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Chemical Composition and Biological Evaluation of the Essential Oil of the leaves of *Psidium Striatulum* in the Amazon Region

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Myrtaceae family is one of the largest and most important of the Brazilian flora. The leaves of most species of this family contain considerable amounts of volatile substances, which make them particularly rich in essential oils. In folk medicine the leaves of many species of this family are used in the most different diseases. *Psidium striatulum* DC species is a variety of this family, popularly known as araçá or araçari in the Amazon region and the best known are guava, guava bush and araçá mirim. In folk medicine they are used in the treatment of diarrhea and infections that cause harm to human health. Thus, this work aims to characterize the chemical profile and biological evaluation of essential oil of the fresh leaves of *Psidium striatulum* DC from Roraima, Brazil. The leaves were collected in the municipality of Boa Vista-RR, on the margin of Rio Branco and submitted to a hydrodistillation process, in a Clevenger, Spell brand double condenser type device for a 2 hour interrupt period. Identification of constituents of the essential oil was performed by comparing the mass spectra obtained by the GC-MS spectra with the NIST11 library and also by comparing the Kovats indexes calculated by GC-FID and literature data. The essential oil chromatogram of *P. striatulum* fresh leaves had 20 peaks. Among the major compounds identified are humulene (13%), α-copaene (8.4%), 1.8-cineole (8.1%), aromadendrene (6.3%) and α-terpinenol (2.1%), globulol (5.5%), β-caryophyllene (5.2%), β-cadinene (4.1%). Microbiological tests of the essential oil at 250 μg mL^{-1} were performed against *Staphylococcus aureus*, ATCC 29212 (47.63 ± 1.58%); *Bacillus cereus*, ATCC 11778 (62.15 ± 0.10%); *Escherichia coli*, ATCC 25922 (0.24 ± 0.05%); *Salmonella typhimurium*, ATCC 14028 (64.03 ± 0.83%) and *C. albicans* (7.33 ± 2.50%) and showed moderate inhibitory activity for the enzyme AChE (53.12 ± 0.53%). The results *in vitro* of the essential oil indicate that they are important in the inhibition of microorganisms, Alzheimer's and can be used *in nature* form, in the form of tea infusion or as aromatic condiment.
Keywords: *Psidium*, Antibacterial, Antifungal, Alzheimer's disease.

1. Introduction

The family Myrtaceae is one of the most significant of the Brazilian flora (Nascimento, 2014.), estimated 140 genera (Senna et al., 2011; Morais, 2014) and approximately 5,760 species (Govaerts al., 2015). In Brazil, there are 23 genera and about 1,000 species of Myrtaceae (Sobral et al, 2015), of these edible species such

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as *araçá*, *goiaba-do-mato*, *araçá-mirim*, in the Brazilian Amazon region is known as *araçari*, but its distribution occurs in other tropical regions of Central and South America (Da Silva et al., 2003). In addition to the dietary use of the fruits, the leaves of this species may contain essential oils (Cerqueira, 2009; Tolkai, 2012), which are generally bioactive.

There are reports of the use in folk medicine of leaves of many species of this family, for example the use of the leaves of *Eugenia brasiliensis* as antirheumatic, use of the seeds and leaves of *E. jambolana* as antidiabetic (Corrêa, 1984), use of the leaves of *Psidium guajava* as antidiarrheals, stimulants, anti-inflammatory and antibacterial, anti-hemorrhagic, treatment on diabetes and intestinal worms (Ramos et al., 2006).

Therefore, the main objective of this work is to verify the chemical composition of the essential oil of the fresh leaves of *Psidium striatulum* collected in the city of Boa Vista, Roraima, Brazil, as well as to verify the antimicrobial potential against fungi and bacteria, as well as inhibitory concentration of the essential oil of the aforementioned species.

