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Isolation and characterization of annonacin from *Annona muricata* L using semipreparative HPLC.

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Abstract

The secondary metabolism of the species *Annona muricata*, popularly known as soursop (Annonaceae), produces a class of substances known as acetogenins. Given the significant pharmacological importance of these secondary metabolites, this work aims to apply chromatographic techniques to isolate and confirm the identity of the main acetogenin in *Annona muricata* L., highlighting the importance of this approach for large-scale production of the active principles of this compound. The ethanolic extract of *A. muricata* seeds was fractionated by classical column chromatography, and the resulting fraction was analyzed by TLC, yielding a fraction rich in acetogenins. From this fraction, 600 mg was isolated by semi-preparative reverse-phase liquid chromatography, yielding 150.52 mg of isolated acetogenin. After isolation, the sample was again analyzed by chromatography in analytical mode to confirm its purity and subjected to NMR for identity confirmation, which revealed it to be annonacin, the major acetogenin of *Annona muricata* L. in both leaves and seeds, corroborating literature data. Thus, this study contributed to the valorization of this species through spectroscopic and chromatographic analysis of its chemical constituents, as well as to the work of natural products chemistry in the prospecting and isolation of these constituents.

Keywords: Annonacin, *Annona muricata*, Nuclear magnetic resonance, High-performance liquid chromatography.

1.Introduction

Natural products chemistry is a multidisciplinary field that explores the biosynthesis, structural elucidation, and pharmacological properties of secondary metabolites derived from living organisms [1]. In Brazil, this discipline is among the oldest and most productive research areas in chemistry, reflecting the country's exceptional biodiversity and rich ethnopharmacological heritage [2]. Brazil harbors approximately 200,000 plant species distributed across diverse biomes, including the Amazon, Cerrado, and Caatinga, many of which are traditionally employed in folk medicine and lack rigorous pharmacological validation [2, 3]. These traditional practices, often transmitted through generations, provide an essential foundation for bioprospecting and the discovery of new bioactive compounds with therapeutic potential [4, 5].

In rural communities of Northeastern Brazil, particularly within the Caatinga biome, medicinal plants remain essential healthcare resources due to limited access to conventional medicine [6]. Among the species most frequently used is *Annona muricata* L. (soursop or graviola), a member of the Annonaceae family [7]. The secondary metabolism of *A. muricata* is characterized by the biosynthesis of a unique class of bioactive polyketides known as acetogenins, which are distributed throughout the leaves, seeds, fruit, and stem bark. These metabolites have attracted significant scientific attention owing to their remarkable biological activities, including anticancer, antiparasitic, and insecticidal effects [8, 9]. According to Formagio et al. [10], the species in this family are characterized by having simple leaves, arranged alternately in the same plane along the branches. They are widely used in folk medicine due to their various pharmacological activities attributed to alkaloids, acetogenins, and flavonoids, the main bioactive constituents found in specific genera of this family. Starting in the 1980s, acetogenins were identified in Annonaceae, generating a new stimulus for the phytochemical study of this family, due to the bioactive nature of this class of natural substances [11].

Advances in chromatographic and spectroscopic techniques have revolutionized the investigation of natural products such as *Annona muricata* by enabling the identification and structural elucidation of their bioactive constituents. Since the pharmacological potential of *A. muricata* is largely attributed to its acetogenins and other secondary metabolites, the application of these analytical methods is essential to isolate, purify, and characterize such compounds from complex plant matrices [12]. Chromatographic methods facilitate the separation and enrichment of minor

constituents, allowing researchers to obtain pure fractions of biologically active molecules, while spectroscopic tools such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) provide detailed information on their molecular structures and stereochemistry. These techniques are therefore indispensable for validating traditional knowledge, correlating specific chemical profiles with biological activities, and ensuring the reproducibility and accuracy of pharmacological studies. Moreover, their use extends beyond natural product research to vital applications in drug discovery, toxicology, and forensic science [13].

The Annonaceae family comprises approximately 120 genera and 2,300 species, of which 29 genera and around 260 species are found in Brazil. Within this family, several species such as *Annona muricata* L., *A. squamosa* L., *A. cherimola* Mill., and their hybrid *A. × atemoya* hold significant economic and medicinal value due to their diverse bioactive constituents [14]. Phytochemical investigations of *Annona* species have revealed a rich array of secondary metabolites, including alkaloids, flavonoids, essential oils, and acetogenins, which contribute to their pharmacological potential [15]. Although early research primarily emphasized the isolation of alkaloids, the identification of acetogenins in the 1980s redirected scientific attention toward this structurally complex and highly bioactive group of polyketides. These compounds, distributed across various plant organs, have since become central to studies integrating chromatographic and spectroscopic methods, which enable their precise isolation, structural elucidation, and biological assessment, thus reinforcing the relevance of the *Annona* genus in natural product and medicinal chemistry [16].

Acetogenins are long-chain fatty acid derivatives biosynthesized through the polyketide pathway. Structurally, they are characterized by a large aliphatic chain containing one to three tetrahydrofuran (THF) rings and a terminal γ -lactone moiety. The first acetogenin, uvaricin, was isolated in 1982 from *Uvaria accuminata*, marking the beginning of extensive research into this family of natural products [17]. Since then, more than 500 acetogenins have been identified in various *Annona* species, including muricatocin, annomuricin, annohexocin, annomutacin, annopentocin, muricapentocin, muricoreacin, cis-annoreticulin, annonacin, and isoannonacin from *A. muricata* [9].

