

RESEARCH ARTICLE

Exploring the chemical composition, in vitro and in silico study of the anticandidal properties of annonaceae species essential oils from the Amazon

Márcia Moraes Cascaes^{1*}, Sílvia Helena Marques da Silva², Mozaniel Santana de Oliveira^{3,4*}, Jorddy Neves Cruz⁵, Ângelo Antônio Barbosa de Moraes⁵, Lidiane Diniz do Nascimento³, Oberdan Oliveira Ferreira³, Giselle Maria Skelding Pinheiro Guilhon¹, Eloisa Helena de Aguiar Andrade^{1,3}

1 Programa de Pós-Graduação em Química, Universidade Federal do Pará, Belém, PA, Brazil, **2** Seção de Bacteriologia e Micologia LabMicol—SABMI Laboratório de Micologia, Instituto Evandro Chagas—IEC/SVS/MS, Ananindeua, Brazil, **3** Laboratório Adolpho Ducke, Coordenação de Botânica, Museu Paraense Emílio Goeldi, Belém, Brazil, **4** Programa de Pós-Graduação em Ciências Biológicas—Botânica Tropical, Universidade Federal Rural da Amazônia and Museu Paraense Emílio Goeldi, Belém, PA, Brazil, **5** Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Universidade Federal do Pará, Belém, PA, Brazil

* cascaesmm@gmail.com (MMC); mozaniel.oliveira@yahoo.com.br (MSO)



OPEN ACCESS

Citation: Cascaes MM, Marques da Silva SH, de Oliveira MS, Cruz JN, de Moraes ÂAB, do Nascimento LD, et al. (2023) Exploring the chemical composition, in vitro and in silico study of the anticandidal properties of annonaceae species essential oils from the Amazon. PLoS ONE 18(8): e0289991. <https://doi.org/10.1371/journal.pone.0289991>

Editor: Guadalupe Virginia Nevárez-Moorillón, Universidad Autonoma de Chihuahua, MEXICO

Received: May 22, 2023

Accepted: July 30, 2023

Published: August 24, 2023

Peer Review History: PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0289991>

Copyright: © 2023 Cascaes et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper.

Abstract

Chemical composition of the essential oils (EOs) from the leaves of five *Annonaceae* species found in the amazon region was analyzed by Gas chromatography coupled to mass spectrometry. The antifungal activity of these EOs was tested against *Candida albicans*, *Candida auris*, *Candida famata*, *Candida krusei* and *Candida tropicalis*. In addition, an in silico study of the molecular interactions was performed using molecular modeling approaches. Spathulenol (29.88%), α -pinene (15.73%), germacra-4(15),5,10(14)-trien-1- α -ol (6.65%), and caryophyllene oxide (6.28%) were the major constituents from the EO of *Anaxagorea dolichocarpa*. The EO of *Duguetia echinophora* was characterized by β -phellanderene (24.55%), cryptone (12.43%), spathulenol (12.30%), and sabinene (7.54%). The major compounds of the EO of *Guatteria scandens* were β -pinene (46.71%), α -pinene (9.14%), bicyclogermacrene (9.33%), and *E*-caryophyllene (8.98%). The EO of *Xylopiya frutescens* was characterized by α -pinene (40.12%) and β -pinene (36.46%). Spathulenol (13.8%), *allo*-aromadendrene epoxide (8.99%), thujopsan-2- α -ol (7.74%), and muurola-4,10(14)-dien-1- β -ol (7.14%) were the main chemical constituents reported in *Xylopiya emarginata* EO. All EOs were active against the strains tested and the lowest inhibitory concentrations were observed for the EOs of *D. echinophora*, *X. emarginata*, and *X. frutescens* against *C. famata* the Minimum Inhibitory Concentration values of 0.07, 0.019 and 0.62 $\mu\text{L}\cdot\text{mL}^{-1}$, respectively. The fungicidal action was based on results of minimum fungicidal concentration and showed that the EOs showed fungicide activity against *C. tropicalis* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$), *C. krusei* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$) and *C. auris* (5 $\mu\text{L}\cdot\text{mL}^{-1}$), respectively. The computer simulation results indicated that the major compounds of the EOs can interact with molecular targets of *Candida* spp.

Funding: The author M.M.C. thanks CAPES for the Phd scholarship process number: [88887.497476/2020-00]. The author A.A.B.d.M. thanks CNPq for the scientific initiation scholarship. The author Mozaniel Santana de Oliveira thanks PDPG-POSDOC - Programa de Desenvolvimento da Pós-Graduação (PDPG) Pós-Doutorado Estratégico, as well as CAPES for the scholars-hip (process number: (88887.852405/2023-00)). The authors thanks Edital 02/2023 - PAPQ/PROPESP - Universidade Federal do Para. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

1. Introduction

Fungal infections pose a threat to global health and annually, more than 150 million serious cases of fungal infections in humans occur worldwide, resulting in approximately 1.7 million deaths a year [1, 2]. The number of fungal species resistant to currently marketed drugs is increasing [3]. Among the microorganisms that cause fungal infections, the most aggravating problems include the resistance of *Candida* species, which cause infections in humans, mainly *C. albicans* [4].

Although several synthetic drugs are commercially available for the control of infections caused by fungi, there is a need to explore new efficient antifungal drugs, which may include naturally occurring compounds such as essential oils (EOs). The antimicrobial action of the different types of essential oils against microorganisms has been subject of several studies [1, 2, 5, 6], and molecular targets that are targeted by commercial drugs for this disease include Sterol 14 α -Demethylase Cytochrome P450 proteins [7].

The EOs are responsible for the characteristic smell and/or flavor of plants and come from their secondary metabolism [8]. From a chemical point of view EOs are organic and volatile substances, composed mostly of terpenoids and phenylpropanoids and generally have a molecular weight of less than 300 Da [6]. Among the constituents present in EOs, terpenoids represent a large group of phytochemicals with promising antimicrobial activity [9]. The interactions between the chemical constituents present in an EO can cause additive, antagonistic or synergistic effects. When the overall effect of the mixture of EO constituents is greater than the sum of the individual actions of these substances, a synergistic effect occurs, while the additive effect results from the combinations of the sum of the effect of each chemical substance. Antagonism is considered when the efficacy of two or more compounds is less than that of one of them [6].

EOs are produced by more than 17,500 species of plants belonging to several families of Angiosperms [8], such as *Annonaceae*. This family is recognized for the economic importance of the fruits, ethnobotany, raw material for cosmetic products, in addition to produce volatile chemical constituents with antimicrobial activity [10, 11]. Some EOs obtained from *Annonaceae* species showed relevant results against fungal microorganisms, such as *Anaxagorea brevipes* Benth. [12], *Annona vepretorum* Mart. [13], and *Duguetia lanceolata* A.St.-Hil [14].

Considering the increase in the incidence of fungal infections in humans, the limitations of available therapeutic strategies and the resistance to certain synthetic drugs, EOs represent promising natural sources for the development of effective antifungal drugs. In this study, the chemical composition and the antifungal activity of EOs from the leaves of some species of *Annonaceae* (*Anaxagorea dolichocarpa* Sprague & Sandwith, *Duguetia echinophora* R.E.Fr., *Guatteria scandens* Diels, *Xylopia emarginata* Mart. and *Xylopia frutescens* Aubl.) were evaluated against *Candida* spp. In addition, were used molecular modeling approaches to investigate how the main compounds of the EOs of these species can interact with Sterol 14 α -Demethylase Cytochrome P450 proteins, molecular targets of *Candida* spp.

2. Materials and methods

2.1. Botanical material

The leaves of *A. dolichocarpa*, *D. echinophora*, *G. scandens*, *X. emarginata* and *X. frutescens* were collected in the municipality of Magalhães Barata (State of Pará, Amazon region, Brazil) in May 2019 (00°47'51.6" S; 047°33'38.4" W). The samples were identified by Dr. Jorge Oliveira, a parataxonomist from the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil. Voucher specimens were deposited at the Herbarium of MPEG under registration code MG-

237513 for *A. dolichocarpa*, MG-237493 for *D. echinophora*, MG-237508 for *G. scandens*, MG-237506 for *X. emarginata* and MG-237492 for *X. frutescens*.

