SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 19, NUM. 08 2023
doi: 10.14808/sci.plena.2023.087201

Caryocar villosum: bioactivity of extracts and oils on phytopathogens

Caryocar villosum: bioatividade de extratos e óleos sobre fitopatógenos

B. C. M. Sousa[1,2]; K. A. Lourido[1]; R. X. Rebelo[1]; S. P. Castro[3]; J. C. N. Matos[3]; A. Sartoratto[4]; T. A. Vieira[1,2]; K. C. F. Castro[1,3]; D. C. Lustosa[1]*

[1]*Institute of Biodiversity and Florests (IBEF), Federal University of Western Pará (UFOPA), 68040-255, Santarém-Pará, Brazil*
[2]*Program in Society, Nature and Development (PPGSND), Federal University of Western Pará (UFOPA), 68040-255, Santarém-Pará, Brazil*
[3]*Program in Biosciences (PPGBIO), Federal University of Western Pará (UFOPA), 68040-255, Santarém-Pará, Brazil*
[4]*Pluridisciplinary Center for Chemical, Biological and Agricultural Research (CPQBA), State University of Campinas (UNICAMP), 13140-000, Campinas-São Paulo, Brazil*

**denise.lustosa@ufopa.edu.br*
(Recebido em 17 de abril de 2023; aceito em 22 de agosto de 2023)

The piquiazeiro has great importance in the Amazon, as well as used in cooking, is used in the cosmetic industry, and empirically as a medicinal product, but little is known about its potential for obtaining fungitoxic substances. This study evaluated the chemical analysis and *in vitro* antifungal activity of ethanolic extracts and oils of *Caryocar villosum*. The chemical profile of the extracts was obtained from the Thin Layer Chromatography (TLC), while the vegetable oil profile was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). Different concentrations of the extracts and vegetable oils were used in biological tests. The experimental design was completely randomized in a factorial scheme with replication. The presence of flavonoids was confirmed in all extracts. The major components of the artisanal oil were oleic acid, palmitic acid, and ethyl oleate, and the major component of the industrialized oil was (Z)-9,17-octadecadienal, (Z)-octadecenal and β-sitosterol. In general, the extract of young leaves and industrialized oil showed the highest reductions in fungal colonies, and the concentrations of 40% of the extracts and 3.75 $\mu L.mL^{-1}$ of oils caused the lowest growth of the challengers.
Keywords: antifungal activity, phytochemical screening, natural products.

O piquiazeiro apresenta grande importância na Amazônia, pois além de utilizado na culinária, é empregado na indústria cosmética, e empiricamente como produto medicinal, mas pouco se sabe sobre seu potencial para obtenção de substâncias fungitóxicas. Este estudo avaliou a análise química e a atividade antifúngica *in vitro* de extratos etanólicos e óleos de *Caryocar villosum*. O perfil químico dos extratos foi obtido por Cromatografia em Camada Delgada (CCD), enquanto o perfil do óleo vegetal foi analisado por Cromatografia Gasosa acoplada à Espectrometria de Massas (GC-MS). Diferentes concentrações dos extratos e óleos vegetais foram utilizadas nos testes biológicos. O delineamento experimental foi inteiramente casualizado em esquema fatorial com replicação. A presença de flavonoides foi confirmada em todos os extratos. Os componentes majoritários do óleo artesanal foram ácido oleico, ácido palmítico e oleato de etila, e o componente majoritário do óleo industrializado foi (Z)-9,17-octadecadienal, (Z)-octadecenal e β-sitosterol. Em geral, o extrato das folhas jovens e o óleo industrializado apresentaram as maiores reduções nas colônias fúngicas, e as concentrações de 40% dos extratos e de 3,75 $\mu L.mL^{-1}$ dos óleos ocasionaram os menores crescimentos dos desafiantes.
Palavras-chave: atividade antifúngica, triagem fitoquímica, produtos naturais.

1. INTRODUCTION

The use of synthetic fungicides to control fungi during storage is not ideal, especially in family farming because they can cause complications or food poisoning, in addition to being inaccessible for practicing in communities with few resources, where grains and other products are consumed during the storage period [1].

Various pathogens are responsible for causing plant disease, including *Bipolaris oryzae* (Breda de Haan) Shoemaker which causes brown spots on rice, and its control is carried out by the

application of chemicals because there are few cultivars available with resistance to the pathogen [2], which increases production costs and damages the environment and human health [3, 4].

Fungi of the *Fusarium* genus can be found and live for long periods in the soil or plant debris, and also in air, water, plants, and insects [5], they adapt very well in tropical and subtropical climates. The diversity of species is wide, being considered one of the most important pathogenic groups [6].

The genus *Pestalotiopsis* comprises species considered cosmopolitan and found in saprophytic, phytopathogenic, and endophytic forms [7]. They cause disease in a variety of cultivars, such as apple, coconut, chestnut, grapevine, guava, and mango trees [8-10]. Signs and symptoms include canker lesions, shoot death, leaf spot, needle rust, tip rust, gray rust, itchy canker, severe chlorosis, and rot, and are also harmful post-harvest fungi [11-15].

Rhizoctonia solani J.G. Kühn causes disease in many cultivated species, being more aggressive in moist soils and at temperatures between 15 and 18 °C [16]. The infection even produces deep cankers in the seedlings, leading to strangulation and causing pre- and post-emergence damping-off [17]. All these diseases affect the quality and productivity of plants.

Extracts and oils obtained from plants generally have low toxicity to mammals and have been investigated for their potential in controlling fungi that cause various plant diseases [18], or in pest control, such as repellents and insecticides [19]. These products can be important in agricultural production, mainly as an alternative to the use of synthetic fungicides [20].

Caryocar villosum (Aubl.) Pers. is a species popularly known in the Amazon as piquiá (piquiazeiro), belongs to the Caryocaraceae family, and has a neotropical distribution, occurring more commonly in the Guianas and Central Amazon [21]. Its fruits can produce substances with antifungal properties, as well as topical anti-inflammatory activity [22]. *In vitro*, studies have shown that aqueous and hydroalcoholic extracts of *Caryocar villosum* have antioxidant action related to their content of phenolic compounds [23].

However, as research and publications on this species are relatively scarce, this study aimed to evaluate the chemical analysis and in vitro antifungal activity of three ethanol extracts and two oils of *Caryocar villosum* on the mycelial growth of disease causing fungi in plant species of socioeconomic importance and occurrence in the region.

