Chapter 14

# *Eleutherine Plicata* – Quinones and Antioxidant Activity

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Additional information is available at the end of the chapter

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## 1. Introduction

Iridaceae is a family of petalloid monocots with 77 genera and more than 1,630 species, which, despite worldwide distribution, has a remarkable concentration in the southern continents. This family has its major center of irradiation in Southern Africa and in Brazil, it is represented by 14 genera and 110 species [1].

*Eleutherine plicata* Herb. is an Iridaceae popularly known in the Amazonian region as marupazinho, marupari, palmerinha, and marupá-pirranga, where it occurs in the form of a clump, with red bulbs like an onion (Figure 1). Its leaves are entire, pleated, and simple; the flowers are colored in white to pink; and the red bulbs are widely used in Brazilian folk phytotherapy, especially in the Amazonian region [2].

The ethnoguided surveys conducted by Barbosa et al. (2009) [3] in the city of Igarapé-Miri and by Jardim et al. [4] in Santa Barbara, both in the Brazilian State Pará, revealed the use of Marupazinho (*Eleutherine plicata* Herb.) to treat diarrhea caused by amoeba and that the people prepare a tea from the bulb of the plant, which is drunk before the meal.

The aqueous extract (AE) of the dried bulbs contains isoeleutherine, which appears as a peak of 99.14% purity in the LC-DAD chromatographic analyses. A buffered aqueous solution containing 2.5 mg of dried AE/mL showed antiamoebic activity, a complete inhibition of the *Entamoeba histolytica* trophozoites growth, in 24 hours [5]. It is remarkable that this solution is less concentrated than the tea (about 3.5 mg/mL), both prepared according to popular knowledge, but normalized according to the Brazilian Pharmacopoeia [6], being indeed sufficient to promote the total annihilation of the tested trophozoites.

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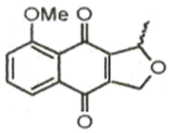
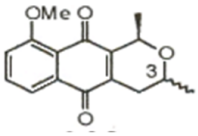
**Figure 1.** *Eleutherine plicata* Herb. (herbaceousborneo.blogspot.com)

The experimental characterization of the antiamoebic activity of the decoction prepared with bulbs of *E. plicata* contributes to validate its alleged popular use. The detection of isoeleutherine in the analyzed decoction can explain partially the reported antiamoebic activity, which can be attributed to the pro-oxidant activity of the substance [7].

*Eleutherine bulbosa* (Mill.) Urb., an accepted name for *E. plicata,* according to Tropicos®[1], is also used by traditional Zulu healers as anti-diarrheal [8] and yields eleutherinone (1), eleutherine (2), and isoleutherine (3), three quinones that show strong antifungal activity [9] Figure 2). Quinones show various pharmacological activities including bactericidal, fungicidal, and antiprotozoal, noting that they cause oxidative stress by inducing the endogenous formation of reactive oxygen species [7]. Quinones are oxygenated aromatic derivatives, characterized by the presence of a diketocyclohexa-1,4-diene residue (para-quinones) or alternatively by a diketocyclohexa-1,3-diene grouping (ortho-quinones) [10].




1 – Eleutherinone




2 – Eleutherine (3-S isomer) 3 – Isoeleutherine (3-R isomer)

**Figure 2.** Chemical constituents isolated from *E. bulbosa* (Mill.) Urb.

Naphthoquinones act under enzymatic influence, accepting an electron to form semiquinone anion radical under catalysis of reduced nicotinamide-adenine dinucleotide 3'-phosphate

1 Tropicos.org. Missouri Botanical Garden. 28 Oct 2014 <http://www.tropicos.org>

(NADPH) cytochrome-P-450-reductase, NADPH cytochrome-b5-reductase, and NADPH ubiquinone-oxidoreductase. The semiquinone anion radical then reduces molecular oxygen to superoxide anion radical ($[O_2]^{\cdot -}$), which in the presence of superoxide dismutase is converted into $H_2O_2$. $[O_2]^{\cdot -}$ under catalysis of transition metals (Fenton Reaction) or reacting with $H_2O_2$ (Haber-Weiss Reaction) generates $[HO]^{\cdot}$ inside the cell. Although $H_2O_2$ is not a free radical, it's a very reactive substance and can also promote the oxidation of certain biomolecules [7].

## 2. Materials and methods

### 2.1. Materials and equipments

#### *2.1.1. Reagents*

Acetone, ethyl acetate, acetonitrile, chloroform, deuterated chloroform, ethanol, hexane, potassium hydroxide, methanol, dimethylsulfoxide (DMSO), silica gel for thin layer chromatography, and silica gel for column chromatography.

