Phytochemical Profile, Antioxidant Potential and Toxicity Evaluation of the Essential Oils from Duguetia and Xylopia Species (Annonaceae) from the Brazilian Amazon

Márcia Moraes Cascaes 1, Ângelo Antônio Barbosa De Moraes 2, Jorddy Neves Cruz 3, Celeste de Jesus Pereira Franco 2, Renan Campos E Silva 1, Lidiane Diniz do Nascimento 1, Oberdan Oliveira Ferreira 4, Tainá Oliveira dos Anjos 5, Mozaniel Santana de Oliveira 2,* and Eloisa Helena de Aguiar Andrade 1,2,4

Abstract: The essential oils (EOs) of Duguetia echinophora, D. riparia, Xylopia emarginata and X. frutescens (Annonaceae) were obtained by hydrodistillation and the chemical composition was analyzed by GC-MS. An antioxidant assay using the ABTS and DPPH radicals scavenging method and cytotoxic assays against Artemia salina were also performed. We evaluated the interaction of the major compounds of the most toxic EO (X. emarginata) with the binding pocket of the enzyme Acetylcholinesterase, a molecular target related to toxicity in models of Artemia salina. The chemical composition of the EO of D. echinophora was characterized by β-phellandrene (39.12%), sabinene (17.08%) and terpinolene (11.17%). Spathulenol (22.22%), caryophyllene oxide (12.21%), humulene epoxide II (11.86%) and allo-aromadendrene epoxide (10.20%) were the major constituents of the EO from X. emarginata. The EO of X. frutescens was characterized by -pinene (20.84%) and α-bisabolol (15.86%). The results of the radical scavenger DPPH assays ranged from 42.22% to 69.38% and the highest percentage of inhibition was observed for the EO of X. emarginata, while for ABTS radical scavenging, the antioxidant capacity of EOs varied from 14.61 to 63.67%, and the highest percentage of inhibition was observed for the EO of X. emarginata. The EO of X. frutescens was characterized by α-pinene (20.84%) and bycliclogermacrene (7.85%). The results of the radical scavenger DPPH assays ranged from 15.87 to 69.38% and the highest percentage of inhibition was observed for the EO of X. emarginata. The EOs obtained from D. echinophora, X. emarginata and X. frutescens showed high toxicity, while the EO of D. riparia was non-toxic. Because the EO of X. emarginata is the most toxic, we evaluated how its major constituents were able to interact with the Acetylcholinesterase enzyme. The docking results show that the compounds are able to bind to the binding pocket through non-covalent interactions with the residues of the binding pocket. The species X. emarginata and X. frutescens are the most promising sources of antioxidant compounds; in addition, the results obtained for preliminary cytotoxicity of the EOs of these species may also indicate a potential biological activity.

Keywords: natural products; Amazon; medicinal plant; volatile compounds; bioactive compounds.
1. Introduction

Essential oils (EOs) are complex mixtures of substances formed in the secondary metabolism of plants [1,2], and the substances present in EOs are intended to protect plants against pests, herbivores, fungi and bacteria [3]. Among these substances, sesquiterpenes, monoterpenes, aldehydes, alcohols, esters, and ketones stand out [4–8]. In aromatic species belonging to the Annonaceae family, compounds belonging to the class of mono and sesquiterpenoids have been identified as predominant [9,10]. Due to the strong demand for pure natural ingredients in various fields, EOs have been widely used all over the world for various applications in industrials sectors, such as food, pharmaceuticals and cosmetics production [11].

The antioxidant activity of EOs is a property of great interest because the EOs may preserve foods, cosmetics, perfumes and other products from the toxic effects of oxidants. Moreover, the ability of EOs to scavenge free radicals may play an important role in prevention of some diseases such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals [12–14]. Furthermore, the Artemia salina Leach assay is a preliminary toxicity test that screens a large number of biosynthesized compounds from plant secondary metabolism and can quickly indicate the potential biological activity of EOs [15]. In general, authors report that the molecular target in toxicity tests with A. salina is acetylcholinesterase, so it is important to investigate the interaction mechanisms using in silico studies [16,17].

