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Chemical Profile of Essential Oil, Extracts and Fractions of *Eugenia pyriformis* Cambess. and its Antioxidant, Cytotoxic and Allelopathic Activities

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HIGHLIGHTS

- *Eugenia pyriformis* essential oil (EO) of the leaf contains linalol, terpinen-4-ol; 2-carene;
- The extract showed *β*-amiryn (**1**) and the porphyrin derivatives;
- The extracts of the leaf and stem showed antioxidant activity;
- The hexane extract of the stem showed allelopathic activity.

Abstract: The chemical investigation of *Eugenia pyriformis*, popularly known as uvaia, led to the isolation of three compounds, the triterpene *β*-amiryn (**1**) and the porphyrin derivatives pheophytin *a* (**2**) and *b* (**3**). Their structures were assigned based on spectroscopic NMR analysis, as well as by comparison with literature data. Most of the compounds found in the essential oil from leaves are terpenes derivatives, such as *o*-cymene; 1,8-cineol; linalol, terpinen-4-ol; 2-carene. Flavonoids, steroids and/or terpenoids, lactones, saponins, tannins and fixed acids were detected in both the leaf and stem crude extracts and fractions generated from the Brazilian *E. pyriformis* plant. According to the results of the DPPH assay, the ethyl acetate fraction of the leaf extract showed higher antioxidant activity than the other samples tested. In the phosphomolybdenum complex assay, the crude ethanol extract of the leaf and stem, and the ethyl acetate fraction of the leaf, showed a higher antioxidant capacity than rutin. It was observed that the chloroform and ethyl acetate fractions of the leaf, and the hexane, ethyl acetate and hydroalcoholic fractions of the stem were toxic to *Artemia salina* brine shrimp with LD_{50} < 1.0 µg/mL. The hexane fraction of the stem was also allelopathic in inhibiting the radicle growth of *Lactuca sativa* seeds. These results adding contributions to chemical and biological studies of this medicinal species.

Keywords: *Eugenia pyriformis*; phytochemical; antioxidant activity; allelopathy.

INTRODUCTION

The plant family Myrtaceae Juss. is widespread in the Americas and Oceania [1], and it consists of 145 genera and 5970 species [2]. In Brazil, this is one the largest plant families with approximately 29 genera and 1192 species distributed throughout the country [3]. Myrtaceae is rich in fruit species such as jabuticaba (*Myrciaria* spp.), pitanga (*Eugenia uniflora* L.), goiaba (*Psidium guajava* L.), araçá (*Psidium Cattleianum* Sabine), cherry-national (*Eugenia retusa* (O. Berg) Nied.), guabiroba (*Campomanesia* spp.), cerejeira-do-mato (*Eugenia involucrata DC*.) and uvaia (*Eugenia pyriformis* Cambess.), among others [4].

The genus *Eugenia* L. belongs to the Myrtaceae family and the tribe Myrteae, it comprises around 1058 species widespread through North, Central and mainly South America [4-6], it is also found in Southeast Asia and Pacific [5]. In Brazil 407 species are described [4]. These plants have nutritional, pharmaceutical and commercial value [7,8]. Studies on *E. beaurepaireana* showed anti-inflammatory, antioxidant activity from the leaves [9,10]; *E. uniflora* presented antioxidant and antimicrobial activity [11] and *E. involucrata* presented cytotoxicity for the B16F10 murine melanoma cells [6].

The species *Eugenia pyriformis* (Figure 1), known as uvaia, is a fruit-bearing tree found in countries of South America such as Argentina, Paraguay and Brazil [12], its leaves have a medicinal property used in folk medicine to treat gout [13,14]. The flavonoids present in the leaves inhibit the enzyme xanthine oxidase, which converts xanthine into uric acid [14].

Studies related to the species such as the performed by Stieven and coauthors [15], evaluating the antimicrobial activity of *E. pyriformis* fruit essential oil, have shown that it is active against pathogenic bacteria *Escherichia faecalis*, *E. coli* and *Staphylococcus aureus*, and encouraged its use in food products to increase their shelf lives [16]. Ethanolic extracts of the leaf and the seed possess useful antimicrobial activity against Gram-positive and Gram-negative bacteria [17]. Additionally, extracts and fractions of the leaf and stem showed antimicrobial activity against pathogenic bacteria and fungi, and further act synergistically with antibiotics to increasing their efficacy [18].

