



Bioactive compounds and antioxidant activity of *Annona muricata* L. fruits located in Manabí, Ecuador

Compuestos bioactivos y actividad antioxidante de frutos de *Annona muricata* L. localizados en Manabí, Ecuador

Compostos bioactivos e actividade antioxidante de frutos de *Annona muricata* L. localizada em Manabí, Equador

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Rev. Fac. Agron. (LUZ). 2024, 41(1): e244107

ISSN 2477-9407

DOI: <https://doi.org/10.47280/RevFacAgron.v41.n1.07>

Food technology

Associate editor: Dra. Gretty R. Ettiene Rojas

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Received: 13-10-2023

Accepted: 17-01-2024

Published: 27-02-2024

Keywords:

Soursop pulp

Polyphenols

Antioxidant activity

Acetogenins

Abstract

The mesoendocarp of *Annona muricata* L. (Annonaceae) has essential minerals, ascorbic acid, dietary fiber, polyphenols, and acetogenins in its composition, which evidences the nutraceutical benefits of this fruit tree. This study aimed to determine the bioactive compounds and antioxidant activity of *A. muricata* fruits located in Manabí, Ecuador. The content of vitamin C, dietary fiber, minerals, polyphenols, flavonoids, antioxidant activity, and the presence of acetogenins were determined. It was observed that the minerals potassium, calcium, sodium, zinc, magnesium, and iron are the major components present in soursop pulp ash. Likewise, relevant content of vitamin C (26.64 mg AA.100 g⁻¹ pulp), total polyphenols (398.79 mg EAG.100 g⁻¹ pulp), flavonoids (192.20 mg EQ.100 g⁻¹ pulp), and high antioxidant activity (318.90 mmol equivalents of trolox.100 g⁻¹ pulp) were evidenced in the fruits of *A. muricata*. The ethanolic extracts obtained from the fruit when applying the Kedde test, presented a pink ring, which is an unequivocal indicator of the presence of acetogenins, which have a proven antineoplastic effect. The mesoendocarp of *A. muricata* located in Manabí, Ecuador has an important content of bioactive compounds: essential minerals (K, Ca, Zn, Mg), vitamin C, polyphenols, presence of acetogenins and high antioxidant activity, properties that reduce the risk of degenerative diseases and cellular aging. Therefore, its consumption is recommended in the daily diet as fresh and processed fruit because it constitutes a valuable nutritional and therapeutic alternative.

Resumen

El mesoendocarpio de *Annona muricata* L. (Annonaceae) presenta en su composición minerales esenciales, ácido ascórbico, fibra dietaria, polifenoles y acetogeninas, lo cual evidencia los beneficios nutracéuticos de este frutal. El objeto de este estudio fue determinar los compuestos bioactivos y la actividad antioxidante de frutos de *A. muricata* localizados en Manabí, Ecuador. Se determinó el contenido de vitamina C, fibra dietaria, minerales, polifenoles, flavonoides, actividad antioxidante y se identificó la presencia de acetogeninas. Se observó que los minerales potasio, calcio, sodio, zinc, magnesio y hierro son los componentes mayoritarios presentes en la ceniza de la pulpa de guanábana. Así mismo, se evidenció un contenido relevante de vitamina C (26,64 mg AA.100 g⁻¹ pulpa), polifenoles totales (398,79 mg EAG.100 g⁻¹ pulpa), flavonoides (192,20 mg EQ.100 g⁻¹ pulpa) y alta actividad antioxidante (318,90 mmoles equivalentes de trolox.100 g⁻¹ pulpa) en los frutos de *A. muricata*. Los extractos etanólicos obtenidos del fruto al aplicar la prueba de Kedde, presentaron un anillo de color rosado, lo cual es un indicador inequívoco de la presencia de acetogeninas, las cuales tienen efecto antineoplásico demostrado. El mesoendocarpio de *A. muricata* localizados en Manabí, Ecuador tiene un importante contenido de compuestos bioactivos: minerales esenciales (K, Ca, Zn, Mg), vitamina C, polifenoles, presencia de acetogeninas y una alta actividad antioxidante, propiedades que disminuyen el riesgo de padecer enfermedades degenerativas y el envejecimiento celular. Por lo tanto, se recomienda su consumo en la dieta diaria como fruta fresca y procesada, debido a que constituye una valiosa alternativa nutricional y terapéutica.

