Infrared spectroscopy, chemical composition and physical-chemical characteristics of the essential oil of red aroeira seeds (Schinus terebinthifolius Raddi) and it is antimicrobial and antioxidant activities

Espectroscopia de infravermelho, composição química e características físico-químicas do óleo essencial de sementes de aroeira vermelha (Schinus terebinthifolius Raddi) e suas atividades antimicrobiana e antioxidante

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Abstract

The aim of this study was to characterize the essential oil (EO) of red aroeira seeds and to evaluate their antimicrobial and antioxidant activities. The EO was extracted by hydrodistillation in a Clevenger-type device with a yield of 9.83% ± 0.31%; it was characterized by Fourier-transform infrared spectroscopy (FTIR), gas chromatography and mass spectrometry (GC-MS) to determine its physical-chemical characteristics. The EO showed acidity of 0.2814 mg KOH g⁻¹, a 1.4763 ± 0.0014 refraction index and density of 0.9365 ± 0.01656 g cm⁻³. Through the FTIR, the absorption bands of the EO indicated the presence of the following components: limonene, delta-3-carene, α-pinene and myrcene. By GC-MS the major compounds found were 4(10)-thujene (44.97%), α-pinene (20.42%), o-Cymene (12.76%) and p-Menth-1-en-4-ol, (R)-(−)-(6.74%). Antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria. In the microdilution method, no inhibiting activity was found in the tested concentrations (serial dilutions from
25.6 to 0.05 μL mL⁻¹) and in the disk diffusion method, inhibition halos were observed only when pure EO was added. Antioxidant activity was evaluated by DPPH (oxidation-reduction reaction) and FRAP (iron ion reduction). In the DPPH, the obtained result was 0.01119 ± 0.0001 μmol g⁻¹ and in FRAP, 13.813 ± 0.02187 μmol g⁻¹, which demonstrates antioxidant activity in the two evaluated methods, indicating the possible application of the essential oil of red aroeira seeds as a natural antioxidant agent.

**Keywords:** Infrared; Gas chromatography; Red aroeira; Antioxidant; Antimicrobial.

**Introduction**

The red aroeira is a tree of the perennial species that can reach up to 15 m in height and up to 30 cm in diameter at chest height. It has tortuous stem and shallow treetop (Neyes et al., 2016). It is present in almost all Brazilian states, such as Sergipe, Paraíba, Alagoas, Pernambuco, Rio Grande do Norte, Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Norte, Santa Catarina and São Paulo. Schinus terebinthifolius Raddi is the scientific name of the red aroeira, also known as Brazilian peppertree and rose-pepper, it belongs to the Anacardiaceae family. It is used as a medicinal plant, demonstrating great potential for new herbal products development (Carvalho et al., 2013). It has antioxidant, anticarcinogen and antimicrobial properties and it can be considered a sustainable source for the development of natural products due to its functional activities (Bendaoud et al., 2010; Salem et al., 2018). According to Bendaoud et al. (2010), the essential oil (EO) of the red aroeira is rich in sesquiterpenes and is a promising source of active compounds with antioxidant properties, which can be used in the food industry as natural antioxidants or even natural flavorings.
The fruits of the red aroeira are reddish, round, small drupes with reniform seeds (Grandi, 2014; Gomes et al., 2013), to which diuretic activity is attributed. The extracted EO demonstrated antioxidant and antimicrobial activity against the pathogenic bacterium *Listeria monocytogenes* (Dannenberg et al., 2016).

According to Vieira et al. (2016) essential oil of red aroeira leaves showed promising antibacterial substances for urinary tract infections and other human diseases. Martinelli et al. (2017) reports that the EO extracted from red aroeira seeds has important antimicrobial properties, which allow its application in food, pharmaceuticals and cosmetics.

The antioxidant potential of aroeira fruits have also been reported. The use of natural antioxidants to replace synthetic antioxidants is increasing in the food and pharmaceutical industries. This can be attributed to the possibility of minimizing collaterals effects of synthetic substances consumption. The secondary metabolites associated with antioxidant activity, such as phenolics, flavonoids, carotenoids, among others, are extremely important because of their benefits to human health, for example, anti-inflammatory and anticarcinogenic action (Limmongkon et al., 2018). However, studies that evaluated the antioxidant activity of the essential oil of aroeira seeds were not found.

