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ANTIOXIDANT AND ANTICHOLINESTERASE ACTIVITIES OF THE ESSENTIAL OIL OF *EUGENIA DYSENTERICA* DC.

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ABSTRACT

This paper describes anticholinesterase and antioxidant activities of *Eugenia dysenterica* (DC). O. Berg (Myrtaceae) essential oil leaves (EOED). EOED were obtained by hydrodistillation using a Clevenger-type apparatus and the products were analyzed by GC-MS and GC-FID. The main constituents of EOED were caryophyllene oxide (66.3%), isoledene (3.9%), 1, 3, 8-*p*-menthatriene (3.5%), mustakone (3.46%) β-phellandrene (1.7%) and selin-11-en-4-α-ol (1.7%). The antioxidant assay was performed based on the formation of Thiobarbituric Acid Reactive Substances (TBARS), hydroxyl radical and nitric oxide production. Performing the Ellman assay it was observed that EOED was able to inhibit the enzyme acetylcholinesterase (AChE) with an IC_{50} = 0.92 $\mu g.mL^{-1}$ promising better value compared with the drug rivastigmine (IC_{50} = 1.87 $\mu g.mL^{-1}$), used in the treatment of Alzheimer's disease. The caryophyllene oxide (the main compound) was tested after purification on the AChE with an IC_{50} = 0.31 $\mu g.mL^{-1}$ Caryophyllene oxide (the majority compound) was tested on the AChE and presented the IC_{50} = 0.31 $\mu g.mL^{-1}$. At concentrations of 0.9, 1.8, 3.6, 5.4 and 7.2 $\mu g.mL^{-1}$ it was found that EOED prevented lipid peroxidation inhibiting amount of TBARS formed in a similar manner to ascorbic acid. In addition, a reduction in the production of hydroxyl radical as well as the production of nitric oxide. To our knowledge this is the first report on compounds from this species that have activity for potentially preventing neurodegenerative disorders.

INTRODUCTION

A variety of essential oil of plants has shown a cetylcholineterase (AChE) inhibitory activity and may be relevant to the treatment of neurodegenerative disorders such as Alzheimer's disease (AD). The essential oils of *Cistus* species have functional properties in prevention of neurodegenerative disorders (1). *Centella asiatica* essential oil and various others essential oils from plant species, for example *Cistus salvifolius* and *Ocimum canum* have shown pharmacological activities relevant to the treatment of cognitive disorders, indicating potential for therapeutic use in disorders such as AD (2). Though recent intensive efforts have been made to understand the mechanism of neurodegeneration involved in AD and to discover new drugs combating the symptoms, at present there is a deficit in the number of efficient and safe therapeutic agents to treat the disease. No new drugs have been approved by the US Food and Drug Administration (FDA) since 2003, likely because the abnormal brain deposits of Aβ and τ-proteins still cannot be considered causes or by-products of the disease (3). Since the approval of galantamine for the treatment of AD patients, the search for new anticholinesterase alkaloids has escalated, leading to promising candidates such as huperzine A (4). Many monoterpenes and sesquiterpenes for example 1,8-cineole, α-pinene, linalool have been cited in promising research due to their potent anticholinesterase activity (5). However, few reports exist that deal with the inhibition of AChE by plant essential oils (6). Acetylcholinesterase inhibitors (IAChE) have therapeutic applications in AD and in addition the central cholinergic system is considered one of the more important neurotransmitter systems involved in the regulation of cognitive functions. Cholinergic neuronal loss in the hippocampal area is the major feature of AD and enhancement of central cholinergic activity by use of is presently the mainstay of pharmacotherapy of senile dementia of Alzheimer type (7, 8). Pharmacological activities from plants and their constituents may be relevant for the treatment of cognitive disorders, including enhancement of cholinergic function in the central nervous system, anti-inflammatory and antioxidant activities (2). A variety of plants has been reported to show AChE inhibitory activity and so may be relevant to the treatment of neurodegenerative disorders such as AD (9). Research of and interest in essential oils is on the increase. Recently our research group published studies with essential oils with, antioxidant and antinociceptive effects from *Citrus limon* Osbeck as studied on mice (10).