Palabras clave: pulpa de guanábana, polyphenols, actividad antioxidante, acetogeninas.

Resumo

O mesoendocarro da *Annona muricata* L. (Annonaceae) tem na sua composição minerais essenciais, ácido ascórbico, fibra alimentar, polifenóis e acetogeninas, o que comprova os benefícios nutracêuticos desta árvore de fruto. O objetivo do estudo foi determinar os compostos bioativos e a atividade antioxidante dos frutos de *A. muricata* localizados em Manabí, Equador. Foi determinado o teor de vitamina C, fibra alimentar, minerais, polifenóis, flavonóides, atividade antioxidante e a presença de acetogeninas. Observou-se que os minerais potássio, cálcio, sódio, zinco, magnésio e ferro são os principais componentes presentes nas cinzas da polpa da graviola. Da mesma forma, foi evidenciado um relevantes teor de vitamina C (26,64 mg AA.100 g⁻¹ de polpa), polifenóis totais (398,79 mg EAG.100 g⁻¹ de polpa), flavonóides (192,20 mg EQ.100 g⁻¹ de polpa) e uma elevada atividade antioxidante (318,90 mmol equivalentes de trolox.100 g⁻¹ de polpa) nos frutos de *A. muricata*. Os extractos etanólicos obtidos do fruto após o teste de Kedde apresentam um anel cor-de-rosa, que é um indicador inequívoco da presença de acetogeninas, que têm um efeito antineoplásico comprovado. O mesoendocarro de *A. muricata* localizado em Manabí, Equador, possui um importante conteúdo de compostos bioativos: minerais essenciais (K, Ca, Zn, Mg), vitamina C, polifenóis, presença de acetogeninas e uma elevada atividade antioxidante, propriedades que reduzem o risco de doenças degenerativas e o envelhecimento celular. Por isso, recomenda-se o seu consumo na dieta diária como fruta fresca e processada, pois é uma alternativa nutricional e terapêutica valiosa.

Palavras-chave: polpa de graviola, polifenóis, atividade antioxidante, acetogeninas.

Introduction

Annona muricata L. (Annonaceae) is a fleshy, juicy fruit that is consumed as a “fresh fruit” and also processed in nectars, ice cream, yogurt, and other types of foods. It has been reported that the mesoendocarp of soursop presents an appreciable content of bioactive compounds: essential minerals, ascorbic acid, dietary fiber (Badrie and Schauss, 2010; Coria-Téllez *et al.*, 2018), and high polyphenol content (Jiménez *et al.*, 2014; Jiménez-Zurita *et al.*, 2017; Vit *et al.*, 2014), which have therapeutic properties: antimicrobial, antiparasitic, anxiolytic, antiulcer, hepatoprotective and as a hypoglycemic agent (Chan *et al.*, 2016; Coria-Téllez *et al.*, 2018; Jiménez-Zurita *et al.*, 2017).

Flavonoids extracted from different vegetative and reproductive organs of the soursop fruit have been evaluated in cell models exposed to different oxidative stimuli to inhibit lipid peroxidation damage (Coria-Téllez *et al.*, 2018).

On the other hand, acetogenins a bioactive compound present in *Annona muricata* leaves and fruits, have been described to have a selective anticancer effect on tumor cells, such as, breast carcinoma, colon, liver, prostatic hyperplasia, and even lymphoma (Hernández-Fuentes *et al.*, 2019; Liaw *et al.*, 2016; Ogbu *et al.*, 2020; Paul *et al.*, 2013).

The above shows the multiple functional properties (nutritional and therapeutic) of the soursop fruit, which raises the alternative of taking advantage of its great biological value (high content of vitamin C, essential minerals, phenolic compounds, and acetogenins). In addition, consumers worldwide have a growing and preferential demand for tropical fruits, since they have components with biological activity that inhibit the oxidation of free radicals, a process that has been associated with the suffering of degenerative diseases and cellular aging (Chan *et al.*, 2016). Therefore, the objective of this research is to determine the bioactive compounds and antioxidant activity of *A. muricata* fruits located in Manabí, Ecuador, which constitutes a contribution to promote the production of this promising fruit tree on a large scale in Manabí, Ecuador, and to stimulate its consumption as a fresh and/or processed fruit worldwide.