2. MATERIALS AND METHODS

2.1 Plant material and extraction methods

The research was registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen, Brazil) under protocol A1B0150. The *Caryocar villosum* extracts were obtained from the bark of the adult tree and the leaves of adult and young trees (Figures 1A and 1B). The plant material was collected during the rainy season in the rural community of Belterra (Pará, Brazil), in November 2013. In this same community, artisanal oil was acquired after boiling the fruit in water (Figure 1C) and scraping the pulp and homogenization of the mass for decantation in a sloping gutter containing cotton at the base by which after five days the vegetable oil was obtained (Figure 1D). Industrialized vegetable oil, also obtained from artisanal extraction of the fruit pulp, was extracted and sold by a homeopathic pharmacy located in the city of Santarém, Pará, Brazil.

Figure 1: Caryocar villosum (Aubl.) Pers. A) Adult Piquiá tree; B) Young piquiá tree; C) Fleshy fruit, dupra type, yellowish-brown color, oblong-globose shape, weight of approximately 500g; D) Longitudinal section showing the edible mesocarp (fruit pulp) and the woody endocarp. Source: B. C. M. Sousa.

After collection, the leaves and bark were washed in running water, and they were immersed in a 2% sodium hypochlorite solution for five minutes, followed by rinsing in distilled sterile water and drying on sterile paper towels. Subsequently, all material was dried at 40 °C for 72 h in a forced-air oven. Once dry, the material was weighed and crushed (Figure 2). The weights of mature leaves collected from adult and young trees were 29.6 g and 25.7 g, respectively. The weight of the crushed bark was 30.0 g.

Figure 2: A) Weighing of a dry sample of leaves and bark; B) Dried and crushed leaves; C) Dried and crushed peels. Source: B. C. M. Sousa.

The crude extracts were obtained by hot extraction, using 500 mL of 96% P.A. ethanol as the solvent. The procedure was performed in a soxhlet apparatus with a total period of eight hours for each extraction (Figure 3). The ethanolic solutions resulting from this process were subjected to a rotary evaporator, and after that the extracts were weighed to obtain yields of 35.2% for the leaves of the adult tree, 21.5% for the leaves of the young tree, and 6.6% for the bark of the adult tree, following an adapted methodology [24].

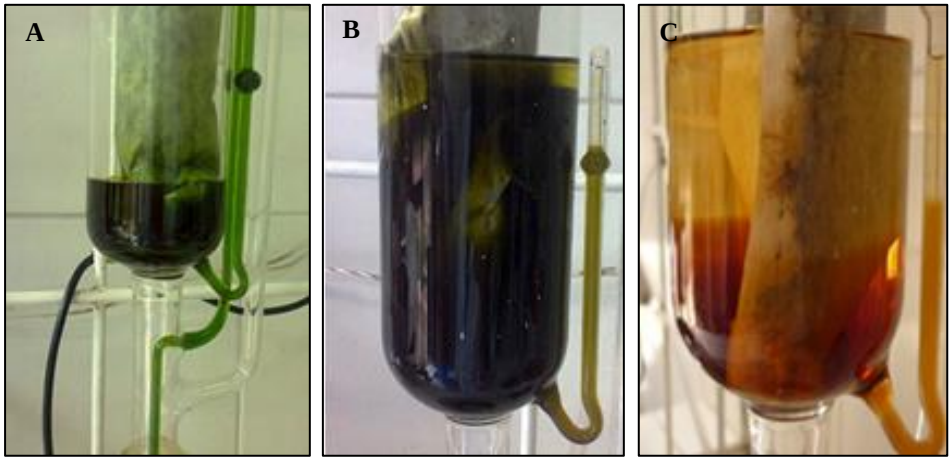

Figure 3: A) Ethanol extraction of young piquiá leaves; B) Ethanol extraction of adult piquiá leaves; C) Ethanol extraction of piquiá adult bark. Source: B. C. M. Sousa.

2.2 Chemical composition of plant extracts and oils

The chemical analysis of the ethanolic extracts obtained from the bark and leaves of *Caryocar villosum* was obtained by Thin Layer Chromatography (TLC). This analysis required the use of aluminum chromatoplates (12 cm x 10.5 cm), silica gel 60 mesh, UV fluorescent indicator in 254 nm, and a layer thickness of 0.20 mm. Aliquots of 100 µL of methanolic solution of the crude extracts were applied at a concentration of 1000 ppm to the chromatographic plate at distances of 0.5 cm each. Standards of phenolic compounds (chlorogenic acid, rutin, quercetin, caffeic acid, and gallic acid) were added to confirm the flavonoids. The plate was eluted using a solvent mixture containing ethyl acetate: formic acid: acetic acid: water (100: 11: 11: 26) until the solvent reached 9.5 cm, and then, it was dried and developed with a methanolic solution with 1% 2-aminoethyl diphenylborinate (w/v), for flavonoids revelation [25]. The retention factors (Rf) were calculated [24].

The chemical analysis of *Caryocar villosum* oils was obtained using Gas Chromatography coupled to Mass Spectrometry (GC-MS), using an HP-6890 chromatograph, HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm), and detector operating at 70 eV with linear scanning in the range of 30 to 500 amu. The chromatographic conditions were: injector temperature at 200 °C, helium as carrier gas (1.0 $mL.min^{-1}$), temperature programming from 60 °C to 240 °C at a rate of 3 $°C.min^{-1}$, and oil injection of 10 µL. The compounds were identified by comparison with the NIST05 library [26].

2.3 In vitro antifungal activity

The effects of extract crude and oils and the fruit pulp of *Caryocar villosum* were evaluated on the mycelial growth of phytopathogenic fungi: *Bipolaris oryzae* (Breda de Haan) Shoemaker obtained from rice (*Oryza sativa* L.) seeds (Figure 4A), *Fusarium* spp. from corn (*Zea mays* L.) seeds (Figure 4B) and bell pepper (*Capsicum annuum* L.) fruits (Figure 4C), *Pestalotiopsis* sp.

from cupuaçu (*Theobroma grandiflorum* (Willd. Ex Spreng.) K. Schum.) leaves (Figure 4D), and *Rhizoctonia solani* J.G. Kühn from soybean (*Glycine max* (L.) Merr.) root (Figure 4E).

