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Biological Evaluation of Essential Oil from Green Fruits of *Psidium striatulum* of the Roraima State, Brazil

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Psidium striatulum is a fruit popularly known as *araçá* or *araçari* in region from Brazilian Amazon. These fruits are widely used in folk medicine for the treatment of diarrhea and infections caused by microorganisms that cause harm to human health. Thus, this work aims to characterize the chemical profile and biological evaluation of the essential oil of the green fruits of *Psidium striatulum* DC. The plant material was collected in the municipality of Boa Vista-RR, on the margin of Rio Branco and submitted to hydrodestillation, in a Clevenger, Spell brand double - condenser type device for 2 hour interrupt period. Identification and quantification of twenty volatile constituents corresponding to 91.9% of its composition was provided through GC-MS and GC-FID, with 8.1% remaining unidentified, with the majority of α-pinene (11.9%), humulene (10.4%), α-copaene (7.1%), globulol (5.7%), δ-cadinene (4.2%) and β-pinene (4.2%). Microbiological tests of essential oil at 250 μg/mL were performed against *Staphylococcus aureus*, ATCC 29212 (67.03±4.06%); *Bacillus cereus*, ATCC 11778 (73.14±2.70%); *Escherichia coli*, ATCC 25922 (11.47±0.28%); *Salmonella typhimurium*, ATCC 14028 (78.43±2.12%) and *C. albicans* (13.18±1.69%) and showed moderate inhibitory activity for the AChE enzyme (44.42%). The results showed that the essential oil had an activity greater than 65% against *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium*, as well as having a moderate inhibitory effect on the enzyme acetylcholinesterase (AChE) in the conduction conditions of the bioassays of the present study.

1. Introduction

Psidium striatulum (Myrtaceae) is a botanical species where its fruit is popularly known as *araçá-mirim* or *araçari*, in the state of Roraima, Brazil. The *Psidium* genus comprises species of nutraceutical importance (Bailão et al., 2015), functional (Manikandan et al., 2013), ecological, socioeconomic, therapeutic (Henriques et al., 2013), and above all for presenting bioactive properties explored in several lines of research (Ponzilacqua et al., 2019). In fact, species of this genus are extensively studied in several areas of knowledge, mainly from the bioprospection of extracts and vegetable oils from their different plant structures. The biological effects of inhibition and suppression against pathogenic microorganisms and activity on metabolic, behavioral and neurochemical parameters, such as action on the enzyme acetylcholinesterase (Li et al., 2017), are also widely reported.

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However, the microorganisms showed resistance to some antimicrobial agents (Faghihi et al., 2019), which encourages the search for natural secondary compounds with antibiotic and antifungal potential, among others. It is also reported that chemical composition investigations show that the leaves (Chalannavar et al. 2013) and the fruits (Oliveira et al., 2018) of the *Psidium* species contain considerable amounts of volatile substances, which make them particularly rich in essential oils with bioactive potential (Silveira et al., 2019).
Studies of phytochemical composition as well as biological activity of the essential oil of the fruits of *Psidium striatulum* are scarce in this line of research. In this sense, to know the properties and characteristics of the chemical races of Psidium Striatulum submitted to the diverse edafoclimatic and ecophysiological conditions, highlighting the quimiotypes present and distributed in the Brazilian Amazonian Savannah, in the state of Roraima, is a core premise for a careful recommendation of the its biotechnological exploitation for different uses, applications and surveys. The objective of this work was to determine the phytochemical composition of immature fruits of *Psidium striatulum* (EO-FP), collected during the summer, using Gas Chromatography of Coupled Mass Spectrometry (GC-MS) and Gas Chromatography (GC-FID), as well as to verify its possible antimicrobial and inhibitory activity on the enzyme Acetylcholinesterase (AChE).

2. Materials and methods

2.1 Collection and records of the genetic material accessed

The fruits of *Psidium striatulum*, in the immature phenological stage, were collected on the banks of Rio Branco - Praia Grande (2°49'48"N, 60°43'13"W), located in the municipality of Boa Vista, state of Roraima, Brazilian Amazonia, during the summer. The climate of this region is characterized as rainy tropical (Aw), according to the types of climatic classification of Köppen. An exsicata was deposited in an indexed herbarium linked to the Federal University of Roraima - UFRR, under number 13619. This access was duly registered in the National Genetic Heritage and Associated Traditional Knowledge Management System-SisGen, according to specific Brazilian legislation, under number A00AA69.

