteristic of leaves of this plant (Tanaka et al., 1986), such as gallic acid, which is considered to be one of the most abundant (Marrero and Morales, 2016).

Table 1. Qualitative and semi-quantitative characterization of the different parts of *T. catappa L.*

Secondary compounds	New leaf	Ripened fruit	Green fruit
Alkaloids	+++	+	+
Phytosterols	-	-	-
Terpenoids	+	+	-
Tanins	+++	+	++
Flavonoids	+	++	++
Carbohydrates	+	+++	+
Saponins	+++	+++	-
Gums and Mucilages	-	-	-
Cardiotonic Glycosides	-	-	+

Legend: absence (–), mild presence (+), mean presence (++), abundant presence (+++).

FTIR signals for the extract of almond GF (*T. catappa L.*) were aromatic compounds (1770-2010 cm^{-1}), OH of phenolic compounds (1066 cm^{-1}), and C=O-C of aromatic ethers (1229 cm^{-1}), indicating a possible presence of polyphenolic acid groups, gallic tannins, saponins and flavonoids. As for GL, these show C-H (2946 cm^{-1}) and C=H (1662 cm^{-1}) pressures, which could be possible indicators of terpenoid chains or phytosterols. Similarly, RF and GF show harmonic signals, which indicate aromatic compounds (1770-2010 cm^{-1}) along with OH signals, which indicate possible phenolic compounds.

In UV spectroscopy of the extracts of GL and GF of the almond (*T. catappa L.*) peaks of 255nm, 294nm, 377nm were obtained, which represent aromatic, nitrogenous compounds that could be tannins, alkaloids and flavonoids; saponins are found at wavelengths of 666 and 662 nm, because of their complex skeletal structures.

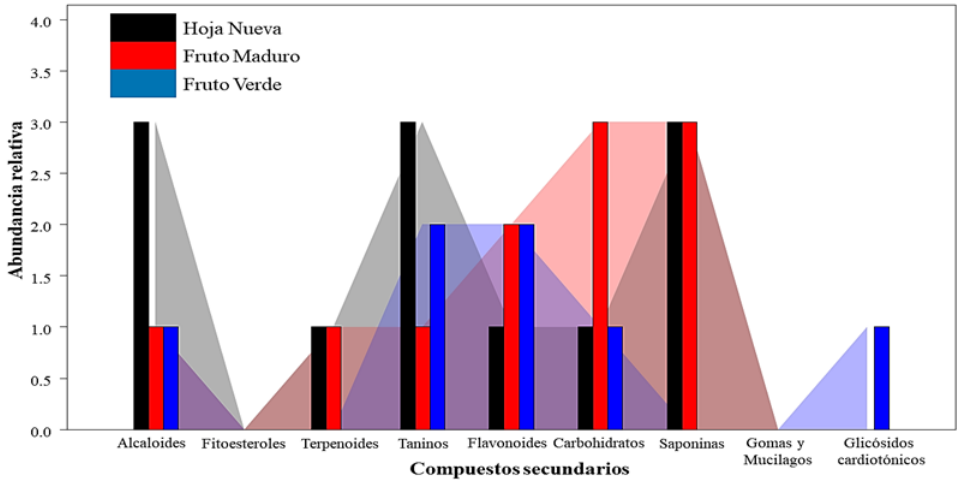

Figure 3. Relative abundance with respect to the semi-quantitative characterization of secondary compounds.

Research confirms that the abundance of polyphenolic compounds is related to bioactive functions, such as antioxidants of great interest at the pharmacogenetic level (Tanaka et al., 1986; Masuda et al., 1999; Lin et al., 2001; Marques et al., 2012). One of the studies that test the possible effectiveness of phenolic compounds (Punicalagin) as antioxidants is the one presented by Chen et al. (2000), where Pu-

nicalagin suppresses the generation of bleomycin-induced intracellular free radicals, identified as superoxide and hydrogen peroxides, avoiding genotoxicity. It has also been reported that punicalagin and punicalin have antihepatotoxic activity on the toxicity induced by acetaminophen (Paracetamol) in rats' liver (Lin et al., 2001).

The Whittaker index only showed a 23.53 % difference between the parts of the plants. GL with RF presented the same groups of secondary compounds. GF with respect to GL and RF presented 27.27 % difference in phytochemical composition. The green fruit presented five groups of secondary compounds, comprising nitrogen compounds (alkaloids) and phenolic compounds (tannins and flavonoids). There was no presence of terpenoids. In RF, six groups belonging to the three major groups were identified. GF presented cardiotonic glycosides with greater abundance of tannins; however, ripened fruit presented terpenoids, with high abundance of carbohydrates and low tannins.