The ethanolic extracts were solubilized in a 1:1 solution (crude extract: distilled and sterilized water). Then, polyvinylpyrrolidone (PVP) was added in a 1:4 (extract/oils: PVP) proportion (previously tested products to confirm that its use does not interfere with the growth of fungi). The solution was filtered and added to a potato-dextrose-agar (PDA) culture medium to obtain concentrations of 10, 20, 30, 40, and 50%. The oils were previously filtered on a Millipore® membrane (0.45 μm) and added to a PDA medium (approximately 45 °C), adjusted to concentrations of 0.25; 0.50; 0.75; 1.25; 2.5; 3.75; and 5.0 $\mu L.mL^{-1}$.

After adding in PDA liquid, the extractives were homogenized and poured into Petri dishes (6 x 1.5 cm). One disk (0.4 cm) containing the fungus structures was placed in the center of each dish. The Petri dishes were incubated at 25 °C with a 12 h photoperiod. The control treatment consisted of fungi only in the PDA medium. The average diameter of the fungal colonies was measured for five days [27].

The experiments followed a completely randomized design (CRD). The factorial scheme for the extracts was 3x6x5 (three extracts; six concentrations, including the control (zero); and five phytopathogens) with three replicates. The factorial scheme for oils was 2x8x5 (two oils; eight concentrations, including control (zero); and five phytopathogens) with four replicates. Data were subjected to normality and homogeneity of variance and analysis of variance (ANOVA). The treatment means were compared by Tukey's test ($p \leq 0.05$), by the statistical program SISVAR 5.6 [28].

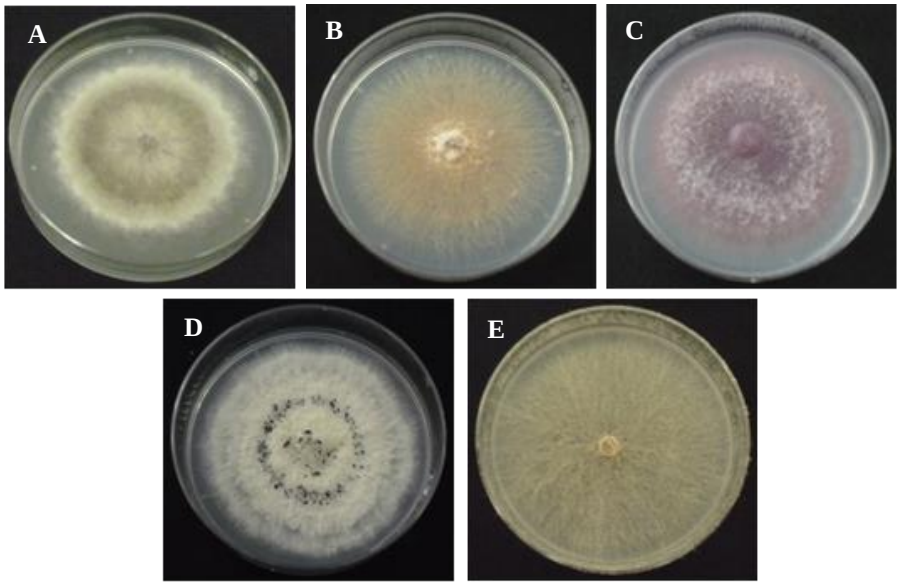

Figure 4: A) Control of Bipolaris oryzae isolated from rice; B) Witness of the genus Fusarium sp. corn isolate; C) Fusarium sp. from bell pepper; D) Witness of the genus Pestalotiopsis sp. isolated from the cupuaçu species; E) Control of Rhizoctonia solani isolated from soybean. Source: B. C. M. Sousa.

3. RESULTS AND DISCUSSION

The TLC analysis confirmed the presence of flavonoids in all ethanolic extracts of *Caryocar villosum*, due to the similarity of retention factors (Rf) found in extracts with at least one of the standards used to identify this class (Table 1).

Plant extracts contain active substances, such as fatty acids, alkaloids, phenols, flavonoids, glycosides, tannins, and terpenoids, which may have a variety of biological activities, including antifungal [29]. The class of flavonoids belonging to the phenolic compounds, and has already been reported in ethanolic extracts from the leaves of *Caryocar brasiliense* [30]. This class

contains substances with diverse biological activities, such as antifungal, antiviral, antibacterial, antiparasitic, immunomodulatory, anti-inflammatory, and antioxidant [31].

Table 1. Retention factor obtained by Thin Layer Chromatography of ethanolic extracts of Caryocar villosum compared to standards for flavonoids.

Standards	Reference *Rf	Bark (adult tree) Rf	Leaves (adult tree) Rf	Leaves (young trees) Rf
Rutin	0.47	-	0.47	0.47
Chlorogenic acid	0.63	-	0.68	0.68
Quercetin	0.86	0.86	-	-
Caffeic acid	0.87	-	-	-
Gallic acid	0.89	-	-	-

*Rf = retention factor.

The artisanal and industrialized oils of *C. villosum* showed variation in their chemical compositions. In total, 14 components were detected in artisanal oil, 10 were identified and three substances had the highest percentages, ethyl oleate (19.9%), palmitic acid (21.5%), and oleic acid (24.7%), considered the majority constituent. The substances (Z)-octadecenal, ethyl hexadecanoate, (Z)-9,17-octadecadienal, (Z)-ethyl octadecenoate, β-sitosterol, squalene, and 14-methyl-pentanoic acid presented percentages below 5.2% (Table 2).

Twelve compounds were detected in the industrialized oil, eight were identified and three substances stood out, β-sitosterol (14.2%), (Z)-octadecadienal (15.5%), and (Z)-9,17-octadecadienal (19.7%), with the latter being the majority. The substances β-tocopherol, (Z)-ethyl octadecenoate, squalene, palmitic acid, and methyl hexadecanoate presented percentages below 5.2% (Table 2).

Table 2. Chemical constituents of artisanal and industrialized oils of Caryocar villosum.

Substance	Artisanal oil		Industrialized oil	
	RT (min)	Rel. %	*RT (min)	*Rel. %
14-methyl-pentanoic acid	24.73	0.8	-	-
methyl hexadecanoate	-	-	24.75	1.3
palmitic acid	25.59	21.5	25.45	1.6
ethyl hexadecanoate	26.05	5.1	-	-
oleic acid	28.81	24.7	-	-
ethyl oleate	29.42	19.9	-	-
(Z)-ethyl octadecenoate	30.69	2.3	30.69	3.9
*n.i.	31.45	2.9	31.46	4.5
*n.i.	-	-	33.54	14.3
(Z)-9,17-octadecadienal	33.60	3.8	33.61	19.7
*n.i.	-	-	34.29	13.5
(Z)-octadecenal	34.35	5.2	34.36	15.5
*n.i.	34.86	3.3	-	-
*n.i.	37.60	5.5	-	-
squalene	39.52	2.1	39.53	1.8
β-tocopherol	-	-	43.05	5.2
*n.i.	43.89	0.6	43.91	4.2
β-sitosterol	49.15	2.3	49.18	14.2
Total		100		99.7

*RT (min) = retention time in mintes; Rel. % = relative percentage; n.i. = not identified.