Eugenia dysenterica (DC). O. Berg and several species of *Eugenia* are used in folk medicine with anti-inflammatory, anti-diarrheic, diuretic and others properties. In Brazil this species is popularly known as 'cagaiteira', with opposite leaves, simple, ovate or elliptical limbo; has edible white flowers (11), holders of laxative properties. Its fruits are consumed raw or in the form of juices, or processed to ice cream and liqueurs and when fermented can produce alcohol and vinegar. Sensory evaluation of fruit wine from cagaita showed over 70% acceptability for colour, flavour and taste for all cagaita

beverages (12, 13). Tea from the leaves is used to combat diarrhea and the bark is used as anti-inflammatory agent. Daily consumption of 'cagaita' (100 g) contributed significantly to the supply the daily requirements of vitamin C (on average 71.0%), vitamin A (on average 7.5%) and folates (on average 7.9%). The 'cagaiteira' has a high pulp yield, reduced total energy value and is considered a source of vitamin C, which an important role in human health (14). This paper describes the anticholinesterase and antioxidant activities of *Eugenia dysenterica* DC. (cagaiteira) essential oil from leaves (EOED). This is the first report on activities of essential oil from leaves of *E. dysenterica.*

MATERIAL AND METHODS

Plant material

E. dysenterica fresh leaves were collected in october 2013 in Uruçuí, Piauí State, Brazil, coordinates [07°14′02″S and 44°33′14″W]. Plant identification was confirmed by Dr. Roseli Farias Melo de Barros, Department of Biology, Piauí Federal University (UFPI), Brazil and a voucher specimen (number 28824) have been deposited at the Graziela Barroso herbarium of the UFPI.

Pure compound, solvents and enzymes

Acetylcholinesterase enzime, ( )-Caryophyllene oxide (95% of purity), ascorbic acid and thiobarbituric acid were purchased from Sigma-Aldrich.

Hydrodistilation of the essential oils

A. The essential oil from the fresh leaves of E. dysenterica was extracted using hydrodistillation for 3 hours with a Clevenger-type apparatus. The essential oil was dried over anhydrous sodium sulphate and the percentage content was calculated on the basis of the dry weight of plant material. The essential oils were stored in a freezer (-20°C) until analyzed. Gas Chromatography with FID (GC-FID) and CG-MS analysis of the essential oils. Gas Chromatography (GC) analyses were carried out using a Shimadzu GC-17A fitted with a flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a ZB-5MS fused capillary column (30 m × 0.25 mm × 0.25 µm film thickness) coated with 5%-phenyl-arylene-95%-methylpolysiloxane. Helium was the carrier gas at 1.0 $mL.min^{-1}$ flow rate. The column temperature program was: 40ºC/3 min, followed by a rate of $4ºC.min^{-1}$ to 240ºC, then a rate of $10ºC.min^{-1}$ to 300ºC, and then 300ºC/3 min. The injector and detector temperatures were 250ºC and 280ºC, respectively. Samples (0.5 µL in CH_2Cl_2) were injected with a 1:50 split ratio. Retention indices were generated with a standard solution of n-alkanes (C_8-C_{20}).

Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

GC/MS analyses were performed on a Shimadzu QP5050A GC/MS system equipped with an AOC-20i auto-injector. A J&W Scientific DB-5MS (coated with 5%-phenyl-

95%-dimethylpolysiloxane) fused capillary column (30 m × 0.25 mm × 0.25 µm film thickness) was used as the stationary phase. MS were taken at 70eV with scan interval of 0.5 s and fragments from 40-500 Da. The other conditions were similar to the GC analysis.

Identification of the constituents

The essential oil components were identified by comparison of i) their retention times (t_R) with those of the same standard compounds (caryophyllene oxide) analyzed under identical conditions, ii) their retention indices (RIs, determined on a DB-5MS column relative to the t_R of a series of *n*-alkanes (C_8-C_{20}), according to Van Den Dool and Kratz (15) with those published in the literature (Van Den Dool and Kratz, 1963) and their mass spectra with those listed in the NIST (05, 05s, 21 and 107) and Wiley 8 mass spectral libraries, and those published in the literature (16).

Acetylcholinesterase inhibition assay

The inhibitory effect of EOED on acetylcholinesterase activity is evaluated by an adaptation of the spectrophotometric method of Ellman (17).