Materials and methods

Fruit selection

A total of 20 healthy plants of *Annona muricata* L., located at 0°41'53.5" south latitude 80°5.617' W" west longitude at 14 m a.s.l. in the Chone Canton, Manabí, Ecuador, were selected. Three (3) soursops per plant were randomly harvested under conditions of physiological maturity, packed in 10 kg bags, and transferred to the Bromatology Laboratory, Department of Agroindustrial Processes, Faculty of Agrosciences, Universidad Técnica de Manabí, Ecuador.

Subsequently, the harvested fruits were washed with water at 25 °C (removal of soil and leaf remains) and immersed in ETHREL® (2 mL.L⁻¹) for 10 min to uniformize maturity, using the method described by Fuenmayor *et al.* (2016). Subsequently, the epicarp was separated from the mesoendocarp of the ripe fruits and stored in a refrigerator (Top Mount Croma | ri-480, Indurama, Ecuador) at 8°C/6 h, to maintain consistency and preserve its conservation.

Obtaining soursop pulp concentrate

The pulp obtained (mesoendocarp) previously refrigerated was placed in properly sterilized plastic containers (approximately 3 kg per container), crushed, and liquefied in an industrial blender

(Osterizer® MAX Reversible model Oster® BLST4655, 1,500 W motor, Ecuador) at 30,000 rpm/5 min, at room temperature (25°C), and scalded (80°C/1 min) in double-coated aluminum containers (Imusa Brand, Ecuador), to inactivate some enzymes. Then, it was cooled in an ice-water bath until it reached approximately 8 °C. Subsequently, the previously scalded mesoendocarp was placed in hermetically sealed plastic bags and stored in a freezer (Indurama Brand; Ecuador), at -5 °C, until it was analyzed in a 24 h period.

General chemical composition

The following variables were determined: pH: a benchtop pH meter (MP-500 Series, SANXYN, Ecuador) was used, and certified buffer solutions were used for calibration. The total soluble solids (°Brix) were determined using the AOAC 22.024 method (1990), in a high-accuracy portable digital refractometer (HI96814, HANNA, Ecuador, ± 0.2 °Brix), with a response time of 1.5 seconds, equipped with a sealed flint glass prism and stainless steel well. Determining moisture and ash content was carried out as established by AOAC 22.008 (1990) for fruits. Proteins were quantified using the micro-Kjeldahl method AOAC 22.008 (1990). For the determination of crude fiber, the method described in AOAC 962.09 was applied (1995).

Mineral content

Metals (Ca, Zn, Mg, Fe, Se, Co, Cr, Cu, Mo) were quantified by atomic absorption spectroscopy (AAS) with flame, using a spectrophotometer (Perkin Elmer model 3030-B, USA), while Na and K were detected by atomic emission spectroscopy (AES) (Perkin Elmer® model 3030-B, USA). The method described by Fernández *et al.* (2007) was used.

Vitamin C

It was determined by applying the method described by Clamens *et al.* (2014). The mesoendocarp (30 g) was crushed in a blender (Osterizer® MAX Reversible model Oster® BLST4655, 1500 W motor, Ecuador). Vitamin C concentration was expressed in mg AA.100 g⁻¹ pulp.

Obtaining ethanolic extracts

A mixture of 1:5 soursop fruit pulp (m/m) and ethanol (Merck, 99% purity) was prepared using a homogenizer (Fisherbrand™ 850, USA). The homogenates obtained were macerated for 24 h without agitation and in the presence of light; They were then centrifuged using a centrifuge (XC-2000 Premiere, USA) at 1008 G for 10 min. Supernatants were used for the quantification of total polyphenols, flavonoids, antioxidant activity, and acetogenin identification.

