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Original Article

Chemical profile and biological potential of essential oil of *Psidium bahianum* Landrum & Funch (Myrtaceae)

Perfil químico e potencial biológico do óleo essencial de *Psidium bahianum* Landrum & Funch (Myrtaceae)

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Abstract

The genus *Psidium,* belonging to the family Myrtaceae, encompasses several plant species rich in essential oils. Several studies involving different research areas have shown promising results for essential oils, which has attracted interest from diverse sectors, including pharmaceutical, food, and cosmetic industries. Thus, the objective of this study was to evaluate the biological activity of the essential oil extracted from *Psidium bahianum* leaves (EOPb) collected in an Atlantic Forest remnant in the northern coast of the state of Bahia, Brazil. EOPb was extracted by steam distillation and analyzed through proton nuclear magnetic resonance (^{1}H-NMR). The free radical scavenging activity was assessed using the DPPH methodology (analyzing the scavenging of the stable free radical 1,1-diphenyl-2-picrylhydrazyl). Antimicrobial disk diffusion assays were conducted; toxicity was determined through assays on sheep erythrocytes and *Artemia salina*. ^{1}H-NMR evaluation showed the presence mainly of monoterpenes and sesquisterpenes. The percentage of antioxidant activity was 18.03±2.53 53 for 125 125 µg mL^{-1}. Antimicrobial assays showed that the essential oil at a concentration of 10 mg mL^{-1} inhibited the growth of the microorganisms *Staphylococcus aureus, Bacillus subtilis*, *B. cereus*, *Micrococcus luteus*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans*, and *C. glabrata*. The toxicity results indicated that concentrations of EOPb at 10, 100, 250, and 500 µg mL^{-1} were not toxic to sheep erythrocytes. The toxicity assay on *A. salina* showed that the lethal concentration for 50% of individuals (LC_{50}) within 24 and 48 hours were 371.48 and 302 µg mL^{-1} of EOPb, respectively, which were considered moderately toxic, whereas the LC_{50} within 72 horas was 33.11 µg mL^{-1}, considered highly toxic. These findings are promising, as the essential oil from *P. bahianum* leaves showed antimicrobial activity against both Gram-positive and Gram-negative bacteria and fungi. The data obtained for the essential oil toxicity in *A. salina* could be tested on different tumor cell lines.

Keywords: antioxidant activity, antimicrobial activity, secondary metabolites, cytotoxicity.

Resumo

O gênero *Psidium,* pertencente à família Myrtaceae é rico em óleos essenciais que vêm sendo utilizados na indústria farmacêutica, alimentícia e na estética. Desse modo, o objetivo geral deste estudo foi avaliar a atividade biológica do óleo essencial de *Psidium bahianum* coletadas em remanescente de Mata Atlântica no Litoral Norte da Bahia, Brasil. O óleo essencial (OEPb) foi obtido das folhas do *P. bahianum* por arraste a vapor d'água. O OEPb foi analisado pela Ressonância magnética nuclear de H^1. A atividade sequestradora de radicais livres foi avaliada pela metodologia de DPPH (analisando a eliminação do radical livre estável 1,1-difenil-2-picrilhidrazil). Foram realizados testes antimicrobianos em difusão em disco, e para a determinação da atividade tóxica realizou-se através dos ensaios em hemácias de carneiro e *Artemia salina.* O estudo de ressonância demonstrou principalmente a presença de monoterpenos, dentre eles o linalol, e sequisterpenos. A porcentagem da atividade antioxidante foi de 18,03 ± 2,53 53 para 125 µg /mL. No ensaio antimicrobiano o óleo essencial inibiu o crescimento dos microrganismos *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Escherichia coli, Aspergillus niger, Candida albicans* e *Candida glabrata* testados na concentração de 10mg/mL. Os resultados da toxicidade demonstraram que nas concentrações de 10 e 100, 250 e 500µg/mL, o óleo essencial não é tóxico para hemácias de carneiro. No teste de toxicidade em *Artemia salina,* os resultados mostram que em 24 e 48 horas da DL50 é de 371,48 e 302µg/mL de óleo essencial sendo considerados moderadamente tóxicos e em 72 horas a DL 50 é de 33,11µg/mL considerado altamente tóxico. Os dados alcançados são promissores, uma vez que o óleo essencial de *P. bahianum* demostrou atividade antimicrobiana para bactérias Gram positiva e negativa e para fungos. Os dados obtidos de toxicidade em *A. salina* para o óleo essencial poderá ser testado em diferentes linhagens tumorais.