Annonaceae has numerous species that produce EOs. This family consists of 2106 species and more than 130 genera concentrated in the tropics. Around 900 species are neotropical, 450 are Afrotropical and the other species are Indomalayan [18]. In the Amazon region it is estimated that there are approximately 268 species [19]. The biological activities described for the EOs of these species include antioxidant [20–22] and cytotoxic activities [23]. Considering the large number of species of Annonaceae occurring in the Amazon region, there are still few studies investigating the chemical composition and the biological activities of the EOs of these species. In this paper, the chemical composition and the antioxidant and cytotoxic properties of the EOs obtained from the Annonaceae species collected in the State of Pará-Brazil (Duguetia echinophora R.E.Fr., D. riparia Huber, Xylopia emarginata Mart. and X. frutescens Aubl) were evaluated. We also studied the interaction of the major compounds of the most toxic EO with the binding pocket of the enzyme Acetylcholinesterase.

It is worth mentioning that there is still no literature available on the biological properties of the EOs from D. echinophora, D. riparia or X. emarginata nor on the chemical composition of the EO of the species D. echinophora. The chemical composition of EOs from D. riparia, X. emarginata and X. frutescens has been evaluated and is characterized by mono and sesquiterpenes [24–26]. The EO from X. frutescens showed interesting anticancer [26] and repellent activities [27]. In folk medicine, this species is known in Brazil as “embira”, “semente-de-embira”, “embira-vermelha” and “pau carne”, and is widely used to treat flu, digestive problems, rheumatism, halitosis, tooth decay and as a bladder stimulant [26,28,29].

The present work provides new information related to the antioxidant potential of EOs from the species D. echinophora, D. riparia, X. emarginata and X. frutescens for use in areas such as food conservation. In addition, we investigate preliminary toxicity that provides important information related to the application of these EOs in potential biological activities.

2. Materials and Methods

2.1. Botanical Material

The leaves of Annonaceae species were collected in the municipality of Magalhães Barata (State of Pará, Amazon region, Brazil) in March 2018 (00°47′51.6″ S; 047°33′38.4″ W). The samples were identified by Jorge Oliveira, a parataxonomist from the Museu Paraense Emílio Goeldi (MPEG), Belém, Pará, Brazil. The voucher specimens were deposited at the Herbarium of MPEG under the registration codes MG-237446 for D. riparia, MG-237477 for D. echinophora, MG-237444 for X. frutescens and MG-237449 for X. emarginata.
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 and then crushed in a knife mill (Tecnal, model TE-631/3, Piracicaba, São Paulo, Brazil). The moisture content was analyzed using a moisture analyzer (Marte, model ID50, São Paulo, Brazil). The EOs were extracted from the leaves of Annonaceae species by hydrodistillation in a glass modified Clevenger-type apparatus [30,31], using 150 g of plant material for each experiment. Hydrodistillations were carried out for 3 h at 100 °C. The obtained EOs were dried over anhydrous sodium sulfate and stored in a freezer at −10 °C. The yields of EOs (%) were calculated based on plant dry weight and expressed in mL/100 g of dried material.

2.3. Analysis of Chemical Profile of Essential Oil

The phytochemical profiles of the EOs were analyzed using chromatography/mass spectrometry (GC/MS) using a Shimadzu QP Plus 2010 GC-MS (Kyoto, Japan) following protocols reported earlier by our research group [32,33]. The retention index was calculated for all volatile constituents using a homologous series of n-alkanes (C8-C40, Sigma-Aldrich, St. Louis, MO, USA) according Van den Dool and Kratz [34], and the compounds were identified by comparing their mass spectrum and retention index with the data from the libraries [35].

2.4. ABTS•+ Radical Scavenging Assay

The ABTS•+ assay was performed according to the methodology adapted from Miller et al. [36], and modified by Re et al. [37]. ABTS•+ was prepared using 7 mM ABTS•+ and 140 mM of potassium persulfate incubated at room temperature without light for 16 h. The solution was then diluted with phosphate-buffered saline until it reached an absorbance of 0.700 ± 0.02 at 734 nm. To measure the antioxidant capacity, 2.97 mL of the ABTS•+ solution was transferred to the cuvette, and the absorbance at 734 nm was determined using a Biospectro SP 22 spectrophotometer. Then, 0.03 mL of the sample was added to the cuvette containing the ABTS•+ radical, and after 5 min, the second reading was performed. The data were expressed as percent inhibition.

2.5. DPPH• Radical Scavenging Assay

The test was carried out according to the method proposed by Blois et al. [38]. To measure the antioxidant capacity, initially, the absorbance of DPPH• 0.1 mM diluted in ethanol was determined. Subsequently, 0.6 mL of DPPH• solution, 0.35 mL of distilled water, and 0.05 mL of the sample were mixed and placed in a water bath at 37 °C for 30 min. Thereafter, the absorbances were determined in a spectrophotometer at 517 nm. The data were expressed as percent inhibition.