In view of the above and considering that there are few scientific studies in the literature on the essential oil of red aroeira seeds, this study aimed to characterize this EO in terms of physical-chemical properties, compounds identification by fourier transform infrared spectroscopy (FTIR) and Gas chromatography–mass spectrometry (GC-MS), and to verify its antimicrobial and antioxidant potential.

2. Materials and Methods

This work was designed based on the hypothetical-deductive method, with the construction of hypotheses from a problem and, through experiments, they are confirmed or refuted as hypotheses (Lakatos & Marconi, 2010).

2.1 Materials

The certified red aroeira seeds were obtained at the company Arbocenter (https://www.sementesarbocenter.com.br/), collected in the city of Rubiácea - SP, 21° 18' 03" S and 50° 43' 37" O.

2.2 Extraction and physicochemical analyses of the essential oil

The EO was extracted in quadruplicate by hydrodistillation in a modified Clevenger type device (Wasicky, 1963). The seeds were crushed in a domestic mill until uniform granulometry was obtained. The material (50 g) was placed in a round bottom flask with 1 L capacity, in which 250 mL of distilled water was added. Distillation was performed for 5 hours. At the end of the extraction, the EO was packed in an amber bottle and stored under refrigeration (8 °C). The EO yield was calculated by relating the obtained mass of EO and the mass of the material used in the extraction (% m m⁻¹).

The EO relative density (mass and volume ratio) was measured by the capillary tube method (Pharmacopoeia, 2010) at 20 °C, in triplicate. The refractive index was measured at 20 °C, in triplicate, using the Abbé-Zeiss refractometer (Pharmacopoeia, 2010). For the acidity analysis, the method described by Moretto (1998) was used, based on the volume spent on KOH titration, expressed in mg of KOH g⁻¹ of essential oil.

2.3 Chemical analysis via Fourier transform infrared spectroscopy (FTIR) and Gas chromatography–mass spectrometry (GC-MS)

The infrared spectrum was obtained with the FTIR in the Agilent Cary® 630 equipment, using the spectral range of 4000 to 500 cm⁻¹, with a 4 cm⁻¹ resolution, 64 scans and reading through the diamond crystal.
The EO samples were submitted to GC-MS analysis in a Thermo machine, Focus GC model with DB5-MS capillary column (30 m x 0.25 mm and 0.25 μm), using helium as drag gas. The temperature was 230 °C in the injector and detector. The initial temperature of the column was 60 °C, it was programmed to increase 3 °C every minute, until the maximum temperature of 220 °C. The injection volume was 1 μL. A mixture of linear alkanes (C4 to C20) was injected into the chromatograph, under the same conditions used in the EO analysis, to estimate retention indexes with linear temperature programming.

2.4 Antimicrobial activity

The broth microdilution method (CLSI, 2009) with adaptations of Franciscato et al. (2018) was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the EO, with the following concentrations: 25.6, 12.8, 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1 and 0.05 μL mL⁻¹. The dilutions were prepared in Mueller-Hinton Broth containing 0.5% Tween 80.

The antimicrobial activity of the essential oils was tested against the following cultures: *Staphylococcus aureus* INCQS 00381 (ATCC 29213), *Staphylococcus aureus* INCQS 00005 (ATCC 14458), *Bacillus cereus* INCQS 00739 (ATCC 14579), *Escherichia coli* INCQS 00033 (ATCC 25922), *Shigella flexneri* INCQS 00152 (ATCC 12022), *Salmonella enterica* serovar Typhi INCQS 00040 (ATCC 19214), *Pseudomonas aeruginosa* INCQS 00099 (ATCC 27853), *Shigella dysenteriae* INCQS 00042 (ATCC 13313). All cultures were obtained from the Reference Microorganism Collection in Sanitary Surveillance - CMRV, FIOCRUZ-INCQS, Rio de Janeiro - Brazil.

Bacterial cultures kept in stock at −20 °C were activated in 3 mL Tryptic Soy Broth (TSB) and incubated at 37 °C for 24 hours. After activation, the cultures were standardized according to the 0.5 McFarland scale, obtaining bacterial suspensions of around 1.0x10⁸ CFU mL⁻¹, with absorbance reading (0.08 to 0.10) in a spectrophotometer at a 625 nm wavelength. After standardisation, each inoculum was diluted 1:10 in saline, followed by further 1:10 dilution in Mueller-Hinton Broth.

