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Evaluation of antibacterial and antioxidant activity of purple araçá essential oil (*Psidium rufum*, Myrtaceae)

[*Avaliação das atividades antibacteriana e antioxidante do óleo essencial do araçá-roxo* (Psidium rufum, *Myrtaceae*)]

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ABSTRACT

The aim of this study was to evaluate the antibacterial and antioxidant activities of essential oil (EO) from fresh leaves of *Psidium rufum*. The EO was extracted by hydrodistillation and identified by gas chromatography coupled to mass spectrometry. The antibacterial activity was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Antioxidant activity was determined by β-carotene/linoleic acid co-oxidation system, 2,2-diphenyl-1-picrylhydrazyl radical scavenging and iron reduction methods. Hydrocarbon sesquiterpenes were the predominant class, indicating 1,8 cineole, α-longipinene as major. The EO was tested against the bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* (MIC = 2,500 µg/mL and MBC = 20,000 µg/mL); *Enterococcus faecalis* (MIC = 2,500µg/mL and MBC > 20,000µg/mL) and *Escherichia coli* (MIC > 20,000µg/mL and MBC > 20,000µg/mL). The EO showed antioxidant potential due to β-carotene/linoleic acid co-oxidation system, with 76.63% of oxidation inhibition (1.0mg/mL) and due to the iron reduction power (*5,38 µmol Fe^{2+}/mg* sample). The results are promising in recommending this species for the development of food, cosmetic and pharmaceutical products.

Keywords: *Enterococcus faecalis, Pseudomonas aeruginosa,*1,8 cineole, *α*-longipinene

RESUMO

O objetivo deste estudo foi avaliar as atividades antibacteriana e antioxidante do óleo essencial (OE) das folhas frescas de Psidium rufum. *O OE foi extraído por hidrodestilação e identificado por cromatografia gasosa acoplada à espectrometria de massas. Foi avaliada a atividade antibacteriana, determinando-se a concentração inibitória mínima (CIM) e a concentração bactericida mínima (CBM). A atividade antioxidante foi determinada pelo sistema de co-oxidação β-caroteno/ácido linoleico, pelos métodos de sequestro do radical 2,2-difenil-1-picrilhidrazil e de redução do ferro. Sesquiterpenos hidrocarbonetos foram a classe predominante, indicando 1,8 cineol, α-longipineno como majoritário. O OE foi testado contra as bactérias* Staphylococcus aureus *e* Pseudomonas aeruginosa *(CIM = 2,500µg/mL e CBM = 20.000µg/mL);* Enterococcus faecalis *(CIM=2,500µg/mL e CBM > 20.000µg/mL) e* Escherichia coli *(CIM > 20.000µg/mL e CBM > 20.000µg/mL). O OE apresentou potencial antioxidante pelo sistema co-oxidação β-caroteno/ácido linoleico com 76,63% de inibição da oxidação (1,0mg/mL) e pelo poder de redução de ferro (5,38 µmol Fe^{2+}/mg amostra). Os resultados são promissores em indicar essa espécie para o desenvolvimento de produtos alimentícios, cosméticos e farmacêuticos.*

Palavras-chave: Enterococcus faecalis, Pseudomonas aeruginosa, 1,8 cineol, α-longipineno

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INTRODUCTION

Brazil, a country of rich biodiversity, has in its territory an invaluable asset of resources, however, very little is known of this diversity (Bresolin and Cechinel, 2003). The Myrtaceae family has approximately 140 genera and more than 3,000 species worldwide, being considered complex from the taxonomic point of view (Gomes *et al*., 2009). This family has leaves with a large number of volatile constituents (Souza and Lorenzi, 2005; Stieven *et al.*, 2009), where they almost always have proanthocyanins, ellagic and gallic acids, as well as produce saponins and less cyanogenic compounds characteristics of native fruit, to which the Araçazeiros belong. They assume prominence because the oils produced by species of this family exhibit insecticide activity, pesticide, nematicide, antifungal, antibacterial, antioxidant and even antiallergic properties , and the presence of flavonoids aids in immunological activity, determining chronic responses in inflammatory processes (Batish *et al.*, 2012; Amarante and Santos, 2013).