2.6. Preliminary Toxicity

The toxicity of the essential oils was tested against larvae of the microcrustacean Artemia salina leach (brine shrimp). The eggs of A. salina (25 mg) were incubated at room temperature (27–30 °C) in an aquarium with artificial salt water composed of a mixture of 46 g of NaCl, 22 g of MgCl2.6H2O, 8 g of Na2SO4, 2.6 g of CaCl2.6H2O, and 1.4 g of KCl in 2.0 L of distilled water. The pH was adjusted to the 8.0–9.0 range using Na2CO3 to avoid the risk of larvae death by lowering the pH during incubation. After 24 h of egg hatching, oil solutions were prepared at concentrations of 100, 50, 25, 10, 5 and 1 µg mL⁻¹ using brine as vehicle and 5% dimethyl sulfoxide as diluent. Ten larvae of A. salina were placed in each tube containing the solution, and the mortality rate of the larvae after 24 h was calculated. The mean lethal concentration (LC50) was estimated using the Probitos statistical method. All the experiments were performed in triplicate using same protocols as described by Rebelo et al. [39].
2.7. In silico analysis

To carry out the in silico study, the molecules spathulenol and caryophyllene oxide (the major constituents present in the EO of *Xylopia emarginata*) were constructed using GaussView 5.5 software [40,41]. Their molecular structures were optimized with B3LYP/6-31G* [42,43] with Gaussian 09 [44]. We used the molecular method to evaluate the compounds interaction mode with Acetylcholinesterase (AChE). For this, we used the Molegro Virtual Docker (MVD) 5.5 [45–47], and the crystal structure used as a molecular target can be found in the Protein Data Bank using the ID: 4M0E [48]. 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 the following parameter settings: number of runs equal to 10, maximum of 1500 interactions, and maximum population size equal to 50. The maximum evaluation of 300 steps with a neighbor distance factor equal to 1 and energy threshold equal to 100 was used during the molecular docking simulation.

2.8. Multivariate Analysis

The multivariate analysis was performed using the Minitab 17® software (free version number 17, Minitab Inc., State College, PA, USA). The chemical constituents of the EOs from the leaves of *D. echinophora*, *D. riparia*, *X. emarginata* and *X. frutescens*, (≥3%), were set as the experimental variables, thus forming a matrix of 4 (samples) × 23 (variables) according to the literature [15,32,33].

3. Results and discussion

3.1. Chemical Composition

The EOs yields from the leaves of the Annonaceae species were 1.76, 0.08, 0.27 and 1.50% for *D. echinophora*, *D. riparia*, *X. emarginata* and *X. frutescens*, respectively. The yield found in this study for the EO of *D. riparia* was close to those found in studies with other species of the *Duguetia* genus (0.1–0.6%) [24]. The EOs yields found for the *Xylopia* species were also very close to those found in others studies [25,26]. The yields and EOs compositions of the species are shown in Table 1.

| Constituents (%) | DE | DR | XE | XF |
|-----------------|----|----|----|----|
| Hexanal         | 5.19| 801| 798| 0.95|
| Hex-(3E)-enol   | 6.95| 844| 845| 1.35|
| Hexanol         | 7.25| 863| 857| 0.85|
| α-Thujene       | 8.30| 924| 925| 4.89|
| α-Pinene        | 11.17| 932| 932| 17.08|
| Camphene        | 11.23| 946| 948| 2.72|
| Sabinene        | 11.98| 969| 974| 2.01|
| β-Pineene       | 12.32| 974| 974| 25.95|
| Myrcene         | 12.57| 988| 991| 3.61|
| α-Phellandrene  | 13.09| 1002| 1002| 1.27|
| δ-3-Carene      | 13.56| 1008| 1011| 0.95|
| α-Terpinene     | 13.89| 1014| 1016| 0.85|
| p-Cymene        | 14.02| 1020| 1022| 0.65|
| Limonene        | 14.32| 1024| 1027| 3.00|
| β-Phellandrene  | 14.79| 1025| 1029| 39.12|
| 1,8-Cineole     | 14.90| 1026| 1031| 3.36|
| (Z)-β-Ocimene   | 14.99| 1032| 1043| 2.60|

Table 1. Yield and chemical compositions of the Annonaceae species essential oils.
Table 1. Cont.

