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Essential oil of the leaves of *Eugenia uniflora* L.: Antioxidant and antimicrobial properties

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ABSTRACT

Essential oil (EO) of the leaves of *Eugenia uniflora* L. (Brazilian cherry tree) was evaluated for its antioxidant, antibacterial and antifungal properties. The acute toxicity of the EO administered by oral route was also evaluated in mice. The EO exhibited antioxidant activity in the DPPH, ABTS and FRAP assays and reduced lipid peroxidation in the kidney of mice. The EO also showed antimicrobial activity against two important pathogenic bacteria, *Staphylococcus aureus* and *Listeria monocytogenes*, and against two fungi of the *Candida* species, *C. lipolytica* and *C. guilliermondii*. Acute administration of the EO by the oral route did not cause lethality or toxicological effects in mice. These findings suggest that the EO of the leaves of *E. uniflora* may have the potential for use in the pharmaceutical industry.

1. Introduction

A growing body of research in biology and medicine has been devoted to reactive oxygen species (ROS). ROS are an inevitable byproduct of cellular respiration, causing oxidation of lipids, nucleic acids, and proteins, and ROS damage is an underlying cause of disease, including cancer, inflammatory, and neurodegenerative diseases (Bakkali et al., 2008). The cells have sophisticated antioxidant regulatory systems to maintain the proper balance of ROS. However, disruption in homeostasis can result in oxidative stress and tissue injury (Halliwell et al., 1995). Thus, it is believed that exogenous antioxidant compounds could be employed to improve situations in which oxidative damage is implicated.

Natural antioxidants are in high demand for application as nutraceuticals, bio-pharmaceuticals, and food additives. In fact, in recent decades, intensive research has been performed for the extraction, characterization and utilization of natural antioxidants, which may serve as potent candidates in combating the aging process (Ozen et al., 2011). Essential oils (EOs) are volatile, natural, complex mixtures composed of secondary metabolites that are commonly concentrated in the leaves, bark or fruits of aromatic plants (Bakkali et al., 2008). They have recently received much attention from researchers due to their multiple functions, such as their antioxidant, antimicrobial, antifungal, antiviral, antinociceptive, and anticancer activities (Bakkali et al., 2008). In the pharmaceutical industry, EOs are employed as medicines or as their coadjutants. In Brazil, examples of successful applications include the EO obtained from *Cordia verbenacea* (Boraginaceae), an anti-inflammatory medicine for topical use (Acheflan®) and clove oil (*Syzygium aromaticum*, Myrtaceae), which is widely used in dental care as a sealing component and as an antiseptic for oral hygiene. As a coadjutant, the EOs are used to promote medicine absorption, increasing penetration in the epidermis due to its lipophilic characteristics (Amorim et al., 2009).

Eugenia uniflora L. (Myrtaceae), known as the Brazilian cherry tree (or pitangueira), is a fruit-bearing tree widely distributed throughout Brazil and is used in popular medicine as a diuretic, anti-rheumatic, anti-febrile, and anti-inflammatory agent and as a therapeutic agent for stomach diseases (Weyerstahl et al., 1988). The Brazilian cherry tree leaves EO has been used by the Brazilian cosmetics industry for its astringent properties, which are associated with its pleasant smell. The main applications are in shampoos, hair conditioners, face and bath soaps, body oils and perfumes (Amorim et al., 2009). EOs obtained from the leaves of *E. uniflora* L. have antifungal (Costa et al., 2010), antibacterial and cytotoxic (Ogunwande et al., 2005), antinociceptive and

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hypothermic properties (Amorim et al., 2009). However, to the best of our knowledge, no study on the antioxidant activity of EO from the leaves of *E. uniflora* L. has been reported so far. Besides, there has been remarkably little research concerning the antimicrobial activity of this EO against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Paracoccidioides brasiliensis*.

Thus, the aim of this study was to evaluate the antioxidant effects of EO from the leaves of *E. uniflora* and to analyze whether it caused acute toxicity when administered to mice. Additionally, studies were performed to evaluate the antimicrobial activity of this EO against bacteria and fungi strains. These experiments were performed to determine the usefulness of EO from the leaves of *E. uniflora* as a phytomedicine.

2. Material and methods

2.1. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTSS^+$), Folin–Ciocalteu's phenol reagent, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), Ethylenediamine-tetraacetic acid (EDTA), ferrous chloride, sodium salicylate, ferrous sulfate, hydrogen peroxide, sodium carbonate and sodium hydroxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals were of analytical grade.

2.2. Animals

Adult male Swiss mice (25–35 g) were used. The mice were kept in separate animal rooms on a 12 h light/dark cycle at a temperature of 22 ± 2 °C and with free access to food and water. The mice were treated according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Pelotas, Pelotas, Brazil.

2.3. Plant material and essential oil extraction

The leaves of the *E. uniflora* plant were collected from a research orchard (germplasm collection of Embrapa Clima Temperado, Pelotas, RS, Brazil) in February and March of 2011 in the morning. The EO of the leaves of *E. uniflora* was extracted by hydro-distillation. The fresh leaves (600 g) were cut with scissors into small pieces and put in a 2000 ml reaction flask. After 3 h of extraction the organic phase was separated and dried over Na_2SO_4. The distillations were performed in triplicate. The chemical composition of the crude essential oil was evaluated using gas chromatography coupled with mass spectrometry (GC–MS).