#### *2.1.2. Equipments*

Stainless steel knives mill (Tecnal®, model TCL-650); ultraviolet (UV) visible (VIS) spectrophotometer spectrum SP 2000; UV chamber 254 and 365nm; analytical balance GEHAKA BK 600; high-performance liquid chromatography system LaChrom7000 Merck-HITACHI® with diode array detector (DAD) and Agilent LiChrospher100 (250 mm × 4.6 mm) column; nuclear magnetic resonance (NMR) spectrometer Plus 300 MHz Variant.

### 2.2. Methods

#### *2.2.1. Collection and identification of botanical material*

The plant material purchased at the Ver-O-Peso Market, Belem, Pará State, Brazil was collected in October 2007 in the same region. The botanical identification occurred by comparison of the prepared exsiccata (Figure 3) to a voucher deposited at the Herbarium of the Emilio Goeldi Museum registered under the number 10543[2].

#### *2.2.2. Processing of plant material and extraction*

Approximately 5 kg of fresh bulbs were sliced and after washing, aeration and selection, dehydrated at room temperature for 2 days. Drying was completed under forced hot air circulation at about 40° C. The dried material was ground in a Wiley knives mill to yield 1.20 kg of herbal drug.

2 The authors acknowledge Prof.Dr.Mario Augusto Gonçalves Jardim by the characterization of the plant material.

**Figure 3.** Voucher specimen of *E. plicata* Herb. prepared for botanical identification.

An ethanol extract (EE) was obtained by successive macerations, using 500 g of the herbal drug and Ethanol 96º GL, until total drug exhaustion. Thereafter, the solvent was removed under reduced pressure in a rotary evaporator.

*2.2.3. Fractionation of ethanol extract*

About 30 g EE were suspended in 500 mL of methanol/water (1:1) and partitioned with Hexane (4 × 100mL) – HF (Hexane Fraction); Chloroform (5 × 100mL) – CF (Chloroform Fraction) and Ethyl Acetate (5 × 100mL) – EAF (Ethyl Acetate Fraction), leaving a residual hydromethanolic solution – RF. Solvents were removed under reduced pressure in rotary evaporator, for water, a lyophilizer was employed.

*2.2.4. Phytochemical screening*

Chemical tests were performed on EE, HF, CF, EAF, and RF based on the Guide for Phytochemical Analysis of Plant Extracts [11] in order to verify the presence of 18 classes of secondary metabolites.

*2.2.5. Thin Layer Chromatography (TLC) analyses*

TLC analyses aiming to corroborate the phytochemical results on EE, HF, CF, EAF, and RF were performed employing silica gel as stationary phase and as eluents hexane/acetone (80:20), chloroform/methanol (90:10), chloroform/methanol/water (70:25:05), chloroform/acetone (99:03) and (99:01), thus the corresponding chromatographic profiles were defined. The obtained chromatograms were observed under visible and ultraviolet light at 254 nm and 365 nm and then sprayed with KOH 10% in methanol to make possible the detection of quinones.

The chromatographic profiles were obtained by determining the retention factor (Rf) and describing the color of each chemical constituent observed as a zone in the chromatograms.

### *2.2.6. Isolation of substances*

Using 2.0 g of lyophilized CF, the first chromatographic separation (A) was performed on a 32–63 μm silica gel column (40 × 2.0 cm) as stationary phase, and chloroform/acetone (99.5:0.5) as mobile phase. The fractions were pooled according their TLC profiles, providing the following samples: A1 = 83 mg (corresponding to the substance **1**); A2 = 380 mg; A3 = 71mg; and A4 = 156 mg.

Sample A2 was chromatographed on a 32–63 μm silica gel column (35 × 1.5 cm), eluted with chloroform/acetone (99.5:0.5) and monitored by TLC, yielding the following fractions: B1 = 175 mg, B2 = 12 mg, B3= 53 mg, and B4 = 74 mg.

Using the same stationary phase in a 20 × 1.5 cm column and chloroform/acetone (99.3:0.7) as eluent, B1 was separated by column chromatography, yielding the following fractions after TLC monitoring: C1 = 8 mg, C2 = 28 mg, C3 = 22 mg, C4=69 mg, and C5 = 15 mg. About 65 mg of C4 were subjected to preparative TLC using normal phase silica gel on a standard chromatoplate (20 × 20 cm), eluted with chloroform/acetone (99:01) to obtain **2**.