Total polyphenols

The method of Singleton *et al.* (1999) was applied, which is based on the colorimetric method with the Folin-Ciocalteu reagent. A calibration curve (0; 0.25; 0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.7 and 0.8 g.L⁻¹) was performed with gallic acid (Sigma, Steinheim, Germany) from a stock solution of 0.1 g.L⁻¹. The results obtained were expressed as mg AG.100 g⁻¹ of soursop pulp.

Total flavonoids

The method of Woisky and Salatino, (1998) was used. Flavonoid concentration was determined by a calibration curve (0; 0.25; 0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.7 and 0.8 g.L⁻¹) with quercetin (Sigma, Germany). The results were expressed as mg Q.100 g⁻¹ of soursop pulp.

Antioxidant activity

The method described by Re *et al.* was applied. (1999). A Trolox calibration curve was constructed at different concentrations (0, 0.25, 0.05; 0.1, 0.2; 0.3; 0.4, and 0.5, 1.25, and 2.5 mM) using an 8 mM Trolox solution (Sigma-Aldrich, Germany) as standard antioxidant.

Antioxidant activity was expressed as µM TEAA.100 g⁻¹ of soursop pulp.

Kedde Test

Two mL of the ethanolic extracts of fruit pulp were added in test tubes, followed by 1 mL of a 2% diluted alcoholic solution of KOH, and 2 mL of 3.5-dinitrobenzoic acid in 2% ethanol was added to the samples. The appearance of a pink coloration is indicative of the presence of acetogenins (lactonic ring) (Laguna-Hernández *et al.*, 2015).

Statistical analysis

The measurements of the variables studied were performed in triplicate and the results obtained were tabulated in an Excel spreadsheet, and descriptive statistics were applied, the results were expressed as the mean ± standard deviation.

Results and discussion

The values obtained in the characterization of the general chemical composition of the fruit pulp of *A. muricata* (soursop) are shown in table 1.

Table 1. Chemical composition of the fruit pulp of *A. muricata*.

Variables	Content
Moisture, %	82.95 ± 1.23
Ash, %	0.69 ± 0.01
pH	3.91 ± 0.01
Fiber, %	0.81 ± 0.02
Protein, %	1.64 ± 0.04
Soluble solids °Brix	15.44 ± 0.99

Results are presented as means ± standard deviation (n = 3)

The moisture content (82.95 %) and ash content (0.69 %) obtained in this study are within the range of values reported (80.93 % to 86.19 %) and (0.22 % to 0.70 %), respectively, for soursop pulp grown or disseminated in different geographical locations (Arrazola-Paternina *et al.*, 2013; Badrie and Schauss, 2010; León-Méndez *et al.*, 2016; Ojeda *et al.*, 2007).

Regarding the pH value obtained (3.91), it is in the published range (3.70 - 4.20) for the pulp of this fruit tree (Arrazola-Paternina *et al.*, 2013; Badrie and Schauss, 2010; García-Soto *et al.*, 2012; León-Méndez *et al.*, 2016; Ojeda *et al.*, 2007).

The protein (1.64 %) and fiber (0.81 %) contents are similar to those published in various studies (Ojeda *et al.*, 2007; Badrie and Schauss, 2010; Arrazola-Paternina *et al.*, 2013; León-Méndez *et al.*, 2016).

The values obtained for total soluble solids (15.44 %) are higher than the value published by Arrazola-Paternina *et al.* (2013): 11.3%; García-Soto *et al.* (2012): 14.38%; León-Méndez *et al.* (2016): 14.10%; but comparable to those reported by Baskaran *et al.* (2015): 15.42% and Ramírez *et al.* (2012): 15.44%.

Mineral composition of soursop pulp

It was observed that the minerals potassium, calcium, sodium, zinc, magnesium, and iron are the main components present in the ash of soursop pulp (table 2). Selenium and cobalt are present as traces; chromium, copper, and molybdenum were not detected. The results obtained are comparable to those reported for soursop pulp (Fernández *et al.*, 2007; Hernandez *et al.*, 2018; León-Méndez *et al.*, 2016; Ramírez *et al.*, 2012).

Table 2. Mineral composition of the fruit pulp of *A. muricata*.

