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Chemical Composition, Antimicrobial and Antioxidant Activities of *Eugenia Dysenterica* DC Essential Oil

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Abstract

Eugenia dysenterica DC is a native species from the Cerrado biome and can be found in several states of Brazil. This study evaluated the chemical composition, antimicrobial and antioxidant activities from the essential oils of a population collected in São Paulo state. Essential oils were obtained by conventional means, and their compositions were analyzed by GC-MS. Screening assays for antimicrobial activity were carried out by the microdilution method and the antioxidant potential was assessed by the DPPH scavenging method. The GC-MS analysis indicated that 52.63% of the essential oil is composed by oxygenated sesquiterpenes and the major compound is (-)-elema-1,3,11(13)-trien-12-ol (24.86%). The antimicrobial assay indicated MIC 42.1 µg/mL for *S. aureus* and MIC > 10000 µg/mL for the other tested microorganisms, Gram negative bacteria and fungi. The oil showed an IC_{50} of 5.4±0.632 mg/mL for in the DPPH assay. The essential oil had a different chemical composition from previous studies. The essential oil did not present a potent antioxidant activity. However, it can be considered a promising antimicrobial agent against *S. aureus*.

Keywords:

Eugenia Dysenterica;
Essential Oil;
Antioxidant Activity;
Antimicrobial Activity;
GC-MS.

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1- Introduction

Myrtaceae is an important plant family in Brazil, where approximately 1000 species are found [1], from these 211 are accounted for Cerrado biome [2]. These species are well known for its edible fruits and are also considered as a source for essential oils. Additionally, several species are traditionally used for medicinal purposes [3]. One of these Cerrado species, *Eugenia dysenterica* DC (also known as "cagaita" or "cagaiteira"), has its fruits widely consumed in this area, both *in natura* or as ingredient for jams and ice creams. The tree is also appreciated for landscaping, due to its numerous flowers, construction (wood) and tannery (barks) [4-5]. In the traditional medicine, its leaves are used to treat diarrhea and dysentery [6]. This activity was evaluated in the essential oil and ethanolic extract from the leaves. The essential oil presented antidiarrheic effect that seems to be linked with its capacity to alter the process of intestinal absorption and/or secretion [7]. The ethanolic extract decreased intestinal motility in induced diarrhea in rats. Therefore, it can be concluded that compounds present in the leaves can have a therapeutic effect in the treatment of diarrhea [8].

Another activity of leaf extract is the gastroprotective effect. Leaf extract could protect the gastric mucosa against ethanol/HCl-induced ulcer in mice and this effect seems to be related to the presence of the condensed tannins in the extract [9]. The extract also showed antiviral activity against simian rotavirus SA11 [10] and molluscicidal activity against *Biomphalaria glabrata*, intermediate host of schistosomiasis [11]. Besides the activities in the gastrointestinal tract, a recent study suggested that the essential oils of *E. dysenterica* leaves possess therapeutic potential in the prevention of neurodegenerative disorders, such as Alzheimer's disease [12]. The whole oil was able to inhibit the

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Acetylcholinesterase enzyme, which has been used in this disease treatment. The natural product was more potent than a drug commonly used (rivastigmine), IC_{50} 0.92 and 1.87 µL/mL respectively. Caryophyllene oxide, the major constituent of oil in that study, was isolated and evaluated as well. This compound was even more potent than the essential oils and the IC_{50} value (0.3 µL/mL) was six times smaller than the reference drug.

Most research on the biological activities of *E. dysenterica* is related to the leaf extracts and little is found for essential oils. In this context and considering that this species can be found distributed in several states of Brazil (Bahia, Federal District, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Piauí, São Paulo and Tocantins) and considering that the populations differ chemically whenever geographical distance exceeds 120 km [13], the objective of this work is to evaluate the chemical composition, antimicrobial and antioxidant activity of the essential oil collected in the state of São Paulo.