| Substance                  | DE  | DR  | XE  | XF  |
|----------------------------|-----|-----|-----|-----|
| Essential Oil Yield (%)    | 1.76| 0.08| 0.27| 1.50|
| **Constituents (%)**       |     |     |     |     |
| γ-Terpinene                | 1.00|     |     |     |
| cis-Hydrate sabinene       | 0.17|     |     |     |
| Terpinolene                | 11.17| 0.39|     |     |
| Linalool                   | 0.30| 1.74|     |     |
| trans-Thujone              |     |     | 0.09|     |
| endo-Fenchol               | 0.33|     |     |     |
| cis-p-Ment-2-en-1-ol       | 0.08|     |     |     |
| α-Campholenal              | 0.38| 0.14|     |     |
| trans-Pinocarveol          | 4.46| 0.36|     |     |
| cis-Verbene                | 0.49| 0.15|     |     |
| Sabina ketone              | 0.27|     |     |     |
| Pinocarvone                | 2.35| 0.16|     |     |
| p-Mentha-1,5-dien-8-ol     | 1.26| 0.11|     |     |
| Umbellulone                | 0.04|     |     |     |
| Terpinen-4-ol              | 1.16| 1.06|     |     |
| p-Cymen-8-ol               | 3.36| 0.71|     |     |
| α-Terpineol                | 0.97|     |     |     |
| Myrtenal                   | 3.24|     |     |     |
| Verbenone                  | 1.62| 0.1 |     |     |
| trans-Carveol              | 0.33|     |     |     |
| Carvone                    | 0.23|     |     |     |
| Geraniol                   | 0.39|     |     |     |
| δ-Elemene                  | 2.32| 4.41|     |     |
| α-Cubebeene                | 0.74| 0.08|     |     |
| α-Ylangene                 | 1.35|     |     |     |
| Isoledeene                 | 0.02|     |     |     |
| α-α-Copaene                | 0.25| 1.07| 0.25|     |
| Geranyl acetate            |     |     | 1.38|     |
| β-Bourbonene               | 0.93|     |     |     |
| β-Elemene                  | 0.74| 0.49| 3.10| 0.54|
| α-Gurjunene                | 0.11| 0.06|     |     |
| (E)-Caryophyllene          | 2.98| 1.56| 0.93| 0.03|
| β-Ylangene                 | 0.72|     |     |     |
| γ-Elemene                  | 0.39|     |     |     |
| Aromadendrene              | 0.75| 0.39|     |     |
| 6,9-Guaiadiene             | 0.06|     |     |     |
| trans-Muurola-3,5-diene    | 0.38|     |     |     |
| α-Humulene                 | 0.73| 1.40| 0.35| 0.10|
| allo-Aromadendrene         | 0.11|     |     |     |
| (E)-9-epi-caryophyllene    | 0.26|     |     |     |
| Dauca-5,8-diene            | 0.29|     |     |     |
| γ-Murolene                 | 3.06|     |     |     |
| Germacrone D               | 1.24| 1.34| 1.08| 3.26|
| β-Selinene                 | 1.61|     |     |     |
| epi-Cubebol                | 0.91|     |     |     |
| γ-Amorphene                | 0.67|     |     |     |
| Viridifloreone             | 0.56|     |     |     |
| Bicyclergermacrene         | 0.21| 7.85|     |     |
| α-Murolene                 | 0.95|     |     |     |
| γ-Cadinene                 | 2.67| 0.13|     |     |
| Cubebol                    | 0.68|     | 0.05|     |
| δ-Cadinene                 | 1.61| 0.38|     |     |
| cis-Calaminene             | 2.06| 4.01| 0.48|     |
Table 1. Cont.