Numerous studies have demonstrated that *A. muricata* extracts and their isolated acetogenins exhibit a broad spectrum of pharmacological activities, including antitumor, cytotoxic,

antimicrobial, antileishmanial, and antiparasitic effects [8, 18]. Several acetogenins, such as annonacinone and crossolone, have shown selective activity against *Leishmania* species. In contrast, others display potent cytotoxicity toward various cancer cell lines, underscoring their potential as lead compounds in anticancer drug development. Furthermore, the traditional therapeutic applications of *A. muricata* for treating hypertension, fever, diabetes, hepatic and cardiac disorders, and gastrointestinal ailments are increasingly corroborated by pharmacological evidence.

Given the pharmacological relevance of acetogenins, particularly their potent cytotoxicity against cancer cell lines, the present study aims to isolate and structurally characterize the principal acetogenin from *Annona muricata* L. using advanced chromatographic and spectroscopic methodologies. The research emphasizes the analytical rigor and reproducibility of chromatographic techniques as essential parameters for the reliable standardization of phytochemical extracts. By optimizing chromatographic conditions to enhance recovery and purity, this work aims to establish an efficient approach for large-scale isolation and identification of annonacin, the major acetogenin found in *A. muricata* leaves and seeds. The study also highlights the importance of integrating chromatographic separation with NMR-based spectroscopic analysis to confirm compound identity and purity. Overall, this study contributes to the chemical and pharmacological valorization of *A. muricata* by providing a reproducible analytical framework for the isolation and standardization of bioactive natural products.

2. Materials and Methods

The main bioactive constituent of extracts obtained from the seeds and leaves of *Annona muricata* L. is annonacin [19]. After preparation of the seed and leaf extracts, each was analyzed by HPLC to compare their chromatographic profiles and select the most suitable extract for subsequent isolation.

2.1 Extraction of Seeds of *Annona muricata* L.

Seeds (3 kg) and leaves (4 kg) of *A. muricata* L. were provided by a local company in Limoeiro do Norte, Ceará, Brazil. The seeds were sun-dried and ground into a coarse powder. The powdered seeds were then macerated in 96% ethanol for 7 days. After maceration, the extracts were filtered and concentrated under reduced pressure using a rotary evaporator, yielding 165 g of ethanolic

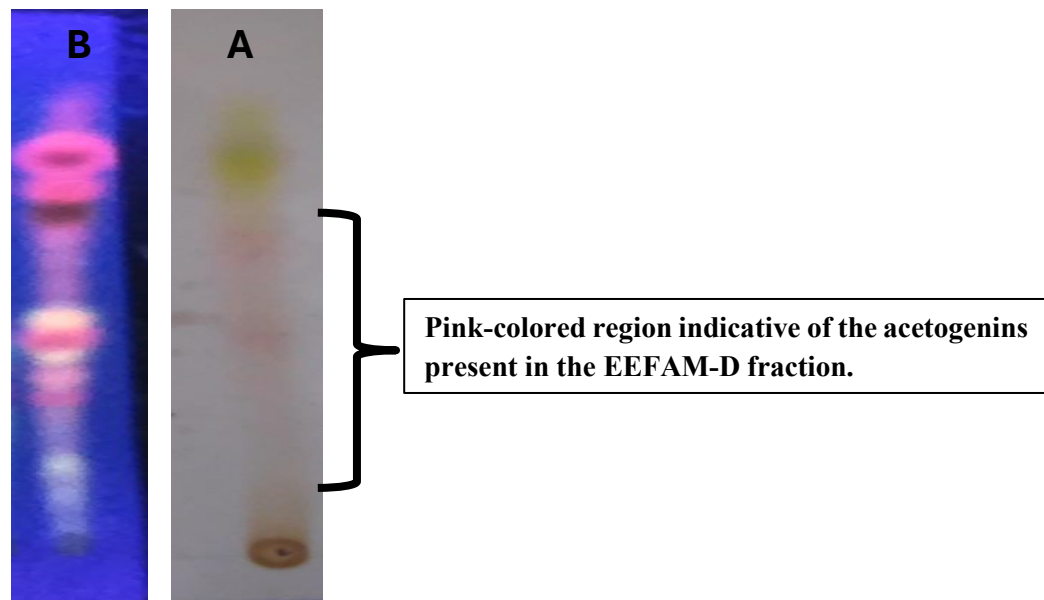
seed extract (E.E.S.A.M). The ethanolic extract was partitioned with chloroform (CHCl_3) and water (1:1) in a 1 L separatory funnel. The aqueous fraction was extracted three times with 400 mL of CHCl_3 . The combined chloroform layers were concentrated under reduced pressure, producing 150 g of the chloroform fraction of E.E.S.A.M.

The chloroform fraction (150 g) was subjected to column chromatography by mixing with 150 g of silica gel 60 (0.063–0.200 mm, 70–230 mesh) and loading on top of a silica gel column (300 g). Fractionation was performed using organic solvents of increasing polarity: hexane, chloroform, ethyl acetate, and methanol. Fractions F4 to F10, eluted with chloroform and ethyl acetate, were analyzed by thin-layer chromatography (TLC) using a solvent system composed of hexane: chloroform: ethyl acetate: acetone: methanol: acetonitrile: water (12:2:4:1:2:5.6:0.1). Spots were visualized using Kedde's reagent (5 mL methanolic 3,5-dinitrobenzoic acid solution mixed with 5 mL of 2 M NaOH), where a pink coloration indicates the presence of γ -lactone-containing acetogenins. Based on TLC analysis, the relevant fractions were pooled, and 1 g of the acetogenin-rich fraction (FRA F4–F10) was obtained. This fraction was further analyzed by analytical HPLC to determine its chromatographic profile.