2.2 Processing of plant material and obtaining essential oil

There were collected 5,250 g and later destined for the Laboratory of Environmental Chemistry of the UFRR, where they were selected, washed in distilled water and duly weighed. Subsequently, still fresh, the fruits were separated in triplicate - obtaining 1,750 g in each sample. In order to obtain the oil and determine the yield, the three samples were submitted to a hydrodistillation process using Clevenger with a double spell condenser for an uninterrupted period of three hours of distillation. The obtained essential oil was dried over anhydrous sodium sulfate and stored at -20 °C, and then each individual sample was packed in amber glass containers, previously sterilized and identified, coated with aluminum foil and kept on refrigeration until the opportunity of the bioassays.

2.3 Phytochemical screening by GC-MS and GC-FID

The identification and quantification of the volatile constituents present in the essential oil of the *Psidium striatulum* (EO-FP) immature fruits were provided by Gas Chromatography of Coupled Mass Spectrometry (GC-MS) and High Resolution Gas Chromatography (GC-FID), respectively, both of which were performed in the Chromatography Laboratory linked to the Chemistry Department of the Federal University of Minas Gerais.

2.4 Antimicrobial Activity Bioassays

After preparation of the working solution and solubilization in dimethylsulfoxide (DMSO) at a concentration of 12.5 mg / mL, the bioassays of antimicrobial activity were carried out for both pathogenic bacteria and yeast by means of the Elisa spectrophotometer with a reading capacity of up to 96 wells, in triplicate, as described by Dos Santos et al., 2015. The microorganisms used in the activity bioassays were: i) *Staphylococcus aureus*: ATCC 29212 (Gram-positive bacteria); ii) *Bacillus cereus*: ATCC 11778 (Gram-positive bacterium); iii) *Escherichia coli*: ATCC 25922 (Gram negative bacterium); iv) *Salmonella typhimurium*: ATCC 14028 (Gram-negative bacteria); and, v) *Candida albicans*: ATCC 18804 (yeast). Ampicillin for bacteria and nystatin for yeast were used as a positive control. The parameters evaluated in relation to the pathogenic microorganisms were: a) Percentage (%) of inhibition at 250 μg mL^{-1}; and b) IC_{50} values (Concentration of the sample which inhibits 50% of the microorganisms present) in μg mL^{-1}.

2.5 Acetilcolinesterase (AChE) inhibition assay

Quantitative evaluation of acetylcholinesterase (AChE) inhibition activity was performed according to the methodology of Ellman (1961), modified by Rhee et al. (2001). This bioassay was performed on microplates of

96 wells. Eserine and galantamine (10 mg mL^{-1}) were used as positive controls while the negative control was performed without inhibitor. The enzyme inhibition percentage was calculated as described by Almeida et al. (2018). The data obtained were treated using Microsoft Origin 6.1 software.

3. Results and discussion

3.1 Phytochemical screening by GC-MS and GC-FID

It was verified by GC-FID and GC-MS that the essential oil of *P. striatulum* (EO-FP) presents a chemical profile having as main constituents of monoterpene and sesquiterpene hydrocarbons, sesquiterperones and monoterpenes and oxygenated alkaloids (Table 1). Among the main compounds identified and quantified that had concentrations above 5% in the essential oil of araçari fruits were α-pinene (12%), humulene (10.4%), α-copaene (7.1%), globulol (5.7%), aromadene (5.1%).

Table 1: Chemical composition (%) of immature fruits of Psidium striatulum (EO-FP), quimiotype collected during the summer in the municipality of Boa Vista, Roraima state, Brazilian Amazon.