### *2.2.7. Characterization of 1 and 2*

About 20 mg of **1** and **2** were dissolved in deuterated chloroform ($CDCl_3$) to be analyzed in a Variant brand Plus NMR Spectrometer. The characterization of these substances was achieved by comparison of their $^1$H- (300 MHz) and $^{13}$C- NMR (75MHz) spectra to those obtained from the same naphthoquinones isolated from other species of *Eleutherine* and reported in the scientific literature.

### *2.2.8. LC profile of EE, CF, 1, and 2*

The LC-DAD profile was recorded using a LaChrom 7000 Merck HITACHI® chromatograph hyphenated to a DAD equipped with a LiChrospher100 Agilent column (250 × 4.6 mm). The mobile phase consisted of ultrapure water and acetonitrile (ACN) as described in Table 1 and was pumped at 1 mL/min. The oven temperature was 26°C (± 1°C) and the detection occurred between 200 nm and 500 nm, this method was adapted from Paramapojn et al. (2008) [12].

| Time (min.) | $H_2O$ | ACN |
|---|---|---|
| 00 | 85 | 15 |
| 10 | 70 | 30 |
| 20 | 50 | 50 |
| 30 | 20 | 80 |

**Table 1.** Gradient composition (in %) of the eluent used to analyze EE, CF, **1,** and **2**.

Aliquots of 50 μL were applied at the following concentrations: **1**, 500 μg/mL; **2,** 1 mg/mL; CF, 2,500 μg/mL; and crude EE, 1 mL.

*2.2.9. Antioxidant activity of EE, 1, and 2*

The antioxidant capacity of EE, Ep1, and Ep2 was evaluated using the free radical 2,2-diphenyl-1-picrylhydrazyl ($[DPPH]^{\cdot+}$) and as reference the substance butylhydroxytoluene (BHT). The reaction was accompanied by color change and the activity is monitored by the decrease in absorbance of the mixture at 517 nm relative to the solvent as blank [13].

## 3. Results and discussion

### 3.1. Botanical identification

The characterization of an exsiccata containing herborized plant material of *Eleutherine plicata* Herb. confirmed the identity of the investigated herbal drug. According to Tropicos®, *Eleutherine bulbosa* is an accepted name for *E. plicata*.

### 3.2. Extraction and fractionation

The ethanol extract, EE, weighed 63 g, from which *circa* 30g provided four fractions by solid/liquid partition: HF = 2.103 g, 7%; CF = 3,224 g, 11%; EAF = 5,551 g, 18%; and RF = 12.494 g, 41%. The process generates a loss in mass of about 25%, partially due to the solubility of the constituents of the extract in the employed solvents and the partition coefficient of them; and to the evaporation of volatile substances inherent to the methods used to obtain and concentrate the fractions.

### 3.3. Phytochemical screening

The positive results of the phytochemical approach of EE and fractions considering the presence of 18 classes of secondary metabolites are shown in Table 2.

The metabolites were detected in fractions according to the polarity of the solvents used in the fractionation, such as steroids and triterpenes, azulenes, anthraquinones and naphthoquinones in HF and CF, which are solvents and metabolites of low polarity. Moreover, saponins, tannins and phenols, and coumarin derivatives present in EAF and RF show middle to high polarities. Reducing sugars, detected only in EE, are metabolites of very high polarity, which in liquid-liquid partition do not migrate to organic layer. saponin, coumarin derivatives, and tannins and phenols were not detected in EE, probably due to their concentration in the crude extract or the occurrence of interference on the reagent used.

### 3.4. Thin Layer Chromatography (TLC) analyses

TLC analyses are used to define the chromatographic profile of extracts and fractions, thereby contributing to the quality control of herbal drugs and their derivatives. In present case,

| METABOLIC CLASSES | SAMPLES | | | | |
|---|---|---|---|---|---|
| | EE | HF | CF | EAF | RF |
| Steroids and Triterpenes | + | + | + | - | - |
| Azulenes | + | + | + | + | + |
| Reducing Sugars | + | - | - | - | - |
| Anthraquinone | + | + | + | - | - |
| Naphthoquinone | + | + | + | - | - |
| Saponin | - | - | - | + | + |
| Phenols and Tannins | - | - | - | + | + |
| Coumarin Derivatives | - | - | - | + | + |

**Table 2.** Metabolic classes detected in derivatives of *E. plicata*.

different chromatographic systems were tested to obtain the chromatographic profile of EE, HF, and CF, wherein normal phase silica gel and chloroform/acetone (99:1) produced chromatograms with very good resolution.