From a pharmacological point of view, studies with gross and compound extracts have proven anti-inflammatory, analgesic, antifungal, antipyretic, hypotensive, antidiabetic and antioxidant activities (Oliveira *et al*., 2006; Armstrong, 2011). Simonetti *et al*. (2016) observed antimicrobial activities in *Psidium* plant statements against *Escherichia coli* and *Listeria monocytogenes*.

According to Daikos *et al*. (2021), in recent years there has been an increase in bacterial species with a high degree of resistance to conventional therapy, such as *P. aeruginosa*, which can present multiresistant phenotypes. Therefore, there is an interest in increasing the knowledge about the inhibitory concentrations of essential oils, in search of a balance between the acceptability and the effectiveness of the antimicrobial action. It is estimated that 20 to 50% of antimicrobial use in humans and 40 to 80% in animals is unnecessary or highly questionable, which leads to a high rate of resistance by bacteria (Beovic, 2006).

The development of natural antioxidants is interesting due to their role in the protection of human cells from damage caused by free radicals and natural compounds rather than synthetic antioxidants, which appear to be preferred in the industry (Mutlu-Ingok *et al*., 2020). Practical uses of these activities are suggested in humans and animals, as well as in the food industry. Since medicinal plants produce a variety of substances with antimicrobial (Alvarenga, *et al*., 2007) and antioxidant properties (Jerônimo *et al*., 2021), research with *Psidium* essential oil is of interest.

The genus *Psidium* originates from the tropical and subtropical Americas and consists of about 100 tree species and shrubs (Landrum and Kawasaki, 1997), of which the most important is the guava (*P. guajava* L.). The genre also includes numerous other species producing edible, logging, and ornamental fruits, with great potential for commercial exploitation. Among these species, *araçazeiros*, *Psidium ruffum*, are deserving of greater attention, especially due to some specific characteristics of their fruits, such as exotic flavor, high vitamin C content and good acceptance by consumers (Manica *et al*., 2000; Pires *et al*., 2002), beyond the presence of α-tocopherol (Barcia *et al*., 2010).

However, research is focused on the extract of fruits and peels, few studies refer to the essential oil (EO) obtained from its leaves, so the proposed study can be a promising field for new discoveries. The objective in this study was to evaluate the antimicrobial and antioxidant activity of the essential oil of the fresh leaves of *P. ruffum*.

MATERIALS AND METHODS

Psidium rufum fresh leaves were collected in the Umuarama region; Northwest Region of the State of Parana, Brazil, in coordinates S23º 46.225 'and WO 53º 16.730', altitude of 391m. A voucher was authenticated and deposited in the Herbarium of Maringá State University under the number HUEM-30716. This specie is registered with the National System of Genetic Heritage Management and Associated Traditional Knowledge (Sisgen) under the registration number A3440b2.

P. ruffum fresh leaves EO was extracted by the hydrodistillation process for two hours using the modified Clevenger device (Gazim *et al*., 2010, 2011; Armstrong, 2011). The EO was removed

from the device with n-hexane, filtered with anhydrous sodium sulfate (NA2SO4) (Simões and Spitzer, 2002), packed in amber bottles, kept under refrigeration at 4ºC until complete evaporation of the solvent (Omolo *et al*., 2004).