2.2. Preparation of botanical material and extraction of essential oils

The leaves of *Annonaceae* species were dried in an air-circulation oven for five days at 35°C, after that they were crushed in a knife mill (Tecnal, model TE-631/3, Brazil). Moisture content was analyzed using a moisture analyzer (Marte, model ID50, Brazil). The EOs were extracted by hydrodistillation in a glass modified Clevenger-type apparatus [15], using 150 g of plant material for each experiment. Hydrodistillations were carried out for 3 h at 100°C. The EOs were dried over anhydrous sodium sulfate and stored in a freezer at -10°C. The yields of EOs (%) were calculated from the plant dry weight and expressed in mL/100 g of dried material.

2.3. Chemical composition analysis

The chemical composition of the EOs were analyzed using chromatography/mass spectrometry (GC/MS) using a Shimadzu QP Plus 2010 GC-MS (Kyoto, Japan) protocols reported earlier by our research group [16, 17]. The retention index was calculated for all volatile constituents using a homologous series of *n*-alkanes (C₈-C₄₀, Sigma-Aldrich, United States) according Van den Dool and Kratz [18], and the compounds were identified by comparing their mass spectrum and retention index with the data from the libraries [19].

2.4. Antifungal activity of the essential oils

2.4.1. Microorganisms. Five fungal species were used to analyze the antifungal activity and includes *Candida albicans* INCQS40175, *C. tropicalis* ATCC 6258, *C. famata* ATCC 62894, *C. krusei* ATCC 13803 and *C. auris* IEC-01. Each microorganism was cultivated in Sabouraud agar at 37°C for 48 h before the beginning of the tests. These tests were performed at the Laboratory of Superficial and Systemic Mycoses of Instituto Evandro Chagas (IEC), Ananindeua, Pará, Brazil.

2.4.2. Agar disc diffusion test. The antifungal activity of the EOs was evaluated using the agar disc diffusion method [20] with modifications. Paper filter discs (6 mm) were impregnated with 20 µL of each EO. The suspensions of the test microorganisms were prepared with 0.45% saline solution (0.5 on the McFarland scale). Each microorganism suspension was spread on the surface of Sabouraud agar culture medium in Petri dishes (15 × 90 mm). Then, paper discs impregnated with the extracts were placed on the surface of the plates inoculated with the microorganisms. The plates were incubated for 48 h at 37°C. After this period, visual readings were taken, observing the presence of growth inhibition zone measured in millimeters, with the help of a millimeter ruler. As a positive control, paper discs were impregnated with a 30 µL nystatin solution. All tests were carried out in duplicate. Inhibition zones ≥ 8 mm indicated that the microorganism was sensitive to the tested essential oil, according to the classification early proposed [21–23].

2.4.3. Broth microdilution: Determination of MIC. The susceptibility of the microorganisms to the Eos was determined by the broth microdilution method recommended by the “US National Committee for Clinical Laboratory Standards” (NCCLS) [24], with adaptations. The microorganisms were cultivated in Sabouraud agar at 37°C for 48 h. From these cultures, cellular suspensions similar to the McFarland scale 0.5 were prepared. In a 96-well plate, serial dilution at a ratio of 2 of each EO to be tested was performed, starting from 10% (20/180 µL), in a final volume of 100 µL. Then, 100 µL of the yeast suspension was added. The final concentration in each well reached 50 µL.mL⁻¹, 25 µL.mL⁻¹, 12.5 µL.mL⁻¹, 6.2 µL.mL⁻¹, 3.1 µL.mL⁻¹, 1.5 µL.mL⁻¹, 0.7 µL.mL⁻¹, 0.3 µL.mL⁻¹, 0.19 µL.mL⁻¹ and 0.0 µL.mL⁻¹. DMSO was used for the

dilutions. After adding the yeast to the previously diluted oils, the plate was incubated at 37°C for 48 h, and at the end, the readings were taken visually, and the lowest concentration of EO capable of inhibiting visible fungal growth was recorded. The test was carried out in duplicate [25].

2.4.4. Broth microdilution: Determination of MFC. After completion of the test and visual reading of the broth microdilution to determine minimum inhibitory concentration (MIC), the test to determine the minimum fungicidal concentration (MFC) was carried out. The test consisted of plating 10 μ L of each dilution in Sabouraud agar and incubating at 37°C for 48 h. After this period, the lowest dilution capable of killing 99.5% of the original inoculum was recorded. The test was carried out in duplicate [25]. To calculate the minimum inhibitory concentrations (MIC), the protocols of Kowalska-Krochmal et al., [26] and minimum fungicidal concentration (MFC) Rex et al., [27], Both using Excel software.

2.5. Molecular docking

The molecular structure of β -phellandrene, cryptone, spathulenol, and pinene were obtained in PubChem; then their structures were optimized with B3LYP/6-31G* [28, 29] using Gaussian09 program [30]. We used the molecular docking method to evaluate the compound's interaction mode with Sterol 14 α -Demethylase Cytochrome P450 (CYP51). For this, we used the Molegro Virtual Docker 5.5 [31, 32], and the crystal structure used as molecular targets can be found in the Protein Data Bank using the ID: 5TZ1 [7]. The MolDock Score (GRID) scoring function was used with a Grid resolution of 0.30 Å and 5 Å radius encompassing the entire connection cavity. The MolDock SE algorithm was used with a number of runs equal to 10, 1500 max interactions, and a max population size equal to 50. The maximum evaluation of 300 steps with a neighbor distance factor equal to 1 and an energy threshold equal to 100 was used during the molecular docking simulation.

3. Results and discussions

3.1. Chemical composition and yield of the essential oils

The chemical composition and the yield of the EOs obtained from *Annonaceae* species are reported in Table 1. The EO yields showed a variation between 0.16 and 1.53% and the highest yield was found for the EO from *X. frutescens* (1.53%).

In another study the yield found for the *X. frutescens* EO was 1.00% [33]. The EO of a specimen of *X. emarginata* collected in the Caxiuanã National Forest, Melgaço, State of Pará State, Brazil showed a higher yield (0.3%) than that described in the present work [34]. The yield in EOs from the fruits of *A. dolichocarpa* was 0.50% [35], while the EO yield in the leaves and fine stems yielded 0.20% and 0.10%, respectively [36].

Comparison between results found in this work with others library indicates that the variation in EO yields of *Annonaceae* species can be correlated with abiotic factors. In general, the EO yield of a plant species varies according to the part, seasonality and geographic distribution, among other factors [37, 38]. Understanding the factors that determine the EO yield of a plant species, especially those of commercial interest, is very important to optimize conditions that can improve it.

In total, 98 chemical constituents were identified in the five analyzed EOs, representing an average of 91.97%. Hydrocarbon monoterpenes were predominant in the EOs of *D. echinophora* (47.24%), *G. scandens* (57.15%) and *X. frutescens* (89.18%), while oxygenated sesquiterpenes were found in greater proportion in the EOs of *A. dolichocarpa* (51.15%) and *X. emarginata* (57.20%).

Table 1. Yield, and chemical composition of the essential oils of *Annonaceae* species.