Many medicinal plants are investigated for their phenolic compound content since, for example, flavonoids and tannins are distinguished by their antioxidant properties and they are known to scavenge free radicals. These can play an important role as defense agents against diseases and biological processes including cancer, aging, inflammation, tissue damage, and even some industrial purposes such as preservation of foods, for instance [15,19]. In addition, their interaction with enzymes, substrates, metal ions, for example, can interfere with microbial metabolism and yield the antimicrobial activity mentioned above [17]. Phenolics have been detected in fruits of *E. pyriformis*, and these showed antioxidant activity [16,19].

Artemia salina is a marine microcrustacean, also called brine shrimp, which is often used as a biological model to evaluate the toxicity of natural products. Linking antifungal, antiviral, antimicrobial, anticancer, trypanosomicidal and parasiticidal activities to the toxicity to *A. salina*, due to the possible effect of the vegetal extracts has been well demonstrated [20]. This can be considered preferential over many bioassays being considered a simple and less expensive preliminary test of cytotoxicity.

Due to the interactions of chemicals released in the environment, the vegetation of a certain area may undergo changes, and this phenomenon is called allelopathy. Species used to examine allelochemicals, such as *Lactuca sativa* L. (lettuce) and *Lycopersicum esculentum* Mill. (tomato) are widely used in growth and germination tests due to their seed sensitivity [21]. The metabolites produced by plants can have defensive activities, preservation and protection from invading neighboring plants, and management of developing crops. These characteristics have bolstered the search for new naturally occurring pesticides [22].

According to the medicinal understanding of *E. pyriformis*, it is an important species target to explore by different approaches. It appears to have therapeutic, nutritional (edible fruits) and economical value. Most studies regarding the species were performed with the fruits, seeds or occasionally some extracts of the leaf. Accordingly, this is a plant to investigate with opportunities to report new discoveries. Therefore, this work aimed the investigation of the phytochemical profile of the species, the identification of compounds and the evaluation of antioxidant activities through DPPH and phosphomolybdenum complex assays, toxicity against *A. salina* brine shrimp and allelopathic effects of crude extracts and fractions of the leaf and stem produced from *E. pyriformis.*

Figure 1. *Eugenia pyriformis* Cambess.

MATERIAL AND METHODS

Identification of the species

Branches and leaves of *Eugenia pyriformis* Cambess. (uvaia, uvaieira, uvaia-do-campo, uvalha and uvalha-do-campo) were collected at the Botanical Garden of Curitiba (Paraná, Brazil) and a voucher specimen was deposited at the Municipal Botanical Museum (MBM) under the collection number MBM 204990, identified by the botanist Osmar dos Santos Ribas and registered in SisGen A0EB51A [23].

Essential oil extraction and analysis

Ground up leaves (200 g) were extracted by hydrodistillation in a Clevenger apparatus modified by Wasicky [24], and the essential oil was collected after 6 h. The major components of essential oil were determined by gas chromatography/mass spectrometry using a Varian CG model 3800 connected to a Varian SATURN 2000 ion-trap spectrometer, and Varian MS-Workstation 6.1.9 software. The carrier gas was helium, and it was used at a flow rate of 1.0 mL/min. A VF-5MS Agilent capillary column (30 m x 0.32 mm I.D. x 1 µm film thickness) was used at a temperature of 50 ºC, increasing at a rate of 2 ºC/min to 90 ºC, then 20 ºC/min to 280 ºC, and held for 5 min at 280 ºC until 45 min. The components were identified by evaluation of the mass spectra and retention times [RT; used in relation to a series of *n*-alkanes to calculate the retention index (RI)], and comparison with the NIST (National Institute of Standards and Technology) database.

Phytochemical analysis

Leaf and stem extraction was carried out by hot maceration to obtain aqueous and 20% v/v hydroalcoholic extracts, performed according to a published protocol [25].