The EOED and caryophyllene oxide were dissolved in methanol to prepare solutions of 10 mg.mL^{-1}. Then, 1.5 µL of the methanol EOED was spotted onto silica gel TLC plate and developed with chloroform: methanol 9:1 after which the enzyme inhibitory activity was detected using Ellman's method "in situ" on the plate (17, 18, 19). The developed plates were sprayed with 1 mM DTNB and 1 mM ATCI in buffer A. The plate was dried for 3-5 minutes and then an enzyme solution of AChE from an electric eel (type VI-s lyophilized, 261 U.mg^{-1} solid, 386 U.mg^{-1} protein) dissolved in buffer A (500 U.mL^{-1} stock solution) was diluted with buffer A to obtain 5 U.mL^{-1} enzyme and was then sprayed on the plate. A yellow background with white spot for inhibiting was visible after about 5 minutes. The observation must be recorded within 15 minutes because they fade after 20-30 minutes. To observe whether the positive results of the extract in TLC or the microplate assay are due to enzyme inhibition or to the inhibition of the chemical reaction between DTNB and thiocholine, (the product of the enzyme reaction), 5 units.mL^{-1} of AChE was premixed with 1 mM ATCI in buffer A and incubated for 15 minutes at 37°C. The enzyme-substrate mixture was used as thiocholine spray. The extract was spotted on the silica gel TLC plate developed as described above and sprayed with 1 mM solution DTNB followed by the thiocholine spray. White spot on a yellow background was observed for false positive extract. The inhibitory effect quantitative of EOED on acetylcholinesterase activity was evaluated using and adaptation of the spectrophometric method of Ellman modified by Rhee (17, 19). Six different concentrations were prepared in triplicate, starting from the EOED and caryophyllene oxide (0.9, 1.8, 2.7, 3.6, 5.4 and 7.2 µg.mL^{-1}). The reaction is monitored for 5 min at 412 nm in spectrophotometer. In test tube is placed 100 µL of sample (concentration 0.1% solution in 50 mM Tris-HCl pH 8, and methanol 10%) was mixed with 100 µL of AChE 0.22 U.mL^{-1} (22 U of enzyme diluted in

100 mL of 50 mM Tris-HCl pH 8, 0.1% BSA) and 200 µL of buffer (50 mM Tris-HCl, pH 8, BSA 0.1%) then incubated the mixture for 5 min at 30°C. Subsequently add, 500 µL of acid 5,5-dithiobis (2-nitrobenzoic acid) - DTNB (concentration of the 3mM in Tris-HCl pH 8, 0.1 M NaCl, 0.02 M $MgCl_2$) and 100 µL of Acetylthiocholine iodide - ATCI (4 mM in water).

The quantitative inhibitory effect of EOED on acetylcholinesterase activity was evaluated using an adaptation of the spectrophometric method of Ellman modified by Rhee (17, 19). Six different concentrations were prepared in triplicate, starting with the EOED and caryophyllene oxide (0.9, 1.8, 2.7, 3.6, 5.4 and 7.2 $\mu g.mL^{-1}$). The reaction was monitored at 412 nm for 5 min in spectrophotometer. 100 µL of sample (concentration 0.1% solution in 50 mM Tris-HCl pH 8, and methanol 10%) was mixed with 100 µL of AChE 0.22 $U.mL^{-1}$ (22 U of enzyme diluted in 100 mL of 50 mM Tris-HCl pH 8, 0.1% BSA) and 200 µL of buffer (50 mM Tris-HCl, pH 8, BSA 0.1%). The mixture is incubated for 5 min at 30°C. Subsequently add 500 µL of DTNB (concentration of the 3mM in Tris-HCl pH 8, 0.1 M NaCl, 0.02 M $MgCl_2$) and 100 µL of ATCI (4 mM in water).