The fruit of the piquiá tree is composed of 65% peel, 30% pulp, and 5% almond. The pulp is composed of 72% oil, 3% protein, 14% fiber, and 11% other carbohydrates [32]. To obtain the fixed oils, it is generally necessary to process the seeds, squeeze them, boil them and let them rest [33], as was the case with obtaining the artisanal oil used in this work, and the final product can be used for pharmacological purposes, industrial and nutritional.

The chemical analysis of the pulp and seeds of *Caryocar villosum* also indicated the presence of palmitic acid (44.63% in the mesocarp and 44.84% in the seeds) and oleic acid (43.66% for the mesocarp and 33.62% for the seeds) [34].

Other species of the genus, such as *Caryocar brasiliense* Cambess., also showed high lipid content in the pulp and almonds, highlighting the presence of fatty acids, predominantly oleic acid (55.87% in the pulp and 43.59% in the almond) and palmitic (35.17% in the pulp and 43.76% in the almond), in addition to components such as stearic, linoleic, palmitoleic acids, among others [35]. For oil from the pulp of *Caryocar coriaceum* Wittm. the presence of oleic acid (56.34%), palmitic acid (27.63%), linoleic acid (5.2%), and linolenic acid (4.42%) was verified [36], and for the seeds of this species the presence of oleic acid (57.15%), palmitic acid (35.53%), stearic acid (4.0%), and linoleic acid (2.38%) was detected [37]. Recent research has shown percentages of 42.47% for oleic acid, 39.49% for palmitic acid, and 10.17% for linoleic acid for *C. coriaceum* seeds [38].

In this sense, the composition of the artisanal oil extracted from the fruits of *C. villosum* presented the major compounds commonly found and described in the literature for the species genus (Figure 5).

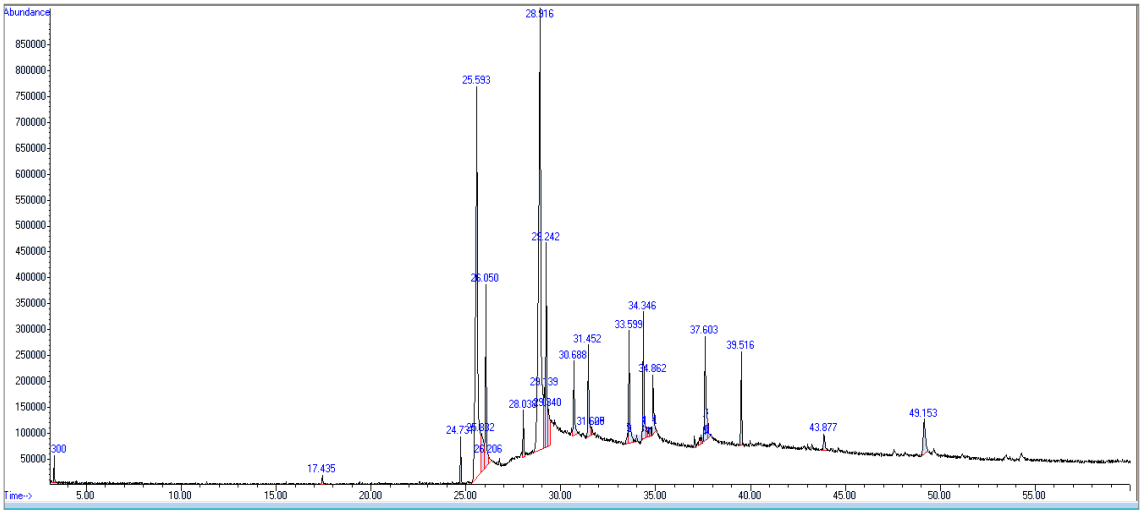

Figure 5: Chromatogram of artisanal Caryocar villosum oil.

The chromatographic profile of industrialized piquiá oil showed differences in the chemical constitution when compared to artisanal oil, such as the absence of oleic acid, suggesting a detailed study of this product (Figure 6). The environment in which the plant species grows, the temperature, the relative humidity of the air, the total duration of exposure to the sun, and the wind regime, influence the chemical composition of the oils and extracts and, consequently, their biological activity [39, 40]. Anthropogenic action also interferes with these factors, from the choice of types of cultivation and processing of plant material to obtaining the final product.

Regarding the biological activities of *Caryocar villosum*, there is a lack of scientific production, with only research using its products for activities such as antioxidant [41], anti-inflammatory [42], larvicide, cytotoxic, photoprotective, and antigenotoxic [43].

In this sense, the data presented in this study are unprecedented regarding the evaluation of the antifungal activity of piquiá extracts and oils.

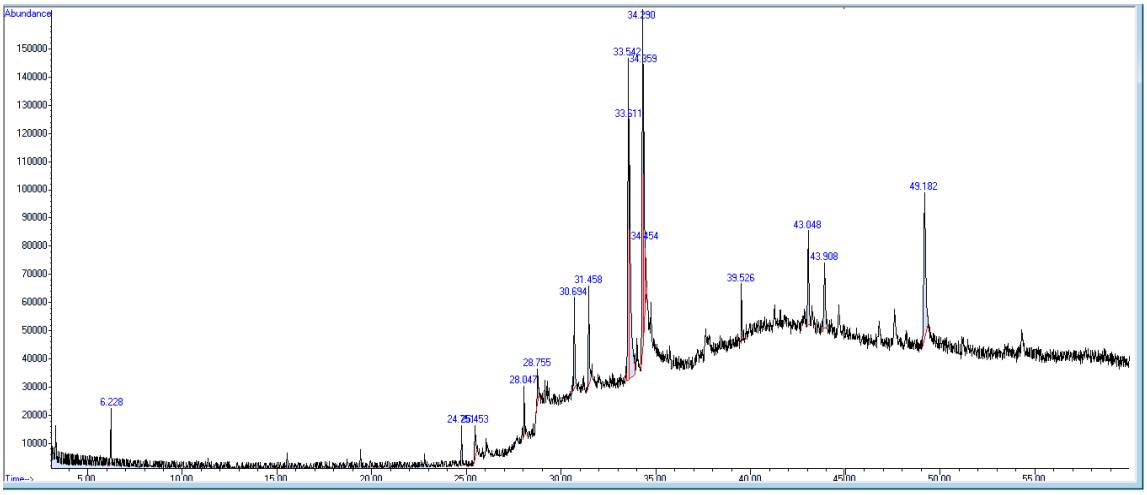

Figure 6: Chromatogram of industrialized Caryocar villosum oil.