Samples were evaluated for the presence of flavonoids using the Shinoda, Taubock and Pacheco reactions by observations of changing colors and fluorescence. Steroids and triterpenoids were detected by the Liebermann-Burchard reaction, and in the face of a positive result, the Keller-Kiliani reactions were performed to test for deoxy sugars and Baljet reaction to identify the presence of pentacyclic lactones. Coumarins were observed in basic medium and detected by blue or yellowish/green fluorescence under UV light. For the anthraquinone research, the Bornträger reaction was carried out, and the positive result was verified by the red staining of free anthraquinones in basic medium.

The aqueous extract was examined for saponins, and the formation of persistent foam was observed after vigorous stirring of the extract. The fixed acids evaluation was performed using Nessler's reactions yielding orange color, and the tannins were detected by reaction with ferric chloride, gelatin and formaldehyde-hydrochloric acid by Stiasny reaction.

Chemical evaluation of the leaves and stem

Extraction, isolation and fractionation

The crude acetone extract was obtained from 840 g of leaf and 1 kg of stem dried in an oven at 40 ºC and extracted with acetone at 30 ºC in a Soxhlet apparatus. The samples were eluted separately in a solvent system in decreasing polarity (water, methanol and acetone) in an octadecylsilyl (RP-18), Lichroprep® (25-40 mesh) filtration process. The methanolic and acetone fractions were concentrated separately and subjected to classical column chromatographic fractionation. Columns of silica gel 60 (0.063-0.02 mm/70-230 mesh), Masherey-Nagel® and aluminum (aluminum oxide 90 neutral), pH 7 ± 0.5, using gradients of petroleum ether and ethyl acetate (4:1 to 2:3). Aliquots were monitored by thin layer chromatography (TLC) using Masherey-Nagel® 60 F254 (0.25 mm) chromatoplates. Final purification was achieved by flash chromatography on neutral alumina [26].

Flash chromatography was performed on a Biotage SP-1 chromatography system using homemade alumina cartridges prepared from column chromatography neutral alumina (Brockmann activity 1) Macherey-Nagel®.

Three compounds (**1**-**3**) were isolated from acetone fraction of the leaves of *E. pyriformis*, subfractions of petroleum ether/ethyl acetate (8:2; 7:3 and 6:4, respectively). The same compounds were identified in the equivalent fractions of the stem. To simplify purification, the waxy precipitate was purified by suspension in 5.2 mL of methanol and stirred for 10 minutes at 60 °C. The suspension was kept at 8 °C overnight, and finally filtered and washed with cold methanol to afford white semi-solid crystals.

NMR analysis

^{1}H and ^{13}C NMR spectra were obtained on Bruker® AC 200 [200 MHz (^{1}H) and 50 MHz (^{13}C)] and JEOL Eclipse 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] spectrometers, and the solvent used was $CDCl_3$.

Extraction and fractionation for assays

The preparation of the crude ethanolic extract from the leaves and stem was done with ethanol at 96 °GL, at 35 °C, in Soxhlet apparatus. The fractions were obtained by liquid-liquid partitioning with solvents in increasing polarity (hexane, chloroform and ethyl acetate), with the remaining fraction being hydroalcoholic [27]. This crude extract and its respective fractions were used to perform the phytochemical analysis and testing the antioxidant, cytotoxic and allelopathic activities.

Antioxidant assays

For the evaluation of the antioxidant activity of crude ethanolic extract and fractions, the DPPH (2,2-diphenyl-1-picryl-hydrazyl) method was used. Vitamin C and rutin were used as positive controls and a mixture of DPPH and ethanol as a negative control. All samples were tested at concentrations of 40, 80, 100, 120, 160 and 200 µg/mL in triplicates [28,29]. Samples which were more active were tested in low concentrations according to the features of the sample. The absorbance analyzed was at 518 nm wavelength and the IC_{50} (Half Maximum Inhibitory Concentration) was determined by linear regression corresponding to three replicates ± standard deviation. The data obtained was analyzed by means of ANOVA and Tukey test (p <0.05).

The phosphomolybdenum complex was also used to evaluate the antioxidant activity, and vitamin C and rutin were used as standard positive controls. The antioxidant activity of each sample and rutin was calculated in relation to vitamin C, the activity of which was considered to be 100%. In the phosphomolybdenum complex antioxidant capacity assay, all samples were tested at 200 µg/mL [29]. The UV absorbance was measured at 695 nm and the activity was calculated compared with the standards values.