A blank is prepared by substituting AChE with 100 µL of buffer (50 mM Tris-HCl buffer pH 8, 0.1% BSA). The reaction is monitored for 5 min at 412 nm and the initial velocity (V_0) recorded. Anticholinesterase activity (%) was calculated using equation 1 (5): Sample V_0 and blank V_0 represents the initial velocities of samples and blank. Inhibition concentration 50% (IC_{50}) values are obtained using Log-Probit. Rivastigmine (commercial acetylcholinesterase inhibitor) is used as positive control at the same concentration of the essential oil.

$$I\ (\%) = (1 - V_0\ Sample / V_0\ Blank) \times 100$$

Equation 1- Anticholinesterase activity (%)

Evaluation of *in vitro* potential against production of thiobarbituric acid reactive substances (TBARS) in essential oil from *E. dysenterica*

Determination of thiobarbituric acid reactive species (TBARS) was performed to quantify the lipid peroxidation level (20). This method was used to determine the EOED, using homogenized egg yolk as a lipid rich substrate (21). Briefly, egg yolk was homogenized (1% w/v) in 20 mM phosphate buffer (pH 7.4). A volume of 1 mL of this homogenate was homogenized with 0.1 mL of EOED, at concentrations of 0.9, 1.8, 3.6, 5.4 and 7.2 $\mu g.mL^{-1}$ of EOED. Lipid peroxidation was induced by adding 0.1 mL of AAPH (2,2-azobis-2-midinopropane, 0.12 $mol.L^{-1}$).

Control was carried out only with the solution (0.05% Tween 80 dissolved in 0.9% saline solution) used to emulsify the substance that was evaluated. Reactions were performed for 30 min at 37°C. After cooling, samples (0.5 mL) were centrifuged with 0.5 mL of trichloroacetic acid (15%) at 1200 g for 10 min. An aliquot of 0.5 mL of the supernatant was mixed with 0.5 mL of thiobarbituric acid (0.67%) and heated at 95°C for 30 min. After cooling, absorbance of the samples was measured on a spectrophotometer at 532 nm.

The results were expressed as the percentage of TBARS was formed by AAPH alone (induced control). Ascorbic acid is used as control in this assay (Ahmad et al., 2015).

Evaluation of EOED in vitro potential against production of hydroxyl radical (OH•)

Production of hydroxyl radical (OH•) was quantified by Fenton reaction. During this reaction, the *in vitro* effect of EOED against the production of OH•, produced by the oxidative degradation of 2-deoxyribose, was determined (22). The principle of the test is to quantify the degradation product of 2-deoxyribose, malonaldehyde (MDA), by its condensation with thiobarbituric acid (TBA). Briefly, the reactions were initiated by the addition of Fe^{2+} ($FeSO_4$) with 6 $mmol.L^{-1}$ final concentration for solutions containing 2-deoxyribose 5 $mmol.L^{-1}$, H_2O_2 100 $mmol.L^{-1}$ and phosphate buffer 20 $mmol.L^{-1}$ (pH 7.2). Concentrations of 0.9, 1.8, 3.6, 5.4, and 7.2 $\mu g.mL^{-1}$ of EOED were added to the system before the addition of Fe^{2+} in order to determine EOED in vitro antioxidant activity against hydroxyl radical formation. The reactions were performed for 15 min at room temperature and they were stopped by the addition of phosphoric acid at 4% (v/v), followed by addition of TBA (1% v/v in NaOH 50 $mmol.L^{-1}$). The solutions were heated in a water bath for 15 min at 95 °C. The absorbance was measured at 532 nm and results were expressed as equivalents of MDA formed by Fe^{2+} and H_2O_2.

Evaluation of essential oil from *E. dysenterica in vitro* potential against production of nitrite ion (NO_2)

. Nitric oxide was generated from the spontaneous decomposition of sodium nitroprussidein 20 mM phosphate buffer (pH 7.4). Once generated, NO interacts with oxygen to produce nitrite ions, which were measured using the Griess reaction (23). The reaction mixture (1 mL) containing 10 mM sodium nitroprusside (SNP) in phosphate buffer and evaluated at concentrations of 0.9, 1.8, 3.6, 5.4 and 7.2 $\mu g.mL^{-1}$ concentrations was incubated at 37°C for 1h. A 0.5 mL aliquot was taken and homogenized with 0.5 mL Griess reagent. The absorbance of the chromophore formed was measured at 540 nm. The extent to which the nitric oxide generated was inhibited was measured by comparing the absorbance values of negative controls (only 10 mM sodium nitro prusside and assay preparations. Results were expressed as percentages of nitrite formed by SNP alone.