Probable substance	RI*	Concentration (%)
Monoterpene hydrocarbons		
α-pinene	980	12
β-pinene	1002	4.2
Mircene	1012	0.4
p-cymene	1033	1.2
Limonene	1036	1.6
Z-β-memmene	1045	3.9
E- β -ocimene	1053	1.1
g-terpinene	1059	0.5
Oxygenated monotherpenes		
1.8-cineol	1037	3.9
terpinen-4-ol	1165	0.4
a-terpinenol	1182	0.8
Sesquiterpene hydrocarbons		
a-copaene	1366	7.1
b-caryophyllene	1408	3.3
Aromadendrene	1428	5.1
Humulene	1443	10.4
Seychellois	1450	2.5
δ-cadinene	1519	4.2
Oxygenated sesquiterpenes		
Globulol	1578	5.7
Viridiflorol	1597	3.2
Alkaloids		
Vinbarbital	1745	1.8
Others		8.1
Total		100

* Retention index

Studies on the yield as well as the chemical composition with oil essences of the fruits of *P. striatulum* are scarce, however the genus *Psidium* is extensively investigated, mainly, the essential oil of the leaves of different species (Chalannavar et al., 2013). When investigating the essential oil of the leaves of *Psidium striatulum* and other species of this genus, Da Silva (2003) observed that Araçá has mainly sesqui and diterpene compounds, composing 7.4% of the samples evaluated in their studies. Another finding was that the main volatile compounds identified in the oils of *P. acutangulum* and *P. striatulum* leaves were similar to those of leaves of *P. guajava*: α-pinene and 1.8-cineole, compounds, also identified and quantified in the oil of the immature fruits of *P. striatulum* of the present research. When investigating the essential oil of leaves of *Psidium laruotteanum*, Medeiros et al. (2018) found that it is rich in monoterpenes and the main compounds identified were p-cymene (19.4-34.8%), 1.8-cineole (6.9-19.2%) and α-pinene (9.2-11.4%), compounds also verified in the immature fruits of Araçá and described in this research. The authors further note that although collected in different locations and years, the composition of the essential oils was very similar to the results of other research, both qualitative and quantitative. This suggests that despite the differences in composition and concentration between the species of *Psidium* and their respective botanical structures, in general, the quimiotypes of this genus reveal qualitative phytochemical profiles that preserve chemical similarities in their constitution. When investigating Mirtaceae, De Cerqueira et al. 2009 observed that α-humulin has a concentration of 9% to 26%, depending on the time of year and plant flora. This secondary metabolite exhibits anti-inflammatory activity and presents the aroma as a striking feature. 1,8-cineol is used in the pharmaceutical industry and has antimicrobial and antioxidant properties (Lee et al., 2005). The most important oil-copaene compound of Copaiba oil (*Copaifera langsdorffii*) and identified in EO-FP, has been widely used in therapeutical processes due to its antimicrobial, anti-inflammatory and cicatrizant action. Globulol is known for its fungitoxic activity and Viridiflorol is reported for its bioactive, anti-inflammatory and anti-oxidant antimycobacterial (Trevizan, et al., 2016).

3.2 Antimicrobial Activity Bioassays

EO-FP showed, Table 2, inhibition greater than 65% against *Staphylococcus aureus, Bacillus cereus* and *Salmonella typhimurium*. An inhibition of less than 15% was also found for *Candida albicans* and *Escherichia coli.* It was observed that none of the treatments presented inhibition superior to the positive controls for the pathogenic microorganisms evaluated, in the conduction conditions of this study.

Table 2: Percentage (%) inhibition of immature fruits of Psidium striatulum (EO-FP), quimiotype collected during the summer in the municipality of Boa Vista, State of Roraima, Brazil, against pathogenic microorganisms.

Sample	*S. aureus*	*B. cereus*	*S. typhimurium*	*E. coli*	*C. albicans*
EO-FP (%)	67.03 ± 4.06	73.14 ± 2.70	78.43 ± 2.12	11.47 ± 0.28	13.18 ± 1.69
Ampicillin (%)	95.76 ± 0.26	92.48 ± 0.52	90.39 ± 0.30	96.64 ± 2.52	-
Nystatin (%)	-	-	-	-	90.18 ± 1.15

Table 3: IC_{50} values of the essential oil of immature fruits of Psidium striatulum (EO-FP), quimiotype collected during the summer in the municipality of Boa Vista, state of Roraima, Brazilian Amazon, and the respective controls positive effects on pathogenic microorganisms.