Toxicity against *Artemia salina*

This evaluation was made in triplicate with the crude ethanolic extract and the hexane, chloroform, ethyl acetate and hydroalcoholic fractions each at concentrations of 1000, 100 and 10 µg/mL. The positive control was quinidine sulfate, and negative control was ethanol [20,30,31]. Data from dead and alive nauplii were analyzed using the Probit statistical method, the LD_{50} (lethal dose to eliminate 50% of the population) and the 95% confidence interval (p<0.05) were determined. Samples were considered active when the LD_{50} had a result lower than 1000 µg/mL.

Allelopathic assay

The crude ethanolic extract and fractions (at the quantities 0.8 mg, 0.4 mg, 0.2 mg, 0.1 mg) were dissolved in ethanol (2 mL) and tested to evaluate the germination and growth of seeds of *Lactuca sativa* L. Petri dishes were embedded in pieces of filter paper (Whatman no. 6) soaked with the solutions prepared and heated at 60 °C for 24 h to evaporate the solvent. Twenty seeds were deposited in a Gerbox box sterilized, and moistened with distilled water (3 mL), being four quadrants of five seeds, where one box was used to analyze the speed of germination index and another box for the growth, the test was performed in duplicate. Gerbox were protected from light with aluminum foil and placed in a chamber germinator at a temperature of 24 °C. The germination was performed for seven days, where germinated seeds were removed. The radicle and hypocotyl size were measured with millimeter paper. The media obtained from the speed germination were submitted to the Skott-Knott test at a 5% probability level. Ethanol and water were used as controls, in order to verify their influence on the preparation and dilution of the samples [32,33].

RESULTS AND DISCUSSION

Essential oil analysis

The main compounds identified in the essential oil of the leaves by GC/MS analysis can be seen in Table 1, such as: γ-terpinene; *o*-cymene; 1,8-cineol; linalool, terpinen-4-ol; 2-carene; terpinolene; di-*tert*-butylacetylene; *ρ*-benzoquinone-2,6-di-*tert*-butyl; retinal and *m*-methoxyphenyl ether along with some unidentified compounds.

The major constituents of the essential oil from leaves in the genus *Eugenia* are terpene derivatives such as monoterpenes and sesquiterpenes, oxygenated monoterpenes and sesquiterpenes. The compounds *β*-caryophyllene and *α*-cadinol were observed in *E. pyriformis* and *E*. *beaurepaireana* by Apel and coauthors [34]. In comparison with another analysis of the essential oil of leaves of *E. pyriformis* by Stefanello and coauthors [35]. Other studies with E. 224 pyriformis identified as major compounds β-caryophyllene, bicyclogermacrene, globulol, and δ-cadinene and 225 terpinene-4-ol as minor compound similar to the current study [36]; the compounds 1,8-cineol and terpinen-4-ol were observed in this study. Limonene (14.8%), nerolidol (11.0%), *α*-cadinol (10.3%), caryophyllene oxide (9.9%) and *β*-pinene (7.1%) were observe as major compounds by Durazzini and coauthors [37], differing from the compounds found in this study, revealing variation in the constituents.

Table 1. Chemical essential oil constituents of leaves of *Eugenia pyriformis* Cambess. Myrtaceae by GC/analysis

Retention time (in minutes)	Compound	Molecular formula
6.145	γ-terpinene	$C_{10}H_{16}$
6.411	*o*-cymene	$C_{10}H_{14}$
6.622	1,8-cineol	$C_{10}H_{18}O$
7.833	linalool	$C_{10}H_{18}O$
9.497	terpinen-4-ol	$C_{10}H_{18}O$
13.377	2-carene	$C_{10}H_{16}$
14.470	terpinolene	$C_{10}H_{16}$
14.531	di-*t*-butylacetylene	$C_{10}H_{18}$
14.938	*ρ*-benzoquinone-2,6-di-tert-butyl	$C_{14}O_{20}H_2$
16.703	retinal	$C_{20}H_{28}O$
16.886	1,2,3,4,5,6-hexahydro-1,1,5,5-tetramethyl-2,4a-metanonaftalen-7(4-*α*-H)-one	$C_{15}H_{22}O$
18.414	*m*-methoxyphenyl ether	$C_{13}H_{12}O_2$
18.656	4*α*,7-methane-4-*α*-H-naft [1,8*α*-*β*] oxirene, octahydro-4,4,8,8-tetramethyl	$C_{13}H_{24}O$
19.083	1,4-methane azulene-7(1H)-one,octahydro-1,5,5,8 -tetramethyl	$C_{15}H_{24}O$