RESULTS AND DISCUSSION

Chemical Composition

Hydrodistillation of the leaves of *E. dysenterica* gave a light-yellowish crude essential oil (EOED), with a yield of 1.45 ± 1.48% (w/w), in relation to the dry weight of the plant material. As shown in Table 1, it was possible to identify 6 compounds (80.67% of the total composition) the sesquiterpenes (75.4%) were the majority (Figure 1, Table 1). The major compounds identified caryophyllene oxide (66.4%) (Figure 2), isoledene (3.9%), 1,3,8-*p*-menthatriene

(3.5%), mustakone (3.5%), β-phellandrene (1,7%) and selin-11-en-4-α-ol (1.7%).

Table 1. Essential oil composition from the leaves of *E. dysenterica.*

Compound	RI[a]	RI[b]	Peak (%)
β-Phellandrene	1030	1025	1.73 ± 0.10
1) 1,3,8-*p*-Menthatriene	1112	1108	3.51 ± 0.11
Isoledene	1374	1374	3.91 ± 0.10
caryophyllene oxide	1583	1582	66.37 ± 2.10
Selin-11-en-4-α-ol	1659	1658	1.69 ± 0.07
Mustakone	1677	1676	3.46 ± 0.04
Monoterpene identified			5.24
Sesquiterpenes identified			75.43
Total identified			80.67

Data are expressed as mean ± SD of three analyses. RI[a] (calc.), retention indices on DB-5MS column calculated according to Van den dool and Kratz (15). RI[b] retention indices according to Adams (16).

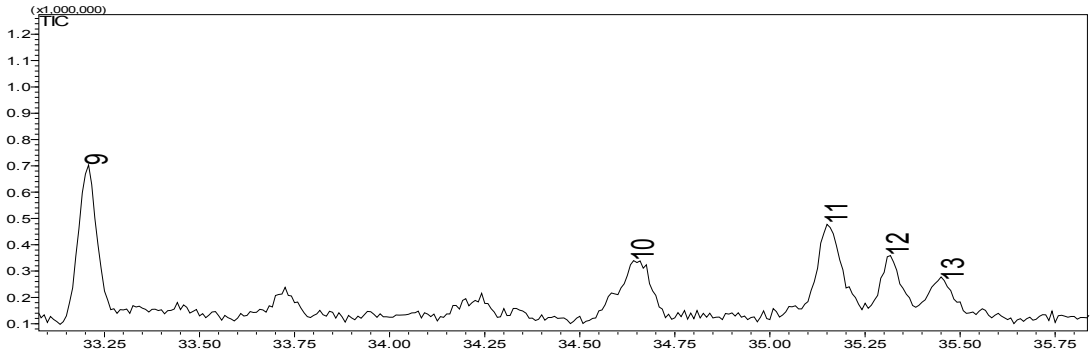

Figure 1. GC-MS of the essential oil of *E. dysenterica*.

Note: 1) β-Phellandrene, 3)1,3,8-*p*-Menthatriene, 5) Isoledene, 8) Caryophyllene oxide, 10) Selin-11-en-4-α-ol, 11) Mustakone, 2,4,6,7,9,12,13,14) Unknown. 7%) and there are also new constituents different from what have been found in other species of *E. dysenterica* collected in another region of Brazil as reported by Costa et al., 2000, β-caryophyllene (14.8%), α-humulene (10.9%) α-terpineol (6.1%), limonene (5.5%), α-thujene (5.6%), In addition to the major constituent caryophyllene oxide (66.37%), β-caryophyllene have been reported in the essential oils of several other species of Eugenia (higher than 20%), indicating that this species is a typical member of the Myrtaceae Family (Costa et al., 2000). The substances isoledene (3.91%), 1, 3, 8-*p*-menthatriene (3.51%), mustakone (3.46%) β-phellandrene (1.73%) and selin-11-en-4-α-ol (1.69%) were identified for the first time as chemical constituents of essential oil from leaves of *E. dysenterica*.

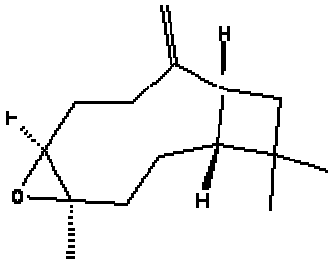

Figure 2. Chemical structure of the caryophyllene oxide.