Essential Oil Yield (%)			A _{dol}	D _{ech}	G _{sca}	X _{ema}	X _{fru}
RI _L	RI _C	Constituents (%)	0.16	0.82	0.24	0.17	1.53
924	920	α -Thujene		0.18			6.05
932	929	α -Pinene	15.73	4.52	9.14		40.12
969	960	Sabinene		7.54			
974	976	β -Pinene		5.13	46.71		36.46
988	985	Myrcene		2.03			0.77
1003	1000	<i>p</i> -Mentha-1(7),8-diene		1.02			
1002	1000	α -Phellandrene					0.64
1014	1008	α -Terpinene		0.37			0.19
1022	1012	<i>o</i> -Cymene					0.30
1025	1019	Sylvestrene			1.30		4.26
1025	1022	β -Phellandrene		24.55			
1054	1052	γ -Terpinene		1.03			0.39
1086	1114	Terpinolene		0.87			
1095	1093	Linalool			0.45		0.66
1102	1100	Perillene		0.12			
1135	1133	<i>trans</i> -Pinocarveol		0.33		1.38	0.35
1136	1134	<i>trans-p</i> -Menth-2-en-1-ol		0.21			
1140	1135	<i>trans</i> -Verbenol	0.27				
1137	1136	<i>cis</i> -Verbenol					0.12
1160	1156	Pinocavone	0.48			0.42	0.15
1166	1162	<i>p</i> -Mentha-1,5-dien-8-ol	1.00			0.46	
1174	1171	Terpinen-4-ol		1.9	0.15		0.29
1183	1180	Cryptone		12.43			
1186	1185	α -Terpineol			0.12	0.13	0.29
1194	1191	Myrtenol	2.05	0.23		2.07	0.55
1204	1206	Verbenone				0.66	
1207	1220	<i>trans</i> -Piperitol		0.12			
1215	1214	<i>trans</i> -Carveol		0.17		0.11	
1224	1249	3-isopropyl-Phenol		2.13			
1227	1303	Phellandral		2.54			
1239	1251	Carvone		0.30			
1249	1248	Geraniol					0.13
1289	1319	<i>p</i> -Cymen-7-ol		1.05			
1290	1285	γ -Terpinen-7-al		0.32			
1335	1333	δ -Elemene			3.39	0.43	1.41
1348	1346	α -Cubebene		0.10	0.17	0.11	
1369	1365	Cyclosativene				0.24	
1373	1369	α -Ylangene				0.35	
1374	1373	α -Copaene	2.50	0.85	2.48	0.30	
1374	1374	Isoledene			0.39		
1379	1377	Geranyl acetate					0.10
1387	1382	β -Bourbonene				0.19	
1389	1388	β -Elemene	2.86	2.43	0.65	1.62	0.18
1417	1438	<i>E</i> -Caryophyllene	0.64	1.33	8.98		
1434	1431	γ -Elemene			2.11	0.41	

(Continued)

Table 1. (Continued)

Essential Oil Yield (%)			A _{dol}	D _{ech}	G _{sca}	X _{ema}	X _{fru}
			0.16	0.82	0.24	0.17	1.53
1439	1437	Aromadendrene				0.16	
1452	1452	α -Humulene	0.46	0.26	0.52	0.13	
1478	1478	γ -Muuroolene				2.16	
1484	1478	Germacrene D			1.75	1.30	0.39
1489	1488	β -Selinene			0.47	1.41	
1492	1491	<i>cis</i> - β -Guaiene				0.61	
1493	1496	<i>epi</i> -Cubebol				1.05	
1495	1499	γ -Amorphene		0.20			
1500	1499	α -Muuroolene				0.89	
1500	1401	Bicyclogermacrene	1.37	0.19	9.33		4.22
1500	1503	α -Muuroolene	0.56	1.47			
1505	1506	β -Bisabolene		0.28			
1505	1507	<i>E,E</i> - α -Farnesene				0.26	
1509	1508	α -Bulnesene		0.17			
1511	1510	δ -Amorphene			0.47		
1513	1511	γ -Cadinene				1.67	
1514	1512	Cubebol	1.67				
1522	1519	δ -Cadinene	0.69	3.28		3.57	
1532	1531	γ -Cuprenene				0.2	
1544	1546	α -Calacorene		0.88		2.37	
1559	1588	Germacrene B			0.47		
1562	1554	<i>epi</i> -Longipinanol		0.14			
1564	1561	β -Calacorene		0.15			
1577	1575	Spathulenol	29.88	12.30	5.26	13.80	0.81
1582	1580	Caryophyllene oxide	6.27	1.96	0.99		
1586	1573	Thujopsan-2- α -ol				7.74	
1590	1581	Globulol					0.14
1592	1593	Viridiflorol			0.19		
1594	1591	Salvia-4(14)-en-1-one				6.45	
1596	1592	Fokienol	2.01				
1600	1606	Rosifoliol			0.19		
1608	1618	Humulene epoxide II	2.89	0.64		4.88	
1618	1646	1,10-di- <i>epi</i> -Cubenol			0.15		
1627	1633	1- <i>epi</i> -Cubenol		0.36			
1630	1631	Muurolo-4,10(14)-dien-1- β -ol				7.14	
1639	1645	<i>allo</i> -Aromadendrene epoxide	2.07			8.99	
1644	1635	α -Muurolol (= Torreyol)			0.76		
1645	1648	Cubenol				0.71	
1648	1650	<i>cis</i> -Guaia-3,9-dien-11-ol					0.22
1652	1658	Himachalol	0.30				
1652	1670	α -Cadinol		0.27			
1658	1672	<i>neo</i> -Intermedeol			1.75		
1668	1671	14-hydroxy-9- <i>epi</i> - <i>E</i> -Caryophyllene		0.21			
1675	1681	Cadalene				1.29	
1676	1682	Mustakone				1.48	
1679	1676	Khusinol				1.78	

(Continued)

Table 1. (Continued)

			A _{dol}	D _{ech}	G _{sca}	X _{ema}	X _{fru}
Essential Oil Yield (%)			0.16	0.82	0.24	0.17	1.53
1685	1689	Germacra-4(15),5,10(14)-trien-1- α -ol	6.65	0.16		0.84	
1690	1690	Z- α -trans-Bergamotol	2.41				
1759	1753	Benzyl benzoate			0.44		
1762	1770	β -Acoradienol					
1765	1772	β -Costol				1.79	
1767	1777	14-oxy- α -Muurolene				0.55	
2042	2042	Kaurene				0.32	
Hydrocarbon monoterpenes			15.73	47.24	57.15	-	89.18
Oxygenated monoterpenes			3.80	21.85	0.72	5.23	2.54
Hydrocarbon sesquiterpenes			9.08	11.59	31.18	19.67	6.20
Oxygenated sesquiterpenes			51.15	16.04	9.73	57.20	1.27
Others			-	-	-	0.32	-
Total			82.76	96.72	98.78	82.42	99.19

RI_C = Calculated retention index; RI_L = Literature retention index; A_{dol} = *Anaxagorea dolichocarpa*; D_{ech} = *Duguetia echinophora*; G_{sca} = *Guatteria scandens*; X_{ema} = *Xylopia emarginata*; X_{fru} = *Xylopia frutescens*.

<https://doi.org/10.1371/journal.pone.0289991.t001>

Spathulenol (29.88%), α -pinene (15.73%), germacra-4(15),5,10(14)-trien-1- α -ol (6.65%) and caryophyllene oxide (6.28%) where the major compounds of EO of *A. dolichocarpa*. The chemical composition described in this work was similar to that described by Andrade and coworkers, in which spathulenol (26.2%), α -pinene (16.8%) and β -pinene (12.3%) were described as the major constituents from the leaves EO of a specimen of *A. dolichocarpa* collected in the same locality [36]. The hydrocarbon monoterpene α -pinene showed significant antifungal activity against *Candida parapsilosis* [39] and *C. albicans* [40]. Antifungal activity of caryophyllene oxide was reported in a previous study [41].

The EO of *D. echinophora* was characterized by β -phellanderene (24.55%), cryptone (12.43%), spathulenol (12.30%) and sabinene (7.54%). Although there are no studies reporting the chemical composition from the EOs of *D. echinophora*, the EOs others species belonging to the genus *Duguetia* were rich on α -pinene, β -pinene, spathulenol, viridiflorene, bicyclogermacrene, caryophyllene oxide, germacrene D, β -caryophyllene and humulene epoxide II [42].

The chemical composition of the EOs from *G. scandens* also being reported for the first time in the literature. This study showed that the monoterpenes β -pinene (46.71%) and α -pinene (9.14%) and the sesquiterpenes bicyclogermacrene (9.33%) and *E*-caryophyllene (8.98%) were the major constituents of the EO of this species. Compounds β -pinene, α -pinene and *E*-caryophyllene have already been reported as the main constituents of *G. hispida* [10], while bicyclogermacrene was described as one of the major constituents of *G. pogonopus* Martius [43] and *G. australis* A.St.-Hi [44]. The positive enantiomers of β -pinene and α -pinene have antimicrobial activity against *C. albicans* [45], and *E*-caryophyllene showed antimicrobial activity against dermatophytic fungi [46]. Antimicrobial activity was also related to EOs that have bicyclogermacrene as major compounds [47].