Phytochemical analysis

Flavonoids were found to be present in the hydroalcoholic extracts of the leaves and stems, as well as chloroform and ethyl acetate fractions, while steroids and/or terpenoids and lactones were present in the hexanes and chloroform fractions. The tests were negative for the presence of coumarins and anthraquinones in the hydroalcoholic extract and fractions. Meanwhile, saponins, tannins and fixed acids were detected in the aqueous extract. The results of the analyses can be seen in Table 2. Phytochemical studies of the hydroalcoholic extracts of *E. pyriformis* showed the presence of alkaloids, flavonoids, tannins, and saponins in the leaf, but only the latter two classes were found in the stems [17]. Flavonoids were seen in leaves of *E. pyriformis*, such as myricitrin, isoquercitrin, hyperoside, quercitrin, myricetin-3-*O*-(2''-*O*-galloyl)-*α*-L-rhamnoside, myricetin-3-*O*-(4''-O-galloyl)-*α*-L-rhamnoside and the aglycone quercetin [38]. Phenolic acids and flavonoids were detected abundantly in the fruit of *E. pyriformis*, predominantly comprising gallic acid, myricetin and quercetin [19,39]. Phytochemical profiling of the genus *Eugenia* also identified flavonoids in the leaves of in *E. uniflora* [11], *E. brasiliensis*, *E. beaurepaireana* (Kiaerskou) Legrand, *E. umbelliflor*a O. Berg [10] and *E. dysenterica* DC. [40]. The leaves of *E. dysenterica* also contained terpenoids and saponins [40].

Table 2. Phytochemical prospection of hydroalcoholic extract and their respective hexanes, chloroform and ethyl acetate fractions and aqueous extract of the leaf and stem of *Eugenia pyriformis* Cambess., Myrtaceae

Chemical groups	**HAF**	**Fractions**		
		HF	**CF**	**EAF**
alkaloids	-	-	-	-
flavonoids	+	-	+	+
steroids/triterpenoids	-	+	+	-
deoxy sugars	-	-	+	-
pentacyclic lactones	-	+	+	-
coumarins	-	-	-	-
anthraquinones	-	-	-	-
	AE			
saponins			+	
tannins			+	
fixed acids			+	

HF=hexanes fraction; CF= Chloroform fraction; EAF= Ethyl acetate fraction; HAF= Hydroalcoholic fraction; AE= aqueous extract

Chemical evaluation of the leaves and stem

Compound identification

Three compounds were isolated from acetone fraction of the leaves of *E. pyriformis,* compound **1** (*β*-amyrin) from the petroleum ether/ethyl acetate (8:2 subfraction) [41,42] and from the elution petroleum ether/ethyl acetate (7:3 and 6:4), the compounds **2** (pheophytin *a*) and **3** (pheophytin *b*), affording 522 mg of **1**, 307.8 mg of **2** and 193.3 mg of **3**. The structures of **1**-**3** are presented in Figure 2 [43,44]. The same compounds were identified in the same fractions of the stem.

This reports the isolation of *β*-amyrin and pheophytins *a* and *b* in the species *E. pyrifromis*. Commonly the compounds *α*-amyrin and *β*-amyrin appear together in a mixture in *E. brasiliensis* and *E. beaupaireana* [9,45], being rarely found separately. In this study it was possible to isolate *β*-amyrin as a major compound, which suggests that the genus and could be considered is a rich source of it, which agrees with the study of Klein and coauthors [46], where they found it as majority.

The reports on the diversity of terpenes in the family Myrtaceae relate to the enzymes terpene synthases (TPS) and oxidosqualene cyclases (OSC), as key protein encoding genes that are involved in the biosynthesis of monoterpenes, diterpenes, sesquiterpenes and triterpenes [48,49]. This agrees with all the

findings reported previously in the literature about *E. pyriformis* and other species from the genus. Therefore, these important results contribute to chemotaxonomic data within the genera.