2.2 Extraction of Leaves of *Annona muricata* L.

Sun-dried leaves of *A. muricata* L. were ground into a fine powder. A total of 500 g of leaf powder was macerated in 4 L of ethanol for 24 h. The extract was filtered and concentrated under reduced pressure using a rotary evaporator, yielding 8.657 g of crude ethanolic leaf extract (E.E.F.A.M). The leaf extract was partitioned with dichloromethane (DCM) and water (1:1). Both fractions were concentrated by rotary evaporation. The aqueous fraction yielded 6.32 g of yellow residue, while the DCM fraction (E.E.F.A.M-D) produced 2.33 g of residue. E.E.F.A.M-D was subjected to TLC (MERCK plates), applying 6 μL per spot. After elution with the same solvent mixture as used for seeds, plates were sprayed with Kedde's reagent. The pink coloration indicated the presence of an α,β -unsaturated γ -lactone, a common feature of acetogenins [20]. In Figure 1, the plate shows a pink-colored region visualized after spraying with Kedde's reagent, confirming the presence of acetogenins in the EEFAM-D fraction.

Representative TLC plates are shown in Figure 1. This fraction was further analyzed by analytical HPLC to obtain its chromatographic profile. This fraction was subjected to analytical HPLC analysis to obtain its chromatographic profile.



Source: Authors, 2024.

Figure 1. TLC analysis of the EEFAM-D fraction from *Annona muricata* leaves after visualization with Kedde's reagent. Pink-colored bands indicate acetogenins containing α,β -unsaturated γ -lactone groups. (A) Full TLC plate; (B) detail of acetogenin-positive region.

2.3 Chromatographic Conditions for Analytical HPLC

For the analysis and isolation of annonacin by high-performance liquid chromatography (HPLC), the procedure described by Gromek, Hocquemiller, and Cavé [21] was adapted. The analyses were performed using a SHIMADZU HPLC system equipped with an SLC-10AVP controller, a UV-VIS SPD-10AVP detector, and an LC-10ATVP isocratic pump. Separation was carried out on an analytical SHIMPACK CLC-ODS M (C18) column (25 cm $\times$ 4 μ m). The mobile phase consisted of methanol and deionized water in a ratio of 87:13 (v/v), with a flow rate of 1.0 mL per minute. The detection wavelength was set at 214 nm, and the column temperature was maintained at 40°C. Each analysis was run for 20 minutes under isocratic conditions. The chromatogram revealed a major peak at a retention time of 13.5 minutes, corresponding to annonacin, the principal acetogenin present in *Annona muricata* L. This analytical profile confirms the successful isolation

of annonacin and demonstrates the reliability of the optimized chromatographic conditions for its detection and purity assessment.

2.4 Chromatographic Conditions for Semi-Preparative HPLC

Isolation of the major acetogenin was performed using a semi-preparative SHIMADZU HPLC system with an SLC-10AVP controller, an SPD-10AVP UV-Vis detector, and an LC-10ATVP isocratic pump. The preparative column used was SUPELCOSIL (ODS), 25 cm × 10 mm, 5 µm particle size. The mobile phase consisted of methanol: deionized water (87:13, v/v), a flow rate of 5 mL/min, an injection volume of 200 µL, detection at 214 nm, and a run time of 20 minutes at room temperature. The major peak eluted at 10 minutes.

2.5 Sample Preparation for Analytical and Semi-Preparative HPLC

For analytical HPLC, 10 mg of each seed FRA (F4–F10) fraction was dissolved in 10 mL of methanol to obtain a 1 mg/mL solution. The 1 mL aliquot of this solution was filtered through a 0.22 µm PVDF syringe filter, and 20 µL of the filtrate was injected into the HPLC system. In parallel, for the analytical profiling of the leaf EEFAM-D fraction, 5 mg of the sample was dissolved in 5 mL of methanol to achieve a 1 mg/mL solution, which was similarly filtered through a 0.22 µm PVDF filter, and 20 µL was injected. For the semi-preparative HPLC isolation of the major acetogenin, 600 mg of the FRA (F4–F10) fraction was dissolved in 20 mL of methanol to prepare a 30 mg/mL solution. The entire solution was filtered through a 0.22 µm PVDF filter prior to injection into the semi-preparative HPLC system, ensuring sample homogeneity and optimal chromatographic performance.