In the microdilution plates, 50 μL of EO at double the concentration was placed into the wells, followed by the addition of 50 μL of inoculum. In this way, the final concentrations of the EOs were obtained with a bacterial concentration of approximately 5.0x10⁵ CFU mL⁻¹. The same cellular concentration was obtained for each bacteria in one microplate, which only contained the culture medium to guarantee bacterial growth (positive control).

The color of the microplates was read after incubation at 37 °C for 24 hours, adding 50 μL of redox indicator (resazurin 0.01%) to each well of the microplate. Final blue staining indicated a negative result and a pink colour indicated a positive result for bacterial growth. The tests were performed in triplicate for each bacterium and the MIC was the lowest concentration in which no bacterial growth was identified in at least two replicates, after the incubation period.

The MBC was determined by the bacterial growth of each microplate well in Muller Hinton Agar plates, before adding resazurin. The non-development of bacterial colony was adopted as a standard for bactericidal activity.

For antimicrobial activity with the adapted disk diffusion test (CLSI, 2018), each standardized bacterial culture at 0.5 McFarland scale, was seeded with the aid of a sterile swab on the surface of the Muller Hinton Agar plates. On these plates, we added sterile discs with 15 μL of the same concentrations of the EO tested in the microdilution method, a disc with 15 μL of saline (control positive) and a disc with 15 μL of the EO itself. The plates were incubated at 37 °C for 24 hours. Then, the halos were measured.

2.5 Antioxidant activity

Two methods were used to evaluate antioxidant potential. The scavenging activity of 2,2-diphenyl-1-picrilhydryl free radicals (DPPH) was determined according to Ravichandran et al. (2012), with some modifications. A stock solution of DPPH
(0.4 mg mL\(^{-1}\)) was prepared, which was diluted with ethanol until the absorbance of 0.80 ± 0.02 at 517 nm (spectrophotometer, UV-VIS Femto, 700 Plus). From this solution, 3.90 ml was mixed with 0.10 ml of the sample. The tubes were kept at room temperature for 30 minutes, in the dark, and the absorbance was read at 517 nm, using ethyl alcohol as blank. The obtained results were calculated as inhibition percentage (%I): \(\%I = (A_0 - A)/A_0 \times 100\), in which \(A_0\) is the initial absorbance of DPPH and \(A\) is the absorbance of each sample after the reaction.

For the ferric reducing antioxidant power (FRAP) the extracts were evaluated following the method described by Benzie and Strain (1996), with some adaptations. The FRAP reagent solution was obtained from a mixture of 25 mL of 0.3 mM acetate buffer solution (pH 3.6), 2.5 mL of a TPTZ solution (2,4,6-tri (2-pyridyl) -1,3,5-triazine) 10 mM dissolved in 40 mM HCl and 2.5 mL of 20 mM ferric chloride. In a test tube, we added 90 μL of each diluted sample, 0.27 mL of distilled water and 2.7 mL of the FRAP reagent. After homogenization, the samples were incubated at 37 °C for 30 min and the absorbance was measured at 595 nm. The calibration curve of the antioxidant activity was prepared with a Trolox solution (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) with concentrations of 20 to 0.75 mM, respectively, with a regression coefficient (\(R^2\)) > 0.99. Thus, the results were expressed in mmol of Trolox equivalent (TE) per g of sample.

3. Results and Discussion

3.1 Yield and physical-chemical properties

The extracted essential oils from the red aroeira seeds presented an average yield of 9.83 ± 3.16 %, a yield higher than what found by Martins et al. (2014), in which the extraction yield of Schinus molle EOs was 1.09 ± 0.22% and 0.91 ± 0.09% (v dry weight\(^{-1}\)) for leaf and fruit EOs, respectively. The obtained yield of the EO in this study was also higher when compared with other authors, who used the same extraction method. Oliveira et al. (2014) obtained a 2.76% yield in red aroeira seeds. Barbosa et al. (2007) reported values of 0.44% (leaves) and 4.65% (seeds), when they extracted EO from leaves and seeds of red aroeira in a 3-hour-period. The observed yield in our study can also be justified by factors such as seed origin (different locations and climates) and extraction time.