Palavras-chave: atividade antioxidante, atividade antimicrobiana, metabólitos secundários, citotoxicidade.

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1. Introduction

Herbal medicines have been used for preventing and treating several diseases since ancient times (Kaur et al., 2011). Plant species from the family Myrtaceae are among those utilized as herbal medicines. This family has a significant number of species, making it one of the largest botanical families (Durães et al., 2015). The fruits of plants of the genus *Psidium*, except the species *Psidium guajava*, commonly known as araçá in Brazil, are edible, with an exotic flavor and high vitamin C content, and are well-accepted by consumers (Bezerra et al., 2006). Furthermore, other parts of these plants are utilized, mainly in folk medicine, in addition to the fresh consumption of their fruits (Diegues and Arruda, 2001).

Psidium bahianum is one of these species and has a geographically restricted distribution in some regions of the state of Bahia, Brazil, mainly in the Atlantic Forest region in the southern region of the state. It is considered an endemic species found in areas of dense ombrophilous forest, mixed ombrophilous forest, and coastal vegetation (Landrum and Funch, 2008; Amaral and Funch, 2019).

Cerqueira et al. (2009), Myrtaceae species are particularly rich in essential oils and have been utilized by the pharmaceutical and food industries to prevent the proliferation of microorganisms. They are used in the cosmetics industry as flavorings and fragrances, either in their raw or refined form, providing purified substances such as limonene, citral, citronellal, eugenol, menthol, and safrole (Bizzo et al., 2009). Durães et al. (2015) reported that the most studied species of the genus *Psidium* are *P. guajava*, *P. cattleianum*e, and *P. guineense*. The chemical composition of these essential oils is essential for determining their biological activities, as many of them share common compounds and may present similar compositions.

Chemotypes present in essential oils can show high variability, with individuals of the same plant species presenting differences in composition and even in the magnitude of their chemical compounds. Therefore, the identification of chemotypes is necessary to correctly direct their uses (Figueiredo et al., 2017).

The activity of essential oils is generally characterized by the action of their primary compound. However, studies have shown that the effects of essential oils do not always follow this pattern, but are the result of the interaction between their compounds, thus producing a new activity (Wolffenbuttel, 2016).

Considering the economic importance of plants of the genus *Psidium*, both for the consumption of their fruits or the use of their essential oils, as well as the lack of studies on the essential oils of *Psidium bahianum,* the present study aimed to evaluate the biological activity of the essential oil extracted from *Psidium bahianum* leaves collected in an Atlantic Forest remnant in the northern coast of Bahia, Brazil.

2. Material and Methods

2.1. Botanical material collection

Young leaves of *Psidium bahianum* were collected in the morning, between 8 and 9 a.m. from a fragment of dense ombrophilous forest in the Atlantic Forest Vegetation Complex at *Campus* II of the State University of Bahia (UNEB) in Alagoinhas, Bahia, Brazil (12°10'42.62"S and 38°24'39.52"W) and taken to the UNEB Experimental Biology Laboratory for essential oil extraction.

2.2. Essential oil extraction

Young leaves were cut and weighed (350 g), and then essential oil was extracted from them through the steam distillation technique for a period of 4 hours and 30 minutes, using a modified Clevenger apparatus, following the methodology of Craveiro et al. (1981). A 2-liter volumetric flask was used.