In the biological tests for *C. villosum* extracts, there was a statistically significant difference for the isolated factors (plant parts, concentration, and phytopathogens), and for all interactions between them ($p \leq 0.05$). An average reduction of 15.9% in the *Bipolaris oryzae* growth was observed at 40% and 50% concentrations of extracts obtained from the bark. The leaf extract obtained from the adult trees, in 40% concentration, inhibited 18.2% of the *B. oryzae* colony. The young tree left extract reduced the growth of this fungus by 13.6% and 25.0% at 40% and 50% concentrations, respectively (Table 3).

In studies of antifungal activity of extracts and oils on the phytopathogens analyzed for *Caryocar villosum*, it was found that rosemary extract (*Rosmarinus officinalis* L.), for example, caused a reduction in the mycelial growth of *Bipolaris oryzae* with increasing concentrations tested [44].

All concentrations of the extracts obtained from bark and leaves from young trees reduced the average diameter of *Fusarium* spp. obtained from corn and bell pepper, respectively. The *C. villosum* bark extract also reduced the mean diameter of *Pestalotiopsis* sp. at all concentrations (Table 3).

Ethanolic extracts of two species of cumaru (*Dipteryx odorata* e *Dipteryx punctata*) were evaluated for antifungal action against *Fusarium* spp. isolates from lettuce and kale, being the concentrations of 40% and 50% of the extract of the endocarps of *D. odorata* the ones that provided the biggest reductions in the average diameter of their colonies. As for *D. punctata*, *Fusarium* sp. lettuce isolate was more sensitive to the action of the leaf extract, obtaining the lowest averages of mycelial growth at concentrations of 30%, 40%, and 50%. And for *Fusarium* sp. obtained from kale, the concentration of 10% of the seeds extract showed a promising result [45].

Different species of the *Fusarium* genus showed distinct inhibition when confronted with extracts of medicinal plants; the cinamomum (*Melia azedarach* L.) extract caused 97% inhibition of *Fusarium proliferatum* (Matsush.) Nirenberg and the combined extracts of river bushwillow (*Combretum erythrophyllum* (Burch.) Sond.) and sawtooth oak (*Quercus acutissima* Carruth.) inhibited *Fusarium verticillioides* (Sacc.) Nirenberg by 96%, *Fusarium proliferatum* by 67% and *Fusarium solani* (Mart.) Sacc. by 56% [1].

For the phytopathogen *Rhizoctonia solani*, the concentration of 30% of the bark extract did not differ from the control treatment; whereas, the other concentrations tested increased mycelial growth of this phytopathogen. Concentrations of 20% and 40% of extracts from the leaves of adult trees were effective in reducing mycelial growth. For the extract obtained from the leaves of young trees, from the concentration of 20%, there was a reduction in the growth of *R. solani*, compared to the control (Table 3). The reduction in the average diameter of the fungus colonies, caused by the extracts of leaves from adult trees, at a concentration of 40%, and from leaves of young trees at a concentration of 50%, was 26.4%.

The ethanol extract of jurubebão fruits (*Solanum grandiflorum* Ruiz & Pav.) showed a fungicidal activity against *Rhizoctonia solani* [46]. The quantity and longevity of the compounds, as well as the relationship between them, can result, at certain times, in greater or lesser inhibition of the phytopathogens; a fact that may be associated with the presence of substances with antifungal activity or with activities that stimulate the growth of phytopathogens [18].

Table 3. Mean diameter of plant pathogen colonies subjected to five concentrations of extracts from different parts of Caryocar villosum.

Parts of the plant	**Concen-tration (%)**	**Mean diameter of plant pathogen colonies (cm)**				
		Bipolaris oryzae (rice)	*Fusarium* sp. (corn)	*Fusarium* sp. (bell pepper)	*Pestalotiopsis* sp. (cupuaçu)	*Rhizoctonia solani* (soybean)
Control	0	4.4 bD	4.9 aC	5.5 aB	5.9 aA	5.3 dB
Bark (adult tree)	10	4.1 bC	4.4 bC	5.4 aB	5.3 bB	7.1 aA
	20	4.1 bC	4.3 bC	5.2 aB	5.2 bB	7.0 aA
	30	4.1 bC	4.4 bC	5.0 bB	5.2 bB	5.6 dA
	40	3.7 cD	4.2 bC	4.9 bB	4.9 bB	6.7 aA
	50	3.7 cD	4.2 bC	4.9 bB	5.1 bB	7.2 aA
Leaves (adult tree)	10	4.6 aC	4.7 aC	5.2 aB	5.2 bB	6.5 bA
	20	4.3 bB	4.6 aB	5.0 bA	5.4 bA	5.0 eA
	30	4.8 aB	4.5 bC	5.1 bB	5.6 aA	6.0 cA
	40	3.6 cD	4.1 bC	4.9 bB	5.5 bA	3.9 gC
	50	4.5 aC	4.4 bC	4.9 bB	5.5 bA	5.4 dA
Leaves (young trees)	10	4.3 bC	4.5 bC	4.9 bB	5.4 bA	5.6 dA
	20	4.1 bD	4.6 aC	5.1 bB	5.8 aA	4.7 fC
	30	4.7 aC	4.4 bC	4.9 bB	5.4 bA	5.0 eB
	40	3.8 cC	4.1 bC	4.8 bB	5.2 bA	4.2 gC
	50	3.3 cC	4.1 bB	4.7 bA	5.1 bA	3.9 gB
***CV%**						4.8

*CV% = coefficient of variation. Means followed by the same lower case letters in the columns and the same upper case letters in the rows are not significantly different by the Tukey test ($p \leq 0.05$).

As for the vegetable oil test, it was found that the reduction in the diameter of the fungus colonies ranged from 0.1 to 0.5 cm for the artisanal oil and from 0.2 to 1.1 cm for the industrialized oil (Table 4).