Figure 4A shows three colored yellow zones with Rfs 0.25, 0.31, and 0.44, respectively, present in EE as well in the fractions HF and CF, and pink colored areas with Rf 0.5 and in 0.62 HF and CF. When the same chromatogram is observed under UV light at 254 nm (Figure 4B), three absorption zones in EE and fractions can be observed with Rfs 0.25, 0.31 and 0.44, respectively, and in HF, one bluish zone by Rf 0.62.

This chromatogram also shows, in EE and fractions, brown colored areas with Rfs 0.25, 0.31, and 0.44, respectively, indicating the presence of naphthoquinones after treatment with KOH 10% in methanol (Figure 4C), which is a reagent to detect quinones [14]. Additionally, three rose colored spots with Rfs 0.5, 0.62, and 0.87, respectively, can be seen in HF.

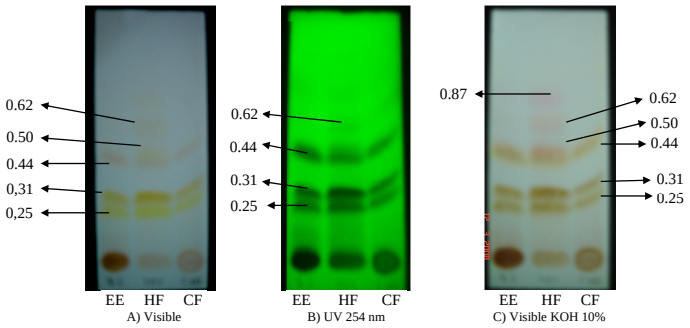



**Figure 4.** Chromatograms on silica gel eluted with chloroform/acetone 99:1; A – observed under visible light; B – under UV 254nm; C – visible light, after KOH 10%.

### 3.5. Isolation of major chemical constituents

Sample A1 (83 mg) appears as an isolated chemical substance when analyzed by TLC; it was named **1** and can be observed in HF fraction with Rf 0.62 (Figure 4A, B, C). From C-4, a sample reacting like a naphthoquinone could be purified by preparative TLC yielding 51 mg of a substance that was named **2** that in the TLC analyses showed Rf 0.44 and can also be observed in Figure 4A, B, C.

### 3.6. Structural characterization of isolated substances

#### *3.6.1. Isoeleutherol*

The $^{1}$H-NMR spectral data of **1** listed in Table 3 shows characteristic signals of aromatic hydrogen at positions C-4, C-6, C-7, and C-8. The aromatic hydrogen H-4 appears as a singlet (δ = 7.860 ppm), H-6 hydrogen (δ = 7.545 ppm) appears coupled with H-7 (δ = 7.399 ppm) making a doublet. Hydrogen H-7 (δ = 7.399 ppm) couples with H-6 (δ = 7,545 ppm) and H-8 (δ = 6.940ppm), appearing as an overlaid double doublet or false triplet. H-8 hydrogen (δ = 6.940 ppm) appears coupled with H-7 (δ = 7.399) as a doublet.

The signal observed as a singlet (δ = 4.108 ppm) refers to the hydrogen atoms of the methoxy group attached to the aromatic ring. There is also a signal of a methyl group (δ = 1.736 ppm), which is attached to C-1 of the furan ring, coupling with H-1 (δ = 5.718ppm), thus appearing as a doublet.

The hydrogen at C-1 (δ = 5.718ppm) couples with the hydrogen atoms of the methyl group at the same position, generating a quartet. Finally, a phenolic hydrogen appears as a singlet at δ = 9.644.

| HYDROGEN | Ep1 δ(ppm) | * ISOELEUTHEROL δ(ppm) | MULTIPLICITY | J (Hz) |
|---|---|---|---|---|
| 1-ME | 1.736 | 1.73 | d | 6.5 |
| 8-OME | 4.108 | 4.11 | s | |
| H-1 | 5.718 | 5.70 | dd | 6.5 |
| H-5 | 6.927 | 6.93 | d | 7.7 |
| H-6 | 7.399 | 7.39 | t | 7.7 |
| H-7 | 7.545 | 7.54 | t | 7.7 |
| H-4 | 7.863 | 7.84 | s | |
| 9-OH | 9.644 | 9.63 | s | |

*From: HARA et al., 1997[15]