3. Results and Discussion

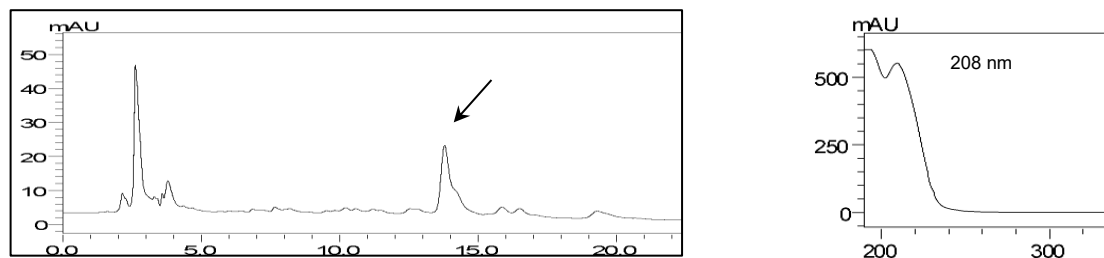
The isolation and identification of acetogenins were successfully achieved through a combination of chromatographic and spectroscopic techniques, including TLC, HPLC, and NMR spectroscopy. These analytical approaches proved effective in confirming the presence and purity of the major acetogenin isolated from *Annona muricata* L. The detailed results and observations obtained at each experimental stage are described in the following sections.

3.1 Preliminary Screening and TLC Analysis

Ethanollic extracts and their corresponding fractions from the leaves and seeds of *Annona muricata* were meticulously analyzed through chromatographic techniques on silica gel plates. The examination revealed distinct chromatographic profiles when visualized under ultraviolet light at wavelengths of 254 nm and 365 nm. Upon application of Kedde's reagent, a striking array of pink to magenta spots emerged, unequivocally indicating the presence of α,β -unsaturated γ -lactone systems. These systems are characteristically associated with Annonaceous acetogenins, which are known for their significant bioactivity.

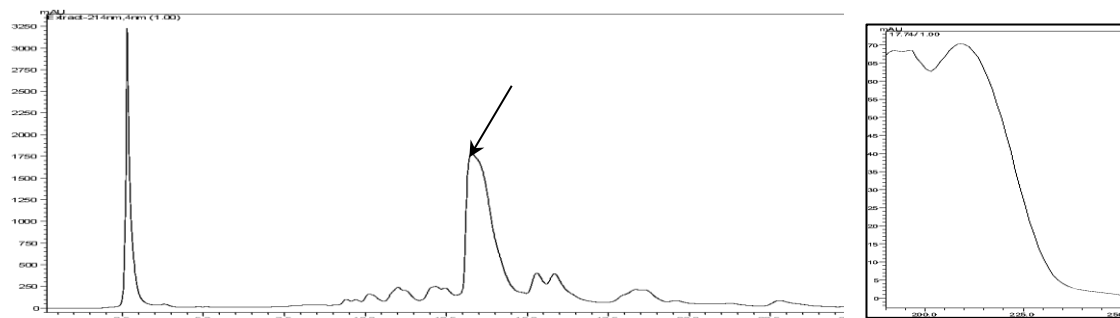
The positive reaction observed in both the leaf and seed fractions was consistent with the celebrated lactone-THF structural motif previously documented by Cavé et al.[21] and Liaw et al.[20]. Among the various seed fractions, particularly those labeled F6 through F9, the color intensity was markedly greater. This observation suggests a superior concentration of acetogenins in these fractions compared to the leaf extract, highlighting the potential of *Annona muricata* seeds as a rich source of these bioactive compounds. Such findings underscore the importance of these extracts in further pharmacological studies and their potential applications in therapeutic contexts.

Figure 2. Analytical HPLC chromatogram of the EEFAM-D fraction from *A. muricata* leaves showing the major constituent peak at 13.52 minutes and its corresponding UV spectrum.



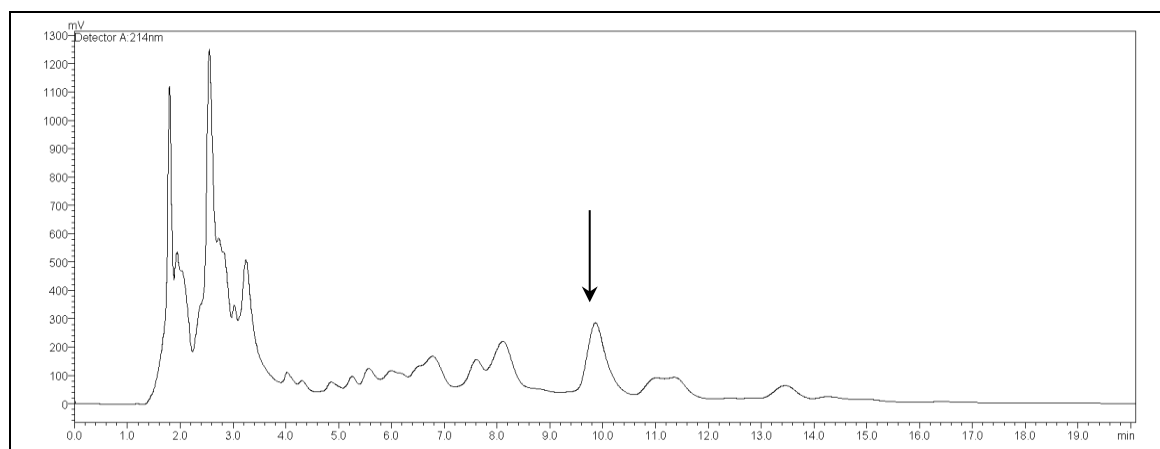
After the injection of this fraction, the acetogenin-rich seed fraction (F4–F10) was analyzed by analytical HPLC, yielding the chromatogram shown in Figure 2. The peak with a retention time of 13.52 minutes corresponds to the major acetogenin present in the fraction.

