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Chemical Composition and Antibacterial Activity of the Essential Oils of the Amazon *Annona* Species

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This study evaluated the chemical composition of essential oils (EOs) obtained through hydrodistillation from four *Annona* species native to the Amazonas state: *Annona amazonica* R.E. Fr. (commonly known as "envireira"), *Annona mucosa* Jacq. ("biribá"), *Annona exsucca* DC. ex Dunal ("araticum"), and *Annona* insignis R.E. Fr. ("araticum"). Additionally, their antibacterial activities were assessed. Using GC-FID and GC-MS, the major compounds identified were β-caryophyllene, from the leaves of *Annona amazonica* (14.7%) and *A. exsucca* (22.2 and 15.3%), γ-muurolene (20.2 and 15.4%) from the leaves of *A. exsucca*, caryophyllene oxide from the leaves of *A. insignis* (35.4%), (Z)-nerolidol from the fruits of *A. mucosa* (38.2%). The EO from *A. exsucca*, rich in γ-muurolene (20.2%), exhibited significant antibacterial activity against the gram-positive bacterium *Streptococcus sanguinis* (MIC: 10 μg.mL^{-1}).

Graphical abstract

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1. Introduction

Annona is one of the most representative genera of Annonaceae in the Brazilian flora because it produces edible fruits of economic value, such as *Annona muricata* L. ("graviola"), *A. mucosa* Jacq ("biribá"), *A. cherimola* Mill. ("cherimólia"), *A. squamosa* L. ("pinha or fruta-do-conde") and *A. atemoya* ("atemóia"), a hybrid between *A. cherimola* and *A. squamosa* [1, 2]. *Annona* species are characterized by their syncarpous fruits that lack an abscission zone between the seed-bearing part and the stalk, which is one of the characters used for sectional classification in *Annona* [3]. In the Amazon, the fruits known as biribá [*Annona mucosa* Jacq. syn. *Rollinia*

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mucosa (Jacq.) Ball.] are widely consumed by the local population and are generally sold and consumed fresh [4, 5].

The chemical composition of the essential oils (EOs) from leaves of *Annona* species and their bioactivities have been investigated in several studies carried out in the different regions of Brazil. In Northeast, the main components identified were *(E)*-caryophyllene (27.4%) and germacrene D (17.1%) of *A. squamosa;* bicyclogermacrene (39.0%), and spathulenol (14.0%) of *A. vepretorum* [6]; bicyclogermacrene (20.3, 45.4%) and (*E*)-caryophyllene (19.9, 14.6%) of *A. salzmannii* and *A. pickelii,* respectively [7], this essential oil of *A. vepretorum* showed a potente trypanocidal activity [6]. Another study with EO of *A. vepretorum* showed neurobiological activity and a predominance of (*E*)-β-ocimene (42.59%) and bicyclogermacrene (18.81%) [8]. The EO of Southeastern species was composed mainly of α-santalene (15.5%) and bicyclogermacrene (12.5%) of *A. acutiflora* [9], nerolidol (57.1%) of *A.crassiflor a* [10], bicyclogermacrene (39.8%) of *A. coriacea* [11], germacrene D (42.82%) and bicyclogermacrene (14.28%) of *Annona × atemoya* graft [12]. The main compounds of *A. sylvatica*. EO from Central-West region were the sesquiterpenes hinesol, z-caryophyllene, β-maaliene, γ-gurjunene, silphiperfol-5-en-3-o and ledol, the anti-inflammatory and anticancer activities this EO was evaluated [13]. Essential oil of *A. foetida* from the Amazon basin showed as major components bicyclogermacrene (35.12%), (*E*)-caryophyllene (14.19%), the antimicrobial activity of these essential oils was also evaluated [14].

In this paper, we report the chemical composition of the EOs of the species *A. amazonica* R.E. Fr. (known in Amazonas as "envireira"), *A. mucosa* Jacq. ("biribá"), *A. exsucca* DC. ex Dunal ("araticum") and *A. insignis* R.E. Fr. ("araticum") and we also report their bactericidal activity.

2. Results and Discussion

Gas chromatography-mass spectrometry (GC-MS) analysis allowed us to determine the chemical composition of the essential oils, with 93.2 to 99.0% of the components identified (Table 1). The chemical components identified in the essential oils obtained from individuals of the four species, including their respective retention indices and percentages, are available in the supplementary material. The number of compounds identified were 59 from the leaves and 60 from the branches of *A. amazonica*; 64 from the fruits of *A. mucosa*; 61 from the leaves of *A. insignis*; 49 and 44 from the leaves of *A. exsucca* collected at different locations. Sesquiterpene hydrocarbons were predominant in the essential oils of the leaves of *A. amazonica* and *A. exsucca*, while oxygenated sesquiterpenes were prevalent in the essential oils of the branches of *A. amazonica*, the fruits of *A. mucosa* and the leaves of *A. insignis*. The essential oil from *A. amazonica* branches also showed a high content of monoterpenes.