| RT   | RI<sub>L</sub> | RI<sub>C</sub> | Essential Oil Yield (%) | DE | DR | XE | XF |
|------|----------------|----------------|-------------------------|----|----|----|----|
| 30.06| 1533           | 1533           | 1.76                    |    |    |    |    |
| 30.25| 1533           | 1534           | 0.08                    |    |    |    |    |
| 30.39| 1537           | 1536           | 0.27                    |    |    |    |    |
| 30.53| 1539           | 1540           | 1.04                    |    |    |    |    |
| 30.67| 1544           | 1540           | 1.47                    |    |    |    |    |
| 30.83| 1548           | 1548           | 0.02                    |    |    |    |    |
| 31.57| 1561           | 1561           | 0.65                    |    |    |    |    |
| 31.97| 1579           | 1579           | 1.87                    | 22.22| 5.63| 2.18|
| 32.28| 1583           | 1583           | 2.49                    | 12.21| 5.63| 0.18|
| 32.51| 1589           | 1589           | 0.61                    |    |    |    |    |
| 32.85| 1596           | 1596           | 2.48                    |    |    |    |    |
| 32.92| 1604           | 1604           | 0.23                    |    |    |    |    |
| 33.09| 1602           | 1602           | 0.10                    |    |    |    |    |
| 33.25| 1608           | 1609           | 0.21                    | 11.86| 1.41|    |
| 33.33| 1630           | 1630           | 4.70                    |    |    |    |    |
| 34.18| 1643           | 1643           | 0.90                    |    |    |    |    |
| 34.36| 1657           | 1657           | 1.20                    |    |    |    |    |
| 34.45| 1661           | 1661           | 1.36                    |    |    |    |    |
| 34.51| 1664           | 1664           | 0.12                    |    |    |    |    |
| 34.71| 1669           | 1669           | 0.83                    |    |    |    |    |
| 34.84| 1672           | 1672           | 0.68                    |    |    |    |    |
| 34.89| 1678           | 1678           | 0.54                    |    |    |    |    |
| 34.93| 1681           | 1681           | 0.30                    |    |    |    |    |
| 35.49| 1694           | 1694           | 0.30                    |    |    |    |    |
| 35.62| 1692           | 1692           | 1.00                    |    |    |    |    |
| 35.94| 1694           | 1694           | 3.36                    |    |    |    |    |
| 36.28| 1695           | 1695           | 0.76                    | 1.40| 0.04|    |
| 39.41| 1768           | 1768           | 0.48                    |    |    |    |    |
| 59.61| 2408           | 2408           | 0.02                    |    |    |    |    |
| 62.38| 2512           | 2512           | 0.02                    |    |    |    |    |

Monoterpenes hydrocarbon: 78.99, 1.80, 8.41, 62.53
Oxygenated monoterpenes: 4.52, 8.50, 27.42, 19.05
Sesquiterpenes hydrocarbon: 8.32, 8.50, 27.42, 19.05
Oxygenated sesquiterpenes: 4.57, 71.76, 22.99, 5.03
Others class: 3.42, 2.30

Total: 96.4, 82.06, 81.69, 95.08

RT: Retention Time; RI<sub>L</sub> = Calculated retention index; RI<sub>C</sub> = Literature retention index; DE: Duguetia echinophora; DR: Duguetia riparia; XE: Xylopia emarginata; XF: Xylopia frutescens.

The chemical compositions of the EOs of D. echinophora, D. riparia, X. emarginata and X. frutescens were characterized by GC-MS, and a total of 22, 19, 59 and 62 components were identified, representing 96.40, 82.06, 81.69 and 95.08% of the total EOs for each species, respectively. The hydrocarbon monoterpenes compounds represented the most abundant class in the EOs of D. echinophora (78.99%) and X. frutescens (62.53%), and the oxygenated sesquiterpenes class characterized the EO of D. riparia (71.76%). The compounds β-Phellandrene (39.12%), sabinene (17.08%) and terpinolene (11.17%) were dominant in the D. echinophora EO, while spathulenol (22.22%), caryophyllene oxide (12.21%), humulene epoxide II (11.86%) and allo-aromadendrene epoxide (10.20%) were the major constituents of the D. riparia EO. The EO of X. emarginata was characterized by spathulenol (5.65%) and caryophyllene oxide (5.63%), and X. frutescens EO was characterized by α-pinene (20.84%) and byciclogermacrene (7.85%). Ion chromatograms are available in the Supplementary Material.
According to data previous published, the chemical compositions of the EOs of Annonaceae species occurring in Brazil are predominantly characterized by substances belonging to the class of mono and sesquiterpenes, and among these compounds, the most abundant are β-elemene, α-pinene, β-pinene limonene, bicyclogermacrene, (E)-caryophyllene, caryophyllene oxide, spathulenol, and germacrene D, [9].

Previous reports have investigated the chemical composition of the EOs from the Annonaceae species described in this work (D. riparia, X. emarginata and X. frutescens). The leaves and fine stems EO of D. riparia, also collected in State of Pará-Brazil, showed spathulenol (46.5%), caryophyllene oxide (28.9%) and α-pinene (6.1%) as their main compounds [24], and quantitative differences were observed for the constituents spathulenol and caryophyllene oxide in relation to the D. riparia EO described in the present work. The EO from the leaves of X. emarginata, collected in Caxiuanã National Forest, Melgaço, State of Pará-Brazil, showed a high percentage of sesquiterpene spathulenol (73.0%) [11], whereas in the present work, this constituent was obtained at a low percentage (5.65%) [25].