Figure 3. Analytical HPLC chromatogram of the FRA (F4–F10) seed fraction of *A. muricata* showing the major constituent peak at 13.52 minutes and its corresponding UV spectrum.



By comparing the chromatographic profiles shown in Figures 2 and 3, it can be concluded that both fractions contain a major acetogenin; however, the FRA (F4–F10) fraction appears to have a higher concentration of this principal compound. Subsequently, both fractions were injected into the HPLC system under semi-preparative conditions. The resulting chromatograms of the leaf and seed fractions (Figure 4) revealed a prominent peak at a retention time of 10 minutes, with a maximum UV absorption at 214 nm. Based on the comparison of these chromatograms, it can be inferred that the FRA (F4–F10) fraction contains a greater amount of the major compound, which is presumed to be annonacin.

Figure 4. Semi-preparative HPLC chromatograms obtained from injections of the EEFAM-D fraction (A; leaves) and the FRA (F4–F10) fraction (B; seeds) of *A. muricata*.



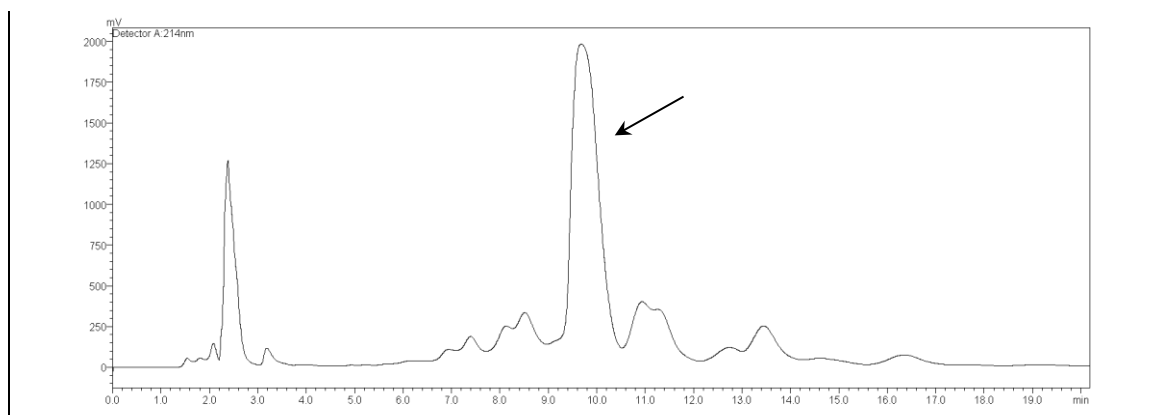
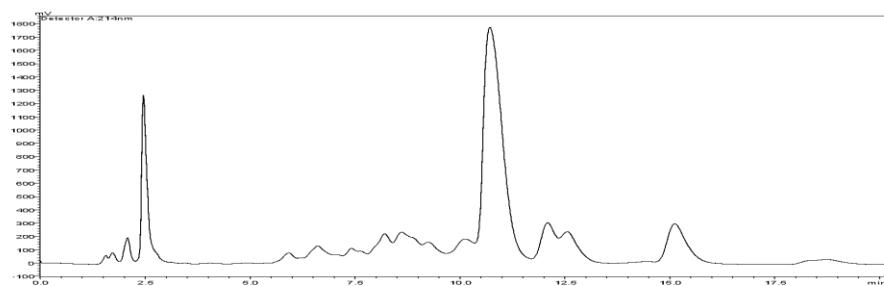


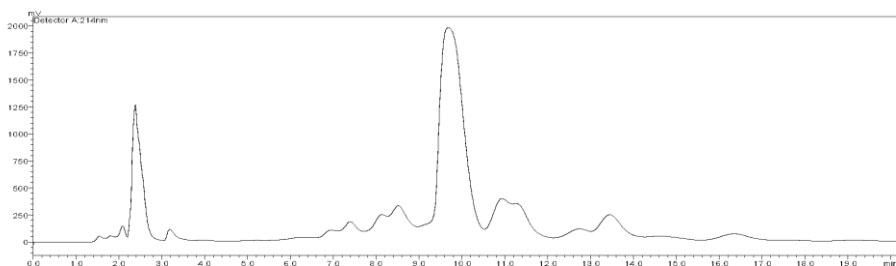
Figure 4 shows the chromatograms obtained from the injections of the EEFAM-D fraction (leaves) and the FRA (F4–F10) fraction (seeds) analyzed under semi-preparative HPLC conditions. Owing to differences in column diameter and flow rate compared with the analytical HPLC system, the retention time of the major acetogenin peak was reduced to approximately 10 minutes. This shift in retention behavior is consistent with the increased flow rate and larger column dimensions typically employed in semi-preparative chromatography, which enhance compound elution efficiency. Based on these findings, the FRA (F4–F10) fraction was selected for semi-preparative isolation, as it exhibited a higher concentration of the major acetogenin.

After identifying the retention time corresponding to the principal acetogenin peak, optimization of the injection volume was performed to establish ideal loading conditions for compound isolation. Sequential injections with increasing sample volumes ranging from 50 μL to 400 μL were carried out, and the resulting chromatograms were compared to assess changes in peak shape, symmetry, and resolution. This comparative analysis enabled the determination of the injection volume that provided the best balance between signal intensity and chromatographic performance, ensuring efficient recovery and purity of the isolated compound. The chromatographic profiles obtained during this optimization process are presented in Figure 5.