2.3. Proton nuclear magnetic resonance (^{1}H-NMR)

Tests for acquiring ^{1}H-NMR spectra were conducted on an INOVA 500 spectrometer operating at 500 MHz, with tetramethylsilane (TMS) as the internal standard. The essential oil was dissolved in deuterated chloroform ($CDCl_3$) containing TMS as an internal reference, with chemical shifts (in ppm) in the range of 0.6 to 7.4 ppm for the analysis of ^{1}H-NMR spectra.

2.4. Assessment of in vitro antioxidant activity

Antioxidant activity was determined by evaluating the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging in 96-well plates, according to Brand-Willians et al. (1995). The essential oil concentration used was 4 mg mL^{-1}. The plates were protected from light and, after 1 hour, they were read on a spectrophotometer (SEAC) at 620 nm; all tests were conducted in triplicates. The percentage of DPPH free radical scavenging (%DPPH-RS) was determined using the following Equation 1:

$$\%DPPH-RS = \left\{ \left[\left(Abs_{control} - \left(Abs_{sample} - Abs_{blank} \right) \right] \times 100 \right\} \; / \; Abs_{control} \right\} \quad (1)$$

where: $Abs_{control}$ is the absorbance of DPPH and ethanol; Abs_{sample} represents the extracts after addition of DPPH; and Abs_{blank} is the absorbance of ethanol.

2.5. Assessment of antimicrobial activity

The essential oil antimicrobial activity was determined using the disk diffusion methodology, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2022). The essential oil was tested on bacterial and fungal strains from the American Type Culture Collection: *Staphylococcus aureus* (ATCC® 6538 ™), *Bacillus subtilis* (ATCC® 6633 ™), *Bacillus cereus* (ATCC® 9592 ™), *Micrococcus luteus* (ATCC® 10240 ™), *Escherichia coli* (ATCC® 94863 ™), *Aspergillus niger* (ATCC® 16404 ™), *Candida albicans* (ATCC® 18804 ™), and *Candida glabrata* (ATCC® 728 ™). Bacteria were cultured on Müeller-Hinton Agar at 37 °C for 24 hours, while fungi were cultured on Sabouraud Dextrose Agar at 37 °C for

48 hours. Subsequently, 5 mm diameter filter paper discs impregnated with 10 mg mL^{-1} of essential oil were applied to the agar inoculated with the test microorganisms. Discs impregnated with 30 µg of tetracycline (Laborclin®) and discs with ciclopirox olamine (0.1%) were used as positive controls in bacterial and fungal assays, respectively. Antimicrobial activity was determined in triplicates, based on the mean measurements (mm) of the inhibitory zone formed around the discs.

2.6. Toxicity assessment

2.6.1. Cytotoxicity: hemolytic activity in sheep erythrocytes

The hemolytic assay was conducted by adding essential oil at concentrations of 10, 100, 250, 500, and 1,000 10 µg mL^{-1} to a 2% suspension with defibrillated sheep blood (Laborclin®). The suspensions were slowly mixed for one minute, in two steps: they were left to rest for 30 minutes after the first agitation; subsequently, they were homogenized and left to kept for 150 minutes at room temperature. The samples were then centrifuged at 3,000 rpm for five minutes. Hemolysis was considered when the plasma fraction exhibited a reddish hue. Distilled water was used as the positive control, while 2.5% dimethyl sulfoxide (DMSO) and 0.9% saline were used as negative controls.

2.6.2. Toxicity in Artemia salina

The toxicity of *P. bahianum* essential oil was evaluated in nauplii of *Artemia salina* Leach. The effects of the essential oil on *A. salina* were assessed following the methodology of Meyer et al. (1982). The assays were conducted in triplicates and consisted of essential oil concentrations (10, 100, and 1,000 µg mL^{-1}) in 6-well plates with 10 *A. salina* nauplii in each well. DMSO and seawater were used as negative controls. Survivors were counted within 24, 48, and 72 hours of exposure to each essential oil concentration. The essential oil toxicity was categorized based on its lethal concentration for 50% of individuals (LC_{50}), according to Clarkson et al. (2004), as follow: non-toxicity (LC_{50})>1,000 µg mL^{-1}); low toxicity (LC_{50} between 500 and 1,000 µg mL^{-1}); moderate toxicity (LC_{50} between 100 and 500 µg mL^{-1}); and high toxicity (LC_{50}<100 µg mL^{-1}).