2.4. Analysis of the EO

The identification and determination of the major chemical constituent ratio of the essential oil was performed by GC–MS. The oil was dissolved in hexane, and the injected sample volume was 1.0 μl. A Shimadzu GC–MS QP2010 and a Polyethylene glycol (Carbowax), model Rtx-Wax (RESTEC) (30 m × 0.25 mm i.d., film thickness 0.25 μm) capillary column were used for the analysis. The temperature was first held at 40 °C, and then raised to 250 °C (10 min, 20 °C/min). The carrier gas was helium at a flow rate of 3 ml/min. The components of the oil were identified based on the comparison of their retention indices and mass spectra with the fragmentation patterns from computer matching with the NIST/EPA/NIH/2005 library.

2.5. Antioxidative activity

2.5.1. Assays without tissue homogenates in the test tube

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical-scavenging assay was performed in accordance with the procedure reported by Choi et al. (2002) with some modifications. The 2,2-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) radical-scavenging activity was evaluated as described by Re et al. (1999). The values are expressed as percentages of DPPH and ABTS radical inhibition compared to the control values (DMSO alone), as calculated from the following equation:

$$I(\%) = [(A_c - A_s/A_c) \times 100],$$

where I is the radical inhibition, A_c is the absorbance of the control reaction and A_s is the absorbance of the sample under analysis.

The Ferric ion reducing antioxidant power (FRAP) was measured according to the method described by Stratil et al. (2006) with slight modifications.

2.5.2. Assays with tissue homogenates in the test tube

2.5.2.1. Tissue preparation. The mice were euthanized by cervical dislocation, and the brain, liver and kidneys were rapidly removed, placed on ice, and homogenized in 50 mM Tris–HCl at pH 7.4 (1/10 w/v), except for the brain that was homogenized in 1/5, w/v). The homogenate was centrifuged for 10 min at 2400 rpm to yield a pellet that was discarded and a low-speed supernatant (S_1) for each tissue, which was used for lipid peroxidation and δ-Ala-D activity assays.

2.5.2.2. Lipid peroxidation assay. Among the lipid peroxidation products used for antioxidant assays, malondialdehyde (MDA) has been most widely used to evaluate the antioxidant activity of chemicals in lipid peroxidation systems. In this work, MDA formation was used as a marker of lipid peroxidation according to the method reported by Ohkawa et al. (1979). In our study, Fe^{2+} and EDTA (at a final concentration of 1.4 mM and 500 μM) were used.

2.5.2.3. Pro-oxidant effect: δ-aminolevulinate dehydratase (δ-Ala-D) activity. δ-Ala-D is a sulfhydryl-containing enzyme and numerous metals and other compounds that oxidized sulfhydryl groups modified its activity. Cerebral, hepatic and renal δ-Ala-D activities were assayed according to the method of Sassa (1982) by measuring the rate of product (porphobilinogen) formation, except that 84 mM PBS (pH 6.4) and 2.5 mM aminolevulinic acid were used.

2.6. In vivo experiments

2.6.1. Acute toxicity

To investigate the potential acute toxicity caused by the leaf essential oil of *E. uniflora* L., the mice received a single oral dose of the EO (10–200 mg/kg) or a vehicle (10 ml/kg of canola oil). After administration, the animals were observed for up to 72 h (at the interval of 24 h) to determine the lethal dose (LD_{50}) of the EO. Body weight gain was recorded as a sign of general toxicity. After 72 h of exposure, the mice were euthanized by cervical displacement, and the brains, livers and kidneys were removed, homogenized and centrifuged. The S_1 was separated and used for *ex vivo* analysis of TBARS and ascorbic acid levels as well as the δ-Ala-D and catalase activities.

2.7. Ex vivo experiments

The low-speed supernatants (S_1) of liver, kidney and brain tissue were used for the thiobarbituric acid-reactive species (TBARS) assay according to the method reported by Ohkawa et al. (1979). The assays were performed as described in the section on *in vitro* experiments, except that the EO was not added to the reaction medium.

The δ-Ala-D activity was assayed according to the method reported by Sassa (1982) using the S_1 of liver, kidney or brain tissue, and the assays were carried out as described in the section on *in vitro* experiments, except that the EO was not added to the reaction medium.

The Catalase (CAT) activity was assayed spectrophotometrically by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the homogenate at 240 nm. The enzymatic activity was expressed in units of U CAT/mg protein.

The vitamin C determination was performed as described by Jacques-Silva et al. (2001) with some modifications and the protein content of the S_1 was measured according to the method reported by Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

2.8. Antimicrobial activity

2.8.1. Antibacterial activity

The following bacterial strains were used in all the antimicrobial assays: *Listeria monocytogenes* 138 ATCC 19117, *Salmonella Typhimurium* ATCC 14028 and *S. aureus* 139 ATCC 27664. All strains were obtained from the American Type Culture Collection 140 (Rockville, MD, USA) and were maintained in soft Tryptic Soy Agar (TSA) at 4 °C.