The EO from the leaves of X. frutescens, collected in Municipality of Capela, Sergipe State, Brazil, had as its major compounds (E)-caryophyllene (31.48%), bicyclogermacrene (15.13%), germacrene D (9.66%), δ-cadinene (5.44%), viridiflorene (5.09%) and α-copaene (4.35%) [26], while the EO from the leaves of the specimen collected in the city of Itabaiana, Sergipe-Brazil, had as its major constituents bicyclogermacrene (23.23%), (E)-caryophyllene (17.24%), β-elemene (6.35%) and (E)-β-ocimene (5.23%) [27].

The chemical composition of EOs can be strongly influenced by several factors, including season, climate, geography, age, genotype, organ, development periods, collection place and even extraction method, etc. [49–51]. Figueiredo and collaborators evaluated the influence of seasonal variation on the EO of Eugenia patrisii Vahl (Myrtaceae) and verified a potential correlation between the content of the main constituents of the essential oil and climatic parameters (temperature, insolation and humidity rate) [52]. The EOs of Flos Chrysanthemi indici, an important medicinal and aromatic plant in China, were obtained by different extraction techniques, hydrodistillation (HD), steam distillation (SD), solvent-free microwave extraction (SFME) and supercritical fluid extraction (SFE), and the authors found that the EO yield, chemical composition and bioactivities varied according to the extraction method used [53]. Some Annonaceae species have shown qualitative and quantitative variability in their EO compositions according to different collection sites. The EOs from the leaves of Annona vepretorum Mart. collected in the State of Sergipe, Brazil, showed bicyclogermacrene, spathulenol and α-phellandrene as the major constituents [54], while another specimen collected in the State of Pernambuco, Brazil, showed α-pinene, limonene, spathulenol and caryophyllene oxide as the compounds with higher percentage [55]. The compounds α-selinene, aristolochene, (E)-caryophyllene and (E)-calamenene were identified as the major constituents of EO from leaves of a specimen of Duqueutia lanceolata collected in the state of Minas Gerais, Brazil [23], while another specimen collected in the State of São Paulo, Brazil, had as its main constituents of the EO the compounds trans-muurola-4(14),5-diene, β-bisabolene, 3,4,5-trimethoxy-styrene and 2,4,5-trimethoxy-styrene [56].

3.2. Multivariate Analyses

Figures 1 and 2 show the correlations between the classes of compounds identified in the different samples according to the multivariate analyses, principal component analysis (PCA) and hierarchical cluster analysis (HCA), respectively. PC1 and PC2 represent the principal components (PC), which contained 39.0% and 32.0% of the variables, respectively, and accounted for 71.0% of the variance in the analyzed data. In the HCA analysis, tree groups were observed that show the similarity between the identified classes. Group I, including the samples of EOs from D. echinophora and X. frutescens showed a similarity of 10.67% (Figure 2) and comprised the compounds β-phellandrene, p-cymen-8-ol, bicyclogermacrene, terpinolene, α-pinene, sabine, myrcene, limonene, β-pinene and α-thujene (Figure 1). Groups II and III contained only one sample each and comprised β-elemene, 1,8-cineol, muurola-4,10(14)-dien-1-β-ol, trans-pinocarveol, myrtenal and γ-muurolene
(X. emarginata EO) and cis-calamene, α-cadinol, mustakone, allo-aromadendrene epoxide, humulene epoxide II, spathulenol and caryophyllene oxide (D. riparia EO), with similarities of 7.18% and 0%, respectively (Figure 2).

Figure 1. Biplot (principal component analysis) for the chemical analysis of the essential oils from Annonaceae species. DE: Duguetia echinophora; DR: Duguetia riparia; XE: Xylopia emarginata; XF: Xylopia frutescens.

