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ANTIFUNGAL POTENTIAL OF BARK EXTRACTS FROM BRAZILIAN SEMI-ARID TREE SPECIES AGAINST *Ganoderma* spp.

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Resumo

Potencial antifúngico de extratos das cascas de três espécies arbóreas do semiárido brasileiro contra Ganoderma *spp*. Espécies arbóreas de ocorrência natural da Caatinga (semiárido brasileiro) possuem características interessantes para o manejo de seus produtos madeireiros e não madeireiros, com destaque para a exploração de seus produtos químicos naturais. O objetivo deste estudo foi avaliar a ação antifúngica de diferentes extratos da casca de *Anacardium occidentale*, *Ziziphus joazeiro* e *Mimosa caesalpiniifolia* na inibição micelial *in vitro* das espécies *Ganoderma lobatum* e *G. multiplicatum*). Os experimentos foram conduzidos em laboratório, cuja metodologia foi a coleta e preparo de amostras de cascas das três espécies; preparação de extratos com extrator *Soxhlet*, com uso de água, etanol e hexano como solventes; e teste do potencial antifúngico de micélios em destruição com os extratos contra fungos do gênero *Ganoderma*. Os extratos hexânicos da casca das três espécies apresentaram atividade antifúngica a *G. multiplicatum*, diferente dos extratos aquosos e etanólicos. Não foram obtidos resultados satisfatórios dos extratos contra *G. lobatum*, apesar dos hexanânicos apresentarem uma pequena ação de destruição micelial do fungo.
Palavras-chave: bioquímica vegetal; ação antifúngica de extrativos; inibição micelial; *Anacardium occidentale*; *Ziziphus joazeiro*; *Mimosa caesalpiniifolia*.

Abstract

Tree native species of the *Caatinga* (a Brazilian semi-arid biome) have characteristics of interest for the use of their wood and non-wood products, especially regarding their natural chemical compounds. The aim of this study was to evaluate the antifungal action of different bark extracts of *Anacardium occidentale*, *Ziziphus joazeiro* and *Mimosa caesalpiniifolia* against *Ganoderma lobatum* and *G. multiplicatum* by *in vitro* mycelial inhibition. The extractions from the bark of the trees were carried out with a Soxhlet extractor, using water, ethanol and hexane as solvents. The potential of mycelia inhibition of each extract was tested against fungi of the *Ganoderma genus.* The hexane extracts of the bark had activity against *G. multiplicatum*, unlike the aqueous and ethanol extracts. Even though the hexane-based extracts had a small mycelial inhibition effect against *G. lobatum*, there were no satisfactory results of extracts against this fungus species.
Keywords: plant biochemistry; antifungal action of extractives; mycelial inhibition; *Anacardium occidentale*; *Ziziphus joazeiro*; *Mimosa caesalpiniifolia*.

INTRODUCTION

Caatinga is one of the most important phytogeographic domains in Brazil, mainly because its biotic characteristics have developed to be acclimated and thrive on the climatic adversities common on the Brazilian semi-arid region which is almost predominant in the Northeast portion of the country (ALBERTIN, 2021). The *Caatinga*'s diverse fauna and flora, that are highly resistant against edaphoclimatic adversities, has great importance in the Brazilian ecosystems. Furthermore, its resources, especially the plant species, are multipurpose, from energy production (firewood and charcoal) to pharmaceutical, industrial and food uses (ALMEIDA *et al*., 2022; BARROS *et al*., 2023).

In this context, several shrub-tree type and tree species present interesting characteristics for their use, ranging from their form of growth to the arrangement and development of branches, regrowth capacity and potential use of wood, as well as the importance of non-timber resources, such as the extraction of chemical compounds and fruits (MEYER-VELTRUP *et al.*, 2017; SILVA *et al.*, 2020). Among these, some tree species that stand out are the cashew tree (*Anacardium occidentale* L., Anacardiaceae), *juazeiro* (*Ziziphus joazeiro* Mart., Rhamnaceae) and *sabiá* (*Mimosa caesalpiniifolia* Benth., Fabaceae), as they have interesting characteristics for the use of their wood and non-wood products, especially for the employment of its natural chemical compounds.