Inhibition of acetylcholinesterase activity

The qualitative results of inhibition of enzyme in TLC showed that the EOED and caryophyllene oxide inhibited the enzyme by the appearance yellow backgrounds with white spots for inhibiting compounds were visible after about 5 minutes. This are the results of the first tests, yellow backgrounds with white spots for inhibiting compounds were visible after about 5 minutes. The best antiacetylcholinesterase activitie was found for *Eugenia dysenterica* essential oil (IC_{50} = 0.92 $\mu g.mL^{-1}$) when compared to *Eucalyptus camaldulensis* (IC_{50} = 18.98 $\mu g.mL^{-1}$), *Ocimum canum* (IC_{50} = 36.16 $\mu g.mL^{-1}$) and *Cistus salvifolius* (IC_{50} = 58.10 $\mu g.mL^{-1}$) (5).

From the Ellman assay it was observed that EOC was able to inhibit the enzyme acetylcholinesterase (AChE) with an IC_{50} = 0.92 $\mu g.mL^{-1}$ (Means from independent experiments were then expressed as means with ± SD. For statistical analyses, $p<0.001$, was considered as statistically significant) with a promising value compared with drug rivastigmine (IC_{50} = 1.87 $\mu g.mL^{-1}$), the conventional AChE inhibitor used in treatment of Alzheimer's disease. In another study, *Eugenia sulcata* essential oil contained monoterpenes known for their anticholinesterase activity (for example α-cubebene and β-copaene), with an inhibitory capacity of the enzyme AChE an value of IC_{50} = 4.66 $\mu g.mL^{-1}$ (24).

caryophyllene oxide was tested on the enzyme acetylcholinesterase and showed a value for CI_{50} = 0.31 $\mu g.mL^{-1}$. In studie are reports of analgesic and anti-inflammatory activities for caryophyllene oxide, constituent of the essential oil of *Eugenia dysenterica* (25).

Testing of antioxidants reactive species with thiobarbituric acid (TBARS).

To evaluate the antioxidant activity of EOED, two other methods were which are based on the ability of a substance scavenge free radicals through direct interaction with a substance reactive molecules, converting the less reactive free radical species and therefore more stable (26). Homogenized egg yolk as a lipid rich substrate was used to determine the antioxidant activity of EOED (21). The TBARS is a complex formed by APPH (soluble water-azo compound is used as free radical generator) and thiobarbituric acid reactive. From the methods used *in vitro*, demonstrated that *E. dysenterica* essential oil was able to reduce the production of free radicals at all concentrations tested, with a better performance in antioxidant TBARS test, which is a method used to quantify the peroxidation which corresponds to a lipid in the cell membrane damage caused by oxidative stress. The , at all concentrations tested, 0.9, 1.8, 3.6, 5.4 to 7.2 $\mu g.mL^{-1}$, were capable of preventing lipid peroxidation inhibiting amount of TBARS 16.07, 14.11,

15.02, 22.07 and 13.95%, respectively, as shown in **Figure 2.**

Similar results were obtained with ascorbic acid as antioxidant used provided standard 12.67% inhibition of TBARS formed. We also found that 50% inhibitory concentration (IC_{50}) of the oil is 1.2 µg.mL^{-1} against the formation of reactive species with thiobarbituric acid (TBARS) with variation margin on the effective concentration 0.3 to 5.8 mg.mL^{-1} (with 95 % confidence interval). There is evidence to indicate that free radicals cause oxidative damage to lipids, proteins and nucleic acids (27). AD is the most common form of dementia, characterized by progressive neurodegeneration. Pathogenetic mechanisms, triggered by β-amyloid (Aβ) accumulation, include oxidative stress, deriving from energy homeostasis deregulation and involving mitochondria and peroxisomes. At severe pathological stages, when senile plaques disrupt cortical cytoarchitecture, antioxidant capacity is gradually lost. Porcellotti and colleaguesIn small quantities antioxidants have great therapeutic potential for some conditions caused by free radicals such as arthritis, Alzheimer's disease, heart disease, aging, cancer, among other (28). Antioxidants may thus be used as neuroprotectores agents, for example: aryl amines and indoles-carotene, lycopene polyenes- carotene, lycopene, retinol selenium containing compounds ebselen. polyphenols- favonoids, stilbenes, and hydroquinone monophenols: tocopherols (vitamin E), 17-estradiol (estrogen), 5-hydroxytryptamine (serotonin), since oxidative damage may be observed before the formation of β-amyloid specific pathological signs (29). The use of antioxidants has been explored in an attempt to slow AD progression and neural degeneration. Given the complex pathology of AD, current strategies for the development of new agents focus on compounds with various powers, and plants are a major source of these compounds (30).