Pheophytin *a* is a degradation product of the chlorophyll and in recent years has been presenting studies that reveal anticancer activity in vitro, being observed photodynamic activity and showed high affinity human mitochondrial translocator protein (TSPO) ligand, which appears in many types of cancerous cells. This study showed that the compound IC_{50} of 22.9 µM is a good target for metastatic alveolar A549 cell lines. [50; 51].

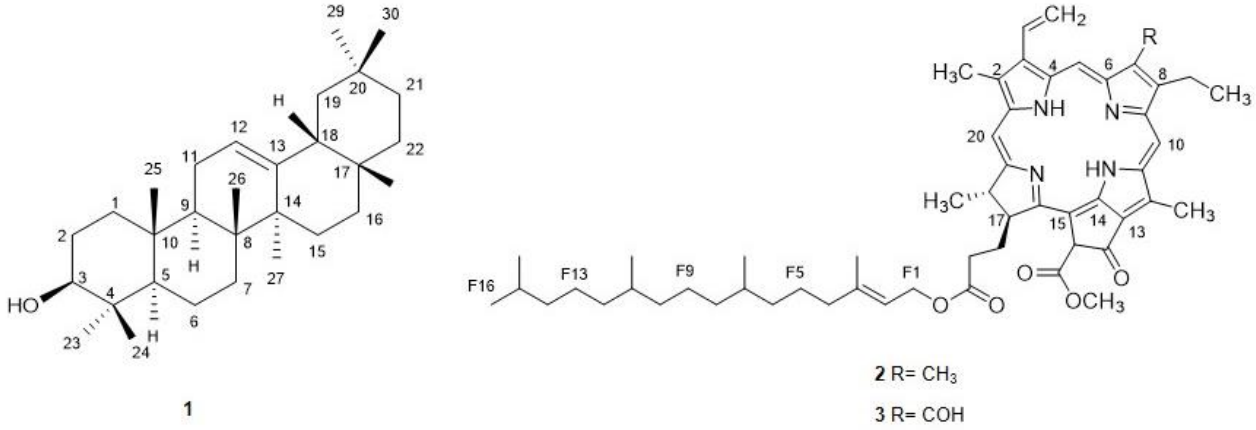

Figure 2. Structures of compounds **1-3** isolated from the leaves and stem of *Eugenia pyriformis* Cambess.

Antioxidant assays

The crude ethanolic extract and its subfractions were tested using the DPPH and the phosphomolybdenum complex for antioxidant activity, the outcome of which are compiled in Table 3. In the DPPH assay all the samples presented antioxidant activity, but the crude ethanolic extract of the leaf (8.83 ± 0.24 µg) and stem (9.22 ± 0.55 µg), the chloroform fractions of the leaf (13.21 ± 0.11 µg) and stem (13.97 ± 0.02 µg), ethyl acetate (4.86 ± 0.02 µg) and hydroalcoholic (9.75 ± 0.20 µg) fractions of the leaf showed antioxidant activity statistically similar to vitamin C (4.36 ± 0.13 µg) and rutin (6.69 ± 0.30 µg).

In the phosphomolybdenum complex antioxidant capacity assay (Table 3) all the samples tested were compared with the vitamin C standard (100%), but none of the samples tested were statistically similar to the activity of this standard. However, when compared to the rutin standard (28.25%), it was observed that the crude ethanolic extract of the leaf (29.31%) and stem (34.15%) and, the ethyl acetate fraction of the leaf (37.05%) were statistically superior to rutin.