As for the physical and chemical characteristics, the essential oil of red aroeira seeds presented 0.2814 mg KOH g\(^{-1}\) acidity and 0.9365 ± 0.01656 g mL\(^{-1}\) density at 20 °C. The property of acidity is directly related to the nature and quality of the essential oil. The lower this index, the better its conservation status (Ial, 2008). Several factors can influence acidity, including the treatment given to seeds during harvest and storage process (Almeida et al., 2011). No acidity parameter was found to compare the EO of red aroeira seeds of this study, but the found value was close to that of patua oil (0.252 ± 0.016 mg mL\(^{-1}\) of NaOH (Alves et al., 2015).

The refraction index of the EO at 20 °C was 1.4763 ± 0.0014, this value is close to that presented by Cole (2008) for the essential oil of fruits of the same species, whose value was 1.4750 at 20 °C. Dourado (2012) reported that the refraction index of the essential oil of red aroeira fruits was 1.484 at 23 °C. Thus, the refraction index of this study is consistent with the literature.

Finally, physicochemical analyses showed a 0.9365 (± 0.01656) g mL\(^{-1}\) relative density, a value close to Cole’s (2008) of 0.9097 g mL\(^{-1}\) at 20 °C and the results of Martins et al. (2014) of 0.872 and 0.825 g mL\(^{-1}\), for leaf and fruit of Schinus molle EOs, respectively.

3.2 Infrared spectroscopy and gas chromatography analysis

FTIR spectroscopy is a non-destructive tool that performs qualitative analyses on the molecular structure of chemical compounds, present in various types of materials (Sivakesava, 2001).
In Figure 1 is the infrared spectrum of EO absorption obtained from red aroeira essential oil and in Table 1 are listed the wavenumbers of the absorption bands, associated with functional groups present in the chemical compounds.

**Figure 1.** Infrared absorbance spectrum of the essential oil of red aroeira seeds. The arrows indicate the wavenumber of the respective absorption band.

![Infrared absorbance spectrum of the essential oil of red aroeira seeds. The arrows indicate the wavenumber of the respective absorption band.](source)

**Table 1.** Wavenumber, functional group, peak intensity, vibration mode and equivalence for the essential oil of red aroeira seeds.

| Wavenumber (cm⁻¹) | Functional group | Peak intensity | Vibration mode | Correspondence |
|-------------------|------------------|----------------|----------------|----------------|
| 2922              | C-H              | Strong         | Stretching     | Majority (Author) |
| 2865              | C-H              | Strong         | Stretching     | Majority (Author) |
| 2826              | C-H              | Strong         | Stretching     | Majority (Author) |
| 1646              | C=C              | Medium         | Stretching     | Majority*; Limonene (Author) |
| 1445              | N-H              | Medium         | Out-of-plane bending | Majority and another elements*; Limonene (Author) |
| 1374              | CH₃              | Strong         | Bending        | Limonene (Author) |
| 862               | Aromatic Ring    | Weak           | Bending        | Limonene (Author) |
| 785               | C-Br             | Strong         | Bending of adjacent 3 H (meta-substituted rings and 1, 2, 3-trisubstituted). | - |

Source: Authors.

The triplet band (2922, 2865 and 2826 cm⁻¹) is referenced as axial deformations to double CH bonds and may represent aromatic alkenes. In addition, CH bonds can be attributed to the majority compounds and other chemical compounds present in the EO of the red aroeira.
The bands in the region of 1646 cm\(^{-1}\) and 862 cm\(^{-1}\) characterize the deformation of tri-replaced carbon in alkenes and the bands in the region of 1445 cm\(^{-1}\) for angular deformation C-H\(_2\). Silva (2015) identified the major compounds limonene, delta-3-carene, alpha-pinene and beta-mycene for the EO of Schinus molle L. of the same genus of the species of this study; the weak signal ‘C=O’ referring to the C=O stretch is present in limonene, delta-3-carene, alpha pinene and myrcene.