EO analysis was by gas chromatography coupled to mass spectrometry - (GC/MS). Using an Agilent 19091S-433 chromatograph. The DB5 Capillary Column (30 m x 0.25 mm x 0.25µm). The column temperature programming was 60°C, remaining for 3 min to 250°C with a heating ramp of 5.0°C/min, remaining at 250ºC for 15 min. The carrier gas was the helium used at a constant pressure of 80 kPa and with linear speed of 1 mL/min to 210°C and with a pressure flow of 25 kPa. Injector temperature: 250°C, the injection was in splitless mode. The conditions for the mass (MS): Source temperature 200°C; interface temperature 250°C; Detection system was by electronic impact to 70 eV; Mass Scan Range, 40-350 amu. In addition to the results obtained by GC/MS, the identification of the compounds was also based on comparing their retention rates (RR) obtained using a homologous series of N-alkans (C7-C30). The mass spectra were compared to the Wiley 275 Libraries library and the literature (Adams, 2017).

EO was evaluated against Gram-positive bacteria *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 and Gram-negative *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, the test was performed using the microdilution method in broth with alterations for natural products (Methods…, 2009).

The bacteria were grown in Petri plates containing blood agar at 36°C for 24 hours. A saline suspension (NaCl at 0.85%) was prepared, with standardization according to the 0.5 scale of MacFarland. Subsequently, these inoculants were diluted 1:10 in saline to obtain a final concentration of 10^7 UFC mL^{-1}.

They were added to the sterile microplate containing 96 wells 100µl of the middle brain heart infusion (BHI) and 100µL of essential oil, prepared at the concentration of 20,000µg.ml-¹, EO was dissolved in 2% Tween 80. Serial dilutions were performed obtaining essential oil concentrations ranging from 20,000µg.ml-¹ to 1.207µg.ml-¹. Then the inoculum (50µL) was added to microlate. The controls: medium (only BHI), negative (BHI and essential oil) and positive (BHI and bacteria) were evaluated. After the microplates were incubated at 35°C for 24 hours. The reading was performed with the addition of 10 µL of TTC aqueous solution (Triphenyl tetrazolium chloride) to 10% in all wells and after 30 minutes to 35°C, the presence or not of reddish coloration was verified. The presence of reddish coloration was considered bacterial growth.

Bacteria colonies were cultivated separately to assess whether there was growth, determining the MBC individually by the subculture of each well. MIC was determined as the lowest concentration in which growth is inhibited and MBC was determined as the lowest concentration capable of inhibiting bacterial growth in subculture.

To determine the free radical scavenging capacity of DPPH, the methodology was evaluated according to Rufino *et al*. (2007). An aliquot of 0.1mL of the different concentrations of the EO of purple araçá (10.0; 7.5; 5.0; 2.5 and 1.0mg.mL^{-1}), with 3.9mL of methanolic solution of DPPH (60µM) were freshly prepared for the activity. For the negative control, 0.1 mL of methanol was used in the DPPH solution (60µM). The mixture was kept in the dark at room temperature for 30 minutes. The absorbance reduction was measured at 515nm in a UV/VIS spectrophotometer. The total antioxidant capacity of extracts and fractions was calculated using a standard solution of quercetin (60µM), as a reference of 100%. From the correlation between absorbance versus concentration of the antioxidant sample, the concentration necessary to reduce 50% of free radicals (IC50) was determined.

The antioxidant capacities of the samples (EO) of purple araçá were evaluated according to Rufino *et al*. (2006b). The reaction can be monitored by spectrophotometry, loss of β-carotene staining at 470nm. 20µL of linoleic acid, 265µL of Tween 40, 25µL of β-carotene solution (20 mg. mL^{-1}) and 0.5 mL of chloroform were added to a beaker protected from light (wrapped in aluminum foil). The solvent was removed using a dryer. Then, the mixture was dissolved in 20mL of deionized water and

oxygenated (by oxygen for 30 min) under vigorous stirring to form an emulsion. The emulsion had the absorbance adjusted to 0.7 at 470nm. The antioxidant activity of the samples was determined by mixing 280µL of emulsion with 20 µL of samples at different concentrations (1.0; 0.75; 0.50 and 0.25mg. mL^{-1}) plotted on 96-well flat-bottomed microplates. The samples were placed in the SpectraMax Plus384 Microplate Reader device, maintained at 40 ºC for 120 minutes with readings taken every five minutes, and the absorbance measured at 470nm. A 615rolox solution (0.2mg. mL^{-1}) was used as a reference standard. Results were expressed as percentage of oxidation inhibition, following Eq. 1 the reduction in the absorbance of the antioxidant system was considered as 100% oxidation. From the absorbance following Eq. 2, the percentage of oxidation was calculated correlated with the absorbance of the sample decreasing with the absorbance of the system, the percentage of oxidation of each sample was subtracted from 100 (Eq. 3) for the percentage of inhibition of oxidation (%).