The *C. villosum* oils analyzed accelerated the growth of *Bipolaris oryzae* and had no significant effect on *Fusarium* spp. There was a reduction in the mycelial growth of *Pestalotiopsis* sp. in the highest concentration of artisanal oil of *C. villosum*. The concentrations of 0.25, 0.75, 1.25, 2.50, and 3.75 $\mu L.mL^{-1}$ of industrialized oil significantly reduced the growth of the fungus *Pestalotiopsis* sp., with the greatest reduction being obtained in the concentration of 2, 50 $\mu L.mL^{-1}$, 9.4% inhibition (Table 4).

Except for 2.50 $\mu L.mL^{-1}$, the other concentrations of artisanal oil of *C. villosum* reduced the mean diameter of *Rhizoctonia solani*. The concentrations of 0.25, 2.50, and 3.75 $\mu L.mL^{-1}$ of industrialized oil reduced the diameter of *R. solani*, with the greatest reduction caused by the industrialized oil at 3.75 $\mu L.mL^{-1}$, with inhibition of 20.7% compared with the control (Table 4).

Oils of copaiba (*Copaifera langsdorffii* Desf.) and castor (*Ricinus communis* L.) (industrialized and artisanal) were evaluated on mycelial growth of five isolates of *Pestalotiopsis* spp. obtained from different tree species. The highest percentages of growth inhibition were observed in colonies submitted to industrialized oils, and there was a reduction of 28.9% and 5.7% for copaiba and castor beans, respectively [47]. The essential oil of *Lippia alba* (Mill) N. E. Brown showed a fungicidal effect from a concentration of 0.50 $\mu L.mL^{-1}$ to a concentration of 1.25 $\mu L.mL^{-1}$ for *Rhizoctonia solani* [48].

In this study, different results were observed for the products tested, with fungistatic effects on the growth of some phytopathogens as well as stimulation on the growth of others. This fact may be related to several factors, such as the part of the plant used, the chemical composition of the products, and the phytopathogen. Fungi may behave differently both in terms of the product used and the lethal dilution or dilution necessary to inhibit their growth [40, 49]. Some phytopathogens are capable of biotransforming compounds from plants, leading to partial inhibition of the fungitoxic effect of the extract, converging to nontoxic substances; however, it is not possible to state whether the detoxification effect can be the cause of mycelial growth stimulation in certain concentrations, when there is partial inhibition in others, requiring further research, including the characterization of the compounds for a better understanding of the mechanisms involved [44].

More in-depth tests are important to confirm the effectiveness of natural products under field conditions, as well as adjust production and dosage, and determine their toxicity to both the environment and human health [50].

Table 4. Mean diameter of plant pathogen colonies subjected to artisanal and industrialized oils of Caryocar villosum in different concentrations.

Oil	**Concentration ($\mu L.mL^{-1}$)**	**Mean diameter of plant pathogen colonies (cm)**				
		Bipolaris oryzae (rice)	*Fusarium* sp. (corn)	*Fusarium* sp. (bell pepper)	*Pestalotiopsis* sp. (cupuaçu)	*Rhizoctonia solani* (soybean)
Control	0.00	4.5 cB	5.3 aA	5.4 aA	5.3 aA	5.3 aA
Artisanal	0.25	5.3 aA	5.4 aA	5.4 aA	5.3 aA	4.8 dB
	0.50	5.3 aA	5.4 aA	5.4 aA	5.3 aA	4.9 cB
	0.75	5.3 aA	5.4 aA	5.4 aA	5.3 aA	5.2 bA
	1.25	5.4 aA	5.4 aA	5.5 aA	5.4 aA	5.1 bB
	2.50	5.3 aA	5.4 aA	5.4 aA	5.5 aA	5.4 aA
	3.75	5.5 aA	5.4 aA	5.2 aA	5.4 aA	5.2 bB
	5.00	5.3 aA	5.3 aA	5.4 aA	5.0 bB	5.2 bB
Industrialized	0.25	4.7 cC	5.4 aA	5.5 aA	5.1 bB	5.2 bB
	0.50	4.9 bB	5.4 aA	5.4 aA	5.3 aA	5.4 aA
	0.75	5.0 bB	5.4 aA	5.4 aA	5.1 bB	5.4 aA
	1.25	5.1 bB	5.4 aA	5.4 aA	5.1 bB	5.3 aA
	2.50	5.2 bB	5.4 aA	5.4 aA	4.8 cC	5.2 bB
	3.75	4.6 cB	5.3 aA	5.2 aA	5.1 bA	4.2 eC
	5.00	5.0 bB	5.3 aA	5.4 aA	5.2 aA	5.3 aA
***CV%**						2.6

*CV% = coefficient of variation. Means followed by the same lower case letters in the columns and the same upper case letters in the rows are not significantly different by the Tukey test ($p \leq 0.05$).

4. CONCLUSIONS

The flavonoid class was confirmed in *Caryocar villosum* extracts by TLC. The presence of chemical marking compounds of the genus *Caryocar*, such as fatty acids and their esters, was observed mainly in artisanal oil. None of the extracts and oils had a fungicidal effect. The reduction or increase in growth varied with plant extracts, oil type, concentration, and phytopathogen. Future research is needed to verify the isolation of substances, chemical analysis, and the spectrum of action through conducting tests *in vitro* and *in vivo* with phytopathogens.