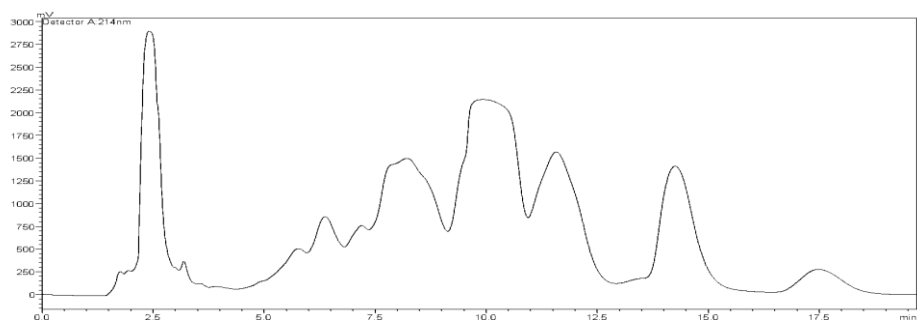
Figure 5. Representation of the injection volume scaling of fraction FRA (F4–F10) for determining the optimal injection volume for isolation using semipreparative HPLC.



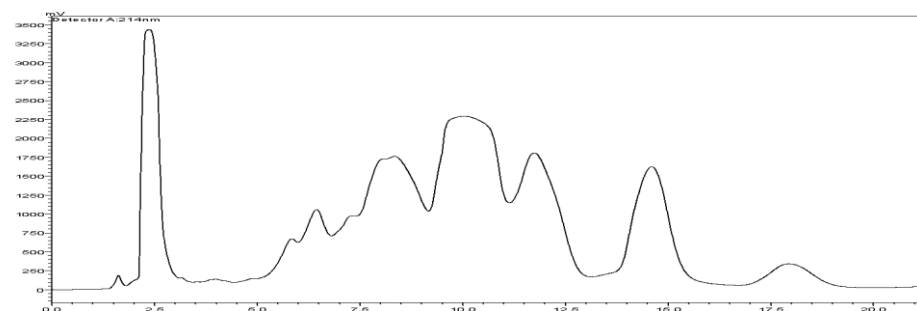
50 µL



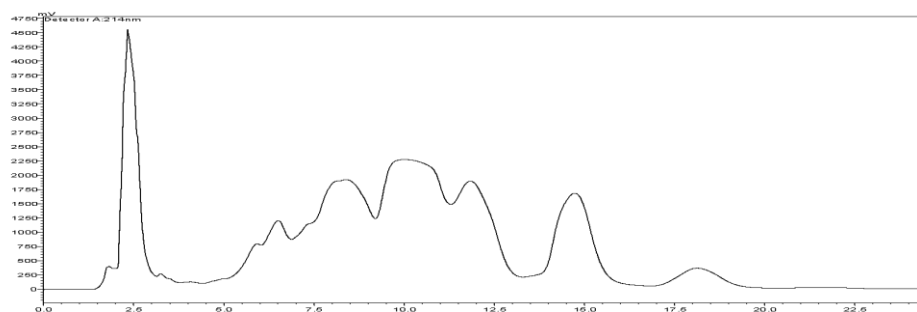
200 µL



250 µL



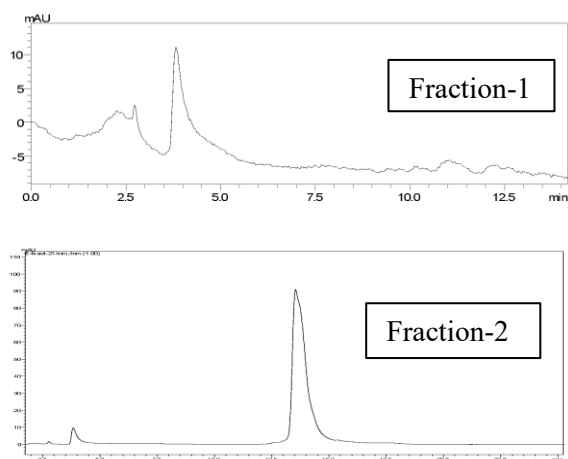
350 µL



400 µL

Based on the comparative chromatographic profiles shown in Figure 5, an injection volume of 200 μL of the FRA (F4–F10) fraction was selected as the optimal condition for semi-preparative isolation. This volume provided a well-defined major peak with good resolution and minimal peak broadening, indicating an effective balance between loading capacity and separation efficiency. Using this optimized volume, semi-preparative HPLC separations were performed, during which three distinct fractions were collected according to their retention times: Fraction 1 (2.5–9.5 minutes), Fraction 2 (9.5–10.2 minutes), and Fraction 3 (10.2–25 minutes). Fraction 2, corresponding to the retention window of the major acetogenin, was expected to contain the predominant compound, likely annonacin. To ensure complete isolation, the semi-preparative procedure was repeated multiple times, totaling 95 injections of 200 μL each, until the entire 20 mL of the sample solution (30 mg/mL) had been processed. The separation criterion was strictly based on retention time, ensuring consistency and reproducibility across all runs.

Following collection, Fractions 1, 2, and 3 were subjected to analytical HPLC analysis to verify the success and purity of the isolation process. The resulting chromatograms, presented in Figures 4 and 5, confirm the effective separation of the major acetogenin component from the other constituents of the extract.