The screening of the antibacterial activity of EO was made by the agar diffusion method according to the National Committee of Clinical Laboratory Standards protocol (NCCLS, 2003). Discs of sulfazotrin and cephalotin were used as positive controls.

The minimum inhibitory concentration (MIC) of the EO was determined using the broth microdilution method, according to the method of NCCLS (2003), with a slight modification.

2.8.2. Antifungal activity

The tested fungal, *Candida albicans, Candida parapsilosis, Candida guilhermondii, Candida globosa, Candida lipolytica, Cryptococcus laurentii* and *Trichosporon asahii*, were obtained from the Department of Microbiology at the Federal University of Pelotas. Fungal strains were maintained on Potato Dextrose (PD) agar. Fungal cultures were subcultured (1% inoculum) in PD broth at 35 °C for at least 2–4 days before being used in the screening assays.

The minimum inhibitory concentration (MIC) of EO was determined according to the method of NCCLS (2002). The EO was tested in concentrations ranging from 500 to 0.85 μg/ml, and the assays were repeated in their entirety to confirm the results. The MIC was recorded as the lowest concentration of the EO that inhibited the fungal growth.

2.9. Statistical analysis

Experimental results were given as the mean ± standard deviation (SD) to show variations among groups. Statistical analyses were performed using one-way ANOVA followed by the Newman–Keuls multiple comparison test when appropriate. All *in vitro* tests were performed at least three times in duplicate. For the *in vivo* assays, six to eight animals were used per group. The IC_{50} values (concentration of sample required to scavenge 50% of the free radicals) were calculated from the graph of the scavenging effect percentage *versus* the compound concentration. Differences were considered statistically significant at a probability of less than 5% ($p < 0.05$).

3. Results and discussion

3.1. Chemical composition of the EO

The essential oil of Brazilian cherry tree leaves was analyzed by GC/MS. The mass fragmentation pattern of the chemical constituents, their molecular range and linear retention indices (LRI) permitted the identification of the major constituents of the EO.

The *E. uniflora* L. EO has oxygenated and non-oxygenated sesquiterpenes as the major constituents, with a prevalence of the non-oxygenated species (Fig. 1). The major components identified were germacrenes **5**–**7** and seline-1,3,7-(11)-trien-8-one oxide **3**. These findings are in partial agreement with other works describing the chemical composition of the essential oil from *E. uniflora* (Weyerstahl et al., 1988; Amorim et al., 2009; Costa et al., 2010; Oliveira et al., 2006).

Despite a slight variation in their chemical compositions, the predominance of sesquiterpenes in *E. uniflora* EOs was confirmed, except in an Argentine specimen, rich in monoterpenes (Lago et al., 2011; Amorim et al., 2009; Weyerstahl et al., 1988; Urbiergo et al., 1987). Concentration changes of these metabolites seem to be due to chemotypes, geographical origin, and seasonality and to the use of different oil extraction methods (Burt, 2004). There is a considerable variety in the concentration of these constituents when comparing essential oils from different origins and even a lack of these compounds. For instance, in a recent study, performed by Lago et al. (2011), selina-1,3,7(11)-trien-8-one **4** was not detected in the leaves EO of *E. uniflora*, in contrast with observed by us and others (Weyerstahl et al., 1988; Amorim et al., 2009; Costa et al., 2010).

3.2. In vitro results

3.2.1. Antioxidative activity

There are many different methods for determining antioxidant function, each of which depends on a particular generator of free radicals, acting by different mechanisms (Huang et al., 2005).

In this study, the EO of *E. uniflora* L. showed DPPH scavenging activity, as observed in Fig. 2a, and the IC_{50} value was 833.3 ± 20.7 μg/ml. This IC_{50} value is comparable with other findings described in the literature for terpenoid-rich EOs, such as *Lycopus lucidus* Turcz and *Thymus algeriensis*, which showed scavenging activity against the DPPH radical with IC_{50} values of 950 and 800 μg/ml, respectively (Yu et al., 2011; Zouari et al., 2011).

Regarding the major compounds of the leaf EO of *E. uniflora* L., there has been remarkably little research about its bioactivity. A literature survey revealed that germacrene D **7** is a strong antioxidant due to its extra cyclic methylene moiety and that β-caryophyllene **2** possesses free-radical scavenging activity as determined by the DPPH assay (Damien et al., 2010). However, the antioxidant effect of essential oils cannot be attributed to their major constituents because minor compounds are likely to play a