5. BIBLIOGRAPHIC REFERENCES

1. Seepe HA, Lodama KE, Sutherland R, Nxumalo W, Amoo SO. *In vivo* antifungal activity of South African medicinal plant extracts against *Fusarium* pathogens and their phytotoxicity evaluation. Plants. 2020 Nov;(9):1668. doi: 10.3390/plants9121668
2. Grohs DS, Menezes VG, Funck GRD, Mundstock CM. Critérios para o manejo de doenças no arroz irrigado. 1. ed. Cachoeirinha (RS): Instituto Rio Grandense do Arroz (IRGA); 2010.
3. Harish S, Saravanakumar D, Radjacommare R, Ebenezar EG, Seetharaman K. Use of plant extract and biocontrol agents for the management of brown spot disease in rice. BioControl. 2008;(53):555-67. doi: 10.1007/s10526-007-9098-9
4. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae pv. oryzae*. Physiol Mol Plant Pathol. 2004 Aug;(65):91-100. doi: 10.1016/j.pmpp.2004.11.008
5. Sharma L, Marques G. *Fusarium*, an entomopathogen- A myth or reality? Pathogens. 2018 Nov;(7):93. doi: 10.3390/pathogens7040093
6. Nikitin DA, Ivanova EA, Semenov MV, Zhelezova AD, Ksenofontova NA, Tkhakakhova AK, et al. Diversity, ecological characteristics and identification of some problematic phytopathogenic *Fusarium* in soil: A review. Diversity. 2023 Jan;(15):49. doi: 10.3390/d15010049
7. Karakaya A. First report of infection of kiwi fruit by *Pestalotiopsis* sp. in Turkey. Plant Dis. 2001 Sep;(85):1028. doi: 10.1094/PDIS.2001.85.9.1028C
8. Maharachchikumbura SSN, Chukeatirote E, Guo LD, Crous PW, McKenzie EHC, Hyde KD. *Pestalotiopsis* species associated with *Camellia sinensis* (tea). Mycotaxon. 2013a Aug;(123):47-61. doi: 10.5248/123.47
9. Maharachchikumbura SSN, Guo LD, Chukeatirote E. A destructive new disease of *Syzygium samarangense* in Thailand caused by the new species *Pestalotiopsis samarangense*. Tropical Plant Pathol. 2013b Jun;(38):227-35. doi: 10.1590/S1982-56762013005000002
10. Maharachchikumbura SSN, Zhang YM, Wang Y, Hyde KD. *Pestalotiopsis anacardiacearum* sp. nov. (Amphisphaeriaceae) has an intricate relationship with *Penicillaria jocosatrix*, the mango tip borer. Phytotaxa. 2013c May;(99):49-57. doi: 10.11646/phytotaxa.99.2.1
11. Crous PW, Summerell BA, Swart L, Denman S, Taylor JE, Bezuidenhout CM, et al. Fungal pathogens of Proteaceae. Persoonia. 2011 Dec;(27):20-45. doi: 10.3767/003158511X606239
12. Maharachchikumbura SSN, Guo LD, Cai L, Chukeatirote E, Wu WP, Sun X, et al. A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Divers. 2012 Sep;(56):95-129. doi: 10.1007/s13225-012-0198-1
13. Zhang YM, Maharachchikumbura SSN, McKenzie EHC, Hyde KD. A novel species of *Pestalotiopsis* causing leaf spots of *Trachycarpus fortunei*. Cryptogam. Mycol. 2012 Sep;(33):1-8. doi: 10.7872/crym.v33.iss3.2012.311
14. Zhang YM, Maharachchikumbura SSN, Tian Q, Hyde KD. *Pestalotiopsis* species on ornamental plants in Yunnan Province, China. Sydowia. 2013 Jun;(65):113-28.
15. Maharachchikumbura SSN, Hyde KD, Groenewald JZ, Xu J, Crous PW. *Pestalotiopsis* revisited. Stud. Mycol. 2014 Sep;(79):121-86. doi: 10.1016/j.simyco.2014.09.005
16. Guerreiro-Gonzáles ML, Rodriguéz-Kessler M, Rodriguéz-Guerra R, González-Chavira M, Simpson J, Sanchez F, et al. Differential expression of *Phaseolus vulgaris* genes induced during the interaction with *Rhizoctonia solani*. Plant Cell Rep. 2011 Aug;(30):1465-73. doi: 10.1007/s00299-011-1055-5
17. Tolêdo-Souza EL, Júnior ML, Silveira PM, Filho ACC. Interações entre *Fusarium solanif*. sp. phaseoli e *Rhizoctonia solani* na severidade da podridão radicular do feijoeiro. Pesq Agropec Trop. 2009 Mar;(39):13-7.
18. Venturoso LR, Bacchi LMA, Gavassoni WL, Conus LA, Pontim BCA, Bergamin AC. Atividade antifúngica de extratos vegetais sobre o desenvolvimento de fitopatógenos. Summa phytopathol. 2011 Mar;(37):18-23. doi: 10.1590/S0100-54052011000100003
19. Spletozer A, Santos C, Sanches L, Garlet J. Plantas com potencial inseticida: enfoque em species amazônicas. Cienc Florest. 2021 Apr-Jun;(31):974-97. doi: 10.5902/1980509832244
20. Camatti-Sartori V, Magrini FE, Crippa LB, Venturin L, Silva-Ribeiro RT, Marchett C. Avaliação *in vitro* de extratos vegetais para o controle de fungos patogênicos de flores. Rev Bras Agroecologia (RBA). 2011 Apr;(6):117-22.
21. Ribeiro JEL, Hopkins JJP, Vicentini A, Sothers CA, Costa MAS. Flora da Reserva Ducke: Guia de identificação das 72 plantas vasculares de uma floresta de Terra-Firme na Amazônia Central. Manuas (AM): INPA; 1999.