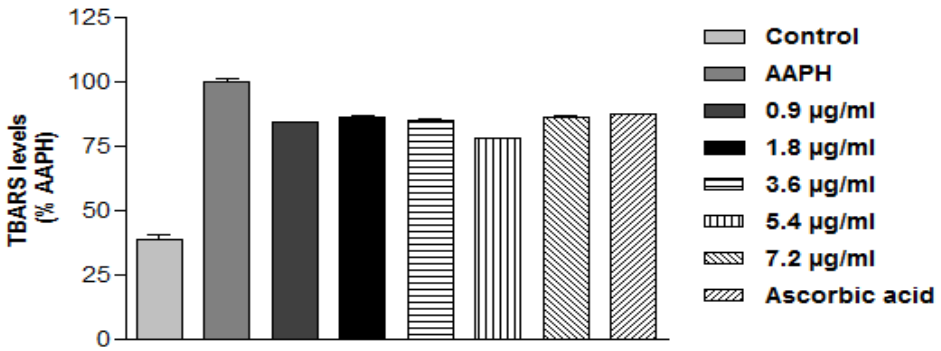

Figure 2. *Antioxidant capacity of E.dysenterica essential oil in different concentrations* (0.9, 1.8, 3.6, 5.4 and 7.2 µg.mL^{-1}*) by the reduction of TBARS levels. The results represent the mean ± S.E.M. of the values of in vitro inhibition, n = 3, of the experiments in duplicate. Ascorbic acid (140 µg.mL^{-1}) was used as standard antioxidant. $p < 0.05$ vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and t-Student–Neuman–Keuls as post hoc test).* $^{a}p < 0.05$ *vs. to AAPH (100% of TBARS levels) (ANOVA and Student–Newman–Keuls as post hoc test).*

Evaluation of EOED in vitro potential against production of hydroxyl radical (OH•)

The radical OH• is more toxic moiety known, since they can oxidise non-specifically all classes of biological macromolecules including lipids, proteins and nucleic acids with virtually limited diffusion rates (31). Therefore OH• may result in oxidative damage that gives rise to various diseases, including arthritis, atherosclerosis, cirrhosis, diabetes, cancer, Alzheimer's disease, emphysema and aging (32).

After antioxidants testing in vitro was observed at all concentrations tested, 0.9, 1.8, 3.6, 5.4 and 7.2 µg.mL^{-1} to hydroxyl radical removal capacity in 73.46, 69.90, 45.09, 52.65 and 58.1%, respectively, as shown in **Figure 3**, results similar to that of ascorbic acid removed 60.96% of the produced hydroxyl radical. IC_{50} was established at 5.814 µg.mL^{-1} against the formation of the hydroxyl radical with a variation range of 253 to 10.39 (with a 95% confidence interval).

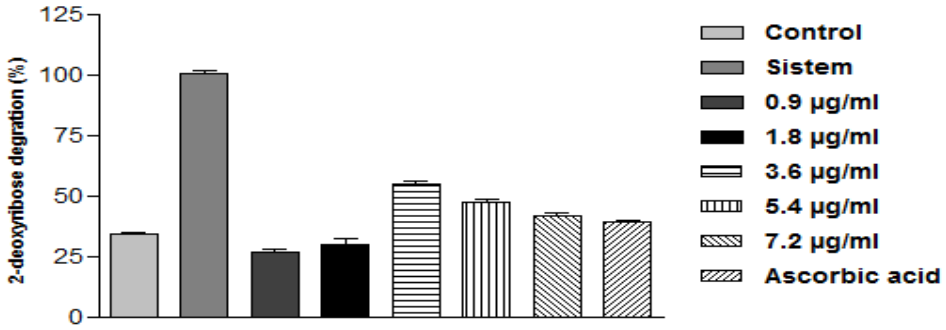

Figure 3. *Antioxidant capacity Eugenia dysenterica essential oil in different concentrations (0.9, 1.8, 3.6, 5.4 and 7.2 µg.mL^{-1}) in the inhibition of 2-deoxyribose degradation by removal of hydroxyl radical. The results represent the mean ± S.E.M. of the values of in vitro inhibition, n = 5, of the experiments in duplicate. Ascorbic acid (140µg.mL^{-1}) was used as standard antioxidant. $p < 0.05$ vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and Student–Newman–Keuls as post hoc test), $^{a}p < 0.05$ vs. (100% of hydroxyl radical) (ANOVA and Student–Newman–Keuls as post hoc test).*