In the list of chemical compounds presented in Table 2, it is also possible to identify the present chemical classes, such as monoterpen hydrocarbons (C\(_{10}H_{16}\)), oxygenated monoterpenes (C\(_{10}H_{18}O\)), sesquiterpene hydrocarbons (C\(_{15}H_{20}\)) and other oxygenated compounds. These chemical classes were grouped according to the identified and quantified compounds, by GC-MS, of essential oil of the red aroeira.

| Compounds                  | LRI   | RI    | Chemical formula | Area % |
|----------------------------|-------|-------|------------------|--------|
| **Monoterpene hydrocarbons** |       |       |                  |        |
| 3-Thuene                   | 923   | 924   | C\(_{10}H_{16}\) | 1.21   |
| alpha-Pinene               | 930   | 930   | C\(_{10}H_{16}\) | **20.42** |
| 4(10)-Thuene               | 971   | 971   | C\(_{10}H_{16}\) | **44.97** |
| beta-Pinene                | 975   | 974   | C\(_{10}H_{16}\) | 3.78   |
| alpha-Myrcene              | 987   | 988   | C\(_{10}H_{16}\) | 0.65   |
| alpha-Cymene               | 1022  | 1021  | C\(_{10}H_{14}\) | **12.76** |
| **Oxygenated monoterpene** |       |       |                  |        |
| cis-beta-Terpineol         | 1067  |       | C\(_{10}H_{18}O\) | 0.28   |
| alpha-Pinene oxide         | 1096  | 1095  | C\(_{10}H_{18}O\) | 0.81   |
| 1-Isopropyl-1-methyl-2-cyclohexen-1-ol | 1122 | 1120 | C\(_{10}H_{18}O\) | 0.28   |
| 2(10) Pinen-3-ol,(1S,3R,5S)-(-)- | 1137 | 1129 | C\(_{10}H_{18}O\) | 0.58   |
| cis-Verbenol               | 1143  | 1137  | C\(_{10}H_{18}O\) | 0.40   |
| Thujan-2-one               | 1152  | 1144  | C\(_{10}H_{18}O\) | 0.42   |
| Pinocarvone                | 1159  | 1160  | C\(_{10}H_{18}O\) | 0.55   |
| 3-Thujanol                 | 1166  | 1164  | C\(_{10}H_{18}O\) | 0.25   |
| **Terpinen-4-ol**          | **1178** | **1182** | C\(_{10}H_{20}\) | **6.74** |
| (R) Myrtenal               | 1192  | 1195  | C\(_{10}H_{20}\) | 1.06   |
| Verbenone                  | 1203  | 1205  | C\(_{10}H_{20}\) | 0.60   |
| p-Menthane,1,2,3,triol      | 1251  |       | C\(_{10}H_{30}O_3\) | 0.96   |
| 4-Isopropyl-5-methylhex-2-yne-1,4-diol | 1271 |       | C\(_{10}H_{18}O_2\) | 0.48   |
| **Sesquiterpenes hydrocarbons** |       |       |                  |        |
| Copaene                    | 1372  | 1370  | C\(_{15}H_{24}\) | 0.42   |
| gamma-Muurolene            | 1471  | 1460  | C\(_{15}H_{22}\) | 0.38   |
| **Oxygenated sesquiterpenes** |       |       |                  |        |
| 6-Isopropyl-3-methyl-7-oxa bicyclo-heptan-2-one | 1391 |       | C\(_{10}H_{18}O_2\) | 0.57   |
| (-) Spathulenol             | 1572  | 1577  | C\(_{10}H_{20}O\) | 0.83   |
| Caryophyllene oxide         | 1577  | 1582  | C\(_{15}H_{20}O\) | 0.56   |
| **TOTAL**                  |       |       |                  | **99.96** |

LRI: Linear retention index.
RI: Kovats retention index.
Source: Authors.

Twenty-four compounds were identified: 13 oxygenated monoterpenes, six monoterpen hydrocarbons, three oxygenated sesquiterpenes and two sequiterpene hydrocarbons. In terms of quantity, the predominant class of compounds was monoterpen hydrocarbons (83.79%), which allows the correlation of this percentage with the peaks of strong intensity of infrared spectroscopy (Table 1), because, according to Portella et al. (2014), monoterpen compounds mainly present absorbance in four regions: 3000 cm\(^{-1}\); from 2800 cm\(^{-1}\) to 1650 cm\(^{-1}\); from 1000 cm\(^{-1}\) to 950 cm\(^{-1}\); and between 900 cm\(^{-1}\) and 850 cm\(^{-1}\).
The major EO compounds of red aroeira seeds were 4(10)-thujene (44.97%), α-pinene (20.42%), o-cymene (12.76%) and terpinen-4-ol (6.74%). Martins et al. (2014) investigated the chemical composition of essential oils of *Schinus molle* L. fruits and identified 16 components, 98.0% of monoterpenes compounds and 0.5% of sesquiterpenes. Most compounds were α-phellandrene (25.9%), limonene (11.7%), β-myrcene (11.1%) and β-phellandrene (10.5%).