3. Results

3.1. Essential oil extraction

The yield of essential oil from approximately 350 g of *Psidium bahianum* leaves subjected to steam distillation was 665 µL (0.19%).

3.2. Essential oil characterization

The ^{1}H-NMR spectral data of the essential oil from *P. bahianum* leaves (EOPb) showed a set of signals (between δ 5.95 and δ 0.79 ppm), denoting the predominance of terpene compounds (Figures 1, 2, 3 and 4). Greater intensity for some signals suggests the presence of a main compound.

3.3. In vitro antioxidant activity

In vitro analysis of DPPH free radical scavenging activity of EOPb showed an antioxidant activity of 18.03±2.53% for 125 µg mL^{-1}.

3.4. Antimicrobial activity

EOPb at a concentration of 10 mg mL^{-1} exhibited antimicrobial activity against all tested bacterial and fungal species, with inhibition zones ranging from 7.3±0.6 to 23.3±2.5 for bacteria and from 9.3±2.3 to 14±2.6 for fungi (Table 1).

The percentages of growth inhibition zones for *Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*, and *B. cereus* were 31.29%, 56.83%, 38.97%, and 33.33%, respectively, compared to the inhibition zones of the positive controls. The growth inhibition zone of the gram-negative bacterium *Escherichia coli* was 34.92% compared to that of the positive control.

EOPb showed better results on fungi than bacteria, with 93.3%, 55.53%, and 59.42% growth inhibition for *Candida albicans, C. glabrata*, and *Aspergillus niger*, respectively, compared to the controls.

3.5. Toxicity

3.5.1. Cytotoxicity: hemolytic activity in sheep erythrocytes

Partial cytotoxicity of EOPb in sheep erythrocytes was found at the highest EOPb concentration (1,000 µg mL^{-1}).

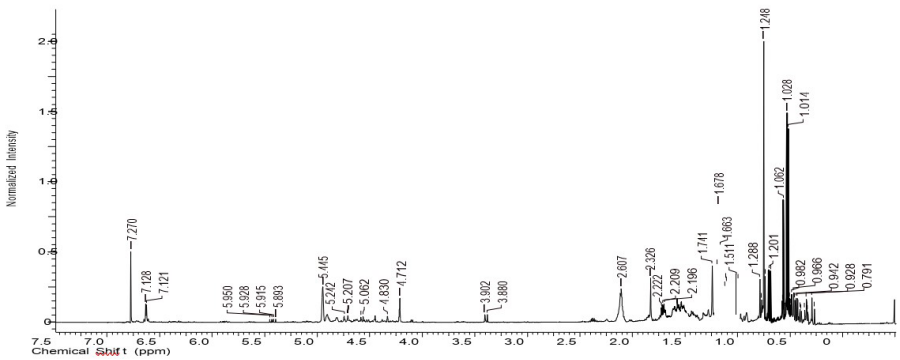

Figure 1. 1H-NMR spectra (500 MHz), CDCl3, in essential oil from *Psidium bahianum* leaves.