2. Material And Methods

1.1 Plant material and essential oil extraction

The fresh leaves of *P. striatulum* were collected on the banks of the Branco river in Boa Vista city, Roraima state, Brazil. The plant material was identified by Marcos Sobral (Herbarium of Universidade Federal de Roraima, UFRR), and a voucher specimen (13619) was deposited in the above mentioned Herbarium. A total amount of 4,710 g of fresh leaves of *P. striatulum* was collected and taken to the Laboratory of Environmental Chemistry of the Nucleus of Research and Post Graduation in Science and Technology (NPPGCT-UFRR), these were properly cleaned with water, followed by distilled water. The samples were separated in triplicate (1,570 g each) to extract the essential oil by hydrodistillation in Clevenger type equipment. The essential oil was dried over anhydrous sodium sulphate and stored at -20 °C before analysis.

1.2 GC-FID and GC-MS analysis

The essential oil of leaves fresh *P. striatulum* was analyzed on a HP 7820A Gas Chromatograph equipped with a flame ionization detector (GC-FID) using a capillary column (HP5 30 m × 0.32 mm × 0.25 microns, Agilent). Column temperature: 70 °C (0 min) at 3 °C min^{-1} up to 240 °C. Gun: 250 °C Split (1:30). FID Detector: 260 °C. Carrier gas: hydrogen at 3 mL min^{-1}. Vol injection: 1 µL. Essential oil was diluted at 1% in chloroform. Data acquisition software used was Compact EZChrom Elite (Agilent). The quantitative analysis was accomplished using standard areas from the chromatograms obtained by GC-FID.

A gas chromatography coupled to mass spectrometry (GCMS) QP2010 ULTRA Shimadzu was used. Column: Rxi-1MS 30 m × 0.25 mm × 0.25 microns (Restek). Column Temp: 70 °C (2 min), 5 °C min^{-1} to 250 °C. Injector: 250 °C Split (1:20), GC-MS interface at 250 °C. MS detector (electron impact at 70 eV) temperature was 250 °C. Carrier gas: helium at 1.5 mL min^{-1}. Vol injection: 1 µL. Essential oil was diluted at 0.1% in chloroform. Data acquisition software used was GC-MS Solution (Shimadzu) together with NIST11 library. Identification of peaks was made by comparison of the mass spectra obtained by GC-MS spectra with the NIST11 library and also by comparing the Kovats indices calculated by GC-FID and literature data.

1.3 Bioactivity assay of essential oil on microorganisms

A pre-inoculum was prepared in which the microorganisms were transferred from the culture medium where they were stored into test tubes containing 3 mL of culture medium (BHI for bacteria and Sabouraud for yeast). The tubes were then incubated in an oven at 37 °C for 36 h. With a micropipette, 500 µL of the pre-inoculum was transferred to test tubes containing sterile distilled water. The tubes were homogenized and the concentration adjusted to 600 nm (bacteria) and 530 nm (yeast), until a transmittance between 74-75% (bacteria) and 75-76% (yeast), corresponding to the 0.5 McFarland standard turbidity, i.e., 10^5 CFU mL^{-1}, thereby obtaining the suspensions of the inoculums used in the bioassay. To prepare the working solution the samples were previously solubilized in dimethylsulfoxide (DMSO) at the concentration of 12.5 mg mL^{-1}. From this solution, an aliquot of 40 µL was removed, which was added to 960 µL of the culture medium used in the bioassay, obtaining the working solution in the concentration of 500 µg mL^{-1}. The bioassays were performed in 96-well plates in triplicate. In the first well 200 µL of the 500 µg mL^{-1} working solution was added. Aliquots of 100 µL were added to the next wells of culture medium per well, then the serial (1:1) microdilution of the sample solution was performed, in this way the concentrations ranged from 250 to 0.98 µg mL^{-1}. Then, 100 µL of standardized microorganism inoculum was added to each well.