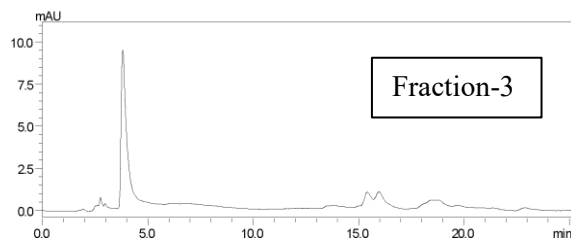
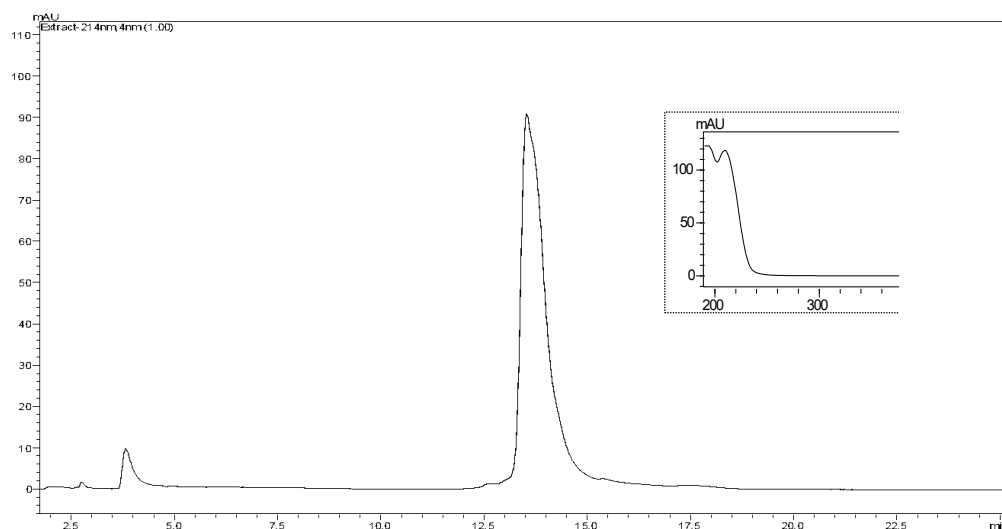


Figure 6. Chromatograms of semipreparative fractions 1 (RT 2.5–9.5 min), 2 (RT 9.5–10.2 min), and 3 (RT 10.2–20 min) obtained from the purification of fraction FRA (F4–F10) of the seed extract.

Fraction 2 was subsequently concentrated under reduced pressure using a rotary evaporator and dried, yielding 150.52 mg of purified material. This amount corresponded to the cumulative recovery from 95 semi-preparative injections of 200 μ L each of the FRA (F4–F10) solution, resulting in an overall yield of 25.09% relative to the initial FRA (F4–F10) fraction. To confirm the purity of the isolated compound, Fraction 2 was subjected to analytical HPLC analysis, as illustrated in Figure 7. For the analytical HPLC assessment, 5 mg of Fraction 2 was accurately weighed and dissolved in 5 mL of methanol, yielding a 1 mg/mL solution. The solution was then filtered through a 0.22 μ m PVDF syringe filter to remove any particulates, and a 20 μ L aliquot of the filtrate was injected into the analytical HPLC system. The resulting chromatogram demonstrated a single dominant peak, confirming the successful isolation and high purity of the major acetogenin compound.

Figure 7. Chromatogram obtained from the injection of fraction 2, representing the main isolated acetogenin and its corresponding UV spectrum.

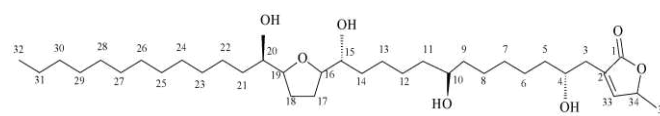


After the isolation and chromatographic profiling of Fraction 2, which contained the principal acetogenin compound, its chemical structure was confirmed using Nuclear Magnetic Resonance (NMR) spectroscopy, specifically through ^1H and ^{13}C NMR analyses. These techniques were selected due to their high sensitivity and reliability in elucidating the molecular framework, proton environment, and carbon skeleton of organic compounds essential for confirming the identity of acetogenins, which often exhibit complex aliphatic and lactonic structures. For the ^1H and ^{13}C NMR analyses, deuterated chloroform (CDCl_3) was employed as the solvent because of its non-protonated nature, excellent solubility for lipophilic compounds, and minimal interference in the spectral regions of interest. The spectra were recorded on a Bruker Avance DRX-500 spectrometer, operating at 500.13 MHz for hydrogen nuclei (^1H) and 125.77 MHz for carbon nuclei (^{13}C) under a magnetic field strength of 11.7440 Tesla. The instrument was equipped with a 5 mm dual probe, optimized for one-dimensional (1D) experiments, allowing precise detection of both proton and carbon resonances.

The resulting ^1H NMR spectrum provided valuable insights into the types and chemical environments of hydrogen atoms present in the molecule, revealing characteristic signals corresponding to methylene, methine, and hydroxyl groups typical of acetogenins. Similarly, the ^{13}C NMR spectrum displayed well-defined resonances indicative of long aliphatic chains, tetrahydrofuran (THF) ring carbons, and the carbonyl carbons of the γ -lactone moiety structural features consistent with known acetogenin frameworks. Together, these spectral data offered

conclusive evidence supporting the successful isolation and structural identification of the major acetogenin compound, as summarized in Table 1, which presents the detailed ^1H and ^{13}C chemical shift assignments.