Oxygenated sesquiterpenes spathulenol (13.8%), *allo*-aromadendrene epoxide (8.99%), thujopsan-2- α -ol (7.74%) and muurolo-4,10(14)-dien-1- β -ol (7.14%) were the main chemical constituents from the EO of *X. emarginata*. The EO from a specimen of *X. emarginata* collected in the Caxiuanã National Forest, Melgaço, State of Pará, Brazil, was characterized by high percentage of sesquiterpene spathulenol (73.00%) [34]. While the specimen collected in

Campo Grande, State of Mato Grosso do Sul, Brazil, showed spathulenol (34.40%), caryophyllene oxide (25.00%), myrtenal (7.7%) and *trans*-pinocarveol (6.3%) as the the major components [48].

The EO of *X. frutescens* was characterized by the monoterpenes α -pinene (40.12%) and β -pinene (36.46%). Previous studies with EO from leaves of a specimen of *X. frutescens* collected in the Municipality of Capela, State of Sergipe, Brazil, presented as major compounds the sesquiterpenes (*E*)-caryophyllene (31.48%), bicyclogermacrene (15.13%), germacrene D (9.66%), δ -cadinene (5.44%), viridiflorene (5.09%) and α -copaene (4.35%) [33]. The EO of another specimen collected in the State of Paraiba, Brazil, was characterized by caryophyllene (23.91%), γ -cadinene (12.48%), β -ocimene (8.19%), cadin-4-en-10-ol (5.78%), δ -cadinene (5.7%), viridiflorol (4.83%) and γ -elemene (4.55%) [49]. While the sesquiterpenes bicyclogermacrene (23.23%), germacrene D (21.16%), (*E*)-caryophyllene (17.24%) and β -elemene (6.35%) were the major constituents of EO of a specimen collected in the Serra de Itabaiana National Park, in the City of Itabaiana, State of Sergipe, Brazil [50].

Although there are qualitative and quantitative differences, the EOs of the five species described in this work were characterized by compounds belonging to the class of mono and sesquiterpenes and this composition is in accordance to other *Annonaceae* species [11, 51, 52].

3.2. Antifungal activity

The antifungal activity of the EOs from the leaves of *Annonaceae* species was estimated in terms of zone of inhibition (in millimeters) in well diffusion assay, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). All EOs were active against the tested microorganisms and the diameter of the halos varied from 7 to 26 mm (Table 2). The EO of *D. echinophora* showed the highest inhibition power (16–26 mm).

Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) are listed in Table 3. The results indicated that the EOs of *D. echinophora*, *X. emarginata* and *X. frutescens* showed a high ability to inhibit the microorganism *C. famata* and exhibited MIC values of 0.07, 0.019 and 0.62 $\mu\text{L}\cdot\text{mL}^{-1}$, respectively. Based on MFC results, the EOs of *D. echinophora*, *X. frutescens* and *X. emarginata* displayed a fungicide activity against *C. tropicalis* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$), *C. krusei* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$), and *C. auris* (5 $\mu\text{L}\cdot\text{mL}^{-1}$), respectively. The results presented by the different fractions of essential oils in the present study may be associated with the presence of different classes of compounds such as hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes, as several literatures have shown that these classes have potential antifungal activities [53–56], being more specific the major compounds identified in essential oils rich in β -phellandrene, cryptone, spathulenol, and β -pinene have shown potential antifungal activities [57–60], furthermore, recent studies on fungal infections reported that the pathogens *C. albicans*, *C. tropicalis*, *C.*

Table 2. In vitro effect of the essential oils of *Annonaceae* species on medically important yeasts using agar disc diffusion method.

Microorganism	Halo Diameter (mm)				
	A _{doi}	D _{ech}	G _{sca}	X _{ema}	X _{fru}
<i>C. albicans</i>	10	16	8	8	10
<i>C. auris</i>	7	20	7	10	9
<i>C. famata</i>	9	26	8	12	12
<i>C. krusei</i>	9	19	8	8	10
<i>C. tropicalis</i>	9	19	-	10	9

D_{ech} = *Duguetia echinophora*; X_{ema} = *Xylopiya emarginata*; X_{fru} = *Xylopiya frutescens*. Experiments performed in duplicate.

<https://doi.org/10.1371/journal.pone.0289991.t002>

Table 3. Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of essential oils of *Annonaceae* species on medically important yeasts; data expressed in $\mu\text{L}\cdot\text{mL}^{-1}$.

Microorganism	D_{ech}		X_{ema}		X_{fru}	
	MIC	MFC	MIC	MFC	MIC	MFC
<i>C. albicans</i>	0.31	> 5	2.5	> 5	5	>5
<i>C. auris</i>	0.62	> 2.5	5	5	5	>5
<i>C. famata</i>	0.07	> 0.31	0.019	> 0.07	0.62	> 2.5
<i>C. krusei</i>	0.31	> 1.25	0.62	> 2.5	2.5	5
<i>C. tropicalis</i>	0.62	2.5	2.5	> 5	5	> 5

D_{ech} = *Duguetia echinophora*; X_{ema} = *Xylopiia emarginata*; X_{fru} = *Xylopiia frutescens*. Experiments performed in duplicate.

<https://doi.org/10.1371/journal.pone.0289991.t003>

krusei, and *C. auris* are related to bloodstream infections, known as invasive candidiasis, and commonly associated with high mortality rates due to the loss of efficiency of antifungal treatments caused by the emergence of resistant forms of pathogens [61]. Alternative treatments capable of controlling such fungal infections may include the EOs which are a source of substances that, isolated or combined, can inhibit or control fungal growth.

Previous studies demonstrated the antifungal activity of the EOs from some *Annonaceae* species against *Candida* spp., however, there is no specific study of the antifungal activity of EOs from *A. dolichocarpa*, *D. echinophora*, *G. scandens*, *X. emarginata* and *X. frutescens*. The EO from the leaves of *A. brevipes*, composed mainly of β -eudesmol (13.16%), α -eudesmol (13.05%), γ -eudesmol (7.54%), and guaicol (5.12%), showed antifungal inhibitory effect against *C. albicans* and *C. parapsilosis* (MIC values of 50.0 to 100.0 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively) [12]. The EO from the leaves of *A. vepretorum*, rich in bicyclogermacrene (43.7%), spathulenol (11.4%), α -phelandrene (10.0%), α -pinene (7.1%), (*E*)- β -ocimene (6.8%), germacrene D (5.8%) and *p*-cymene (4.2%), exhibited antimicrobial activity against *C. albicans* and *C. tropicalis*, with MIC values the 5000 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively [13]. *D. lanceolata* EO composed mainly of β -elemene (12.7%), caryophyllene oxide (12.4%) and β -selinene (8.4%), inhibited the growth of *C. albicans* (MIC values 60.0 $\mu\text{g}\cdot\text{mL}^{-1}$) [14].

The results found in the present paper indicated that those EOs tested could be a source of bioactive substances with antifungal activity, since terpenes and their derivatives was the class of compounds identified in the highest proportion. These compounds exhibit several pharmacological activities, including antimicrobial activity, and are part of the mechanisms of oxidative phosphorylation and oxygen uptake, which are extremely important for microbial survival, the interaction between terpene and microorganism causes alteration in cellular respiration resulting in the uncoupling of oxidative phosphorylation [9]. However the mode of action must be better understand once that the tested EOs are a complex mix of mono and sesquiterpenes, which can act synergistically or antagonistically.

3.3. Molecular docking

Computer-aided drug design has supported the investigation of new drugs against various diseases worldwide. This paper mainly used molecular modeling approaches to investigate how major compounds of essential oils from *Annonaceae* species can interact with molecular targets of *Candida* sp. The compounds used in the essential oil were the majority compounds; we consider that the majority of compounds have a concentration greater than 5% of the oil composition [62–64].