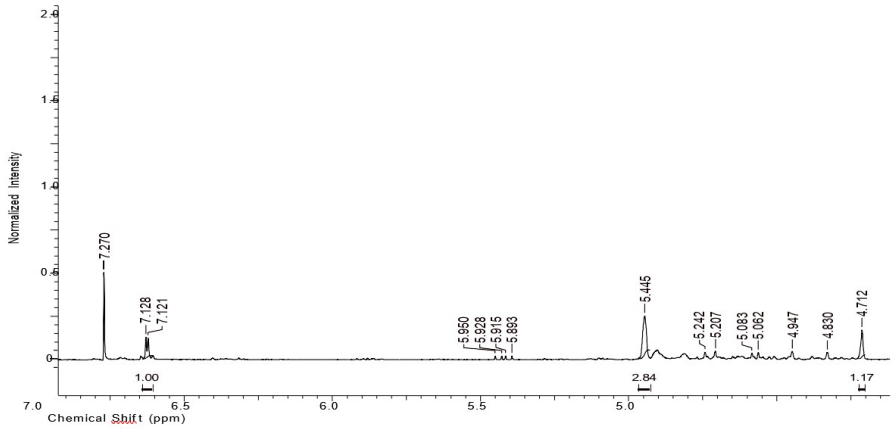

Figure 2. Magnification of signals between 7.27 and 4.71 from 1H-NMR spectra (500 MHz), CDCl3, in essential oil from *Psidium bahianum* leaves.

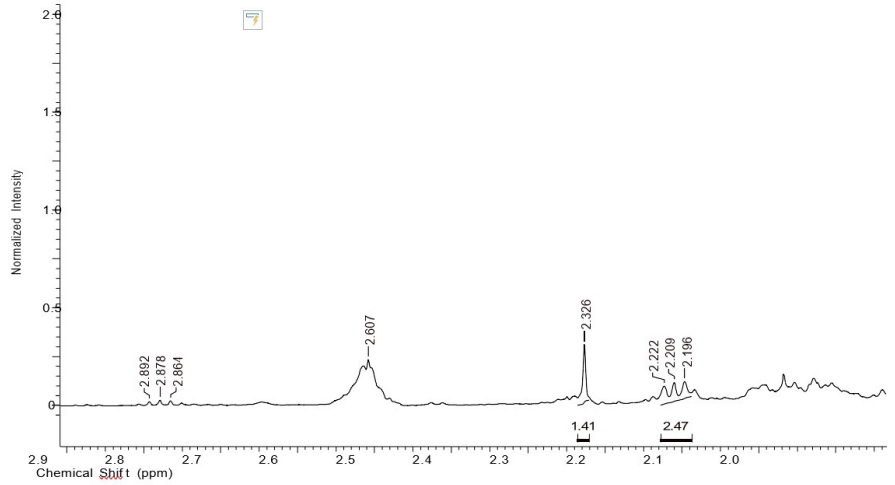

Figure 3. Magnification of signals between δ 2 and δ 2.9 ppm from 1H-NMR spectra (500 MHz), CDCl3, in essential oil from *Psidium bahianum* leaves.

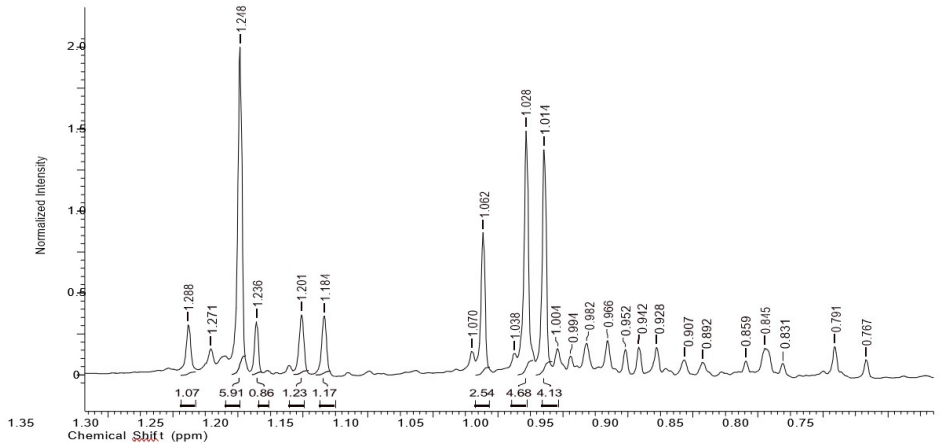

Figure 4. Magnification of signals between δ 1.36 and δ 0.75 ppm from 1H-NMR spectra (500 MHz), CDCl3, in essential oil from *Psidium bahianum* leaves.