Figure 2. Dendrogram presenting the relational similarity of the chemical composition of the essential oils from Annonaceae species. DE: Duguetia echinophora; DR: Duguetia riparia; XE: Xylopia emarginata; XF: Xylopia frutescens.
3.3. Antioxidant Capacity

The antioxidant potential of the EOs from Annonaceae species was evaluated based on their ability to scavenge stable free DPPH• (2,2-diphenyl-1-picrylhydrazyl) and ABTS•+ (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals; the results are shown in Figure 3. The results of the DPPH assays ranged from 15.87 to 69.38% and the highest percentage of inhibition was observed for the EO of Xylopia emarginata, characterized by spathulenol (5.65%) and caryophyllene oxide (5.63%). For ABTS radical scavenging, the antioxidant activity of the EOs ranged from 14.61 to 63.67%. The species Xylopia frutescens showed the higher antioxidant capacity by the ABTS•+ assay. This may be due to the presence of α-Pinene (20.84%) and β-Pinene (25.95%), the major components present in this EO. Possibly, the antioxidant activity of the X. emarginata EO can also be attributed to its main components which are described as antioxidants [57,58]. The high free radical scavenging effect of this sample may be related to the fact that the combination of the numerous organic chemical constituents present in EOs have a synergistic effect, increasing the biological activity or, conversely, an antagonistic effect [59]. In addition, bioactive compounds belonging to the monoterpenoid class have antioxidant activity, as reported in the literature [60].

![Figure 3. ABTS•+ and DPPH• radical scavenging assay and Trolox equivalent antioxidant capacity of essential oils. Values are expressed as mean and standard deviation (n = 3) of Trolox equivalent antioxidant capacity.](image)

Other studies investigating EOs of the Duguetia and Xylopia genera identified antioxidant effects. The EO of Xylopia sericea A. St.-Hil. showed significant antioxidant activity using DPPH (IC\(_{50}\) 49.1 µg·mL\(^{-1}\)), β-carotene/linoleic acid bleaching (IC\(_{50}\) 6.9 µg·mL\(^{-1}\)), TAC (IC\(_{50}\) 78.2 µg·mL\(^{-1}\)) and TBARS (IC\(_{50}\) 80.0 µg·mL\(^{-1}\)) methods [20]. The EO of Duguetia lancelata St. Hil. branches showed antioxidant effects using a DPPH assay (EC\(_{50}\) 159.4 µg·mL\(^{-1}\)), Fe\(^{3+}\) reduction (EC\(_{50}\) 187.8 µg·mL\(^{-1}\)) and inhibition of lipid peroxidation (41.5%); the authors suggest that caryophyllene oxide is one of the active compounds found in this EO [21].

3.4. Cytotoxic Activity of Essential Oils

The toxicity of the EOs from Annonaceae species was measured in terms of LC\(_{50}\) (lethal concentration) with two negative control groups (control 1:10 nauplii and artificial sea-water with DMSO 0.1%; control 2: 10 nauplii and artificial seawater) and one positive control (K\(_{2}\)Cr\(_{2}\)O\(_{7}\), 50 µg·mL\(^{-1}\)). The values are shown in Table 2. Values of LC\(_{50}\) < 80 µg·mL\(^{-1}\) are considered highly toxic [15,61,62]; values of LC\(_{50}\) within the range 80 to 250 µg·mL\(^{-1}\) are moderately toxic; and LC\(_{50}\) > 250 µg·mL\(^{-1}\) are considered as low toxicity or non-toxic [63].
The EOs of *D. echinophora*, *X. emarginata* and *X. frutescens* showed high toxicity, whereas the EO of *D. riparia* showed low toxicity or was non-toxic. The major compounds from the EOs of *X. emarginata* (spathulenol and caryophyllene oxide) [64,65], *D. echinophora* (β-phellandrene and terpinolene) [66,67] and *X. frutescens* (α-pinene and byciclogermacrene) [68,69] showed cytotoxic effects and these results indicate that the cytotoxic potential observed for the EOs tested may be related to the presence of these secondary metabolites.

| Essential Oil          | LC₅₀ (µg mL⁻¹) |
|------------------------|----------------|
| *Duguetia echinophora* | 28.00 ± 0.30   |
| *Duguetia riparia*     | 310.80 ± 0.70  |
| *Xylopia emarginata*   | 26.72 ± 0.17   |
| *Xylopia frutescens*   | 54.36 ± 0.20   |
| Positive control (K₂Cr₂O₇) | 50.00 ± 0.00  |

Values are expressed as mean and standard deviation (n = 3).