Sample (µg/mL)	*S. aureus*	*S. typhimurium*	*C. albicans*	*B. cereus*
EO-FP (%)	28.62	18.69	-	24.74
Ampicillin (%)	1.66	4.20	-	1.90
Nystatin (%)	-	-	7.53	-

In the literature, investigations with bioactivity of the essential oil of P. striatum fruits were not found, aiming at the pathogenic microorganisms studied in this research. However, studies with different species and botanical structures through different methods of extracting the secondary metabolites of these Myrtaceae are extensively investigated. Fernandes et al. (2012) to evaluate the antimicrobial activity of the aqueous extract of *Psidium guinean*, araçá field, combined with ampicillin, amoxicillin, cefoxitin, ciprofloxacin and meropenem against twelve strains of *Staphylococcus aureus*, demonstrated that this extract is associated with beta-lactam antimicrobials, fluoroquinolones and carbapenems act to synergistically inhibit this pathogenic bacteria, varying between 50 and 87.5% inhibition among the evaluated treatments. When investigating the bioactivity of the essential oil of leaves of *Psidium guajava*, Souza Prestes et al. (2011) found no effect on *Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*. This indicates bioactive potential of the EO-FP quimiotype and the expansion of systematic studies of biological activity, isolated and/or combined with antimicrobials, from this natural product with samples that can be obtained at different times of the year or elsewhere of the state of Roraima to verify if these factors influence the activity of the secondary compounds. Other studies have also shown that *Pisidium saline* oils, obtained at 3 times of the year, have significant antifungal activity against three species of *Candida albicans* and may act in synergism with fluconazole (De

Macêdo et al., 2018). The concentration of the 50% inhibitory sample (IC_{50}) of the microorganisms present at the 250 µg/mL dose was also found in Table 3.

3.3 Acetilcolinesterase (AChE) inhibition assay

The percentage of inhibition of (AChE) obtained in this study was greater than 40% in the immature fruits of *P. striatulum*, Table 4, and had a positive control of galantamine (91.71%) as a reference for treatment of Alzheimer's disease. In this context, Vinutha et al. (2007) proposes the inhibitory potential on the activity of the enzyme acetylcholinesterase (AChE), such as: i) potent inhibitors - above 50%; ii) moderate - between 30% and 50%; and, iii) weak - below 30%. Therefore, the essential oil studied in this work has a moderate inhibitory potential against AChE.

Table 4: Percentage (%) inhibition of the essential oil of immature fruits of Psidium striatulum (EO-FP), quimiotype collected during the summer in the municipality of Boa Vista, Roraima State, Brazilian Amazon, and the respective positive controls on the activity of the enzyme acetylcholinesterase (AChE).

Sample	Inhibition (%)	Coefficient of variation
EO-FP	44.417 ± 0.358	0.008
Galantamina	91.710 ± 0.380	0.004

Bioactivity investigations of the essential oil of fruits of *P. striatulum* on the enzyme Acetylcholinesterase were also not identified. In this sense, Penido et al. (2017) evaluated the combination of the best inhibitory and antioxidant activities of acetylcholinesterase in six species distributed in the Northeast region of Brazil as the best potential sources of therapeutic agents against Alzheimer's disease, among them *Psidium guajava*. Oliveira et al. (2018), when analyzing the effects of *P. cattleianum* fruit extract on metabolic, behavioral and neurochemical parameters in rats submitted to different diets, found that the diet with red arachis prevented the increase of acetylcholinesterase activity in the prefrontal cortex. Finally, more robust investigations with EO-FP activity are recommended to confirm the moderate or superior effect of this natural product on AChE both in vitro and in vivo.

4. Conclusion

The effect of the main constituents of *Striatulum psidium* (EO-FP) essential oil: α-pinene (12%); humulene (10.4%); α-copaene (7.1%); globulol (5.7%); and aromadendene (5.1%), associated with the other secondary constituents identified, were responsible - under the conditions of the present study, for the activity superior to 65% against *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium*, as well as promoted a moderate effect (44.47%) on the enzyme acetylcholinesterase. However, further studies are needed to establish the therapeutic safety and efficacy of its secondary bioactive metabolites as a possible pharmaceutical agent. In view of this, systematic investigations related to the biological activity of EO-FP - isolated and / or combined with antimicrobials and other chemotypes are suggested, especially to evaluate the possible synergistic effect of these compounds.

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