22. Xavier WKS, Medeiros Jr B, Lima CS, Favacho HA, Andrade EHA, Araújo RNM, et al. Topical anti-inflammatory action of *Caryocar villosum* oil (Aubl.) Pers. J Appl Pharm Sci. 2011 May;(1):62-7.
23. Chisté RC, Freitas M, Mercadante AZ, Fernandes E. The potential of extracts of *Caryocar villosum* pulp to scavenge reactive oxygen and nitrogen species. Food Chem. 2012 Dec;(135):1740-9. doi: 10.1016/j.foodchem.2012.06.027
24. Taube Jr PS, Castro KCF, Barata LES. Experimentos de Química. Santarém (PA): UFOPA; 2014.
25. Wagner H, Bladt S. Plant drug analysis: A thin layer chromatography atlas. Berlin: Springer; 2001.
26. Nist05. MS Library and Search Program. 2; Scientific Instrument Services, Inc. Version; 2005.
27. Sousa BCM, Lustosa DC, Lourido KA, Rebelo RX, Vieira TA. Controle alternative de *Fusarium* spp. com quarto oleos vegetais. Cadernos de Agroecologia. 2016 May;(10):11542-7.
28. Ferreira DF, Software Estatístico SISVAR: versão 5.6. Minas Gerais: CNPQ/UFLA; 2010.
29. Andrade-Bustamante G, Cervantes-Díaz L, Aíl-Catzim CE, Toro-Sánchez CLdel, Borboa-Flores J, Preciado-Rangel P, et al. Potential of oregano and cachanilla extracts for the control of phytopathogenic fungi in tomato fruits. Inf Tec Econ Agr. (ITEA). 2018 Dec;(114):344-52. doi: 10.12706/itea.2018.020
30. Bezerra JCB, Silva IA, Ferreira HD, Ferri PH, Santos SC. Molluscicidal activity against *Biomphalaria glabrata* of Brazilian Cerrado medicinal plants. Fitoterapia. 2002 Aug;(73):428-30. doi: 10.1016/s0367-326x(02)00121-1
31. Moraes GV, Jorge GM, Gonzaga RV, Santos DA. Potencial antioxidante dos flavonoides e aplicações terapêuticas. Res Soc Dev. 2022 Oct;(11):e238111436225. doi: 10.33448/rsd-v11i14.36225
32. Shanley P, Serra M, Medina G. Frutíferas e plantas úteis na vida amazônica. 2. ed. rev. ampl. Brasília (DF): CIFOR; 2010.
33. Schmal B. Óleos da Amazônia os cheiros da floresta em vidrinhos: Manejo comunitário de produtos florestais não-madeireiros e fortalecimento local no município de Silves – AM. Manuaus (AM): IBAMA/PróVárzea; 2006.
34. Tostes LCL, Lins ALFA, Santos AHO, Guimarães JRS, Ferreira AMS, Dias MRL. Anatomical aspects and phytochemical potential of *Caryocar villosum* (Aubl.) Pers. (pequiá). Braz J Dev. 2019 Nov;(5):25807-29. doi: 10.34117/bjdv5n11-235
35. Lima A, Silva AMO, Trindade RA, Torres RP, Mancin-Filho J. Composição química e compostos bioativos presentes na polpa e na amêndoa do pequi (*Caryocar brasiliense* Camb.). Rev Bras Frutic. 2007 Dec;(29):695-8. doi: 10.1590/S0100-29452007000300052
36. Lima MT, Maia GA, Guedes ZBL, Oriá HF. Composição de ácidos graxos da fração lipídica do piqui (*Caryocar coriaceum* Wittm.). Ciência Agronômica. 1981;(12):93-6.
37. Oliveira MLM, Nunes-Pinheiro DCS, Tomé AR, Mota EF, Lima-Verde IA, Pinheiro FGM, et al. In vivo topical anti-inflammatory and wound healing activities of the fixed oil of *Caryocar coriaceum* Wittm. seeds. J Ethnopharmacol. 2010 May;(129):214-9. doi: 10.1016/j.jep.2010.03.014
38. Lima JR, Souza ACR, Magalhães HCR, Pinto CO. Pequi kernel oil extraction by hydraulic pressing and its characterization. Rev Bras Frutic. 2020 Jun;(42):e-456. doi: 10.1590/0100-29452020456
39. Morais SM, Catunda Jr FEA, Silva ARA, Martins Neto JS, Rondina D, Cardoso JHL. Atividade antioxidante de óleos essenciais de espécies de Croton do Nordeste do Brasil. Quim Nova. 2006 Oct;(29):907-10. doi: 10.1590/S0100-40422006000500004
40. Oliveira JD, Alves DKM, Miranda MLD, Alves JM, Xavier MN, Cazal CM, et al. Chemical composition of essential oil extracted from leaves of *Campomanesia adamantium* subjected to different hydrodistillation times. Cienc Rural. 2017;(47):e201511131. doi: 10.1590/0103-8478cr20151131
41. Roxo M, Peixoto H, Wetterauer P, Lima E, Wink M. Piquiá Shells (*Caryocar villosum*): A fruit by-product with antioxidant and antiaging properties in *Caenorhabditis elegans*. Oxid Med Cell Longev. 2020 Aug;(2020):7590707. doi: 10.1155/2020/7590707
42. Soares GL, Hilgenberg LCR, Batista GR, Souza MKJ, Ferreira RCM, Silva IS, et al. *Caryocar villosum* attenuates inflammation by inhibiting CXCL1 activation and peripheral hyperalgesia through opioid pathway modulation. Acta Amaz. 2022 Jul-Set;(52):245-53. doi: 10.1590/1809-4392202200552
43. Batista GR, Soares GL, Sousa GF, Moura CCV. Phytochemical prospection and biological activities of the Amazonian species *Caryocar villosum*. Enciclopédia Biosf. 2021 Dec;(18):22-35. doi: 10.18677/EnciBio_2021D40
44. Silva WR, Moreira-Nuñes V, Gaviria-Hernandéz V, Gonçalves VP, Azambuja RHM, Farias CRJ. Fungitoxicidade de extratos vegetais e óleo essencial de alecrim no crescimento micelial e esporulação de *Bipolaris oryzae*. Magistra, Cruz das Almas. 2018 Aug;(29):257-65.
45. Sousa BCM, Castro SP, Lourido KA, Kasper AAM, Paulino GS, Delarmelina C, et al. Identification of coumarins and antimicrobial potential of ethanolic extracts of *Dipteryx odorata* and *Dipteryx punctata*. Molecules. 2022 Sep;(27):5837. doi: 10.3390/molecules27185837
46. Lima RA, Ferreira Neto M. Atividade antifúngica do extrato etanólico dos frutos de *Solanum grandiflorum* sobre *Rhizoctonia solani in vitro*. Rev Saúde e Pesq. 2014 Jan-Abr;(7):103-8.

47. Sousa BCM, Lustosa DC, Vieira TA. Atividade antifúngica de óleos vegetais artesanais e industrializados sobre fitopatógenos de espécies florestais. Cadernos de Agroecologia. 2013 Dec;(8):14335.
48. Sousa BCM, Castro SP, Lourido KA, Chiara D, Castro KCF, Lustosa DC. Óleo essencial de erva-cidreira sobre fitopatógenos de espécies florestais e agrícolas. Agroecossistemas. 2018 Nov;(10):206-215. doi: 10.18542/ragros.v10i1.5212
49. Santos ACA, Rossato M, Serafini LA, Bueno M, Crippa LB, Sartori VC, et al. Efeito fungicida dos óleos essenciais de *Schinus molle* L. e *Schinus terebinthifolius* Raddi, Anacardiaceae, do Rio Grande do Sul. Rev Bras Farmacogn. 2010 May;(20):154-9. doi: 10.1590/S0102-695X2010000200003
50. Sousa BCM, Gomes DA, Vieira TA, Lustosa DC. Bioactivity of ethanolic extracts of *Dipteryx punctata* on *Colletotrichum musae*. Agronomy. 2022 Sep;(12):2215. doi: 10.3390/agronomy12092215