Cavalcanti et al. (2015) investigated the chemical composition of essential oils extracted from fruits of *Schinus molle* L. and *Schinus terebinthifolius* Raddi. In the EO of *Schinus terebinthifolius* Raddi the most frequent chemical compounds were α-pineno (44.9%), germacreno D (17.6%) and β-pineno (15.1%); and in the EO of *Schinus molle* L they were β-pineno (36.3%), α-pineno (20.3%), germacren D (12.1%) and spathulenol (11.4%). The variation of chemical compounds in essential oils depends on many factors, such as variety, part of the plant, climatic conditions, drying conditions (if applied) and extraction methods (Ribeiro-Santos et al., 2017).

Although terpinen-4-ol was only the fourth compound (6.74%) of the EO of red-aroeira seeds in this study, it represented 50.2% of the oxygenated monoterpenes. Sökmen et al. (2003) suggested that oxygenated monoterpenes were responsible for the high antimicrobial activity of the essential oil of *Achillea sintenisii* and the fractions of monoterpenes and sesquiterpene hydrocarbons were considered inert.

### 3.3 Antimicrobial activity

Antimicrobial activity was not observed in the tested concentrations in the microdilution method. Thus, a MIC > 25.6 μL mL⁻¹ was established against the microorganisms tested in this study. In line with the MIC result, MBC was also considered > 25.6 μ L mL⁻¹. There was bacterial growth in all wells of the microplate, except for the negative controls of the first column. However, the antimicrobial potential of this essential oil was previously evaluated by Dourado (2012), who used the fruits of the red aroeira for oil extraction. The author found antimicrobial activity against *Staphylococcus aureus* at a minimum concentration of 10.92 μg mL⁻¹ and 2.73 μg mL⁻¹ against *Escherichia coli* and *Listeria monocytogenes*. In Silva’s study (2015), the essential oil of red aroeira green fruits presented 1000 μg mL⁻¹ MIC against *Candida albicans*, but inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella Enteritidis* (MIC > 2000 μg mL⁻¹) was not observed.

In the study by Cole et al. (2014), the EO of red aroeira fruits, with predominance of monoterpenes, showed antibacterial activity against all tested strains, gram-positive bacteria *Corynebacterium* sp. (3.55 μg mL⁻¹), *Bacillus* sp. (7.11 μg mL⁻¹) and *Nocardia* sp. (7.11 μg mL⁻¹), as well as Gram-negative bacteria *Enterobacter* sp. (56.86 μg mL⁻¹), *Enterobacter agglomerans* (28.43 μg mL⁻¹), *Escherichia coli* (28.43 μg mL⁻¹) and *Klebsiella oxytoca* (28.43 μg mL⁻¹). Gram-positive species were more sensitive to the EO, which is likely due to the lower structural complexity of their cell wall.

In the disc diffusion method, inhibition halos were observed only when pure essential oil was added (Table 3); the largest halo was visualized against *Shigella flexneri* (17 mm). According to the classification for microbial sensitivity to essential oils proposed by Ponce et al. (2003), microorganisms are considered non-sensitive when they have inhibition halos of less than 8 mm; sensitive with halos from 9 to 14 mm; very sensitive with halos of 15 to 19 mm; and extremely sensitive with halos greater than 20 mm. Thus, in this study it was possible to observe antimicrobial sensitivity to pure red aroeira essential oil in 75% of the tested bacteria. Melo et al. (2014) also did not detect inhibition of pure red aroeira EO (composed mainly of monoterpenes–78%), with inhibition halos smaller than 8 mm for *Escherichia coli*, *Salmonella Enteritidis* and *Staphylococcus aureus*.

The results of antimicrobial activity of essential oil of red aroeira seeds in this study differed from other studies; however, the percentage of possible metabolites with antimicrobial properties (e.g., oxygenated monoterpenes) was low in our EO. Secondary metabolites produced by plants vary according to different cultivation conditions, climate and environmental
stress. Thus, it is possible that the red aroeira plants used to harvest the seeds for the EO could be in conditions that did not favor the production of such components.