Four controls were performed: control of microorganism growth (to verify cell viability); the blank, which consists of the sample solution at the same concentrations evaluated, replacing the inoculum with sterile

distilled water; positive control (the working solution is replaced by a commercial antibiotic) and the sterility control of the culture medium containing 100 µL of culture medium and 100 µL of sterile distilled water. The microplates were incubated in an oven at 37 °C and after 24 hours the plate reader was read at 490 nm.
The maximum DMSO concentration allowed in this assay was respected according to the literature (Zacchino, Gupta, 2007). Final DMSO concentration used in the assay was 2%. This concentration does not affect microbial growth. Besides, DMSO was added to the control. Therefore, any effect from DMSO was discounted from the final inhibition rates.
The antibiotics used for the quality control of the assays were: ampicillin, for bacteria and nystatin, for yeast, whose working solutions were prepared as previously described for the samples tested. The samples were tested on the following microorganisms: yeast type *Candida albicans* (ATCC18804), two Gram-positive bacteria *Staphylococcus aureus* (ATCC 29212) and *Bacillus cereus* (ATCC 11778) and two Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028).

3. Results and discussion

The chromatographic and spectrometric analyzes of the essential oil of fresh leaves of P. striatulum allowed the identification of 20 components, representing 90.7% of the total substances and 9.3% not identified (Table 1). Among the major compounds identified were α-humulene (13%), α-copaene (8.4%), 1,8-cineol (8.1%), aromadendrene (6.3%) and α-terpinenol (5.7%). According to Cerqueira (2009) and Stefanello (2010) the constituents identified (Table 1) are found in the different species of Myrtaceae, but in varied concentrations. This is due to several factors, such as climate, soil, water and seasons (Silva et al., 2003; Ostrosky et al., 2008; Oliveira et al., 2012, Eherlet et al., 2013).

Table 1: Identification and concentration of chemical components in essential oil of leaves of P. striatulum.

Probable substance	RI*	Concentration (%)
Monoterpene hydrocarbons		
α-pinene	980	2
β-pinene	1012	3
Mircene	1002	0.5
p-cymene	1033	1.1
Limonene	1059	2.6
Z-β-ocimene	1036	0.5
E- β-ocimene	1045	0.2
g-terpinene	1059	1.9
Oxygenated monotherpenes		
1.8-cineol	1037	8.1
terpinen-4-ol	1165	2.1
α-terpinenol	1182	5.7
Sesquiterpene hydrocarbons		
α-copaene	1366	8.4
β-caryophyllene	1408	5.2
Aromadendrene	1428	6.3
α-Humulene	1443	13.3
Seychelene	1450	3.6
δ-cadinene	1519	4.1
Oxygenated sesquiterpenes		
Globulol	1578	5.5
Viridiflorol	1597	2.7
Alkaloid		
Vinbarbital	1745	2.3
Others		9.3
Total		100

* Retention index

Some of the chemical components mentioned in the previous paragraph, such as α-humulene, for example, present a concentration of 9 to 26% (Cerqueira, 2009), this chemical component exhibits anti-inflammatory activity (Fernandes et al., 2007), in addition to its bioactivity has remarkable aroma used by the perfume industry (Affonso et al., 2012). Another chemical component of medicinal interest is 1.8-cineol, as it has

antimicrobial, antioxidant properties (Lee, 2005) and bioinsecticide (Sukontason et al., 2004). Another chemical component is α-copaene also for medicinal use, has antimicrobial, anti-inflammatory and cicatrizant activity (Brito et al., 2005).

According Silva (2003) the constituents identified in the oil of the leaves of *P. striatulum* show similarities with the oil of *P. guajava*, which are found in the Amazon and that the constituents of the Myrtaceae family, vary within the same species (Silva et al., 2003; Ostrosky et al., 2008; Oliveira et al., 2012). The majority of substances *P. striatulum* collected in Boa Vista, Roraima, Brazil, differ from studies by Silva et al. (2003), one of the differences is the composition and the variation of the concentration of the constituents, it was found the presence of the constuents seychelene and vinbarbital that was not verified in the literature for the genus *Psidium* and these may be probably a chemotype of the species under study.

There are five main components identified in this study according to chromatogram of the essential oil of *P. striatulum* leaves (Figure 1).