Table 1. Mean growth inhibition zones (10 mg mL^{-1}) by essential oil from *Psidium bahianum* leaves.

Inhibition zone diameter (mm)			
Microorganism	**ATCC®***	**Mean±Standard deviation**	**Antibiotic ** **
Escherichia coli	94863 ™	7.3 ± 0.6	21
Bacillus cereus	9592 ™	10 ± 1	30
Bacillus subtilis	6633 ™	11.3 ± 1.5	29
Micrococcus luteus	10240 ™	23.3 ± 2.5	41
Staphylococcus aureus	6538 ™	9.7 ± 0.58	31
			Antimycotic * **
Aspergillus niger	16404 ™	9.3 ± 2.3	15.7
Candida albicans	18804 ™	14 ± 2.6	15
Candida glabrata	728 ™	8.3 ± 0.6	15

*ATCC®: American Type Culture Collection; ** Tetracycline (30 µg) (Laborclin®); *** Ciclopirox olamine (0.1%).

3.5.2. Toxicity in Artemia Salina

EOPb toxicity in *A. salinas* showed a dose-dependent effect regarding the exposure time at a concentration of 1,000 mg mL^{-1}. The results indicated that the LC_{50} were 371.48, 302, and 33.11 µg mL^{-1} within 24, 48, and 72 hours of exposure to OEPb.

4. Discussion

Few studies on the genus *Psidium*, specifically those known as araçá trees, have evaluated the yield of their essential oils. Castelo et al. (2010) found an essential oil yield of 0.13% for *Psidium myrsinites*, whereas Santos et al. (2014) found a mean yield of 0.42% for *Psidium oligospermum* essential oil. These are similar results to those found in the present study.

Morais (2009) stated that the age and stage of developmental stage of the plant can affect the total amount of secondary metabolites produced and the relative size of these compounds. Younger plant tissues generally present high biosynthetic activity, resulting in increased production of several compounds, including essential oils. Additionally, Costa et al. (2013) reported that the time required for essential oil extraction may vary according to the species, location, and specific anatomy that stores the essential oil. Some factors after collecting the plant material also affect the yield and quality of essential oils, as well as the drying and processing of the material. However, it is important to highlight that the essential oil evaluated in the present study was extracted from fresh leaves.

There is no consensus among some studies regarding medicinal species regarding the collection time of the plant material and the essential oil contents and chemical compositions. Different results have been found for each species, making it difficult to find a pattern for essential oils of aromatic plants (Oliveira et al., 2012).

Studies on *P. guajava* essential oils have found monoterpenes and sesquiterpenes as their main compounds (Lima et al., 2011; Satyal et al., 2015; Soliman et al., 2016). Signals from ^{1}H-NMR spectra between δ 5.95 and δ 5.06 ppm (typical of olefinic hydrogens) were observed in *P. myrsinites* essential oil, resulting from the presence of linalool (Castelo et al., 2010). A doublet at δ 1.021 ppm (J 7.0 Hz), which is characteristic of the methyl hydrogen of the typical component of the isopropyl group, was observed for cadinol, α-pinene, δ-cadinene, and monoterpenes present in plants of the genus *Psidium* (Cornwell et al., 2000).

Comparing the signals from ^{1}H-NMR for OEPb with those obtained for *P. myrsinites*, the presence of the monoterpene linalool stood out (Castelo et al., 2010). Identifying other substances was not possible, as studies on essential oils from other *Psidium* species performed identification analyses by gas chromatography-mass spectrometry.

Silva et al. (2021) conducted a review study covering 18 species of *Psidium* and found 110 records of essential oils with significant variability in volatile compounds according to seasonality and collection locations. Monoterpenes and sesquiterpenes such as *p*-menthane, pinane, bisabolane, germacrane, caryophyllene, cadinane, and aromadendrane-type skeletons were among these compounds.