The essential oil of *Annona amazonica* was mainly composed of sesquiterpenes (95.1%), which the sesquiterpene hydrocarbon β-caryophyllene (14.7%) the main component. This sesquiterpene was also found in high percentages in the EO of *A. exsucca* (22.2 and 15.3%), which showed some variation in the levels of predominant sesquiterpene hydrocarbons, such as *β*-caryophyllene, *γ*-muurolen, *β*-elemene and *γ*-muurolene, in leaves collected at different locations (INPA and UFAM) (Table 2). The oxygenated sesquiterpene caryophyllene oxide was the predominant compound (35.4%) in the essential oil of *A. insignis* leaves, while (*Z*)-nerolidol (38.2%) was the major constituent in *A. mucosa* fruits. In *Annona amazonica* branches, the sesquiterpenes represented 58.0% of the total oil and the major constituents were the monoterpenes linalool (11.4%) and *o*-cymene (8.7%), in addition to the oxygenated sesquiterpene α-cadinol (10.0%), highlighting the difference in the composition of the essential oil in different parts of the same individual. These are the first reports of studies on EOs of *A. amazonica*, *A. insignis* and *A. mucosa*.

Table 1. Relative percentages of chemical constituents identified from essential oils of *Annona* spp.

Types of chemical constituents	AaF	AaG	AMFr	AeF-I	AeF-U	AiF
			%			
Monoterpenes hydrocarbons	0.7	18.8	0.7	6.3	0.9	1.7
Oxygenated monoterpenes	3.2	21.8	19.8	1.7	0.3	0.7
Sesquiterpene hydrocarbons	68.2	23.2	14.0	81.1	77.7	35.0
Oxygenated sesquiterpenes	26.9	34.8	60.1	10.4	20.5	55.8
Not identified	1.0	1.4	5.4	0.5	0.6	6.8
Total identified	**99.0**	**98.6**	**94.6**	**99.5**	**99.4**	**93.2**

AaF - *A. amazonica* leaves; AaG - *A. amazonica* branches; AmFr - A. *mucosa* fruits; AeF-I - *A. exsucca* leaves INPA; AeF-U - *A. exsucca* leaves UFAM; AiF - *A. insignis* leaves

Table 2. Major constituents of the essential oils of *Annona* spp

Species	Plant part	Majority Constituents (%> 5)
Annona amazonica	leaves**	β-caryophyllene (14.7%), *cis*-β-guaiene (6.6%), caryophyllene oxide (6.3%), α-copaene (5.7%), α-amorphene (5.4%). β-copaene (5.2%).
	branches**	linalool (11.4%), α-cadinol (10.0%), *o*-cymene (8,7%), γ-muurolene (5.7%)
Annona exsucca	leaves *	β-caryophyllene (22.2%), β-elemene (17.2%), γ-muurolene (15.4%), α-humulene (5.3%)
	leaves **	γ-muurolene (20.2%), β-caryophyllene (15.3%), β-elemene (9,9%), caryophyllene oxide (5,1%)
Annona insignis	leaves *	caryophyllene oxide (35.4%), β-caryophyllene (12.8%)
Annona mucosa	fruits*	(*Z*)-nerolidol (38.2%), *iso*-verbanol (11.6%)

* collected on the INPA campus (Manaus,Amazonas), ** collected on the UFAM campus (Manaus, Amazonas).

In this analysis, β-caryophyllene was the major component in the leaves of *Annona amazonica* and *A. exsucca* This sesquiterpene hydrocarbon has been found as a major constituent in leaves of *Annona* species collected in Brazil [5], as well as in leaves of species from other botanical families [15,16]. The variation in the contents of the predominant hydrocarbon sesquiterpene in *Annona exsucca* that was collected at INPA and UFAM may be related to the collection period. Cascaes et al. 2022 [17] verified the possible influences of climatic variables on the chemical composition of the EOs in *A. exsucca* and climatic parameters were obtained during two collection periods (March and September). Higher levels of β-caryophyllene, germacrene D, β-elemene, bicyclogermacrene, and α-humulene were observed in March and a higher content of linalool and sylvestrene was observed in September.

In the essential oil of *A. insignis* leaves, caryophyllene oxide was predominant (35.4%). The literature reports high levels of this oxygenated sesquiterpene in species of *Guatteria* (Annonaceae) [18,19]. (Z)-Nerolidol was found in high levels in the fruit of *Annona mucosa* (38.2%). Nerolidol (*trans* and a *cis* form), also known as peruviol, is a naturally occurring sesquiterpene alcohol present in the EO of various plants with a floral odor. This sesquiterpene is a fragrance ingredient used in cosmetics, fine fragrances, shampoos, soaps and other personal care and household cleaning products, which has generated interest in investigating its toxic effects [20,21].

The samples of essential oil from leaves of *Annona exsucca* and *A. insignis* were subjected to assay against six strains of bacteria but the antibacterial activity was more expressive for samples of *A. exsucca* rich in γ-murolene (20.2%), β-caryophyllene (15.3%) and β-elemene (9,9%); *A. insignis* rich in caryophyllene oxide (35.4%) and β-caryophyllene (12.8%) against the gram-positive bacteria *S. sanguinis* (MIC 10 μg.mL^{-1}) and *S. aureus* (MIC 80 μg.mL^{-1}), respectively.