Antioxidant potential evaluation in nitric oxide removal (NO)

In our studies, in vitro, was also possible to determine the 50% inhibitory concentration (IC_{50}) of the sample 0.1684 µg.mL^{-1} against the formation of nitrite radical, with variation margin on the effective concentration from 0.06630 to 0.4276 µg.mL^{-1} with 95% confidence interval, was observed at all concentrations tested, 0.9; 1.8; 3.6; 5.4 and 7.2 µg.mL^{-1} to remove nitrite metabolic capacity in 44.62; 43.45; 48.97; 51.09 and 47.05% as shown in Figure 4, results similar to that of ascorbic acid removed 36.73%.

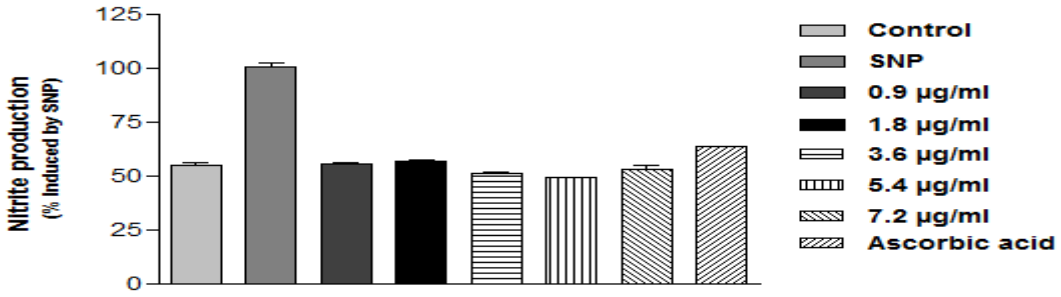

***Figure 4**: Nitric oxide (NO) scavenging assay. Antioxidant capacity of E. dysenterica essential oil in different concentrations (0.9, 1.8, 3.6, 5.4 and 7.2 µg.mL^{-1}) against the formation of nitrite ions generated. The results represent the mean ± S.E.M. of the values of in vitro inhibition, n = 5, of the experiments in duplicate. Ascorbic acid (140µg.mL^{-1}) was used as standard antioxidant. $p < 0.05$ vs. control (0.05% Tween 80 dissolved in 0.9% saline) (ANOVA and Student–Newman–Keuls as post hoc test), $^{a}p < 0.05$ vs. SNP (100% of nitrite ions) (ANOVA and Student–Newman–Keuls as post hoc test).*

Antioxidants comprise a broad and heterogeneous family of compounds that share the common task of interfering with (stopping, retarding, or preventing) the oxidation (or autoxidation) of an oxidizable substrate (33). Numerous physiological and biochemical processes in the human body may produce oxygen containing free radicals and other reactive oxygen or nitrogen species as by-products (25). Overproduction of such radicals can cause oxidative damage to biomolecules, eventually leading to many diseases, such as atherosclerosis, cancer, diabetes, or inflammatory conditions and pain (34).

CONCLUSION

The main constituents of *Eugenia dysenterica* DC. (cagaiteira) essential oil of water extract from leaves were caryophyllene oxide (66.37%), isoledene (3.91%), 1,3,8-*p*-menthatriene (3.51%), mustakone (3.46%) β-phellandrene (1,73%) and selin-11-en-4-α-ol (1,69%). The pure caryophyllene oxide (the main compound) was tested on the AChE and presented $IC_{50} = 0.31$ µg.mL^{-1}. This is the first report, that the essential oil from leaves *E. dysenterica* exhibits antioxidant effects preventing lipoperoxidation and acetylcholinesterase activity. The results are promising and so it is suggested that they will be followed up with *in vivo* assay.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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