**Table 3.** Data of $^{1}$H-NMR analysis of Ep1 compared to authentic isoeleutherol.

The $^{13}$C-NMR spectral data of **1** listed in Table 4 shows the presence of 14 carbon atoms. The signals with δ = 19.34 and δ = 56.77 are characteristic of methyl carbon atoms of C-11 and C-10, respectively, and the signal at δ = 76.80 is characteristic of carbon C-1. The signals at δ = 126.79, δ = 123.82, δ = 116.67, and δ = 106.44 correspond to the carbons C-4, C-5, C-6 and C-7, respectively. The carbon C-3 of furan ring that is double bonded to an oxygen atom shows a signal at δ = 170.69 and the resonance of non-substituted aromatic carbon atoms such as C-4a and C-8a appears at δ = 137.37 and δ = 117.66. The resonance at δ = 156.42 refers to C-8 where a methoxy group is attached, while the signal at δ = 149.20 corresponds to C-9 bonded to the hydroxyl group. Finally, the carbon atoms C-3a, C-9a of the furan residue condensed with an aromatic ring resonate at δ = 126.04 and δ = 128.07, respectively.

| CARBON/ POSITION | Ep1 δ(ppm) | * ISOELEUTHEROL δ(ppm) |
|---|---|---|
| C-1 | 76.80 | 76.64 |
| C-3 | 170.69 | 170.55 |
| C-3a | 126.04 | 125.92 |
| C-4 | 126.79 | 126.61 |
| C-4a | 137.37 | 137.24 |
| C-5 | 123.82 | 123.67 |
| C-6 | 116.67 | 116.50 |
| C-7 | 106.44 | 106.33 |
| C-8 | 156.42 | 156.61 |
| C-8a | 117.66 | 117.54 |
| C-9 | 149.35 | 149.20 |
| C-9a | 128.07 | 127.94 |
| C-10 | 56.57 | 56.43 |
| C-11 | 19.35 | 19.18 |

*From: HARA et al., 1997[15]

**Table 4.** Data of $^{13}$C-NMR analysis of Ep1 compared to authentic isoeleutherol.

The very close correspondence of the $^{1}$H- and $^{13}$C-NMR spectral data of **1** to those found in the literature (Tables 3 and 4) allows to infer that the isolated substance is isoeleutherol (Figure 5), which has been isolated from *Eleutherine americana* Merr. et Heyne by Hara et al. (1997) [15] being this the first report of its occurrence in *E. plicata* Herb.

Hara et al. (1997) [15] found that isoeleutherol did not inhibit the enzyme topoisomerase II DNA dependent, but significantly hinders the HIV replication in H9 lymphocytes.

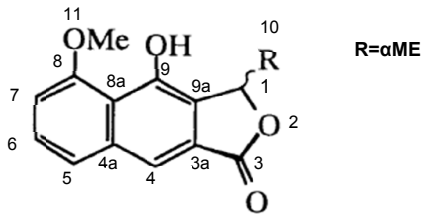



**Figure 5.** Chemical structure of isoeleutherol.

Since isoeleutherol seems to be very stable and the major chemical constituent in EE, it could be used as a chemical marker of *E. plicata* and its derivatives.

### *3.6.2. Isoeleutherine*

Substance **2** was analyzed by $^{1}$H-NMR and its structure was characterized by comparison of the obtained spectral data to those reported in the literature. Table 5 shows the resonance values of H-6, H-7, and H-8 from the aromatic ring δ = 7.73, δ = 7.64, and δ = 7.27, respectively, with identical coupling constant $J_{6H\text{-}7H}$=$J_{7H\text{-}8H}$=6.7Hz. The signal of H-6 appears as a doublet since it couples with H-7, which in turn appears as a false triplet due the coupling with H-6 and H-8.

The hydrogen atoms of the methyl group attached to C-1 appear as a doublet at δ = 1:54 ($J_{H1\text{-}CH3}$=6.7Hz) and that one bonded to C-3 at δ = 1:33 ($J_{H3\text{-}CH3}$=6.1Hz).

| HYDROGÊN | Ep2 δ(ppm) | * ISOELEUTHERINE δ(ppm) | MULTIP. | J (Hz) |
|---|---|---|---|---|
| 1-H | 5.00 | 5.01 | q | 6.7 |
| 1-ME | 1.53 | 1.53 | d | 6.7 |
| 3-H | 3.95 | 3.96 | m | |
| 3-ME | 1.33 | 1.34 | d | 6.1 |
| 4-αH | 2.69 | 2.68 | dd | 3.5–19.0 |
| 4-βH | 2.23 | 2.23 | dd | 11.0–19.0 |
| 6-H | 7.73 | 7.74 | d | 6.7 |
| 7-H | 7.64 | 7.64 | t | 6.7 |
| 8-H | 7.27 | 7.27 | d | 6.7 |
| 9-OME | 4.00 | 4.00 | s | |