Furthermore, to communicate our results *in silico* and what was observed experimentally in the treatment of candidiasis, we selected molecular targets that are the target of commercial

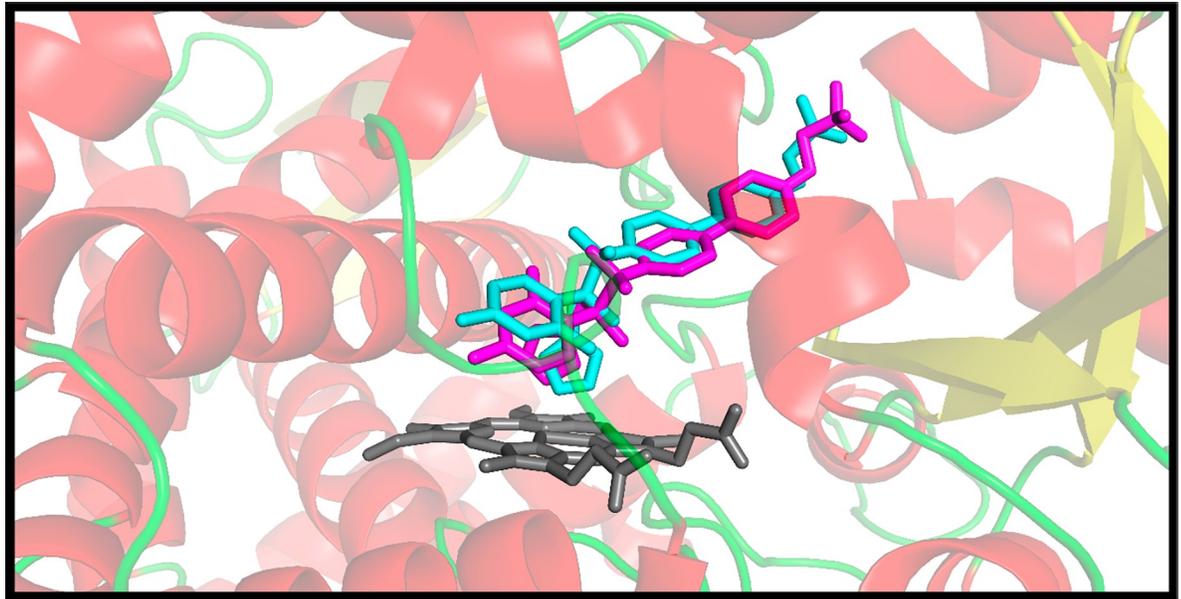


Fig 1. The structure was obtained by redocking (magenta), overlapping the crystallographic structure (cyan) of CYP51 complex.

<https://doi.org/10.1371/journal.pone.0289991.g001>

drugs for this disease. We used the proteins Sterol 14 α -Demethylase Cytochrome P450 as molecular targets because they are targets of commercial drugs such as triazoles that inhibit ergosterol biosynthesis (fluconazole, itraconazole, voriconazole, posaconazole) [65–67].

Before proceeding with molecular docking, we first evaluated whether the docking protocol can reproduce *in silico* the binding mode of interaction of the crystallographic ligand. For this purpose, each crystallographic ligand was redocked using the template docking feature implemented in the Molegro Virtual Docker 5.5 program. The fitness evaluation redocked pose was evaluated by considering the RMSD values and docking scores. According to the literature, a docking protocol is suitable for thorough investigations when the RMSD between the crystallographic ligand and the redocked ligand has a value equal to or less than 2 angstroms [68–70]. The RMSD value between the crystallographic and redocked ligand showed a slight deviation reaching a value of 1.38 Å (Fig 1). Therefore, our docking protocol demonstrated to reproduce an experimentally obtained binding mode.

Then the β -phellandrene, cryptone, spathulenol, and pinene compounds were docked in the sterol 14 α -demethylase cytochrome P450 binding pocket, and the chemical interactions responsible for the interaction with the active site can be seen in Fig 2.

The compound β -phellandrene established hydrophobic interactions with residues of Leu376, His 377, Phe 233, Leu121, Met508, Pro230 and Val510, while van der Waals interactions were formed with Val509, Tyr505, Ser507, Ser506, Phe380, Tyr118, Ser378 and Pro375. Cryptone was also able to interact with residues of Met508, Tyr118, Phe380, Phe233, and Leu 121, while interactions with Ile379, Leu376, Pro375, Val509, Ser378, His377, and Tyr64 were all van der Waals interactions. Spathulenol formed more van der Waals interactions than hydrophobic interactions. Van der Waals interactions were formed with Thr122, Met508, Phe126, Gly307, His310 and Thr311; the hydrophobic interactions were with Tyr118, Leu121, Phe233, Leu376, Tyr132 and Phe228. Pinene interacted with residues of Mey508, Pro230, Leu121, Phe233, His377, and Leu376 through hydrophobic interactions, and van der Waals interactions were formed with Phe380, Tyr64, Ser378, Val509, Pro375, Val510, and Ser507.

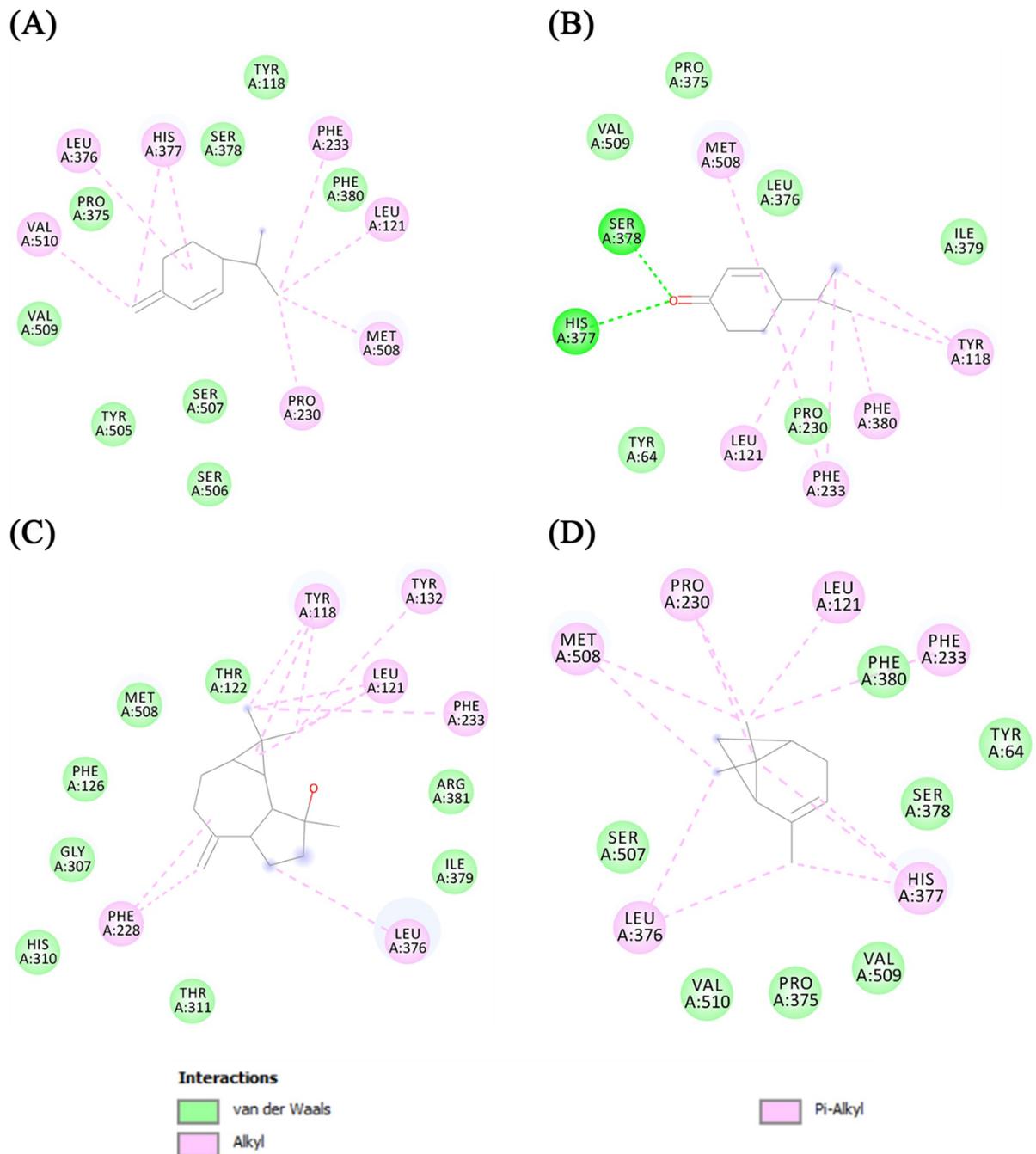


Fig 2. Representation of the interactions established with the binding pocket of Sterol 14 α -Demethylase Cytochrome P450 with the compounds (A) β -Phellandrene, (B) Cryptone, (C) Spathulenol and (D) β -Pinene.