Table 1. ^1H and ^{13}C NMR data of the isolated acetogenin.



Annonacina		
Carbono	δ ^1H (J em Hertz)	δ ^{13}C
1		174.8
2		131.2
3a	2.38; 1H, m	33.5
3b	2.51; 1H, m	
4	3.8; 1, m	70.0
5-8	1.2-1,7, m	22-38
9	1.2-1,7, 2H, m	22-38
10	3.59; 1H, m	71.9
11	1.2-1,7, 2HH, m	22-38
12-14	1.2-1,7, 6H, m	22-38
15	3.45; 1H, m	74.4
16	3.8; 1H, m	82.7
17a	1.97; 1H, m	29
17b	1.64; 1H, m	29
18a	1.97; 1H, m	29
18b	1.64; 1H, m	29
19	3.8; 1H, m	82.9
20	3.45; 1H, m	74.3
21-31	1.2-1.7; 22H, m	22-38
32	0.87; 3H	14.3
33	7.2; 1H	152.1
34	5.04; 1H	78.2
35	1.4; 3H	19.2

Following the confirmation of compound purity by analytical HPLC and subsequent characterization through ^1H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, the identity of the isolated compound was definitively established by comparison with spectral data previously reported in the literature [22]. The isolated compound was identified as annonacin, an acetogenin belonging to the Annonaceae family, as previously proposed by Liaw et al. [20]. Considering that cancer is a disease with complex etiology and pathophysiology, the search for

new and effective antitumor agents remains of profound clinical importance. In this context, the exploration and development of bioactive natural compounds such as annonacin offer valuable therapeutic potential, providing more selective and efficient treatment alternatives with reduced side effects and lower cost compared to conventional therapies [23].

According to Leng et al. [23], traditional cancer therapies such as radiotherapy and chemotherapy often lack specificity, resulting in collateral damage to healthy tissues and causing severe adverse effects. In contrast, naturally derived antitumor substances, such as annonacin, tend to exhibit greater selectivity toward cancer cells, making them attractive alternatives or adjuncts to current treatment regimens.

Given the broad spectrum of biological activities exhibited by acetogenins, particularly their ability to modulate cancer cell metabolism and induce apoptosis, the use of semi-preparative liquid chromatography proved to be an effective and reliable strategy for their isolation. The purified annonacin obtained through this approach not only serves as a reference standard for further analytical and pharmacological studies but also provides a foundation for the preparation of standardized extracts containing defined concentrations of acetogenins. Such standardized preparations can facilitate more targeted biological evaluations, contributing to the development of novel natural product-based therapeutic strategies against cancer.

Conclusion

Through the combined application of chromatographic techniques ranging from Thin Layer Chromatography (TLC) to High-Performance Liquid Chromatography (HPLC) it was possible to obtain fractions rich in acetogenins from both the leaves and seeds of *Annona muricata* L., and to successfully isolate the major acetogenin present in the ethanolic seed extract. Subsequent Nuclear Magnetic Resonance (NMR) analyses of ^1H and ^{13}C spectra confirmed that the isolated compound corresponded to annonacin, the predominant acetogenin in extracts of *A. muricata*, in agreement with findings previously reported for other species of the *Annona* genus. Once isolated and structurally characterized, annonacin can serve as a reference standard for quantitative analyses of extracts derived from *Annona* species and other plants containing this bioactive molecule. Moreover, its isolation enables more specific pharmacological applications, allowing for detailed evaluation of its direct biological and therapeutic effects.

Based on the isolation process described herein, it can be estimated that approximately 1 gram of pure annonacin can be obtained from 2 kilograms of *A. muricata* seeds, demonstrating the practicality and efficiency of the methodology developed in this study. Overall, the integration of chromatographic and spectroscopic techniques proved to be highly effective in the investigation and characterization of natural products, enabling the successful isolation of annonacin and paving the way for its application in the development of standardized plant extracts and targeted pharmacological studies. This work highlights the importance of combining advanced analytical methods with natural product research to promote the discovery and rational utilization of bioactive compounds from medicinal plants.

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Author Contributions

A.A.S. Silva: Conducted extraction, chromatographic procedures, and data analysis.
R.H.A.H. Lima: Assisted with chromatography, sample preparation, and data interpretation.
M. Muthuvel: Provided methodological support and contributed to HPLC optimization.
I.M. Lima: Assisted with NMR acquisition and interpretation. F.N.P. Mendes: Assisted with plant material processing and extract preparation. Í.G.P. Vieira: Conceptualization, project supervision, manuscript review, and final approval of the version to be submitted.
All authors read and approved the final manuscript.

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Ethics declarations

Ethics

The plant material (*Annona muricata* L) used in this study was obtained as a donation from the company Fruta Boa, Limoeiro do Norte, Ceará, Brazil. The plant material originated exclusively from legally cultivated sources, and no wild plant collection was performed for this research.

The collection, donation, and use of the plant material complied with local and national regulations, in accordance with the legislation of the Brazilian Ministry of Agriculture and Livestock (MAPA) under Law No. 10,711 of August 5, 2003, which establishes the National System of Seeds and Seedlings.

All necessary permissions and authorizations applicable to cultivated plant material were duly observed, and no additional specific collection permits or licences were required for this study.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Data availability

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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