Reduction of absorbance=Ainitial-Alast (Eq. 1)
% of oxidation=Reducion Asample x 100/ (Reduction A) system (Eq. 2)
% of protection=100-(% Oxidation) (Eq. 3)

This method was performed as described by Rufino *et al*. (2006a). To prepare the FRAP reagent, 25mL of acetate buffer (0.3 M), 2.5mL of aqueous solution of 2,4,6-Tris(2-pyridyl)-striazine (TPTZ - 10 mM), 2.5mL of aqueous ferric chloride solution (20 mM) and 3mL of distilled water. The reagent solution consisted of 10µL of samples (OE of araçá) at different concentrations (1.00; 0.75; 0.50 and 0.25µg. mL^{-1}), 290µL of FRAP reagent in each well of the microplate. The mixture was placed in the SpectraMax Plus384 Microplate Reader device and kept at 37°C for 30 minutes. Absorbance was read at 595 nm. Using a standard curve of ferrous sulfate (0 – 2000µM) the percentage of antioxidant activity was calculated. The antioxidant activity was expressed in µM ferrous sulfate mg^{-1} of the sample.

All tests were performed in triplicate. The results were submitted to analysis of variance (ANOVA), and the differences between the means determined by Duncan's test ($p \leq 0.05$) by the SPSS Statistics 22 program.

RESULTS

The chemical identification of the EO of the fresh leaves of *P. rufum* revealed the presence of 41 compounds, considering only the compounds with a relative area above 0.30% (Table 1). The major classes were hydrocarbon sesquiterpenes (70.01%) followed by oxygenated monoterpenes (28.08%), where the main compounds identified were 1,8-cineole (19.36%) and α-longipinene (19.00%).

In addition to the chemical constitution, the antibacterial potential of this EO against four microorganisms showed: *S. aureus* (MIC = 2,500µg.mL^{-1} and MBC = 20,000µg.mL^{-1}); *E. faecalis* (MIC = 2,500µg.mL^{-1} and MBC > 20,000µg.mL^{-1}); *P. aeruginosa* (MIC= 2,500µg.mL^{-1} and MBC= 20,000µg.mL^{-1}) and *E. coli* (MIC > 20,000µg.mL^{-1} and MBC > 20,000µg.mL^{-1}).

Regarding the antioxidant activity by the DPPH method, the EO of *P. rufum* was able to reduce the stable DPPH radical to yellow diphenylpicrylhydrazine, where the concentration required to reduce 50% of free radicals was 4.74±1.82mg mL^{-1}, about 474 times higher than quercetin, positive control, which makes evident the reduced antioxidant activity of this oil by this method (Table 2), presenting an antioxidant activity of 17.73 % at a concentration of 1.00 mg mL^{-1}.

The result of the antioxidant activity by the reduction of iron for the EO of *P. rufum* (Table 3) was calculated from the equation of the straight line obtained by the standard curve of ferrous sulfate (R^2 = 0.999), where it was possible to quantify the concentration of Fe^{2+} present in solution, being 5.38±0.63µM ferrous sulfate mg^{-1} of essential oil, a value only 0.6 times lower than the positive control (Trolox) 9.17µM ferrous sulfate mg^{-1}.