<https://doi.org/10.1371/journal.pone.0289991.g002>

4. Conclusions

This study reports the chemical composition and antifungal activity of five *Annonaceae* EOs against standard strains associated with human infections, namely *C. albicans*, *C. auris*, *C. famata*, *C. krusei* and *C. tropicalis*. The EO yield varied from 0.16 to 1.53% and the GC/MS analysis of the EOs showed that monoterpenes were predominant in the EOs of *D. echinophora*

(47.24%), *G. scandens* (57.15%) and *X. frutescens* (89.18%), while sesquiterpenes characterized the EOs of *A. dolichocarpa* (51.15%) and *X. emarginata* (57.20%). The chemical composition found in this work is in agreement with other species of *Annonaceae*, characterized by mono and sesquiterpenes. All EOs were active against the tested strains of *Candida*. The EOs of *D. echinophora*, *X. emarginata* and *X. frutescens* showed a high ability to inhibit the microorganism *C. famata* (MIC values of 0.07, 0.019 and 0.62 $\mu\text{L}\cdot\text{mL}^{-1}$, respectively). The results of MFC showed that the EOs of *D. echinophora*, *X. frutescens* and *X. emarginata* were found to display a fungicide activity against the species *C. tropicalis* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$), *C. krusei* (2.5 $\mu\text{L}\cdot\text{mL}^{-1}$) and *C. auris* (5 $\mu\text{L}\cdot\text{mL}^{-1}$), respectively. The presence of compounds belonging to the class of the terpenes in the EOs may explain the antifungal action of the tested EOs through synergistic or antagonistic effects, however, there is a need to carry out *in vivo* clinical trials focusing on the antimicrobial activity of these EOs. The molecular docking study suggested that the major compounds of the EOs of *Annonaceae* species can interact with molecular targets of *Candida* spp.

Acknowledgments

The author M.M.C. and M.S.O thanks CAPES for the Ph.D, and Postdoctoral scholarship, respectively. The authors thanks Edital—PAPQ/PROPESP—Universidade Federal do Para.

Author Contributions

Conceptualization: Márcia Moraes Cascaes.

Formal analysis: Márcia Moraes Cascaes, Silvia Helena Marques da Silva, Jorddy Neves Cruz, Ângelo Antônio Barbosa de Moraes, Lidiane Diniz do Nascimento, Oberdan Oliveira Ferreira.

Supervision: Mozaniel Santana de Oliveira, Giselle Maria Skelding Pinheiro Guilhon, Eloisa Helena de Aguiar Andrade.

Validation: Giselle Maria Skelding Pinheiro Guilhon, Eloisa Helena de Aguiar Andrade.

Visualization: Mozaniel Santana de Oliveira, Giselle Maria Skelding Pinheiro Guilhon, Eloisa Helena de Aguiar Andrade.

Writing – original draft: Márcia Moraes Cascaes.

References

1. Nazzaro F, Fratianni F, Coppola R, De Feo V. Essential oils and antifungal activity. *Pharmaceuticals*. 2017; 10: 1–20. <https://doi.org/10.3390/ph10040086> PMID: 29099084
2. Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D. Fungal infections in humans: The silent crisis. *Microb Cell*. 2020; 7: 143–145. <https://doi.org/10.15698/mic2020.06.718> PMID: 32548176
3. Negri M, Salci TP, Shinobu-Mesquita CS, Capoci IRG, Svidzinski TIE, Kioshima ES. Early state research on antifungal natural products. *Molecules*. 2014; 19: 2925–2956. <https://doi.org/10.3390/molecules19032925> PMID: 24609016
4. Benzaid C, Belmadani A, Djeribi R, Rouabhia M. The effects of mentha × piperita essential oil on *C. Albicans* growth, transition, biofilm formation, and the expression of secreted aspartyl proteinases genes. *Antibiotics*. 2019;8. <https://doi.org/10.3390/antibiotics8010010> PMID: 30704020
5. Mutlu-Ingok A, Devecioglu D, Dikmetas DN, Karbancioglu-Guler F, Capanoglu E. Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules*. 2020; 25: 4711. <https://doi.org/10.3390/molecules25204711> PMID: 33066611
6. Huang X, Lao Y, Pan Y, Chen Y, Zhao H, Gong L, et al. Synergistic antimicrobial effectiveness of plant essential oil and its application in seafood preservation: A review. *Molecules*. 2021; 26: 1–13. <https://doi.org/10.3390/molecules26020307> PMID: 33435286

7. Hargrove T.Y., Friggeri L., Wawrzak Z., Qi A., Hoekstra W.J., Schotzinger R.J., et al. Structural analyses of *Candida albicans* sterol 14 α -demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol biosynthesis. *J Biol Chem.* 2017; 292: 6728–6743. <https://doi.org/10.1074/jbc.M117.778308>
8. Wińska K, Mączka W, Łyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential oils as antimicrobial agents—myth or r1. Wińska K, Mączka W, Łyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential oils as antimicrobial agents—myth or real alternative? *Molecules.* 2019; 24(11):1–21. eal alternative? *Molecules.* 2019; 24: 1–21.
9. Mahizan NA, Yang SK, Moo CL, Song AAL, Chong CM, Chong CW, et al. Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. *Molecules.* 2019; 24: 1–21. <https://doi.org/10.3390/molecules24142631> PMID: 31330955
10. Aciole SDG, Piccoli CF, Duque JEL, Costa E V, Navarro-silva MA, Marques FA, et al. Insecticidal activity of three species of *Guatteria* (Annonaceae) against *Aedes aegypti* (Diptera: Culicidae). *Rev Colomb Entomol.* 2011; 37: 262–268.
11. Cascaes MM., Carneiro OS., Do Nascimento L. D.; Moraes A. A. B.; Oliveira M. S.; Cruz J. N.; et al. Essential Oils from Annonaceae Species from Brazil: A Systematic Review of Their Phytochemistry, and Biological Activities. *Int J Mol Sci.* 2021; 22: 2–23.
12. De Alencar DC, Pinheiro MLB, Pereira JLDS, De Carvalho JE, Campos FR, Serain AF, et al. Chemical composition of the essential oil from the leaves of *Anaxagorea brevipes* (Annonaceae) and evaluation of its bioactivity. *Nat Prod Res.* 2016; 30: 1088–1092. <https://doi.org/10.1080/14786419.2015.1101103> PMID: 26586465
13. Costa E V., Dutra LM, Nogueira PCL, Moraes VRS, Salvador MJ, Ribeiro LHG, et al. Essential oil from the leaves of *Annona vepretorum*: Chemical composition and bioactivity. *Nat Prod Commun.* 2012; 7: 265–266. PMID: 22474976
14. Sousa O V., Del-Vechio-Vieira G, Alves MS, Araújo AAL, Pinto MAO, Amaral MPH, et al. Chemical composition and biological activities of the essential oils from *Duguetia lanceolata* St. Hil. barks. *Molecules.* 2012; 17: 11056–11066. <https://doi.org/10.3390/molecules170911056> PMID: 22976469
15. de Oliveira MS, Cruz JN, Ferreira OO, Pereira DS, Pereira NS, Oliveira MEC, et al. Chemical Composition of Volatile Compounds in *Apis mellifera* Propolis from the Northeast Region of Pará State, Brazil. *Molecules.* 2021; 26: 3462. <https://doi.org/10.3390/molecules26113462> PMID: 34200300
16. Franco C de JP, Ferreira OO, Antônio Barbosa de Moraes Â, Varela ELP, Nascimento LD do, Percário S, et al. Chemical Composition and Antioxidant Activity of Essential Oils from *Eugenia patrisii* Vahl, *E. puniceifolia* (Kunth) DC., and *Myrcia tomentosa* (Aubl.) DC., Leaf of Family Myrtaceae. *Molecules.* 2021; 26: 3292. <https://doi.org/10.3390/molecules26113292> PMID: 34072598
17. Silva SG, da Costa RA, de Oliveira MS, da Cruz JN, Figueiredo PLB, Brasil D do SB, et al. Chemical profile of *Lippia thymoides*, evaluation of the acetylcholinesterase inhibitory activity of its essential oil, and molecular docking and molecular dynamics simulations. Bonizzoni M, editor. *PLoS One.* 2019; 14: e0213393. <https://doi.org/10.1371/journal.pone.0213393> PMID: 30849129
18. van Den Dool H, Kratz P. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *J Chromatogr A.* 1963; 11: 463–471. [https://doi.org/10.1016/S0021-9673\(01\)80947-X](https://doi.org/10.1016/S0021-9673(01)80947-X) PMID: 14062605
19. Adams RP. Identification of Essential Oil Components by Gas Chromatography/mass Spectroscopy. Allured Publishing Corporation; 2007. Available: <https://books.google.com.br/books?id=9ut3PQAACAAJ>
20. Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement; Clinical and Laboratory Standards Institute. Wayne, PA, USA; 2005.
21. Celikel N, Kavas G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. *Czech J Food Sci.* 2008; 26: 174–181. <https://doi.org/10.17221/1603-cjfs>
22. 1 da Silva Ana Cristina Rivas, Lopes Paula Monteiro, Mariana Maria Barros de Azevedo DCMC, 1 CSA, Alviano and DS. Inhibitory parameters of essential oils to reduce a foodborne pathogen. *LWT—Food Sci Technol.* 2005; 38: 565–570. <https://doi.org/10.1016/j.lwt.2004.07.012>
23. Ponce AG, Fritz R, Del Valle C, Roura SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT—Food Sci Technol.* 2003; 36: 679–684. [https://doi.org/10.1016/S0023-6438\(03\)00088-4](https://doi.org/10.1016/S0023-6438(03)00088-4)
24. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. 2005.
25. Ferreira OO, da Silva SHM, de Oliveira MS, Andrade EH de A. Chemical Composition and Antifungal Activity of *Myrcia multiflora* and *Eugenia florida* Essential Oils. *Molecules.* 2021; 26: 7259. <https://doi.org/10.3390/molecules26237259> PMID: 34885839

26. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens*. 2021; 10: 165. <https://doi.org/10.3390/pathogens10020165> PMID: 33557078
27. Rex JH, Alexander BD, Andes D, Arthington-Skaggs B, Brown SD, Chaturvedi V, et al. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 3th ed. *Clin Lab Stand Inst*. 2008; 28: 0–13. Available: https://clsi.org/media/1461/m27a3_sample.pdf
28. Becke AD. Density-functional thermochemistry. III. The role of exact exchange. *J Chem Phys*. 1993; 98: 5648–5652. <https://doi.org/10.1063/1.464913>
29. Lee C., Yang RGP W. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys Rev B*. 1988; 37: 785–789. <https://doi.org/10.1103/physrevb.37.785> PMID: 9944570
30. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, et al. *Gaussian 09*. Wallingford CT; 2009. pp. 2–3.
31. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. *J Med Chem*. 2006; 49: 3315–3321. <https://doi.org/10.1021/jm051197e> PMID: 16722650
32. Lima A. de M., Siqueira A.S., Möller M.L.S., Souza R.C. de, Cruz J.N., Lima A.R.J., da Silva R.C., et al. In silico improvement of the cyanobacterial lectin microvirin and mannose interaction. *J Biomol Struct Dyn*. 2022; 40: 1064–1073. <https://doi.org/10.1080/07391102.2020.1821782> PMID: 32990187
33. Ferraz RPC, Cardoso GMB, Da Silva TB, Fontes JEDN, Prata APDN, Carvalho AA, et al. Antitumour properties of the leaf essential oil of *Xylopia frutescens* Aubl. (Annonaceae). *Food Chem*. 2013; 141: 542–547. <https://doi.org/10.1016/j.foodchem.2013.02.114> PMID: 23768347
34. Maia JGS, Andrade EHA, Carla A, Silva M, Oliveira J, Carreira LMM, et al. Leaf volatile oils from four Brazilian *Xylopia* species. 2005; 474–477. <https://doi.org/10.1002/ffj.1499>
35. Fournier G, Hadjiakhoondi A, Charles B, Leboeuf M, Cavi A. Volatile Components of *Anaxagorea dolichocarpa* Fruit. *Biochem Syst Ecol*. 1994; 22: 605–608.
36. Andrade Eloisa Helena A., Jorge Oliveira M das GBZ. Volatiles of *Anaxagorea dolichocarpa* Spreng. & Sandw. and *Annona densicoma* Mart. Growing Wild in the State of Pará, Brazil. *FLAVOUR Fragr J*. 2007; 22: 158–160. <https://doi.org/10.1002/ffj>
37. Almas I, Innocent E, Machumi F, Kisinza W. Effect of Geographical location on yield and chemical composition of essential oils from three Eucalyptus species growing in Tanzania / Asian Journal of Regular article Effect of Geographical location on yield and chemical composition of essential oils fr. *Asian J Tradit Med*. 2019; 14: 1–12.
38. Da Cruz E de NS, Peixoto LDS, da Costa JS, Mourão RH V., do Nascimento WMO, Maia JGS, et al. Seasonal Variability of a Caryophyllane Chemotype Essential Oil of *Eugenia patrisii* Vahl Occurring in the Brazilian Amazon. *Molecules*. 2022; 27: 2417. <https://doi.org/10.3390/molecules27082417> PMID: 35458612
39. Nóbrega JR, Silva D de F, Andrade Júnior FP de, Sousa PMS, Figueiredo PTR de, Cordeiro LV, et al. Antifungal action of α -pinene against *Candida* spp. isolated from patients with otomycosis and effects of its association with boric acid. *Nat Prod Res*. 2021; 35: 6190–6193. <https://doi.org/10.1080/14786419.2020.1837803> PMID: 33094646
40. Barros DB de, Lima L de O e, Silva LA, Fonseca MC, Diniz-Neto H, Rocha WP da S, et al. Antifungal effect of α -pinene alone and in association with antifungals against *Candida albicans* strains Efecto. *Res Soc Dev*. 2022; 11: e58711427748. <https://doi.org/10.33448/rsd-v11i4.27748>
41. Yang D, Michel L, Chaumont JP, Millet-Clerc J. Use of caryophyllene oxide as an antifungal agent in an in vitro experimental model of onychomycosis. *Mycopathologia*. 1999; 148: 79–82. <https://doi.org/10.1023/a:1007178924408> PMID: 11189747
42. dos Santos Albert C.1, Nogueira Mateus L.2, de Oliveira Felipe P.2, Costa Emmanuel V.1 and DPB. Essential Oils of *Duguetia* Species A. St. Hill (Annonaceae): Chemical Diversity and Pharmacological Potential. *Biomolecules*. 2022; 12: 1–14.
43. FONTES JEN, FERRAS RPC, Britto ACS, Carvalho AA, Moraes MO, Pessoa C, et al. Antitumor effect of the essential oil from leaves of *guatteria pogonopus* (Annonaceae). *Chem Biodivers*. 2013; 10: 722–729. <https://doi.org/10.1002/cbdv.201200304> PMID: 23576358
44. Siqueira CAT, Serain AF, Pascoal ACRF, Andreazza NL, De Lourenço CC, Góis Ruiz ALT, et al. Bioactivity and chemical composition of the essential oil from the leaves of *Guatteria australis* A.St.-Hil. *Nat Prod Res*. 2015; 29: 1966–1969. <https://doi.org/10.1080/14786419.2015.1015017> PMID: 25710362
45. DS A. C. R., Lopes P. M. L, Azevedo M. M. B., Costa D. C. M., Alviano C. L., D. S. A. Biological Activities of α -Pinene and β -Pinene Enantiomers. *Molecules*. 2012; 17: 6305–6316. <https://doi.org/10.3390/molecules17066305> PMID: 22634841