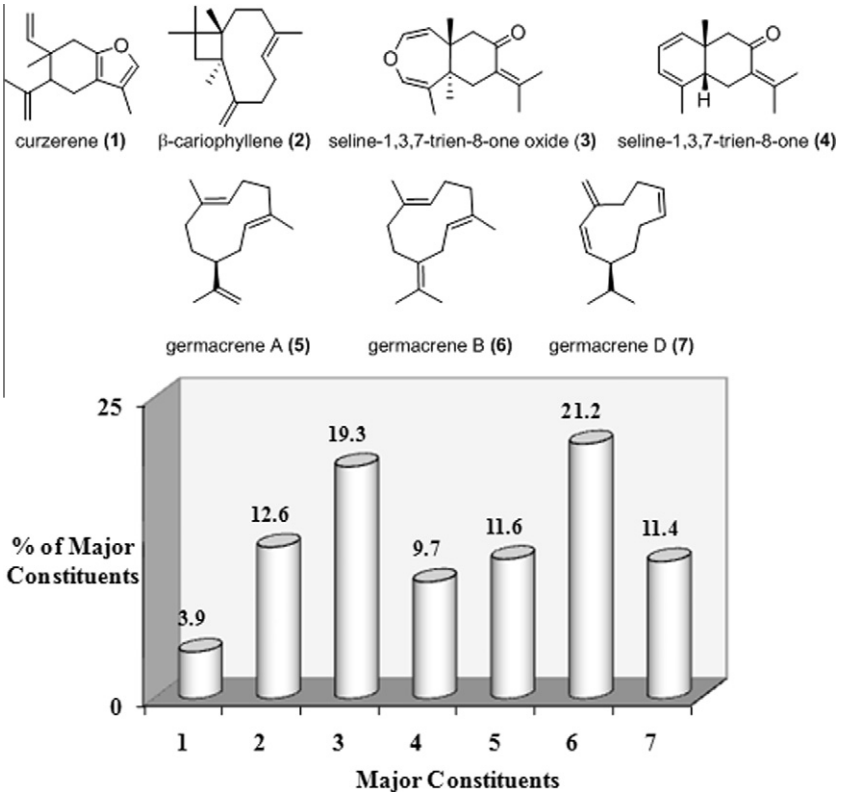

Fig. 1. Chemical composition of the *Eugenia uniflora* L. essential oil.

significant role in the observed activity, and synergistic effects have also been reported (Peschel et al., 2006).

The ABTS coloring method is another commonly used assay to evaluate the antioxidant activity of different substrates *in vitro*. Reduction of $ABTS^{\cdot+}$ radical cations can be even more efficient than that of $DPPH^{\cdot}$ (Barreca et al., 2011). The ABTS radical scavenging activity of *E. uniflora* L. leaf EO is depicted in Fig. 2b, and this result demonstrated that the EO has a good ability to scavenge ABTS radicals, displaying an IC_{50} value of 8.1 ± 0.20 μg/ml.

Comparing the IC_{50} values of the ABTS (8.1 μg/ml) with those of DPPH (833 μg/ml) assays, it is possible to propose that the *E. uniflora* L. essential oil was more potent in the ABTS assay, suggesting that the mechanism of its antioxidant activity is principally based on single electron transfer.

Many reports have demonstrated that the reducing power of natural plant extracts and essential oils might be strongly correlated with their antioxidant activities (Stratil et al., 2006). Thus, based on this evidence, the FRAP assay was also used to determine the reducing power of the EO of the leaves of *E. uniflora* L. to elucidate the relationship between its antioxidant effect and its reducing power. As shown in Fig. 2c, the EO exhibited ferric-reducing ability, and the reducing power was improved by increasing its concentration. This result is in agreement with the findings from the ABTS assay and confirms that the antioxidant power of the EO can be due to electron transfer.

The correlation coefficient was also established among the different antioxidant activity assays by a linear regression analysis. Among the methods used for quantifying the antioxidant activity, the correlations between ABTS, DPPH and FRAP are shown in Table 1. The correlation coefficients of the DPPH radical scavenging capacity and both the FRAP and ABTS assays taken together were 0.96 ($p < 0.001$) and 0.94 ($p < 0.001$), respectively. The FRAP and ABTS assays also showed significant correlations ($R^2 = 0.80$). In addition, the significant correlation ($R \geqslant 0.80$) between the IC_{50} values (DPPH and ABTS) and the values of the reducing power suggested that the components present in the EO that are capable of scavenging DPPH and ABTS radicals are also able to reduce ferric ions.

Table 1
Linear correlation coefficients, R^2, for the relationship between the DPPH, ABTS and FRAP assays for the leaf EO of *Eugenia uniflora* L.

	DPPH (IC_{50})	ABTS (IC_{50})
ABTS (IC_{50})	0.94***	–
FRAP (at 100 μg/ml)	0.96***	0.80***

Asterisk represents significant effect ($p < 0.001$).

Furthermore, it has been demonstrated that the three different assays employed (DPPH, ABTS and FRAP), even though they are all ET-based (electron transfer) rather than HAT-based (hydrogen atom transfer) antioxidant tests, may yield different results, owing to their individual mechanisms and kinetics of radical inactivation and to the different pH values at which they are carried out (Huang et al., 2005).

Excessive production of free radicals can generate a lipid peroxidation chain reaction and lipid peroxidation, which are responsible for pathological disorders. In this study, we demonstrated that Fe^{2+}/EDTA can induce lipid peroxidation in mice tissue homogenates *in vitro* (data not shown). However, in the present study, *E. uniflora* L. EO at all the tested concentrations did not reverse damage caused by the Fe^{2+}/EDTA system on TBARS in the liver, kidney and brain (data not shown).