Table 3. Inhibition halo of red aroeira essential oil at different concentrations.

| Microorganisms                          | Inhibition halo (mm) |
|----------------------------------------|----------------------|
|                                        | Positive control (saline) | Concentrations of 25.6 to 0.05 μL mL⁻¹ | Essential oil pure |
| Staphylococcus aureus (ATCC 29213)     | -                     | -                                   | 11               |
| Staphylococcus aureus (ATCC 14458)     | -                     | -                                   | 10               |
| Bacillus cereus (ATCC 14579)           | -                     | -                                   | 10               |
| Escherichia coli (ATCC 25922)          | -                     | -                                   | 11               |
| Shigela flexneri (ATCC 12022)          | -                     | -                                   | 17               |
| Salmonella enterica serovar Typhi (ATCC 19214) | -                     | -                                   | 07               |
| Pseudomonas aeruginosa (ATCC 27853)    | -                     | -                                   | -                |
| Shigela dysenteriae (ATCC 13313)       | -                     | -                                   | 11               |

Source: Authors.

3.4 Antioxidant activity

The compound diversity present in essential oils (EOs) can exhibit different mechanisms of antioxidant action, thus, the use of more than one type of assay is common in the evaluation of antioxidant activity.

In this study, the diluted essential oil (12 mg mL⁻¹) promoted a DPPH free radical scavenging, with an inhibition percentage of 46.25 ± 0.51%. Although we did not find studies on antioxidant activity in the aroeira seed essential oil, the value obtained in our study was similar to what Oliveira et al. (2020) found in the aroeira fruit EO (42.68 ± 0.05%). Martins et al. (2014) found lower values of DPPH inhibition in leaf and fruit EOs of Schinus mole, 4.8% and 5.5%, respectively. The result obtained in this in vitro test of the aroeira seed EO may be promising, once free radical species are responsible for activating several pro-inflammatory transcription factors involved in inflammatory diseases (Silva et al., 2017), which can be proved by future in vivo tests.

In the analysis of antioxidant potential of essential oils, factors such as sample solubility at different testing systems and stereo selectivity of the radicals used in the methods may influence results (Veras et al., 2020). According to previous studies (Wu et al., 2020), the scavenging mechanism of DPPH radicals is hydrogen atom transfer, so, the resulting antioxidant radical may either propagate or terminate radical chain reaction. This method is widely used in the characterization of antioxidant activity of essential oils (Martins et al., 2014; Wu et al., 2020). However, data on FRAP assay, based in the reduction of ferrous ion, is scare for essential oils. According to our data, the FRAP methodology could be used to measure antioxidant activity of essential oils and the result for aroeira seed EO was 13.81 ± 0.02 mmol TE g⁻¹. Although literature did not provide data on this assay, this result reinforces that EOs are an alternative to chemical preservatives and that they can be used in food conservation with a positive impact on consumer’s health (Dima et al., 2016).

The major compound of the aroeira vermelha seed essential oil was 4(10)-thujene, a bicyclic monoterpene also known as sabine. Ruberto and Baratta (2000) described the high antioxidant activity of this compound using the TBARS method (thiobarbituric acid reactive substances); mild activity was found when determining the rate of conjugated diene formation from linoleic acid. According to them, α-pinene, the second major compound found in red aroeira essential oil (20.5%)—also exhibited mild activity in the TBARS method.
Additionally, the monoterpene hydrocarbon α-cymene, which accounts for 13% of the essential oil content, is known for its high antioxidant activity against hydroxil radicals, although it is mild to low against DPPH and ABTS radicals (Nie et al., 2020).

Regarding the most abundant oxygenated monoterpene found in the essential oil of the aroeira seed, terpinen-4-ol, an in vivo study performed by Souza et al. (2018) pointed out that long-term exposure to this substance reduced TBARS levels in the liver and lipid hydroperoxide in the liver and kidney of silver catfish (Rhamdia quelen), demonstrating its antioxidant property. These four compounds (4(10) -thujene, α-pinene, α-cymene and terpinen-4-ol) account for 85% of the essential oil content of red aroeira seeds and may explain the high antioxidant activity observed in this study.

4. Conclusion

The results of this study show that the essential oil of red aroeira seeds has antioxidant properties, which may be related to the strong presence of terpenoids such as 4(10) -thujene, α-pinene α-cymene and terpinen-4-ol. This characteristic, associated with the pleasant aroma of this oil, makes it an excellent candidate for use as an antioxidant agent in food preparations. Due to the facts mentioned about the results of antimicrobial activities, further studies are needed to better understand the amount of oxygenated monoterpenes.

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