Table 1. Chemical composition and area (%) *Psidium rufum* essential oil

Peak	[A]Compound	[a]calculated RI	[b]Relative area %	Identification Methods
	Hydrocarbon monoterpenes			
1	Sabinene	967	t	a, b, c
2	β-pinene	980	0.61	a, b, c
3	β-felandrene	1008	1.29	a, b, c
	Oxygenated monoterpenes			
4	1,8-cineole	1018	19.36	a, b, c
5	Cis-sabinene hydrate	1029	0.71	a, b, c
6	Trans-sabinene hydrate	1044	0.92	a, b, c
7	Camphene hydrate	1050	0.34	a, b, c
8	α-terpineol	1086	1.91	a, b, c
9	Fragranol	1146	t	a, b, c
10	Trans-geraniol	1160	0.96	a, b, c
11	Geraniol	1176	3.90	a, b, c
	Hydrocarbon sesquiterpenes			
12	γ-elemene	1247	0.35	a, b, c
13	α-cubebene	1436	1.78	a, b, c
14	α-longipinene	1500	19.00	a, b, c
15	α-ylangene	1505	t	a, b, c
16	α-copaene	1515	t	a, b, c
17	Isoledene	1531	1.41	a, b, c
18	β-patchouli	1539	0.32	a, b, c
19	β-elemene	1557	2.57	a, b, c
20	Longifolene	1575	4.23	a, b, c
21	Trans-caryophyllene	1579	3.35	a, b, c
22	α-gurjunene	1583	0.57	a, b, c
23	β-cedrene	1589	t	a, b, c
24	α-guaiene	1592	0.52	a, b, c
25	Aromadendrene	1597	0.35	a, b, c
26	α-humulene	1664	1.50	a, b, c
27	α-patchouli	1710	2.54	a, b, c
28	γ-curcumene	1713	3.78	a, b, c
29	β-selinene	1717	0.47	a, b, c
30	α-amorfene	1725	3.45	a, b, c
31	γ-selinene	1730	t	a, b, c
32	Zingiberene	1743	0.33	a, b, c
33	Valencene	1743	1.45	a, b, c
34	α-selinene	1773	5.09	a, b, c
35	Bicyclogermacrene	1783	t	a, b, c
36	α-muurolene	1787	t	a, b, c
37	Epizonarene	1791	t	a, b, c
38	β-bisabolene	1800	0.60	a, b, c
39	Trans-α-bisaboleno	1885	0.76	a, b, c
40	γ-cadinene	1903	1.70	a, b, c
41	δ-cadinene	1908	0.88	a, b, c
42	Cis-calamenene	1915	1.07	a, b, c
43	Cis-α-bisaboleno	1921	2.01	a, b, c
44	α-cadinene	1926	2.67	a, b, c
45	Germacrene B	1928	2.60	a, b, c
46	Longipinene	1931	1.60	a, b, c
47	Allo-aromadendrene	1938	0.78	a, b, c
48	Calarene	1944	0.54	a, b, c
49	Eremofilene	1944	0.47	a, b, c
50	Aristolene	1947	1.31	a, b, c
	Total identified		99.99	
	Hydrocarbon monoterpenes		1.90	
	Oxygenated monoterpenes		28.08	
	Hydrocarbon sesquiterpenes		70.01	

[a]Compounds listed in order of elution by HP-5MS column (5% phenylmethylsiloxane). [b]**RI=** Retention index calculated using C_7 – C_{30} *n*-alkanes in HP-5MS UI column. [c]**MS=** identification based on the comparison of mass spectra found in NIST 11.0 libraries (Adams, 2017). Relative area (%): percentage of the area occupied by the compounds in the chromatogram. **t=**trace.