3.2.2. Pro-oxidant effect: δ-Ala-D activity

δ-Ala-D is a sulfhydryl-containing enzyme that is inhibited by a variety of sulfhydryl reagents (Sassa, 1982). In this study, the Brazilian cherry EO did not alter the enzyme activity in liver, kidney and brain of mice.

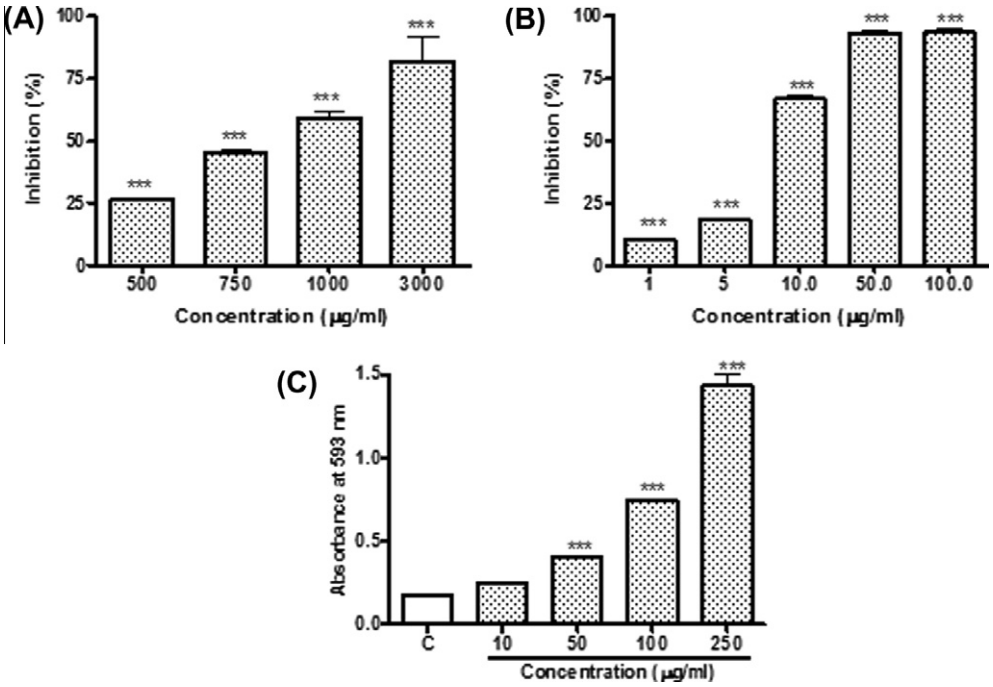

Fig. 2. Antioxidant activities of the leaf EO of *Eugenia uniflora* L.: (A) DPPH radical-scavenging activity. The values are expressed in percentage of inhibition in relation to a control without essential oil. The mean value of absorbance (517 nm) of the control is 0.73 ± 0.30. (B) ABTS radical-scavenging activity. The values are expressed in percentage of inhibition in relation to a control without essential oil. The mean value of absorbance (734 nm) of the control is 0.58 ± 0.02. Each value is expressed as a mean ± SD ($n = 4$). (C) Ferric ion reducing antioxidant power (FRAP). The values are expressed in absorbance; the mean value of absorbance (593 nm) of the control is 0.17 ± 0.02. Each value is expressed as the mean ± SD ($n = 4$). ***$p < 0.001$ when compared with the respective control without essential oil (one-way ANOVA followed by the Newman–Keuls multiple comparison test when appropriate).

Table 2
Effect of acute treatment with leaf EO of *Eugenia uniflora* L. on the δ-Ala-D and catalase activities and on the ascorbic acid levels.

Dose (mg/kg)	δ-Ala-D (nmol PBG/mg protein)			Catalase (Ucat/mg protein)			Ascorbic acid (μg AA/g tissue)		
	Liver	Kidney	Brain	Liver	Kidney	Brain	Liver	Kidney	Brain
0 (control)	28.6 ± 9.0	1.35 ± 0.20	1.27 ± 0.50	1.33 ± 0.18	0.54 ± 0.11	0.25 ± 0.01	762.2 ± 6.60	733.9 ± 4.51	766.4 ± 6.61
10	25.60 ± 8.20	1.40 ± 0.18	1.40 ± 0.56	1.63 ± 0.32	0.70 ± 0.08	0.35 ± 0.05	755.6 ± 3.72	731.6 ± 6.65	754.0 ± 1.29*
50	24.20 ± 6.80	1.23 ± 0.14	1.53 ± 0.46	2.10 ± 0,26	0.72 ± 0.08	0.37 ± 0.09	755.5 ± 5.33	733.5 ± 6.10	759.7 ± 2.53
100	27.11 ± 3.00	1.47 ± 0.28	1.31 ± 0.51	1.80 ± 0.45	0.76 ± 0,08	0.33 ± 0.06	755.1 ± 6.60	734.3 ± 5.60	753.5 ± 5.75**
200	15.20 ± 9.50	1.30 ± 0.31	1.24 ± 0.56	1.82 ± 0.75	0.66 ± 0.13	0.35 ± 0.13	757.7 ± 4.10	732.6 ± 5.05	759.5 ± 0.80

The values were analyzed by one-way ANOVA, each value is expressed as the mean ± SD ($n = 6$). Asterisks represent significant effects ($p < 0.05$; **$p < 0.01$) compared with the respective control.