46. Sobrinho ACN, de Morais SM, de Souza EB, Albuquerque MRJR, dos Santos HS, de Paula Cavalcante CS, et al. Antifungal and Antioxidant Activities of *Vernonia Chalybaea* Mart. ex DC. Essential Oil and their Major Constituent β -caryophyllene. *Brazilian Arch Biol Technol*. 2020; 63: 1–11. <https://doi.org/10.1590/1678-4324-2020190177>
47. Fontenelle ROS, Morais SM, Brito EHS, Brilhante RSN, Cordeiro RA, Nascimento NRF, et al. Antifungal activity of essential oils of Croton species from the Brazilian Caatinga biome. *J Appl Microbiol*. 2008; 104: 1383–1390. <https://doi.org/10.1111/j.1365-2672.2007.03707.x> PMID: 18298533
48. Lago JHG, Reis AA, Martins D, Cruz FG, Roque NF. Composition of the leaf oil of *Xylopia emarginata* mart. (Annonaceae). *J Essent Oil Res*. 2005; 17: 622–623. <https://doi.org/10.1080/10412905.2005.9699013>
49. de Souza ILL, Correia AC de C, Araujo LC da C, Vasconcelos LHC, Silva M da CC, Costa VC de O, et al. Essential oil from *Xylopia frutescens* Aubl. reduces cytosolic calcium levels on guinea pig ileum: Mechanism underlying its spasmolytic potential. *BMC Complement Altern Med*. 2015; 15: 1–10. <https://doi.org/10.1186/s12906-015-0849-3> PMID: 26376723
50. Nascimento AMD, Maia TDS, Soares TES, Menezes LRA, Scher R, Costa E V., et al. Repellency and Larvicidal Activity of Essential oils from *Xylopia laevigata*, *Xylopia frutescens*, *Lippia pedunculosa*, and Their Individual Compounds against *Aedes aegypti* Linnaeus. *Neotrop Entomol*. 2017; 46: 223–230. <https://doi.org/10.1007/s13744-016-0457-z> PMID: 27844468
51. Brito MT, Ferreira RC, Beltrão DM, Moura APG, Xavier AL, Pita JCLR, et al. Antitumor activity and toxicity of volatile oil from the leaves of *Annona leptopetala*. *Brazilian J Pharmacogn*. 2018; 28: 602–609. <https://doi.org/10.1016/j.bjp.2018.06.009>
52. Bomfim LM, Menezes LRA, Rodrigues ACBC, Dias RB, Gurgel Rocha CA, Soares MBP, et al. Antitumour Activity of the Microencapsulation of *Annona vepretorum* Essential Oil. *Basic Clin Pharmacol Toxicol*. 2016; 118: 208–213. <https://doi.org/10.1111/bcpt.12488> PMID: 26348780
53. Costa TR, Fernandes OFL, Santos SC, Oliveira CMA, Lião LM, Ferri PH, et al. Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *J Ethnopharmacol*. 2000; 72: 111–117. [https://doi.org/10.1016/s0378-8741\(00\)00214-2](https://doi.org/10.1016/s0378-8741(00)00214-2) PMID: 10967461
54. Moghaddam M, Mehdizadeh L. Chemical Composition and Antifungal Activity of Essential Oil of *Thymus vulgaris* Grown in Iran against Some Plant Pathogenic Fungi. *J Essent Oil Bear Plants*. 2020; 23: 1072–1083. <https://doi.org/10.1080/0972060X.2020.1843547>
55. Benomari FZ, Andreu V, Kotarba J, Dib MEA, Bertrand C, Muselli A, et al. Essential oils from Algerian species of *Mentha* as new bio-control agents against phytopathogen strains. *Environ Sci Pollut Res*. 2018; 25: 29889–29900. <https://doi.org/10.1007/s11356-017-9991-4> PMID: 28866759
56. Pilar Santamarina M, Roselló J, Giménez S, Amparo Blázquez M. Commercial *Laurus nobilis* L. and *Syzygium aromaticum* L. Merr. & Perry essential oils against post-harvest phytopathogenic fungi on rice. *LWT—Food Sci Technol*. 2016; 65: 325–332. <https://doi.org/10.1016/j.lwt.2015.08.040>
57. Zahra G, Khadijeh B, Hossein D, Ali S. Essential oil composition of two *Scutellaria* species from Iran. *J Tradit Chinese Med Sci*. 2019; 6: 244–253. <https://doi.org/10.1016/j.jtcms.2019.07.003>
58. Karakaya S, Yilmaz SV, Özdemir Ö, Koca M, Pınar NM, Demirci B, et al. A caryophyllene oxide and other potential anticholinesterase and anticancer agent in *Salvia verticillata* subsp. *amasiaca* (Freyn & Bornm.) Bornm. (Lamiaceae). *J Essent Oil Res*. 2020; 32: 512–525. <https://doi.org/10.1080/10412905.2020.1813212>
59. Mesquita PRR, Nunes EC, Santos FN dos, Bastos LP, Costa MAPC, de M. Rodrigues F, et al. Discrimination of *Eugenia uniflora* L. biotypes based on volatile compounds in leaves using HS-SPME/GC-MS and chemometric analysis. *Microchem J*. 2017; 130: 79–87. <https://doi.org/10.1016/j.microc.2016.08.005>
60. Elgat WAAA, Kordy AM, Böhm M, Černý R, Abdel-Megeed A, Salem MZM. Eucalyptus camaldulensis, Citrus aurantium, and Citrus sinensis Essential Oils as Antifungal Activity against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Fusarium culmorum*. *Processes*. 2020; 8: 1003. <https://doi.org/10.3390/pr8081003>
61. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. *Nat Rev Dis Prim*. 2018; 4: 1–20. <https://doi.org/10.1038/nrdp.2018.26> PMID: 29749387
62. Diniz Do Nascimento L, Antônio Barbosa De Moraes A, Santana Da Costa K, Marcos J, Galúcio P, Taube PS, et al. Bioactive Natural Compounds and Antioxidant Activity of Essential Oils from Spice Plants: New Findings and Potential Applications. *Biomol* 2020, Vol 10, Page 988. 2020; 10: 988. <https://doi.org/10.3390/biom10070988> PMID: 32630297
63. Muzammil S, Neves Cruz J, Mumtaz R, Rasul I, Hayat S, Khan MA, et al. Effects of Drying Temperature and Solvents on In Vitro Diabetic Wound Healing Potential of *Moringa oleifera* Leaf Extracts. *Molecules*. 2023. <https://doi.org/10.3390/molecules28020710> PMID: 36677768

64. de Almeida RBM, Barbosa DB, do Bomfim MR, Amparo JAO, Andrade BS, Costa SL, et al. Identification of a Novel Dual Inhibitor of Acetylcholinesterase and Butyrylcholinesterase: In Vitro and In Silico Studies. *Pharmaceuticals*. 2023. <https://doi.org/10.3390/ph16010095> PMID: 36678592
65. McCarthy MW, Kontoyiannis DP, Cornely OA, Perfect JR, Walsh TJ. Novel agents and drug targets to meet the challenges of resistant fungi. *J Infect Dis*. 2017; 216: S474–S483. <https://doi.org/10.1093/infdis/jix130> PMID: 28911042
66. Scorzoni L, de Paula e Silva ACA, Marcos CM, Assato PA, de Melo WCMA, de Oliveira HC, et al. Antifungal therapy: New advances in the understanding and treatment of mycosis. *Front Microbiol*. 2017; 8: 36. <https://doi.org/10.3389/fmicb.2017.00036> PMID: 28167935
67. Ramakrishnan J, Rathore SS, Raman T. Review on fungal enzyme inhibitors-potential drug targets to manage human fungal infections. *RSC Adv*. 2016; 6: 42387–42401. <https://doi.org/10.1039/c6ra01577h>
68. Galucio NC da R, Moysés D de A, Pina JRS, Marinho PSB, Gomes Júnior PC, Cruz JN, et al. Antiproliferative, genotoxic activities and quantification of extracts and cucurbitacin B obtained from *Luffa operculata* (L.) Cogn. *Arab J Chem*. 2022; 15: 103589. <https://doi.org/10.1016/j.arabjc.2021.103589>
69. Rego CMA, Francisco AF, Boeno CN, Paloschi M V., Lopes JA, Silva MDS, et al. Inflammasome NLRP3 activation induced by Convulxin, a C-type lectin-like isolated from *Crotalus durissus terrificus* snake venom. *Sci Rep*. 2022; 12: 1–17. <https://doi.org/10.1038/s41598-022-08735-7> PMID: 35304541
70. Almeida VM, Dias ÊR, Souza BC, Cruz JN, Santos CBR, Leite FHA, et al. Methoxylated flavonols from *Vellozia dasypus* Seub ethyl acetate active myeloperoxidase extract: in vitro and in silico assays. *J Biomol Struct Dyn*. 2022; 40: 7574–7583. <https://doi.org/10.1080/07391102.2021.1900916> PMID: 33739225