Table 2. Antioxidant activity (%) and IC_{50} (mg mL^{-1}) by the Radical 2,2 diphenyl-1-picrylhydrazyl (DPPH) method, from purple Araçá EO

Samples	AA (%) (Quercetin 60 µM)				IC_{50} ($mg.mL^{-1}$)
	1.00	0.75	0.50	0.25	
Purple araçá EO	$17.73^{a} \pm 2.11$	$13.10^{b} \pm 0.97$	$9.04^{c} \pm 0.16$	$7.61^{c} \pm 0.81$	$4.74^{B} \pm 1.82$
Quercetin	-	-	-	-	$0.01^{A} \pm 0.01$

Values are the mean ± standard deviation of the experiment performed in triplicate. The statistical analysis used was analysis of variance (ANOVA), and the differences between the means determined by Duncan's test ($p \leq 0.05$) by the SPSS Statistics 22 program. For antioxidant activity (%), different letters (lowercase) in the same row indicate that there was a significant difference between the results ($p \leq 0.05$). For the IC50, different letters (uppercase) in the same column indicate that there was a significant difference between the values ($p \leq 0.05$); AA: Antioxidant activity; IC_{50}: Concentration of EO purple araçá that inhibited 50% of the DPPH radical; Positive controls for DPPH: Quercetin (0.010 mg. mL^{-1}).

Table 3. Antioxidant activity by the Iron Reducing Power (FRAP) of the purple araçá EO

Sample	µM ferrous sulphate mg of sample^{-1}
Purple araçá EO	$5.38^{b} \pm 0.63$
Trolox	$9.17^{a} \pm 0.01$

Values are the mean ± standard deviation of the experiment performed in triplicate. The statistical analysis used was analysis of variance (ANOVA), and the differences between the means determined by Duncan's test ($p \leq 0.05$) by the SPSS Statistics 22 program. Different letters in the same column indicate that there was a significant difference between the results ($p \leq 0.05$).

The most expressive result in the antioxidant activities was obtained by the method of oxidation inhibition by the *β*-carotene/linoleic acid co-oxidation system. In Figure 1, the lack of antioxidant potential can be accompanied by a reduction in the absorbance of the negative control (without antioxidant) which went from 0.633 to 0.110 after 120 minutes. On the other hand, the EO at a concentration of 0.25mg.mL^{-1}, showed a final absorbance 1.35 times lower than the EO at a concentration of 1.00 mg.mL^{-1} (Figure 1). The different *P. rufum* EO dilutions were higher than the positive control (Trolox), which reduced the absorbance to 0.504 at the end of the test.

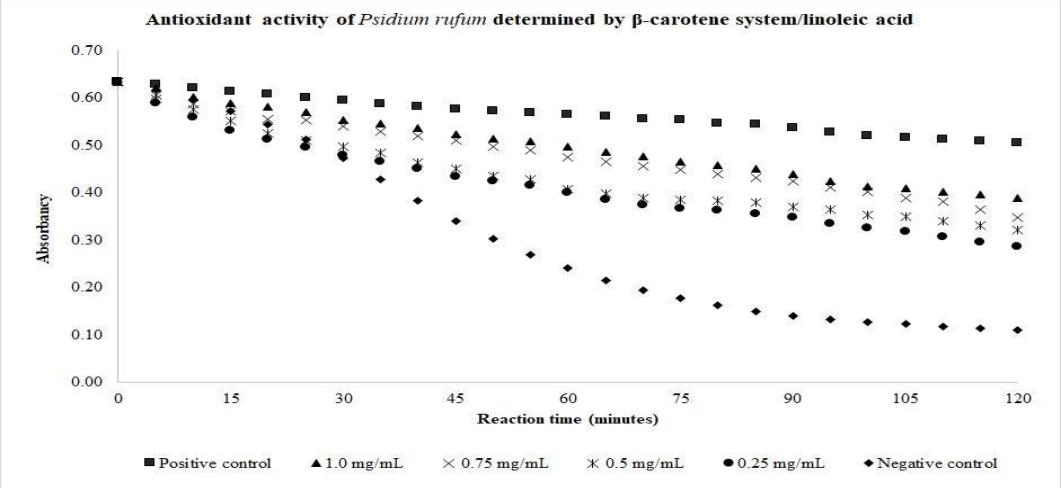

Figure 1. Antioxidant activity of essential oil from fresh leaves of *Psidium rufum* by the method of inhibition of oxidation (%) by the co-oxidation system β-carotene/linoleic acid.