3.3. In vivo experiments

3.3.1. Acute toxicity

A single oral administration of *E. uniflora* L. leaf EO at 10, 50, 100 and 200 mg/kg did not cause death in any animal when compared to the control group (canola oil). The LD_{50} value obtained for the EO certainly is greater than 200 mg/kg given that lethality was not observed at that dose. The administration of EO at these doses did not cause significant reduction in the body weight when compared to the control group (data not shown).

3.4. Ex vivo experiments

3.4.1. Lipid peroxidation

The levels of TBARS after oral exposure to Brazilian cherry EO changed at doses between 10 and 200 mg/kg, demonstrating the significant ability of the EO to reduce lipid peroxidation in the kidney. However, the acute exposure of EO in mice did not have any effect on the liver and brain (Fig. 3). These data suggest that the EO does not cause any oxidative stress in mice tissue after acute treatment, thought it reduces the levels of TBARS in mice kidneys.

3.4.2. δ-Ala-D activity

The hepatic, renal and cerebral δ-Ala-D activities were not affected by treatment with *E. uniflora* L. EO. This is the first report on the toxicological parameters of this EO, and the data show that it is not toxic to the liver, brain and kidney of mice. Ogunwande et al. (2005) study the toxicity of the EO from the leaves and fruits of *E. uniflora* L. in other experimental model and the results revealed cytotoxicity towards the PC-3 and Hep G2 human tumor cell lines and complete inhibition of the growth of Hs578T.

3.4.3. Catalase activity

CAT activity was not altered in the liver, kidney or brain of mice treated with the EO at any of the tested doses when compared to the control group.

3.4.4. Vitamin C levels

Ascorbic acid is always considered a marker of oxidative stress, and a decrease in its content might indicate an increase in oxidative stress (Jacques-Silva et al., 2001). Exposure to *E. uniflora* L. EO did not alter vitamin C levels in the liver, kidney or brain.

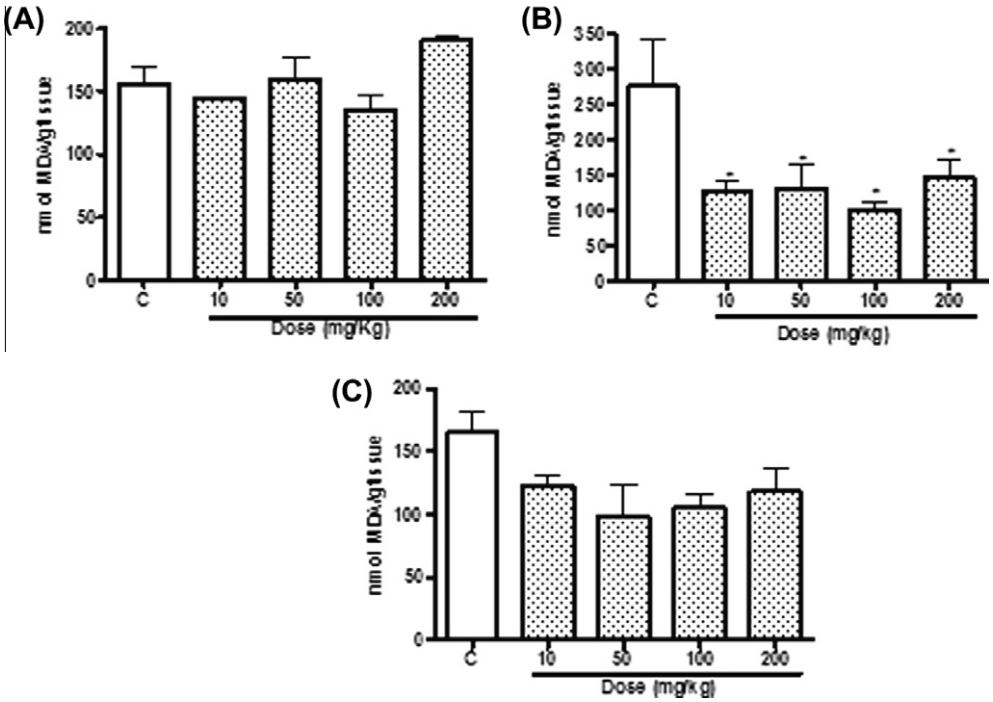

Fig. 3. Effect of the leaf EO of *Eugenia uniflora* L. on lipid peroxidation in the liver (A), kidney (B) and brain (C) of mice. Each value is expressed as the mean ± SD ($n = 6$). Asterisks represent significant effects (*$p < 0.05$) compared with the respective control (C) without essential oil by one-way ANOVA followed by the Newman–Keuls multiple comparison test when appropriate.

Table 3
Antibacterial activity of leaf EO of *Eugenia uniflora* L.