Essential oils and their components have been studied for their antimicrobial activities and various mechanisms of action have been proposed, however some mechanisms of antibacterial activity remain poorly studied [22]. Alcantara et al., (2017) reported the antibacterial activity of essential oils extracted from *Xylopia aromatica* and *Guatteria blepharophylla* against *Streptococcus sanguinis* and *Staphylococcus aureus*. These essential oils presented molecular diversity of the general composition but the sesquiterpene caryophyllene oxide was predominant in *X. aromatica* while *G. blepharophylla* a higher content of sesquiterpene spathulenol, monoterpenes *trans*-pinocarveol and dihydrocarveol were observed [18]. These results allied to obtained in our study with the species *Annona exsucca* and *A. insignis* show the possibility of synergy between the components to benefit the activity in *S. sanguinis* and *S. aureus*.

3. Material and Methods

3.1. Plant material: collection and extraction of the essential oils

The plant samples were collected from individuals at the Universidade Federal do Amazonas (UFAM) and National Institute for Amazonian Research (INPA) in the city of Manaus, Amazonas, Brazil (List 1). The samples were dried at room temperature under reduced light, then ground in a grinder and submitted to hydrodistillation (in triplicate) for four hours using a modified Clevenger-type apparatus. The resulting distilled oils were dried over anhydrous sodium sulphate (Merck) and stored in amber glass bottles in a refrigerator (4 °C).

List 1. Collection data of *Annona* species and essential oil yields.

Species	Plant part	Collection site/ month	registration code	Yield (%)
Annona amazonica	Leaves	UFAM/Setember	HUAM 9237	0.22 ± 0.04
	And branches			0.13 ± 0.00
Annona exsucca	Leaves	UFAM/Setember	HUAM 9228	0.24 ± 0.01
	Leaves	INPA/June	INPA 245126	0.21 ± 0.03
Annona insignis	Leaves	INPA/June	INPA 245130	0.16 ± 0.00
Annona mucosa	Fruits	INPA/January	INPA 245127	0.15 ± 0.02

3.2. Analysis of the essential oils by gas chromatograph coupled to a mass spectrometer

The determination of the chemical profiles of the essential oils was performed in a gas chromatograph coupled to a mass spectrometer (QP 5000, Shimadzu) equipped with an OV-5 MS capillary column (30 m x 0.25 mm x 0.25 μm) and used helium as the capillary gas. The system was operated in full scan mode with electron impact (70 eV), and ranged from 40 to 450 m/z. The injector was kept at 220 °C, with a carrier gas flow rate of 1:20 and temperature programming of 60 °C – 240 °C (3 °C min^{-1}). Samples of the oil were diluted in ethyl acetate, and 1 μL of solution was injected.

For the quantitative analysis, a gas chromatograph with a flame ionization detector (Shimadzu, CG-2010) was used. The system was equipped with an OV-5 capillary column, helium as the carrier gas, injector at 250 °C, detector at 290 °C, 1:20 split and the same temperature program as the GC-MS system.

Each essential oil was analyzed in triplicate via GC-FID and GC-MS. The retention indices (KI) were calculated in relation to the elution times of essential oil compounds and a series of *n*-alkanes (C9-C24, Sigma, St. Louis, MO, USA), co-injected with the sample in GC-FID. The constituents were identified with the data set of retention indices, and mass spectra were compared with the data found in the literature [23] and the NIST 12, NIST62 and WILEY 139 databanks.

3.3. Antibacterial assay

The bacterial strains used for screening were Gram-positive *Staphylococcus aureus* (ATCC6538), *Enterococcus faecalis* (ATCC29212) and *Streptococcus sanguinis* (ATCC10556) and Gram-negative *Pseudomonas aeruginosa* (ATCC9027), *Salmonella enterica* (ATCC13076) and *Escherichia coli* (ATCC 8739). The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) on 96-well culture plates using the microdilution method, starting from a solution at a concentration of 20 mg.mL^{-1} in 10% DMSO. A metabolic test was performed by adding 10 μL of resazurin (1%). A solution of chlorhexidine digluconate (2%) served as the positive control, and the negative control was the same solution of DMSO 10% used to solubilize the essential oils. The samples

of essential oil from *Annona exsucca* and *A. insignis* were tested in triplicate and resazurin was used as an indicator of bacterial growth.

4. Conclusions

The study of the volatile constituents of four species of *Annona* occurring in the state of Amazonas contributes to the knowledge of the aromatic flora of the Campus of the Federal University of Amazonas (UFAM) and the National Institute for Amazonian Research (INPA) in addition to aggregate chemical knowledge to the Annonaceae family of Amazon region.

Supporting Information

Supplementary material is available.

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Author Contributions

Maria da Paz Lima designed the study and supervised the phytochemical analyses. All authors participated in the formal analysis, investigation, methodology and validation. All authors contributed to writing the manuscript.

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