By measuring the percentage of oxidation inhibition by the β-carotene/linoleic acid co-oxidation system (Table 4 and Fig. 1), it is verified that the EO of *P. rufum* leaves was effective at all concentrations, maintaining its antioxidant potential throughout the experiment 76.63% (1mg.mL^{-1}) and 57.02% (0.25mg. mL^{-1}).

Table 4. Antioxidant activity of essential oil from fresh leaves of *Psidium rufum* by the method of inhibition of oxidation (%) by the co-oxidation system β-carotene/linoleic acid (BCLA)

	Concentrations (mg.mL-1)			
Sample	1.00	0.75	0.50	0.25
Purple araçá EO	76.63a ± 1.27	69.10b ± 0.63	62.77c ± 1.18	57.02d ± 1.21

Values are the mean ± standard deviation of the experiment performed in triplicate. The statistical analysis used was analysis of variance (ANOVA), and the differences between the means determined by Duncan's test ($p \leq 0.05$) by the SPSS Statistics 22 program. Different letters on the same line indicate that there was a significant difference between the results ($p \leq 0.05$); Positive control : Trolox (0.2mg.mL-1).

DISCUSSION

The characteristics of the essential oil of *P. rufum* obtained in this study were compared with the obtained in the literature (Table 5). The production and characteristics of essential oil can vary depending on the genetic, regional, and climatic characteristics of the plant (Baser and Buchbauer, 2010; Jeribi *et al*., 2014).

Table 5. Characteristics of the EO of *P. rufum* obtained and its biological potential

Compound	Area (%)	Biological potential	Reference
1,8-cineol	19.36	Antiedematogenic, anti-inflammatory, gastroprotective, antimicrobial against S. aureus, E. coli and S. typhimurium and repellent against Sitophilus granarius and Sitophilus zeamai. They exhibit strong growth-inhibiting effect on plants and are involved in competition for plants.	Hammer *et al*., 1999; Obeng-Ofori *et al*., 1997; Yoneyama and Natsume, 2010 and Martins *et al*., 2017; Vidal *et al*., 2017
Geraniol	3.90	Antioxidant, antimicrobial activity (Candida and Staphylococcus sp)	Farhath *et al*., 2013; Prasad and Muralidhara, 2017; Lira *et al*., 2020
α-longipinene	19.00	Antifungal (C. albicans, in Caenorhabditis elegans and Aspergillus niger)	Sakata and Miyazawa, 2010; Manoharan *et al*., 2017
Longifolene	4.23	Anti-inflammatory, antimicrobial (Staphylococcus aureus and Escherichia coli), antitumoral tumor cell lines A-549	Bourgou *et al*., 2010
Trans-caryophyllene	3.35	Anti-inflammatory, antispasmodic	Fernandes *et al*., 2007; Silva *et al*., 2012

In recent years, there has been an increase in bacterial species with a high degree of resistance to conventional therapy (Daikos *et al*., 2021; Qin *et al*., 2022). Therefore, there is an interest in increasing the knowledge about the inhibitory concentrations of essential oils, in search of a balance between the acceptability and the effectiveness of the antimicrobial action. It is estimated that 20 to 50% of antimicrobial use in humans and 40 to 80% in animals is unnecessary or highly questionable, which leads to a high rate of resistance by bacteria (Beovic, 2006).

Plant essential oils have been shown to be effective in controlling the growth of a wide variety of microorganisms, including filamentous fungi, yeasts, and bacteria. Practical uses of these activities are suggested in humans and animals, as well as in the food industry, since medicinal plants produce a variety of substances with antimicrobial properties (Alvarenga, *et al*., 2007).