Bacteria	Inhibition zone (mm)		
	Eugenia uniflora	Sulphadiazine	Cephalotine
L. monocytogenes	18 ± 3.2*	30	24
S. aureus	26 ± 7.0***	36	40
E. coli	10 ± 0.6	28	32
S. dysinteriae	NA	30	26
P. aeruginosa	8 ± 0.5	22	27
S. enteritidis	NA	44	28
A. hidrophylla	13 ± 3.0	20	24

The values were analyzed by one-way ANOVA, followed by the Newman–Keuls multiple comparison test, each value is expressed as the mean ± SD ($n = 3$). Asterisks represent significant effects ($^{*}p < 0.05$; $^{***}p < 0.001$) when compared with the respective diameter of the paper disk (6 mm).
NA – non active.
The concentrations of EO, sulphadiazine and cephalotine used were 4.35 mg/disk, 5 and 5 μg/disk, respectively.

Table 4
Antifungal activity of leaf EO of *Eugenia uniflora* L.

Fungi	MIC (μg/ml)[a]
C. albicans	208.3 ± 72.1
C. globosa	187.5 ± 72.1
C. guilhermondi	109.4 ± 31.2
C. parapsilosis	208.3 ± 72.1
C. lipolytica	93.7 ± 36.1
T. asahii	312.5 ± 125
C. laurenthi	208.3 ± 72.1

Each value is expressed as mean ± SD ($n = 3$).
[a] MIC = minimal inhibitory concentration.

3.5. Antimicrobial activity

In this study, we screened the antimicrobial activity of the *E. uniflora* L. leaf EO against eight bacteria in the disk diffusion assay (Table 3). For comparison, sulphadiazine and cephalotine were used as standards. The EO showed activity statistically different against two gram positive strains: *L. monocytogenes* and *S. aureus* when compared with the diameter of the disk (negative control). However, when the Gram-negative strains were subject to the EO, no activity was observed. This gram-positive-specific activity was also observed for other EOs (Lago et al., 2011; Tiwari et al., 2009; Gutierrez et al., 2008; Burt, 2004). In other study, however, the EO of *E. uniflora* L was not gram-specific, presenting antibacterial activity against *Bacillus cereus* (gram-positive), *S. aureus* (gram-positive), *E. coli* (gram-negative) and *P. aeruginosa* (gram-negative) (Ogunwande et al., 2005).

In agreement with our results from the disk diffusion assay, the results on the minimum inhibitory concentration (MIC) determination indicated that the EO had the lower MIC values for *S. aureus* (0.8 mg/ml) and *L. monocytogenes* (1.04 mg/ml), two gram-positive bacteria. Delamare et al. (2007) studied the antibacterial activity of EO of *Salvia officinallis* cultivated in South Brazil and according to this study, the MIC against *S. aureus* was between 5.0 and 10.0 mg/ml. Thus, the EO of *E. uniflora* is more effective on the growth inhibition of *S. aureus* than the *S. officinallis* EO. Another essential oil with antibacterial activity against *S. aureus* and *L. monocytogenes* is the EO of Brazilian species of *Cunila*. Among the studied species, the *Cunila galioides* showed lower values of MIC: 0.62 and 1.25 mg/ml for *S. aureus* and *L. monocytogenes*, respectively (Sandri et al., 2007).

In our study about antifungal activity, EO presented MIC values ranging from 93.7 to 312.5 μg/ml (Table 4). The antifungal activity of *E. uniflora* EO was also evaluated against *C. albicans* and *C. parapsilosis* by others (Lago et al., 2011), showing MIC values of 1800 and 3750 μg/ml, respectively, while in our study MIC values of 208 μg/ml were founded for both strains (Table 4). The presence of germacrene **5**–**7** and seline-1,3,7-trien-8-one **4** have been reported as being responsible by the antifungal activity of *E. uniflora* EO (Costa et al., 2010).