2- Materials and Methods

2-1-Plant Material

Eugenia dysenterica DC leaves were collected at Reserva Biológica and Estação Experimental Fazenda Campininha, Mogi-Guaçu, SP, Brazil (22˚15′ to 22˚16′ Sand 47˚8′ to 47˚12′W). Taxonomic identification was performed by Dr. Inês Cordeiro from Instituto de Botânica de São Paulo, where a voucher specimen has been deposited in its Herbarium (Brumati 213).

2-2-Essential Oil Extraction

E. dysenterica leaves were dried at room temperature and the essential oil extraction was performed by steam distillation in a Clevenger-type apparatus for 3 hours. After the extraction, the oils were collected, dried over anhydrous sodium sulfate, weighted and kept at −25°C until the analysis. The yield was calculated based on plant dry weight (w/w).

2-3-CG-MS Analysis

Qualitative analysis of essential oils from the leaves of *E. dysenterica* was performed in an Agilent6890 Series GC (Agilent, Santa Clara, CA, USA) interfaced with a 5973 series quadrupole MS detector (Agilent, Santa Clara, CA, USA), equipped with a DB-5 column (30 m × 0.25 mm i.d. × 0.25 µm) (Agilent J&W, Santa Clara, CA, USA). Chromatography conditions were as follows; over temperature: initially held at 40 °C for 1 min and subsequently increased to 240 °C at 3 °C/min; carrier gas: He at a flow rate of 1 mL/min; injector and detector temperature: 250 °C, electron ionization: 70 eV. The components were identified by comparing retention indices (evaluated in relation to the retention times of a series of *n*-alkanes) and mass spectra with those reported in the literature [14, 15].

2-4-Antimicrobial Activity

The antimicrobial activity was evaluated using microdilution method [16] against the microorganisms Candida albicans (ATCC 10231), Escherichia coli (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), Staphylococcus aureus (ATCC 6538) and *Aspergillus brasiliensis* (ATCC 16404). The inoculum was prepared from microbial suspension (standardized in 0.9% saline solution and McFarland turbidity scale) and liquid culture media (TSB for the bacteria and SDB for the yeast and fungi). The final concentration was 2 × 103 colony-forming units (CFU)/mL in each well of the microplate (Corning, New York, NY, USA). Dilutions of the essential oil were prepared using the solvent DMSO/MeOH (1: 1). Growth (inoculum), sterility (culture media) and solvent (DMSO/ MeOH) controls were also performed. The incubation period was 48 h at 25 °C for C. albicans, 72 hours at 25 °C for *A. brasilensis* and 24 h at 35 °C for the other microorganisms. The plates were read in a spectrophotometer (BiotekSynergy HT Biotek, Winooski, VT, USA) at 630 nm. For *A. brasiliensis* the determination of the inhibition was visual, considering the presence or absence of growth. Minimum inhibitory concentration (MIC) was considered the lowest dilution value of essential oil that was able to totally inhibit microbial growth.

2-5-In Vitro Antioxidant Activity

Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method [17]. The assay was performed on 96-well microplates (Corning, New York, NY, USA) using DPPH methanol solution at the final well concentration of 0.06 mg/mL. The essential oil was solubilized in methanol and a serial dilution was made. Quercetin was used as positive control. The DPPH solution was considered the sample control, methanol was considered the blank sample. The reaction occurred for 30 minutes, protected from the light, then the plates were read at 517 nm in a spectrophotometer (Biotek Synergy HT Biotek, Winooski, VT, USA). IC_{50} value calculation was based on the equation of the linear regression curves of the inhibition for each sample.

3- Results

3-1-Chemical Composition of Essential Oil

The leaf essential oil from *E. dysenterica* presented a yield of 0.064%±*0.005*. *E. dysenterica* essential oil consisted of at least 31 compounds from which 19 were identified. The amount of co-eluting compounds in the sesquiterpene range made it difficult to atribute a structure to all the peaks. The compounds, their retention indices, and the percentage of each oil constituent are listed in Table 1.