*From: HARA et al., 1997[15]

**Table 5.** Data of $^{1}$H-NMR analysis of Ep2 compared to authentic isoeleutherine.

The spectral data listed in Table 5 when compared with that from the literature permit to deduce that **2** corresponds to isoeleutherine (Figure 6), a naphthoquinone already isolated from *Eleutherine bulbosa* Mill [9] and from *E. americana* Merr. by Hara et al. (1997) [15]. This is the first report of the occurrence of isoeleutherine in *E. plicata*.

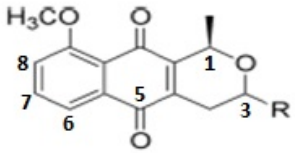



**Figure 6.** Chemical structure of isoeleutherine.

### 3.7. Chromatographic profile by LC-DAD

The LC-DAD profile of EE and CF was obtained using the method developed by Paramapojn et al. (2008) [12] with modifications and the best chromatograms were registered at 250 nm. EE profile shows two peaks of high intensity with retention time – Rt = 18.93 min and 20.83 min, with areas of 45,854,675 and 60,180,902 and a purity of 99.97% and 99.72%, respectively (Figure 7).

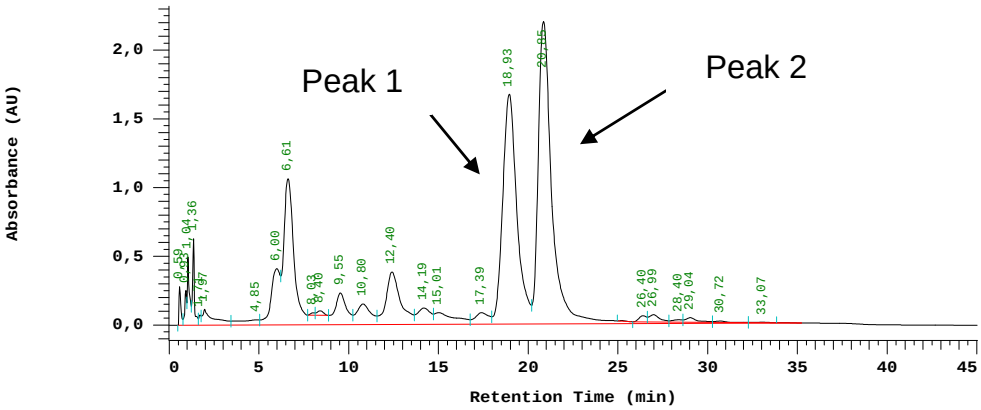



**Figure 7.** LC-DAD profile of EE registered at 250 nm.

CF chromatogram shows two peaks with high intensity at 19.12 min (Peak 1) and 21.18 min (Peak 2), with areas of 7,813,739 and 1,900,571 and purity of 98.29% and 99.75%, respectively (Figure 8).

The isolated isoeleutherol was also analyzed by LC-DAD under the same conditions as EE and CF, generating a peak at 21.71 min with 3,641,711 area and purity of 99.92% (Figure 9).