The durability of wood against weathering and time is closely associated with the wood's chemical composition. The higher the extractive content is with antibiotic potential in a given organ of the tree, the greater

it is its resistance to the attack of xylophagous organisms, either by the infestation of pests and pathogens or through the deterioration of woody materials in the nutrient cycling process. The natural resistance of wood against xylophagous agents is also influenced by the environment to which it is subjected, as well as the use of wood preservatives products that prevent its deterioration by various agents (FERNÁNDEZ-COSTA, 2017). The most common cause of wood deterioration is rot, a decomposition process that is often caused by living agents, with fungi being one of the main groups responsible for wood deterioration.

Therefore, there is a need to obtain new products to ensure wood preservation against biodeterioration, especially ones produced by sustainable processes, as well as and to encourage the use and development of new technologies for forest products. Thus, the aim of this study was to evaluate the antifungal action of different bark extracts of *Anacardium occidentale*, *Ziziphus joazeiro* and *Mimosa caesalpiniifolia* against *Ganoderma lobatum* and *G. multiplicatum* by *in vitro* mycelial inhibition.

MATERIAL AND METHODS

Sample collection and preparation of extracts

A. occidentale, *Z. joazeiro* and *M. caesalpiniifolia* were selected due to their wide occurrence and distribution in the *Caatinga* biome. Young branches of three healthy plants per species were sampled and the bark of these branches was separated and cut using a blade to produce a composite sample for each species. The samples were then and took into a drying oven at 65 ± 5ºC until dehydration (BEZERRA NETO; BARRETO, 2011). Subsequently, the material (with about 10% moisture) was grinded using a knife mill (Willey type) and then sifted with a 2 mm particle selection sieve.

A Soxhlet system was used for the extraction, with distilled water, ethanol absolute PA and hexane PA as solvents in order to obtain extracts with different chemical characteristics for each species. Approximately 200 grams of bark (per species) were used for one liter of solvent. Considering the extractive contents for each of these solvents in the bark of the three arboreal species, as described by Silva *et al.* (2021), the approximate concentrations of the extracts are listed in Table 1. All treatments had a total extraction time of 2 hours each.

Table 1. Approximate concentration of extracts obtained from the three *Caatinga* species.
Tabela 1. Concentração aproximada dos extratos obtidos a partir de três espécies arbóreas da Caatinga.

Solvent	Concentration of extracts (g.L^{-1})		
	A. occidentale	***Z. joazeiro***	***M. caesalpiniaefolia***
Water	3.7	4.0	3.2
Ethanol	4.2	4.5	5.1
Hexane	6.3	7.6	6.1

Reference: Silva *et al.* (2021)

Bioassays and antifungal test with extracts and data analysis

Two fungi species of the *Ganoderma genus*, *G. lobatum* (URM 386) and *G. multiplicatum* (URM 6975) were obtained from the Mycotheca URM collection of the Federal University of Pernambuco. These fungi were subcultured in Petri dishes, with acidified Sabouraud Agar culture medium, through applications of fungal structures in the centre of the dishes using a needle-type platinum loop. The dishes were incubated in a bacteriological incubator at room temperature (25 ± 2ºC) for 15 days.

To evaluate the toxicologic potential of the extracts on the mycelial inhibition of the fungi, the antibiogram-type bioassay methodology was used (OLIVEIRA *et al.*, 2008). For each Petri dish containing a single fungal colony-forming unit (grown in the middle of the dish), which already occupied more than 1/3 of the dish, 50 µL of each extract (aqueous, ethanolic and hexane-based) was applied in a 5 mm diameter perforation in the centre of the colony.

The Petri dishes were incubated at 25 ± 2ºC for up to 15 days. Assays were performed in triplicates. After adding the extracts, their effects were evaluated by daily measurements of the inhibition halo, recording, when present, the average radius from the centre of the perforation ([larger radius + smaller radius]/2), in cm, of each triplicate.

Blank tests were also performed, using distilled water, ethanol PA and hexane PA, to assess the antifungal action of pure solvents, relating them to the results obtained with the extracts. The resulting data from the observation of halos created by pure solvents and extracts was evaluated. The analysis of variance (ANOVA) was performed using Tukey's mean test with a 95% probability. Data was also evaluated for homogeneity of variances using the Cochran test. All statistics were performed using RStudio software version 3.6.3 (R CORE TEAM, 2021).