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

Compounds	RI[a]	RI (lit.)[b]	%
linalool	1098	1096	0.67
α-terpineol	1193	1188	1.24
α-copaene	1373	1376	3.82
β-caryophyllene	1414	1419	1.59
α-humulene	1452	1454	2.81
α-bulnesene	1510	1509	0.56
δ-cadinene	1515	1523	5.33
N.I.	1531	-	0.59
α-calacorene	1537	1545	2.07
N.I.	1545	-	0.95
N.I.	1550	-	1.27
N.I.	1565	-	0.77
caryophyllenyl alcohol	1571	1572	2.57
caryophyllene oxide	1577	1583	2.46
N.I.	1582	-	0.96
N.I.	1590	-	4.61
N.I.	1595	-	6.23
ledol	1599	1602	1.52
humulol	1603	1618	1.91
junenol	1607	1619	6.24
α-corocalene	1612	1623	1.02
N.I.	1616	-	0.92
muurola-4,10(14)-dien-1-β-ol	1624	1631	3.89
N.I.	1628	-	2.60
(-)-elema-1,3,11(13)-trien-12-ol	1637	1673	24.86
N.I.	1657	-	9.01
germacra-4(15),5,10(14)-trien-1-α-ol	1665	1686	0.62
N.I.	1669	-	5.67
N.I.	1678	-	1.56
amorpha-4,9-dien-2-ol	1692	1700	0.59
10-nor-calamenen-10-one	1695	1702	1.09
Total identified			64.86
N.I.			35.14
Oxygenated monoterpenes			1.91
Oxygenated sesquiterpenes			45.75
Sesquiterpenes hydrocarbons			17.2

[a] Retention index on DB-5 column; [b] Literature values [14,15]. N.I. = not identified.

3-2-Antimicrobial Activity

The antimicrobial activities of the essential oil of *E. dysenterica* were evaluated against *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. brasiliensis*.The most sensitive organism was *S. aureus*, presenting the lowest MIC (minimum inhibitory concentration) value.The MIC value for all the microorganisms are presented in Table 2. The values on the table correspond to thefinal well concentration.

Table 2. Minimum inhibitory concentration (MIC) of the essential oil from *E. dysenterica* leaves.

Microorganism	MIC±SD(µg/mL)
S. aureus	42.1±1.92
E. coli	> 10,000
P. aeruginosa	> 10,000
C. albicans	> 10,000
A. brasiliensis	> 10,000

SD – Standard deviation.

3-3- In Vitro Antioxidant Activity

The essential oil antioxidant activity was expressed as half maximal inhibitory concentration (IC_{50}) of the stable radical DPPH and the results are presented in Table 3. The concentration values presented correspond to the final well concentration.The antioxidant potential was compared to that obtained for quercetin, as positive control.

Table 3. Antioxidant activity (IC_{50}) by DPPH method of the essential oil from *E. dysenterica* leaves.

Sample	IC_{50} ±SD (mg/mL)
E. dysenterica	5.4±0.6
	IC_{50} ±SD (µg/mL)
Quercetin[a]	4.3±0.9

[a] Positive control .SD – Standard deviation.

4- Discussion

The yield obtained for the leaf essential oil from *E. dysenterica* collected in the São Paulo Cerrado (0.064%) was much lower than those previously found in other locations such as Piauí 1.48% [12], Minas Gerais, 0.34% [7], Brasília 0.25% [18] and Goiás 0.15% [6]. The chemical analysis allowed to identify 19 compounds and most of them are sesquiterpenes, divided in oxygenated 45.75% and hydrocarbons 17.2%. This result was similar to previous studies, where sesquiterpenes were the main class [6, 7, 12, 13, 17-19]. However, sesquiterpene hydrocarbons were predominant in most of the studies. Variations in essential oil yields and chemical composition among different populations can be caused by several factors such as the season of the year in which the collection occurred, the nutrients present in the soil [20] as well as the distance between the populations [13].