Mineral	mg.100 g pulp
Potassium (K)	49.23 ± 0.43
Zinc (Zn)	38.11 ± 0.24
Sodium (Na)	28.75 ± 0.18
Magnesium (Mg)	21.34 ± 0.30
Calcium (Ca)	19.14 ± 0.31
Iron (Fe)	9.24 ± 0.12
Selenium (Se)	< 1.06
Cobalt (Co)	< 0.98
Chromium (Cr)	ND
Copper (Cu)	ND
Molybdenum (Mo)	ND

Results are presented as means ± standard deviation (n = 3). ND: Not Detected

The mineral contents present in fruits probably vary due to the effect of the climatic conditions where the crops are located, phenology (biological cycles), maturity stage, edaphic conditions, frequency of fertilization and irrigation (Hernández *et al.*, 2018; Jiménez *et al.*, 2017; Nam *et al.*, 2017).

On the other hand, it was evidenced that the analyzed soursop pulp has essential minerals in its constitution, which play vital roles in physiological and metabolic processes in living beings (Oboh *et al.*, 2015). It is worth highlighting the high content of zinc, a trace element that contributes to the strengthening of the immune system, and potassium, a key macro-element because it acts by controlling the osmotic processes of the cardiovascular system (Oboh *et al.*, 2015).

Vitamin C content, total polyphenols, flavonoids, and antioxidant activity of *A. muricata* fruit pulp

The results obtained for vitamin C content, total polyphenols, flavonoids, and antioxidant activity in the pulp of *A. muricata* are presented in table 3.

Table 3. Phytochemical content and antioxidant activity of ethanolic extracts of *A. muricata* fruit pulp.

Phytochemicals	Content
Vitamin C (mg AA 100 g ⁻¹ pulp)	26.64 ± 1.01
Total polyphenols (mg EAG.100 g ⁻¹)	398.79 ± 14.91
Total flavonoids (mg EQ.100 g ⁻¹)	192.20 ± 8.50
Antioxidant activity (trolox equivalent mmol.100 g ⁻¹)	318.90+ 10.58

Results are presented as means ± standard deviation (n = 3)

It was evidenced that the vitamin C content (26.64 mg AA.100 g⁻¹ pulp) is within the ranges reported by Badrie and Schauss. (2010): 20.90-26.76 mg AA.100 g⁻¹ pulp; García-Soto *et al.* (2012): 11.76-26.67 mg AA.100 g⁻¹ pulp and León-Méndez *et al.* (2016) 21.10-27.44 mg AA.100 g⁻¹ pulp; but higher than those described by Ojeda *et al.* (2007): 19.40-21.56 mg AA/100 g⁻¹ pulp; Clamens *et al.* (2014): 14-13 mg AA.100 g⁻¹ pulp and Arrazola-Paternina *et al.* (2013): 5-7 mg AA.100 g⁻¹ pulp and other products such as: *Persea americana* (8 mg AA.100 g⁻¹ pulp), *Citrullus lanatus* (10 mg AA.100 g⁻¹ pulp), *Musa paradisiaca* (10 mg AA.100 g⁻¹ pulp), and *Solanum melongena* (2 mg AA.100 g⁻¹ pulp) (Latham, 2002); however, it is lower than the ranges

published by Fuenmayor *et al.* (2016): in fruits harvested from plants grafted on *A. muricata* (33.1 - 28 mg AA.100 g⁻¹ pulp), *A. glabra* (24.4 - 24.1 mg AA.100 g⁻¹ pulp) and *A. montana* (37.4 - 25.8 mg AA.100 g⁻¹ pulp), and at the value reported by Ramírez and Pacheco (2011): 33.24 mg AA.100 g⁻¹ pulp.

It has been shown that the variation of vitamin C content in the mesoendocarp of *A. muricata* is closely linked to the stages of fruit growth (Clamens *et al.*, 2014), rootstock selection (propagation), and fertilization frequency (Fuenmayor *et al.*, 2016).