Table 3. Antioxidant activity by DPPH reduction and phosphomolybdenum complex capacity assay of the leaf and stem (crude extract and fractions) of *Eugenia pyriformis* Cambess. Myrtaceae

	DPPH				**Phosphomolybdenum complex**	
Samples	IC_{50} (µg) ±SD	Tukey test	IC_{50} (µg) ±SD	Tukey test	%	
vitamin c	4.36± 0.13	a1			100	
rutin	6.69 ± 0.30	a1			28.25	
	Leaf		**Stem**		**Leaf**	**Stem**
CE	8.83 ± 0.24	a1	9.22 ± 0.55	a1	29.31	34.15
HF	212.53 ± 62.68	a4	63.88 ± 0.31	a2	19.58	12.08
CF	13.21 ± 0,11	a1	13.97 ± 0,02	a1	16.00	24.00
EAF	4.86 ± 0.02	a1	65.89 ± 4.34	a2	37.05	13.25
HAF	9.75 ± 0.20	a1	156.73 ± 5.83	a3	19.93	11.06

Samples classified in the same group do not differ statistically, from the standard. IC_{50} = Half Maximal Inhibitory Concentration, SD = standard deviation. CE= crude extract; HF=hexanes fraction; CF= Chloroform fraction; EAF= Ethyl acetate fraction; HAF= Hydroalcoholic fraction

The positive results in both tests demonstrate that *E. pyriformis* extract is an effective free radical scavenger. The results of an earlier study also supported this conclusion by showing that the ethanolic extract of the leaves *E. pyriformis* had a great antioxidant activity using DPPH and ORAC tests [52].

Studies regarding the antioxidant activity of the crude ethanolic extract and fractions of the leaf and stem of *E. brasiliensis*, *E. beaurepaireana* and *E. umbelliflora* using the DPPH test, potential reducer and inhibitor of lipid peroxidation showed that the first two species had higher antioxidant activity than *E. umbelliflora* in the crude extract and fractions of the leaf and stem, but a higher activity was observed in the leaf fractions, which contains phenolic compounds [10]. Ethanol: water (70:30 v/v) and ethyl acetate (EtOAc) extracts of the leaves and fruits of *E. pyriformis* showed antioxidant activity with (DPPH•), 18.8 ± 0.4 and 8.4 ± 0.4 µg mL^{-1} and peroxyl radical (ROO•), 6.4 ± 0.2 and 4.9 ± 0.4 µg mL^{-1} [38].

Ethanolic extracts of *E. jambolana* leaf Lam. and *E. uniflora* were studied by Santos and coauthors [52], who evaluated the antioxidant activity using DPPH. The *E. jambolana* species presented high activity compared to *E. uniflora* due to the presence of phenolic compounds. Noting the results obtained with the crude ethanolic extract of *E. pyriformis* also has high antioxidant activity. The ethanolic extract of leaves of *E. catharinae* showed antioxidant activity under DPPH assay, and also contains flavonoids [53]. The same can be observed in our study, which is possible to see on Table 2 the presence of flavonoids in the hydroalcoholic, chloroform and ethyl acetate fractions, and in the aqueous extract to have saponins and tannins. All these compounds are well known to have great antioxidant activities.

Toxicity against *Artemia salina*

The chloroform (LD_{50} = 201.36 µg/mL) and ethyl acetate (LD_{50} = 292.52 µg/mL) fractions of the leaves were toxic against *A. salina* and the hexanes (LD_{50} = 217.04 µg/mL), ethyl acetate (LD_{50} = 115.21 µg/mL) and hydroalcoholic (LD_{50} = 115.21 µg/mL) fractions of the stem also showed similar activity. The toxicity assay using *A. salina* showed that the chloroform, ethyl acetate and hexane fractions of the leaf and ethyl acetate and hydroalcoholic fractions of the stem were toxic with LD_{50} <1000 µg/mL (Table 4). The essential oil of *E. pyriformis* was tested against *A. salina*, showing a ($LC_{50=}$ 125.64 µg mL^{-1}) to kill 50% of the population of the shrimp [39]. Antifungal, antiviral, antimicrobial, and antiparasitic activities have been correlated with toxicity to *A. salina*, due to many possible effects of applying plant extracts [21]. According to the results the species could be tested in different assays, such as those mentioned above, to investigate new biological activities. Furthermore, crude extracts and fractions of the leaves and stem of *E. pyriformis* of the present work have showed antibacterial and antifungal activities in the results reported earlier by de Souza and coauthors [18].