β-caryophyllene (15-37 %), caryophyllene oxide (66.3 %) and cis-β-ocimene (19.14 %) were the major compounds previously identified. In the present study, cis-β-ocimene was not found and β-caryophyllene was present in very low amounts (1.59%), and even considering its oxidation product (caryophyllene oxide, 2.46%), this compound still would not reach the previous rates. The major compound found in our study was the oxygenated sesquiterpene (-)-elema-1,3,11(13)-trien-12-ol (Fig. 1), with almost 25% of the total essential oil composition. This compound has already been isolated from the wood essential oil of *Thujopsis dolobrata* Sieb et Zucc, an endemic species from Japan, demonstrating inhibitory activity for Na^+/K^+-ATPase and lipopolysaccharide-induced nitric oxide production and it may also be an alternative to the treatment of allergic disorders mediated by IgE [21].

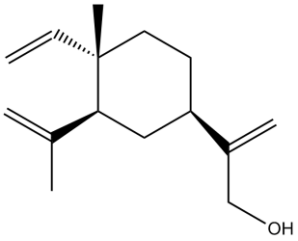

Figure 1. Chemical structure of (-)-elema-1,3,11(13)-trien-12-ol.

The essential oil of *E. dysenterica* was evaluated for its antimicrobial activity against five microorganisms. At the concentration of 10,000 µg/mL, the essential oil was not able to totally inhibit the growth of *E. coli*, *P. aeruginosa*, *C. albicans* and *A. brasiliensis*. The result obtained for *C. albicans* agrees with previous studies [6], however the other microorganisms had not yet been evaluated. Normally, only plant extracts that can completely inhibit microbial growth at concentrations below 100 µg/mL are considered interesting for the development of new antimicrobial agents [16]. *S. aureus* was the most sensitive microorganism, with a minimum inhibitory concentration (MIC) of 42.1 µg/mL. Gram negative bacteria have a cell wall composed of mucopolysaccharides that prevents the contact of the hydrophobic essential oil constituents with the bacterial cell membrane, therefore, the essential oils generally are more active against gram positive bacteria [7]. The activity found for *S. aureus* with the *E. dysenterica* oil was more potent than the MIC's reported for other *Eugenia* species, such as *E. adstringens* Cambess (119.2 µg/mL), *E. beaurepaireana* (Kiaersk) D. Legrand (1,110 µg/mL) and *E. brasiliensis* Lam. (156.2 µg/mL) [16].

Biological redox reactions and environmental factors (pollution, smoke, sun light) generate free radicals and the excess of this substances can cause damage in the living organisms [22]. Therefore, free radical scavenging agents find application in the treatment of diseases such as atherosclerosis and cancer and in the decreasing of the aging process. They also can be useful in the food industry, increasing the shelf-life of food products [23]. Due to the abundance of oxygenated compounds, the antioxidant activity of the essential oil was also evaluated. The *E. dysenterica* essential oil was able to quench the DPPH radical with an IC_{50} of 5.4 mg/mL. Compared with other *Eugenia* species, such as *E. uniflora* (IC_{50}= 833µg/mL) [24] and *E. caryophyllata* (IC_{50}= 0.2 µg/mL) [25], *E. dysenterica* presented the weaker activity. Using as reference *Gingko biloba* (Egb 761) extract, used medicinally duo to its recognized antioxidant activity (IC_{50}= 44.7 µg/mL) [26], it is possible to state that the essential oil of *E. dysenterica* cannot be considered a potent antioxidant agent.

5-Conclusion

The essential oil was composed for the most part by oxygenated sesquiterpenes. This result was different from most of the previous studies accomplished in the other states of the country and might be an indication of different chemotypes for this species. The antioxidant activity can be considered weak comparing to other Eugenia species and *Gingko biloba*. However, this essential oil presented a potent activity against *S. aureus* making this oil a potential antimicrobial agent against this microorganism. Further studies evaluating the antibacterial effect including synergism with other antimicrobials can be interesting.

6- Funding

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7- Conflict of Interest

The authors declare no conflict of interest.

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