According to the MIC results, it was observed that *E. coli* was not inhibited up to the highest concentration tested, while *S. aureus, E. faecalis* and *P. aeruginosa* showed inhibitory activity at 2,500µg.mL^{-1}. Regarding the bactericidal concentration, for *E. faecalis* and *E. coli* it was not possible to determine the MBC up to the maximum concentration tested. *S. aureus* and *P. aeruginosa* did not show bacterial growth in their cultures, being defined as MBC = 20,000µg.mL^{-1}.

Studies have shown how *E. coli* is increasingly resistant to antibiotics, the pathogenicity of *E. coli* strains is related to the expression of virulence factors found in genetic elements called plasmids, which may explain the fact that this microorganism has been the only one with MIC above 20,000µg.mL^{-1}. In Canada, in a study with 2,483 cattle, it was found that 2.1% transmitted *E. coli* resistant to cefoxitin (Mulvey *et al*., 2009) and in the United States, a change in the resistance pattern of these microorganisms isolated from mastitis was observed of cows, with resistance to two or more antimicrobials in different combinations (Srinivisan *et al*., 2007). There are numerous examples of the increase in antimicrobial resistance in veterinary medicine, in several animal species, and many of the microorganisms are resistant to antimicrobials for human use, which is worrying, since isolated bacteria can be a reservoir of resistant genes, with a role in dissemination of this resistance to pathogenic and commensal bacteria (Srinivisan *et al*., 2007).

According to Sartonatto *et al*. (2004), MICs between 50-500µg.mL^{-1} are considered to have high activity; moderate activity for MICs between 600-1500µg.mL^{-1} and weak activity for MICs above 1500µg.mL^{-1}. According to the results obtained by the MIC, it was possible to verify that the EO of *P. rufum* showed high antibacterial activity against the Gram-positive bacteria *S. aureus* (ATCC 29213), *E. faecalis* (ATCC 29212) and *P. aeruginosa* (ATCC 27853) (Gram-negative).

The antioxidant efficiency of an essential oil is mainly attributed to its major components, although it can also be caused by the synergistic effect of the minor components, as well as the possible interaction between the compounds (Tian *et al*., 2014).

P. rufum EO showed greater antioxidant activity by the FRAP method (5.38 µM ferrous sulphate mg of sample^{-1}) (Table 3). This method evaluates the ferric ion reduction capacity of a given sample (Rufino *et al*., 2006a), being used to measure the antioxidant capacity of fruits (Sucupira *et al*., 2012).

Rufino *et al*. (2010), using the method of oxidation inhibition (%) by the β-carotene/linoleic acid co-oxidation system, classified the antioxidant activity as being high when the percentage of oxidation inhibition is greater than 70%, intermediate when is between 40 and 70% and low when the oxidation inhibition percentage is less than 40%. Based on this classification, this study found a high antioxidant capacity for EO of purple araçá at a concentration of 1 mg/mL (76.63%) (Table 4). According to Sucupira *et al*. (2012), this method has been used to analyze various food matrices, mainly fruits and seeds rich in lipids.

The purple araça EO showed low antioxidant potential by the DPPH method with (IC_{50} = 4.74 mg mL^{-1}) when compared to the positive control quercetin (IC_{50} = 0.001mg mL^{-1}) (Table 2). EO are generally poorly soluble in aqueous and methanolic solutions such as those used in DPPH method. Thus, the lower molecular movement of

these solvents probably reduces the reactive antioxidant capacity of EO when compared to the antioxidant activity in non-polar solvents, such as those used by the BCLA method. Thus, chemical interactions between essential oil and solvents may explain the lower antioxidant activity of *P. rufum* EO by DPPH methods and the higher activity by BCLA. The results found in this study suggest the use of EO in the food industry.

CONCLUSION

The results of this research showed the potential promising of essential oils for the development of new antimicrobials, considering the resistance to residues left by antibiotics. The essential oil of purple araçá exerted antimicrobial action on the microorganisms tested. In addition, EO of purple araçá showed antioxidant activity when using the antioxidant method by the β-carotene/linoleic acid co-oxidation system.

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