Table 4. Index of lethality against *Artemia salina* for the active samples from *Eugenia pyriformis* Cambess. Myrtaceae, of the leaf and stem (crude extract and fractions)

Samples	LD_{50} (µg/mL)	95% Confidence interval (µg/mL)	LD_{50} (µg/mL)	95% Confidence interval (µg/mL)
quinidine	50.12	(35.80-70.16)		
		Leaf		**Stem**
CE	>1000	-	>1000	-
HF	>1000	-	217.04	(196,80-240,25)
CF	201.36	(154.54-262.37)	>1000	-
EAF	292.52	(244.87-349.44)-	115.21	(113,64-201,22)
HAF	>1000	-	115.21	(113,64-201,22)

CE= crude extract; HF=hexanes fraction; CF= Chloroform fraction; EAF= Ethyl acetate fraction; HAF= Hydroalcoholic fraction. LD_{50} = Dose required to induce 50% lethality

Studies carried out with the ethanolic extract of *E. uniflora* to evaluate the toxic activity against *A. salina* resulted in the observation of activity at LD_{50} >1000 µg/mL, the extract being the extract few active and with insignificant value [55], unlike the leaf fractions (chloroform and ethyl acetate) evaluated in *E. pyriformis*, in which toxic agents were observed. In *E. braziliensis,* the crude ethanol extract and fractions of the leaves were active against *A. salina* [46].

Allelopathic assay

There were no significant allelopathic results observed on the germination and growth on the seeds of *L. sativa* from the crude ethanolic extract of the leaf and its respective fractions. However, the hexanes fraction of the stem inhibited the radicle's growth in all the concentrations tested (0.1; 0.2; 0.4; 0.8 mg/mL), which shows this fraction could have an allelochemical influence on the growth of plants in the nature (Table 5). This fraction tested positive for triterpenoids in the phytochemical analysis, and some literature reports note that these types of molecules inhibited germination and radicle growth [54,55]. Although triterpenoids were also detected in the leaf fraction, which was not active in the present investigation, it is suggested that the allelopathic activity observed by others could exist due to a synergistic interaction with compounds there present but not in the leaves studied here.

In an allelopathic test performed with the aqueous extract of *E. dysenterica* on seeds of *L. sativa*, Giotto and coauthors [57] did not observe any action on germination, but on growth allelopathic effects were demonstrated. In the study of Sausen and coauthors [58], the aqueous leaf extract of *E. involucrata* DC. presented effects of modulating the germination and growth of *L. sativa*, meaning that the species could hamper the establishment of new plants in the environment, and as a consequence facilitate the initial establishment of this species over others.

Table 5. Allelopathic activity of the hexanes fraction (HFS) of the stem of *Eugenia pyriformis* Cambess. Myrtaceae against lettuce seeds (*L. sativa*) germination.

Treatment (mg)	Repetion	Radicle (average mm)
		HFS
0.8	1	31.20 a2
	2	31.60 a2
	3	30.00 a2
	4	26.40 a2
0.4	1	29.00 a2
	2	30.00 a2
	3	29.40 a2
	4	29.20 a2
0.2	1	30.40 a2
	2	31.40 a2
	3	24.00 a2
	4	30.00 a2
0.1	1	29.00 a2
	2	30.00 a2
	3	29.40 a2
	4	29.20 a2
Controls		
W	1	33.80 a1
	2	36.80 a1
	3	36.40 a1
	4	33.00 a1
E	1	36.40 a1
	2	34.00 a1
	3	38.20 a1
	4	41.60 a1

* Scott-Knott's test showed that the mean treatment differed significantly from the average control. HFS= hexanes fraction stem; W= water; E= ethanol

CONCLUSION

The current research enriches the knowledge of chemical diversity and provides data for the chemotaxonomic studies within the genus *Eugenia*, reporting also the constituents from the essential oil, which altogether complements previous studies. Additionally, this work showed that *E. pyriformis* hosts a great antioxidant potential all fractions tested for the DPPH assay. In the phosphomolybdenum complex antioxidant capacity assay the crude ethanolic extract of the leaf and stem and, the ethyl acetate fraction of the leaf exhibited superior activity to rutin. This material further demonstrated brine shrimp toxicity, which

makes it an interesting species to be investigated in a deeper context, and it also has allelopathic properties. This suggests that more studies related to the potential of this plant in different allelopathic tests, ecological interactions with other species, and applications in agriculture, for example, are worth investigating.

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