Toxicity tests in *A. Salina* performed with *Coriandrum sativum* L. (*Apiaceae*) showed an LC₅₀ value of 23 µg mL⁻¹ [70], which is similar to those obtained in the present work for *D. echinophora* and *X. emarginata* EOs. Oliva and coworkers evaluated toxicity of the EOs from some medicinal plants, and the results showed a decreasing activity in the brine assay of *Aloysia polystachia* (*Verbenaceae*) (LC₅₀ 6459 µg mL⁻¹), *Aloysia triphylla* (*Verbenaceae*) (LC₅₀ 1279 µg mL⁻¹), *Minthostachys verticillata* (*Myrtaceae*) (LC₅₀ 1848 µg mL⁻¹), and *Schinus poligamus* (*Anacardiaceae*) (LC₅₀ 1179 µg mL⁻¹), that were considered non-toxic [71]. Other authors have also reported the toxicity of essential oils from a variety of plants [17,72,73].

The essential oils of *Duguetia* species have been studied by using the *A. salina* bioassay. The EOs from the leaves, underground heartwood and underground stem bark of *Duguetia furfuracea* (A. St.-Hil.) Saff. showed potent activity against *A. salina* larvae (LC₅₀ 6.01, 7.79 and 9.98 µg mL⁻¹, respectively) and the leaf EO from *D. lanceolata* also showed potent activity against the same larvae (LC₅₀ 0.89 µg mL⁻¹) [23]. In another study, the EOs of *D. lanceolata* showed toxicity against *A. salina* with LC₅₀ values of 49.0 µg mL⁻¹ (2 h of hydrodistillation extraction) and 60.7 µg mL⁻¹ (4h of hydrodistillation extraction) [74].

3.5. Molecular Docking

Molecular modeling approaches have been used to investigate how natural compounds interact with molecular targets of pharmacological interest [75–78]. One of the tools used has been molecular docking, which can provide insights into how these compounds interact with the binding pocket of proteins. Here, we use this approach to assess how the major compounds of the EO from *X. emarginata* interact with the AChE active site, as this target is closely related to the toxicity mechanism observed in the *A. salina* assays [79,80]. Spathulenol formed hydrophobic interactions with various residues such as Ser293, Phe297, Trp286, Tyr72, Tyr341, and Phe338. A hydrogen bond was established with Ser293. Caryophyllene oxide established pi-alkyl hydrophobic interactions with Trp286, Tyr341 and Tyr337 (Figure 4). The interaction between spathulenol and caryophyllene with the active site of AChE has already been described [58] and this could be the likely mechanism responsible for the cytotoxicity of the EO from *X. emarginata*. 
poligamus (Anacardiaceae) (LC50 1179 µg·mL\(^{-1}\)), that were considered nontoxic [71]. Other authors have also reported the toxicity of essential oils from a variety of plants [17,72,73]. The essential oils of Duguetia species have been studied by using the A. salina bioassay. The EOs from the leaves, underground heartwood and underground stem bark of Duguetia furfuracea (A. St. -Hil.) Saff. showed potent activity against A. salina larvae (LC50 6.01, 7.79 and 9.98 µg·mL\(^{-1}\), respectively) and the leaf EO from D. lanceolata also showed potent activity against the same larvae (LC50 0.89 µg·mL\(^{-1}\)) [23]. In another study, the EOs of D. lanceolata showed toxicity against A. salina with LC50 values of 49.0 µg·mL\(^{-1}\) (2h of hydrodistillation extraction) and 60.7 µg·mL\(^{-1}\) (4h of hydrodistillation extraction) [74].

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Figure 4. (A) Binding pocket of interaction of compounds with AChE. Molecular interactions established by (B) spathulenol and (C) caryophyllene oxide with the AChE active site.

4. Conclusions

The present study presents new insights concerning the chemical composition, antioxidant activity and preliminary toxicity of some Annonaceae EOs. Essential oils obtained from D. echinophora, X. emarginata and X. frutescens showed high toxicity, compared with EO obtained from D. riparia, which showed low toxicity or was non-toxic. The cytotoxicity test against A. salina can be considered as a good preliminary assessment of bioactive compounds, and may indicate a potential biological activity. The docking results elucidated the interaction mode of the major compounds of X. emarginata EO, spathulenol and caryophyllene, with the active site of the enzyme Acetylcholinesterase. The greatest capacities to scavenge DPPH and ABTS radicals were found in the essential oils of X. emarginata and X. frutescens, respectively, and the main constituents of the EO of this species may play the main role in the observed antioxidant capacity; however, the impact of less abundant constituents also should be considered.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox11091709/s1, Figure S1: Ions-chromatogram relating to the chemical profile of essential oils from different species of Annonaceae Xylopia.

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