4. Conclusion

Essential oils are, from the chemical point of view, quite complex mixtures composed of several tens of components, and this complexity often makes it difficult to explain the properties of the EO. However, essential oils are a source of natural components with promising pharmacological properties, and for this reason, research into their properties is ongoing. The essential oil of the leaves of *E. uniflora* L. showed antioxidative activity in three different assays, establishing authenticity for the results. Furthermore, the essential oil presented a LD_{50} higher than 200 mg/kg in mice and antimicrobial activity against bacteria and fungi strains. More studies are necessary to elucidate the mechanism of action of the *E. uniflora* L. EO aiming future applications as a phytomedicine.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Aebi, H., 1984. Catalase '*in vitro*'. Methods Enzymol. 105, 121–127.
Amorim, A.C.L., Lima, C.K.F., Hovell, A.M.C., Miranda, A.L.P., Rezende, C.M., 2009. Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora* L. (Brazilian Pitanga). Phytomed. 16, 923–928.
Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of 287 essential oils: a review. Food Chem. Toxicol. 46, 446–475.
Barreca, D., Bellocco, E., Caristi, C., Leuzzi, U., Gattuso, G., 2011. Elucidation of the flavonoid and furocoumarin composition and radical-scavenging activity of green and ripe chinotto (*Citrus myrtifolia* Raf.) fruit tissues leaves and seeds. Food Chem. 129, 1504–1512.
Burt, S., 2004. Essential oil: their antibacterial properties and potential applications in foods – a review. Int. J. Food Microbiol. 94, 223–253.
Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M.Y., Park, S.H., Kim, S.K., 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163, 1161–1168.
Costa, D.P., Alves Filho, E.G., Silva, L.M.A., Santos, S.C., Passos, X.S., Silva, M.R.R., Seraphin, J.C., Ferri, P., 2010. Influence of fruit biotypes on the chemical composition and antifungal activity of the essential oils of eugenia uniflora leaves. J. Braz. Chem. Soc. 21, 851–858.
Damien, D.H.J., Christina, F.A., Jose, B.Â.G., Stanley, D.G., 2010. *In vitro* evaluation of antioxidant activity of essential oils and their components. Flav. Frag. J. 15, 12–16.
Delamare, L.A.P., Moschen-Pistorello, I.T., Artico, L., Tti-Serafini, L., Echeverrigaray, S., 2007. Antibacterial activity of the essential oils of *Salvia officinallis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100, 603–608.
Gutierrez, J., Rodrigues, G., Barry-Ryan, C., Bourke, P., 2008. Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables. Antimicrobial and sensory screening. J. Food Prot. 71, 1846–1854.
Halliwell, B., Murcia, M.A., Chirico, S., Aruoma, O.I., 1995. Free radicals and antioxidants in food and in vivo: what they do and how they work. Crit. Rev. Food Sci. Nutrit. 35, 7–20.
Huang, D.J., Ou, B.X., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841–1856.
Jacques-Silva, M.C., Nogueira, C.W., Broch, L.C., Flores, E.M., Rocha, J.B.T., 2001. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacol. Toxicol. 88, 119–125.
Lago, J.H.G., Souza, E.D., Mariane, B., Pascon, R., Vallin, M.A., Martins, R.C.C., Baroli, A., Carvalho, B.A., Soares, M.G., Santos, R.T., Sartorelli, P., 2011. Chemical and

biological evaluation of essential oils from two species of myrtaceae-*Eugenia uniflora* L. and *Plinia trunciflora* (O. Berg) Kauser. Molecules 16, 9827–9837.
Lowry, O.H., Rosemburg, N.J., Farr, A.L., Roudall, R., 1951. Protein measurement with folin–phenol reagent. J. Biol. Chem. 193, 265–275.
NCCLS (National Committee of Clinical Laboratory Standards), 2003. 328 Antimicrobial Disk Susceptibility Test. Approved Standards. third ed. M2–A8.
NCCLS (National Committee of Clinical Laboratory Standards), 2002. In: National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: proposed standard. M27–A2.
Ogunwande, I.A., Olawore, N.O., Ekundayo, O., Walker, T.M., Schmidt, J.M., Setzer, W.N., 2005. Studies on the essential oils composition, antibacterial and cytotoxicity of *Eugenia uniflora* L. Int. J. Arom. 15, 147–152.
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Oliveira, A.L., Lopes, R.B., Cabral, F.A., Eberlin, M.N., 2006. Volatile compounds from pitanga fruit (*Eugenia uniflora* L.). Food Chem. 99, 1–5.
Ozen, T., Demirtas, I., Aksit, H., 2011. Determination of antioxidant activities of various extracts and essential oil compositions of *Thymus praecox* subsp. skorpilii var. skorpilii. Food Chem. 124, 58–64.
Peschel, W., Sánchez-Rabaneda, F., Diekmann, W., Plescher, A., Gartzy, I., Jiménez, D., 2006. An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem. 97, 137–150.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 26, 1231–1237.
Sandri, I.G., Zacaria, J., Fracaro, F., Delamare, A.P.L., Echeverrigaray, S., 2007. Antimicrobial activity of the essential oils of Brazilian species of the genus of Cunila against foodborne pathogens and spoiling bacteria. Food Chem. 103, 823–828.
Sassa, S., 1982. Delta-aminolevulinic acid dehydratase assay. Enzyme 28, 133–145.
Stratil, P., Klejdus, B., Kuban, V., 2006. Determination of total content of phenolic compounds and their antioxidant activity in vegetables – evaluation of spectrophotometric methods. J. Agric. Food Chem. 54, 607–616.
Tiwari, B.K., Valdramidis, V.P., O'Donnell, C.P., Muthukumarappan, K., Bourke, P., Cullen, P.J., 2009. Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 57, 5987–6000.
Urbiergo, G., Taher, H.A., Talenti, E.C., 1987. Chemical composition of essential oil of *Eugenia Uniflora*. An. Ass. Quim. Argent. 75, 377–379.
Weyerstahl, P., Marschall-Weyerstahl, H., Christiansen, C., Oguntimein, O., Adeoye, A.O., 1988. Volatile constituents of *Eugenia uniflora* leaf oil. Planta Med. 54, 546–549.
Yu, J., Lei, J., Zhang, X., Yu, H., Tian, D., Liao, Z., Zou, G., 2011. Anticancer, antioxidant and antimicrobial activities of the essential oil of *Lycopus lucidus* Turcz. Var. hirtus Regel. Food Chem. 126, 1593–1598.
Zouari, N., Fakhfakh, N., Zouari, S., Bougatef, A., Karray, A., Neffati, M., Ayadi, M.A., 2011. Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae). Food Bioprod. Proc. 89, 257–265.