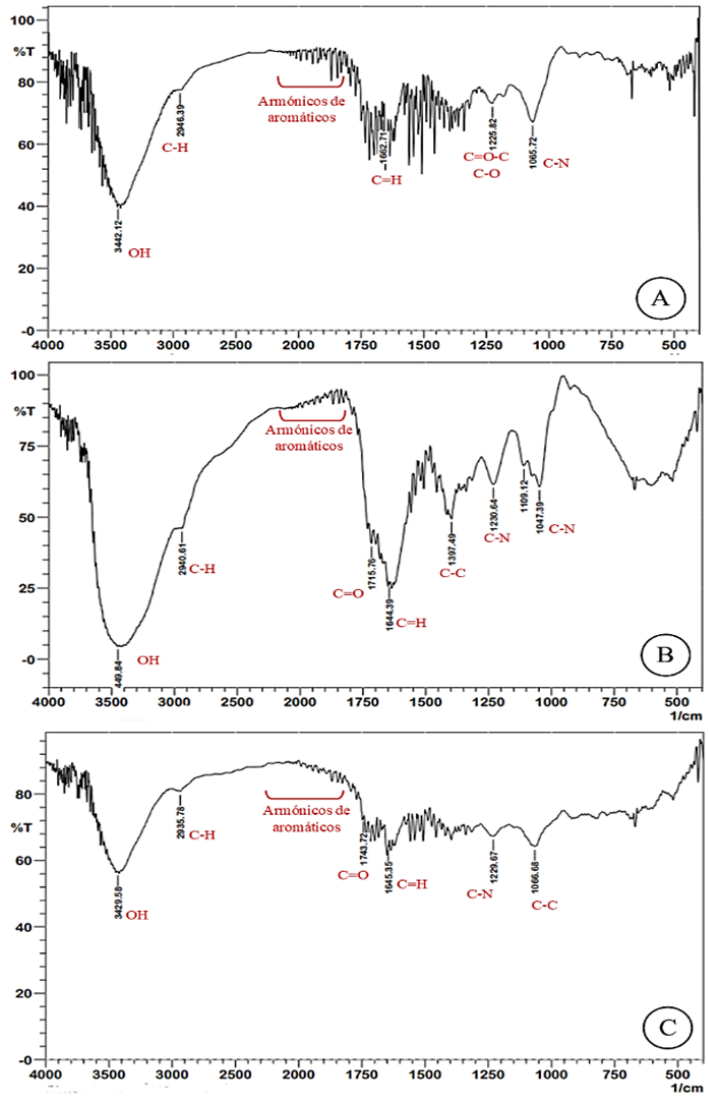

Figure 4. FTIR of the different plant parts of *T. catappa L.* A. New leaf B. Ripened fruit C. Green fruit.

Variations in fruit are common in maturation processes where higher carbohydrate or sugar content is produced; there is also an increase in the production of volatile or aromatic compounds (terpenoids) and a decrease in others, in this case tannins (Arrieta et al., 2006). The presence of flavonoids is also important as they give color to the vegetable parts and indicate maturation. In the case of almond fruit, it decreases the green color (chlorophyll) and increases the yellow color associated with a type of flavonoid (anthocyanin) (Ronald, 2011).

These variations agree with what was reported above, where a considerable increase in carbohydrates is observed in the mature state, being one of the predominant compounds, followed by terpenoids and phenolic compounds (Ratnasooriya and Dharmasiri, 2000; Santos et al., 2016). These carbohydrate values have shown that almond fruit has a high energy value; similarly, they have been reported to provide basic substances for the growth and maintenance of body functions (Lima, 2012). At present, it is related with the presence of saponins. Therefore, they have been used in various products as raw material in the enrichment of existing formulations in the food industry (Nagappa et al., 2003; Lima, 2012; Chanda et al., 2013).

4 Conclusions

In this study, the first phytochemical composition was achieved of the ethanolic extracts of green leaves, green fruits and ripened fruits of *T. catappa L.* in Panama. The semi-quantitative characterization or abundance of phytochemical compounds changes for each part, and nitrogen compounds (alkaloids) and phenolic compounds (tannins, flavonoids) were observed with greater abundance. Low abundance of terpenoids and absence of phytosterols, cardiotonic glycosides, gums and mucilage were detected.

Considering the phytochemical composition of *T. catappa L.* reported in other studies and its similarity with what was found in this work, it can be considered as a potential resource for the health, since it has a great ethnobotanic and pharmacological value in the food industry of Panama.

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