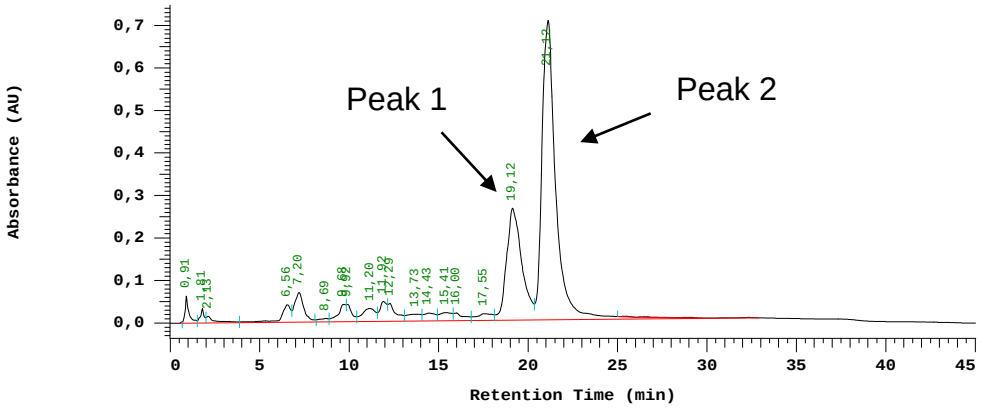



**Figure 8.** LC-DAD profile of the chloroform fraction registered at 250 nm.

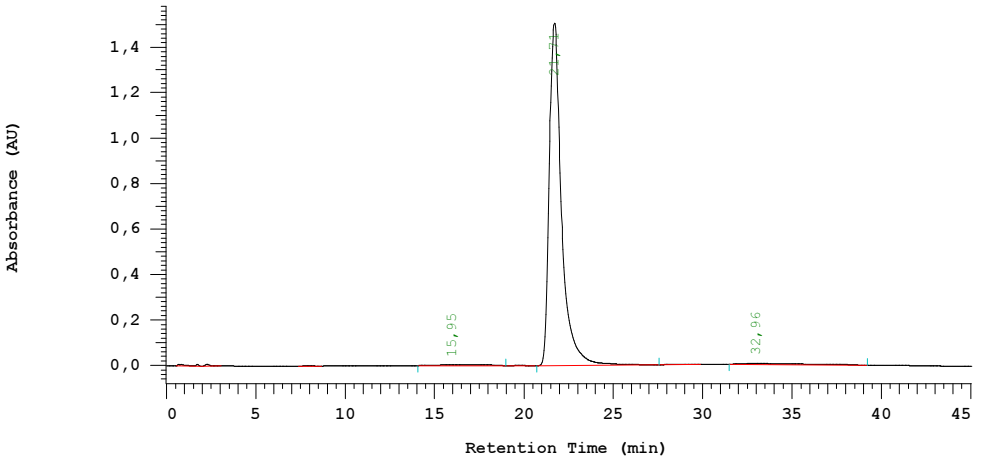



**Figure 9.** HPLC chromatogram of isoeleutherol at a wavelength of 250 nm.

The same procedure was adopted to analyze the obtained isoeleutherine, producing the LC-DAD profile, showed in Figure 10, where a peak at 18.13min with area 24,727,851 and purity of 99.14% is registered.

The reverse survey feature in the library of the chromatograph shows correlations of 97.40% and 99.89% between the UV spectrum of the peak 01 in EE (Figure 7) and in CF (Figure 8) and that of isoeleutherine. Similarly, the peak 02 in EE (Figure 7) and in CF (Figure 8) showed a correlation of 99.84% and 99.98%, respectively, between the UV spectra of both the peaks and that of isoeleutherol.

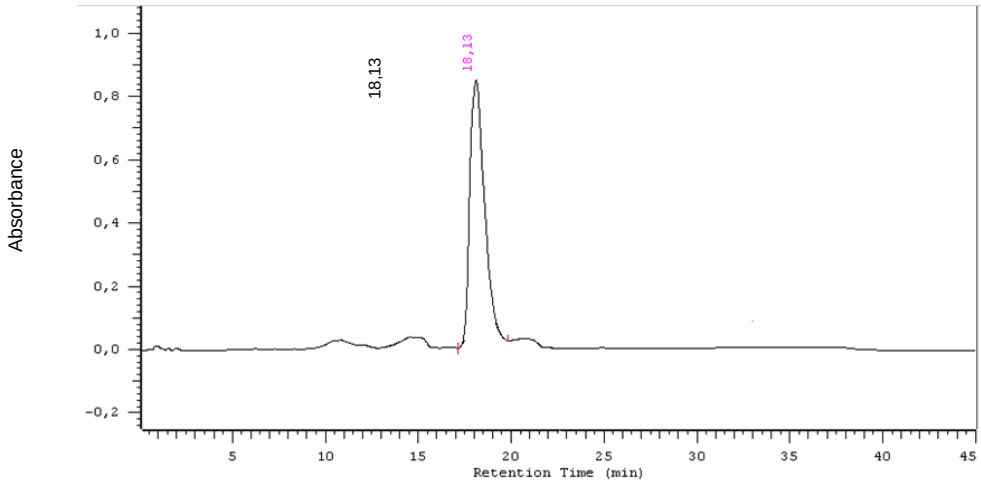



**Figure 10.** LC-DAD profile of isoeleutherine registered at 250 nm.

### 3.8. Antioxidant activity of EE, isoeleutherol, and isoeleutherine

Figure 11 and Table 6 show the evaluation of the antioxidant activity of EE, isoeleutherol, and isoeleutherine on DPPH in comparison to the results obtained for BHT used as standard. The antioxidant activity of isoeleutherol (Ep1) appears in concentrations up from 4 μg/mL; for EE, 5 μg/mL; and isoeleutherine (Ep2), 6 μg/mL; while BHT showed activity in concentrations above 1 μg/mL.