According to Branco et al. (2023), few studies evaluating the antioxidant activity of essential oil from leaves of species of the genus *Psidium* are found. Similar results were found for the essential oils of *P. cattleianum* (16.19±2.33 for 100 µg mL^{-1}) (Scur et al., 2016) and *P. guajava* (half-maximal inhibitory concentration (IC_{50}) of 460.37±1.33 µg mL^{-1}) (Lee et al., 2012). Nascimento et al. (2018) found higher antioxidant activity of the *P. guineense* essential oil (IC_{50} of 26.13 µg mL^{-1}) compared to other species. Regarding the antioxidant activity by percentage of DPPH free radical scavenging (%DPPH-RS), the higher the %DPPH-RS, the greater the free radical scavenging activity. Contrastingly, the lower the IC_{50}, the higher the activity of scavenging 50% of the free radicals.

The analysis of antimicrobial activity showed promising results against the evaluated microorganisms. *Staphylococcus aureus* is a gram-positive bacterium that is naturally present in the microbiota of humans, residing especially in the nostrils and perineum, however, it is a threatening pathogen due to its resistance to antibiotics (Otto, 2010). According to Ferreira et al. (2006), species of the genus *Staphylococcus* have shown resistance to

certain antibiotics; one of the reasons for this resistance is the indiscriminate use of these drugs.

Micrococcus luteus is a bacterium present in the environment and in the human transient microbiota and can be found on the skin, presenting association with the occurrence of infections such as abscesses, pneumonia, septic arthritis, meningitis, bacteremia, and septic shock in immunocompromised patients (Trabulsi and Alterthum, 2008). Segundo Boldock et al. (2018), this microorganism on human skin acts as a pro-infectious agent, enabling the establishment of pathogens such as *S. aureus*.

The genus *Bacillus* are saprophytic, aerobic, gram-positive bacteria, presenting a rod shape. They can be found in soil, water, dust, and air and are considered allochthonous. Few species are pathogenic (Hoa et al., 2000). *Bacillus cereus* is a bacterium involved in food deterioration and is connected to the production of enterotoxin and, consequently, food poisoning (Silva et al., 2018).

Escherichia coli is a gram-negative bacterium naturally present in the intestine of humans and animals; however, some strains are classified as pathogenic and with potential to cause aggressive diseases, including hemorrhagic colitis that can progress to hemolytic uremic syndrome (Amani et al., 2010; Forsythe, 2010).

Aspergillus niger is one of the most studied filamentous fungi in the biotechnology field (Frisvad et al., 2011). Yeasts belonging to the genus *Candida* are classified as opportunistic pathogens present in the mucous membranes of humans; their imbalance can lead to the development of infections known as candidiasis (Álvares et al., 2007).

Candida albicans is the most common causative agent of mucosal infections and systemic infections, accounting for approximately 70% of fungal infections worldwide; it is the main cause of invasive infections, with a mortality rate of approximately 40% (Morad et al., 1996; Chen et al., 2020; Basmaciyan et al., 2019). *Candida glabrata* is the second most frequently isolated candidiasis agent and accounts for approximately 15% to 25% of invasive clinical cases (Kounatidis et al., 2018)

The findings described in the present study indicate for the first time the antimicrobial activity of the essential oil from *P. bahianum* leaves, presenting growth inhibition zones varying ranging from 7 to 23 mm for bacteria and from 8 to 14 mm for fungi. Hanif et al. (2018) found antifungal activity of essential oil from *P. guajava* leaves against fungi such as *A. niger, A. flavus, Fusarium solani,* and *Rhizopus solani,* obtaining inhibition zones between 13 and 15 mm, whereas growth inhibition zones of the bacteria *S. aureus*, *E. coli*, *Streptococcus pyogene*, and *Bacillus subtilis* were between 6 and 15 mm.

Dias et al. (2022) found antifungal activity of essential oil from fresh leaves of *P. myrtoides,* with higher antifungal activity for the oil obtained from leaves collected during the dry season compared to that from leaves collected during the rainy season. They reported that the main compounds found were the monoterpenes β-caryophyllene (20.0% and 32.9%) and limonene (10.4% and 19.8%) in essential oils from leaves collected during the rainy and dry seasons, respectively.