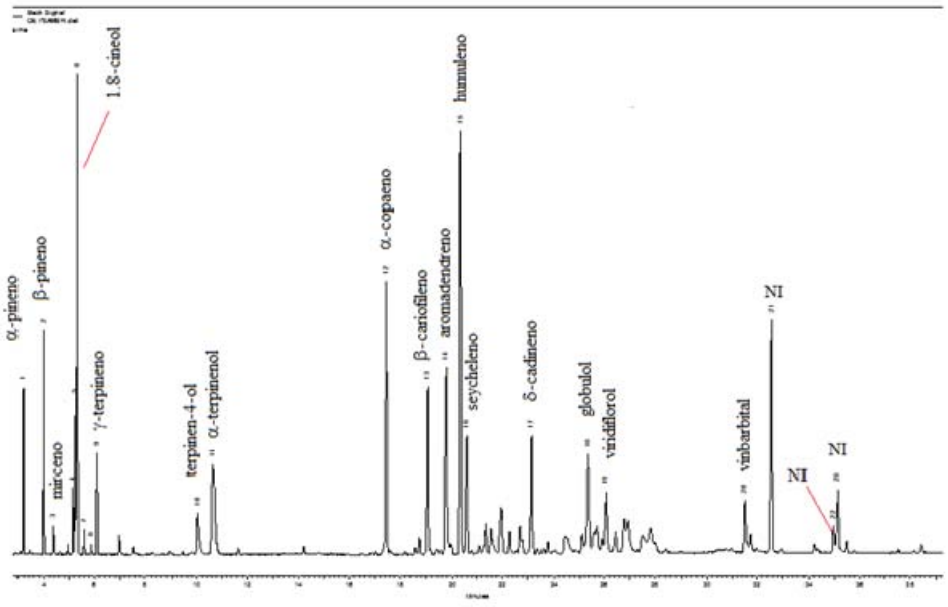

Figure 1: Chromatogram of the essential oil of fresh leaves of P. striatulum. NI = Not identified

3.1 Minimum Inhibitory Concentration (MIC)

Through the Minimal Inhibitory Concentration (MIC) it was possible to observe good results (Table 2) of the bioactivity of the essential oil of the fresh leaves of *P. striatulum* on *S. typhimurium* and *B. cereus*, and with determination of the IC_{50} it was possible to confirm the best concentration of inhibition at 250 μg mL^{-1}, but did not reach 50% inhibition (Table 3).

Table 2: Inhibitory evaluation of essential oil of P. striatulum fresh leaves on microorganisms at 250 μg mL^{-1}.

Sample	Inhibition on microorganisms (%)				
	S. aureus	***B. cereus***	***S. typhimurium***	***E. coli***	***C. albicans***
PSAMBH	47.63 ± 1.58	62.15 ± 0.10	64.03 ± 0.83	0.24 ± 0.05	7.33 ± 2.50
Ampicilin	95.76 ± 0.26	92.48 ± 0.52	90.39 ± 0.30	96.64 ± 2.52	-
Nystatin	-	-	-	-	90.18 ± 1.15

Table 3: Determination of IC_{50} for P. striatulum essential oil at 250μg mL^{-1}.

Sample	***S. aureus***	***S. typhimurium***	***C. albicans***	***B. cereus***
PSAMBH	-	29.78	-	38.08
Ampicilin	1.66	4.20	-	1.90
Nystatin	-	-	7.53	-

The essential oil of *P. striatulum* presented inhibition against the tested microorganisms. However, the inhibition was less than 50%, and a higher activity on *S. typhimurium* followed by *B. cereus* may not be an

exceptional result, essential oil is a raw material without isolated chemicals, which may possibly occur synergisms that favor the inhibition presented.

4. Conclusions

The chemical composition of the essential oil from fresh leaves of *Psidium striatulum* presents 1.8-cineol, α–humulene, α-copaene, globulol, aromadendrene and viridiflorol, which in the literature are natural phytochemical compounds that act as antioxidants, contribute to the reduction of blood pressure, levels of cholesterol and blood sugar. The *in vitro* test of the essential oil revealed inhibition of microorganisms *Salmonella typhimurium*, *Bacillus cereus* and against *Staphylococcus aureus*, besides being promoter of the potent effect on the acetylcholinesterase enzyme. 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 essential oil isolated and/or combined with antimicrobials and other chemotypes, news research are suggested for the production of functional foods.

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