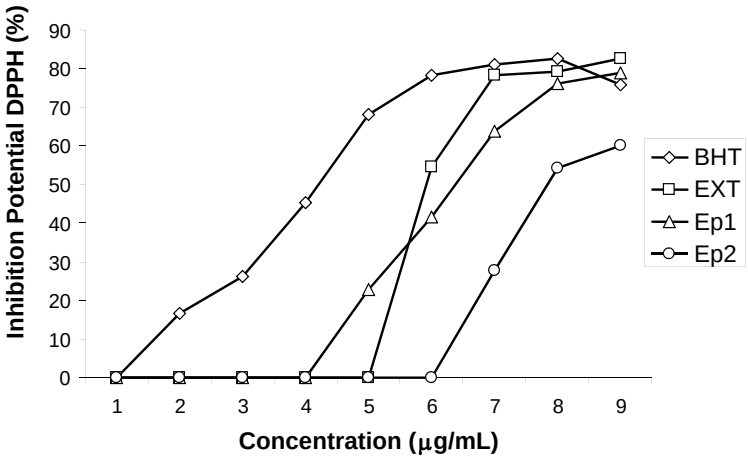



**Figure 11.** Evaluation of antioxidant activity of EE, and isoeleuterol, and isoeleutherine against DPPH.

Among the tested samples, isoeleutherol showed the best antioxidant activity considering its Inhibition Concentration value (Table 6), followed by EE and isoeleutherine. However, when the $IC_{50}$ values of isoeleutherol and BHT are compared, stay clear that the antioxidant activity of isoeleutherol is about 5× lower.

| SAMPLE | $IC_{50}$ |
|---|---|
| BHT | 17.83 |
| EE | 94.72 |
| ISOELEUTHEROL | 84.63 |
| ISOELEUTHERINE | 281.04 |

**Table 6.** Determination of $IC_{50}$ of EE, isoeleutherol, isoeleutherine and BHT.

The antioxidant activity of isoeleutherol, higher than that observed for EE and isoelutherine, may be attributed to the hydroxyl group at C-9; this phenolic residue may act as free radical scavenger and sometimes as chelating agent of metal ion with effective action, mainly in preventing lipid oxidation, acting both on the initiation step as on the propagation step of this oxidative process.

Table 6 shows that EE has a higher $IC_{50}$ than isoeleutherol, but lower than that of isoeleutherine. This fact can find explanation in the presence of tannins in EE, which are polyphenols that have adequate chemical structure for the capture of free radicals, contributing to an effective antioxidant capacity of the sample. It is noteworthy to mention that the presence of naphthoquinones in EE may antagonize the activity of tannins since these secondary metabolites can induce oxidative stress or show pro-oxidant capacity, leading EE to present a very low antioxidant activity. Indeed, isoeleutherine presents a negligible antioxidant activity as expected from its structural characteristics, but its occurrence in aqueous extract may be a reason for the antiamoebic activity detected in a previous work [5] and described for *E. bulbosa*, a synonym of *E. plicata* according to TROPICOS®[3], 30 years before today [16].

## 4. Conclusions

Isoeleutherol and isoeleutherine described before in other *Eleutherine* species were isolated from the chloroform fraction of EE and characterized by $^{1}$H- and $^{13}$C-NMR. Their HPLC analyses allow confirmation that both are present in the herbal drug, dried bulbs of *E. plicata*. Isoeleutherol seems to be the major chemical constituent in EE and thus can be indicated as a chemical marker for the quality control of this plant species and its derivatives. The occurrence of isoeleutherine in aqueous antiamoebic extract indicates this substance to be used in the quality control of the extract and derivatives, mainly if the substance can be linked to the

3 tropicos.org an information system of Missouri Botanical Garden's electronic databases, Access: 21 Sep. 2014.

reported activity. The isolation and characterization of isoeleutherol and isoeleutherine, which show well-described pharmacological activities, justify the potential of *E. plicata* Herb. to originate a phytomedicine to face neglected diseases, such as Amoeba infection, and probably explain the reason why this plant finds a wide popular use in Amazonian countries.

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