The content of determined total polyphenols (398.79 mg EAG.100 g⁻¹ pulp), (table 3), is higher than the reported value (332.40 mg EAG.100 g⁻¹ pulp) for soursop pulp located in the municipality of Mara, Venezuela (Clamens *et al.*, 2014) and the average value published by García-Soto *et al.* (2012): 161.25 mg EAG.100 g⁻¹ pulp for fruits obtained with different forms of propagation in the same locality. Significant contents of total polyphenols (941.40 mg EAG.100 g⁻¹ pulp) have been reported in the mesocarp of *A. muricata* (Vit *et al.*, 2014), which shows that this fruit tree is an important source of these phytochemicals.

It is important to note that polyphenols act as antioxidants by inhibiting cellular biological oxidation, contributing to reducing the risks of hypertension, cancer, diabetes, cerebrovascular diseases, and cellular aging (McDougall, 2016).

The flavonoid content (Table 3) obtained in the present study (192.20 mg EQ.100 g⁻¹ pulp) is lower than the value reported by Vit *et al.* (2014) for soursop pulp (574 mg EQ.100 g⁻¹). These differences probably depend on the cultivar, soil conditions, fruit maturity, propagation forms, and agronomic practices (Clamens *et al.*, 2014; Ramírez *et al.*, 2012; Sandoval *et al.*, 2014).

It has been reported that the flavonoids present in soursop pulp are: the luteolin flavone, the most abundant, followed by the flavonol myricetin and the flavone apigenin; which are found in greater proportion than the values observed for *Musa paradisiaca*, *Mangifera indica*, *Carica papaya*, and *Ananas comosus* (Sandoval *et al.*, 2014). Flavonoids (phenolic compounds) of natural origin have biological activity and beneficial effects on the development of chronic degenerative diseases (Chan *et al.*, 2016).

The soursop pulp studied has an antioxidant activity (318.90 mmol equivalent of trolox.100 g⁻¹ pulp), (Table 3), higher than the values reported for the mesocarp of soursop fruits located in the municipality of Mara, Zulia, Venezuela (Fuenmayor *et al.*, 2016), but analogous to those reported for fruits of *A. muricata* in a range that varies between 306.00 – 321.87 mmol equivalent of trolox.100 g⁻¹ pulp (Vit *et al.*, 2014). It has been shown that antioxidant activity is affected by agroecological conditions, genetic aspects, production techniques, maturity periods, storage conditions, and temperature, including post-harvest management (Fuenmayor *et al.*, 2016).

The content of phytochemicals present in these fruits has been associated with the antioxidant activity exhibited by the pulp of *A. muricata* (Clamens *et al.*, 2014; Correa *et al.*, 2012; Hernandez *et al.*, 2018; Jiménez *et al.*, 2014; Vit *et al.*, 2014).

Identification of acetogenins in fruit pulp of *Annona muricata* (soursop)

It was evidenced that the ethanolic extracts of soursop pulp when performing the Kedde test, present a pink ring (Figure 1), which is an unequivocal indicator of the presence of acetogenins, which present in their structure a characteristic lactonic ring responsible for the coloration (Laguna-Hernández *et al.*, 2015).

These compounds are a group of bioactive secondary metabolites present particularly in the Annonaceae family and mainly in the genus *Annona*, both in fruits, seeds, and leaves (Gavamukulya *et al.*, 2014; Liaw *et al.*, 2016).



Figure 1. Identification of the presence of acetogenins in the pulp of the fruit of *Annona muricata* (soursop) by applying the Kedde test.

Acetogenins (ACGs) have antiproliferative effect in cancer cell lines. It has been described that the distance between the tetrahydrofuran group and the lactonic ring must be 13 carbons to exhibit high and optimal anticancer activity, a structural conformation that has been observed in ACGs obtained from fruits and mainly from leaves of *A. muricata* (Gavamukulya *et al.*, 2014; Liaw *et al.*, 2016; Paul *et al.*, 2013; Ogbu *et al.*, 2020).

Conclusions

The mesoendocarp of *Annona muricata* (soursop) located in Manabí, Ecuador has an important content of bioactive compounds: essential minerals (K, Ca, Zn, Mg), vitamin C, polyphenols; presence of acetogenins and high antioxidant activity, properties that reduce the risk of degenerative diseases and cellular aging. Therefore, its consumption in the daily diet as fresh and processed fruit constitutes a valuable nutritional and therapeutic alternative.

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