The antimicrobial activity of active compounds in essential oils can be explained by the lipophilic nature of the monoterpenes contained in them. Monoterpenes act by disrupting the microbial cytoplasmic membrane, resulting in the loss of membrane impermeability (Jing et al., 2014).

The results found showed varied degrees of antimicrobial activity. The *P. bahianum* essential oil presented bioactive compounds conferring distinct antimicrobial properties, including inhibitory effects on the growth of fungi and gram-positive and gram-negative bacteria.

Costa (2002) reported that certain chemical groups originating from the secondary metabolism of plants can exhibit hemolytic activity. Therefore, the World Health Organization (OMS, 1998) recommends quality control for all productions that use plants as raw or related materials.

According to Hirota et al. (2012), evaluating the toxicity of a chemical compound is essential for determining the effects that a substance can cause in an organism. Therefore, toxicity evaluation is an essential step to define the viability of a new product.

Studies on cytotoxicity in red blood cells by species of the genus *Psidium* corroborate the results obtained in the present study. Costa et al. (2023) evaluated *P. glaziovianum* in a hemolysis assay and found a low potential of its essential oil to cause cell lysis. Few studies are found evaluating cytotoxicity in erythrocytes caused by essential oils from species of the genus *Psidium* and they are mostly based on extracts of leaves, stems, or fruits obtained using organic solvents (Pereira et al., 2020) and tested on different cancer cell lines (Hosseini et al., 2023; Ashraf et al., 2016; Alam et al., 2023). However, studies evaluating the effects of these essential oils on normal cells are essential to ensure the safe use of these products.

Rocha et al. (2021) evaluated the toxicity of different leaf and stem extracts of *P. baihanum* obtained using different solvents, following the same methodologies as the present study. They found no or low toxicity in red blood cells, denoting that this species has potential for use in phytotherapy.

The methodology described by Meyer et al. (1982), used in the test with *Artemia salina* in the present study, is considered a reference for establishing a correlation between toxicity in *A. salina* and antitumor activity. According to McLaughlin et al. (1998), assays with *A. salina* are as accurate or even superior compared to those with human tumor cell lines and can be used as a pre-evaluation method instead of more expensive assays, and as a pesticide evaluation method.

The determination of toxicity in *A. salina* proposed by Cavalcante et al. (2000) is based on the survival or death of individuals. The lethal concentration for 50% of evaluated individuals (LC_{50}) could be assessed according to the number of dead microcrustaceans. According to Meyer et al. (1982), a sample is considered active in terms of toxicity when it presents an LC_{50} lower than 1,000 μg mL^{-1}; therefore, the findings of the present study are relevant for the determination of LC_{50}, as it was lower than 1,000 μg mL^{-1} for obtaining toxic action in all tested periods.

According to the classification by Clarkson et al. (2004), the essential oil from *P. bahianum* leaves is moderately toxic within 24 and 48 hours, whereas it is highly toxic within 72 hours of exposure to LC_{50}. Therefore, considering the low cytotoxicity of *P. bahianum* essential oil in erythrocytes, the

correlation between the toxicity action of natural products on *A. salina* and toxicity in different cancer cell lines, and that cancer is one of the leading causes of death worldwide, studies on natural products represent an important tool for evaluating anticancer agents.

The compounds in the essential oil from *Psidium bahianum* leaves (OEPb) showed antimicrobial activity. Data on cytotoxicity in red blood cells showed the feasibility of using OEPb, as different OEPb concentrations were non-toxic. Additionally, toxicity in *Artemia salina* indicated that OEPb can be tested on different immortalized cell lines to evaluate its potential antitumor activity.

The findings of this study denote that the bioactive molecules in the *P. bahianum* essential oil have potential for use in phytotherapy, either in their original form or through some of their isolated compounds. Therefore, further investigations and new approaches are necessary.

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