RESULTS

The results of the antifungal activity of the pure solvents are shown in Table 2. Only hexane had activity against *G. multiplicatum*, with the other solvents not showing any inhibition effect on *G. lobatum* and *G. multiplicatum*.

Table 2. *In vitro* mycelial inhibition activity of blank test (distilled water, ethanol and hexane) against fungi of the *Ganoderma* genus.
Tabela 2. Atividade de inibição micelial *in vitro* do teste em branco (água destilada, etanol e hexano) contra fungos do gênero *Ganoderma*.

Fungus	Solvent	Active	Bigger Inhibition Halo (Days)	Inhibition Halo Diameter (mm)	Standard Deviation
G. lobatum	Water	No	-	-	-
	Ethanol	No	-	-	-
	Hexane	No	-	-	-
G. multiplicatum	Water	No	-	-	-
	Ethanol	No	-	-	-
	Hexane	Yes	1	5.8	1.472

The hexane extracts from the three arboreal species had mycelial inhibitory effect on both fungal species. However, the other extracts did not produce antibiosis results against these fungi (Table 3).

Table 3. *In vitro* mycelial inhibition activity of aqueous, ethanolic and hexane-based extracts of *Anacardium occidentale*, *Ziziphus joazeiro* and *Mimosa caesalpiniifolia* bark against fungi of the *Ganoderma* genus.
Tabela 3. Atividade de inibição micelial *in vitro* de extratos aquosos, etanólicos e hexanólicos de cascas de *Anacardium occidentale*, *Ziziphus joazeiro* and *Mimosa caesalpiniifolia* contra fungos do gênero *Ganoderma*.

Fungus	Tree Species	Solvent	Active	Bigger Inhibition Halo (Days)	Inhibition Halo Diameter (mm)	Standard Deviation
G. lobatum	*A. occidentale*	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	6.0 b	0.632
	Z. joazeiro	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	6.2 b	0.753
	M. caesalpiniifolia	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	6.0 b	0.000
G. multiplicatum	*A. occidentale*	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	11.2 a	3.710
	Z. joazeiro	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	10.7 a	2.503
	M. caesalpiniifolia	Water	No	-	-	-
		Ethanol	No	-	-	-
		Hexane	Yes	1	10.3 a	4.131

Means followed by the same letter do not differ statistically among themselves by Tukey's test ($p > 0.05$)

When visually comparing the actions of pure solvents with the results produced by the extracts, both distilled water and the aqueous extracts did not show antifungal activities, as verified with the aqueous extracts on *G. lobatum* (Figure 1) and *G. multiplicatum* (Figure 2).

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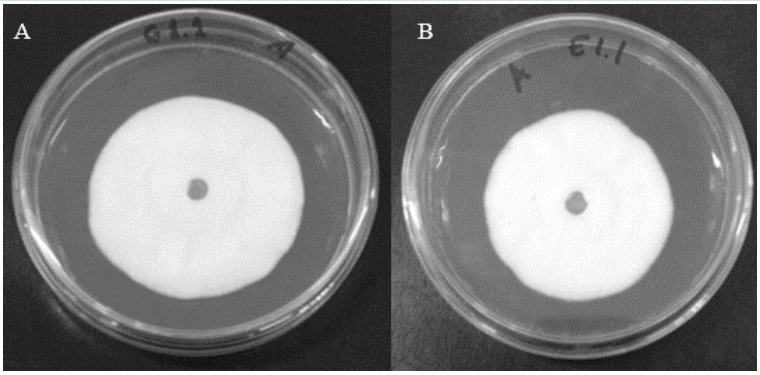

Figure 1. Action of aqueous extracts against *G. lobatum*. (A. action of only distilled water; B. action of the *Mimosa caesalpiniifolia* aqueous extract).
Figura 1. Ação dos extratos aquosos contra *G. lobatum.* (A. ação da água; B. ação do extrato aquoso de *Mimosa caesalpiniifolia*).

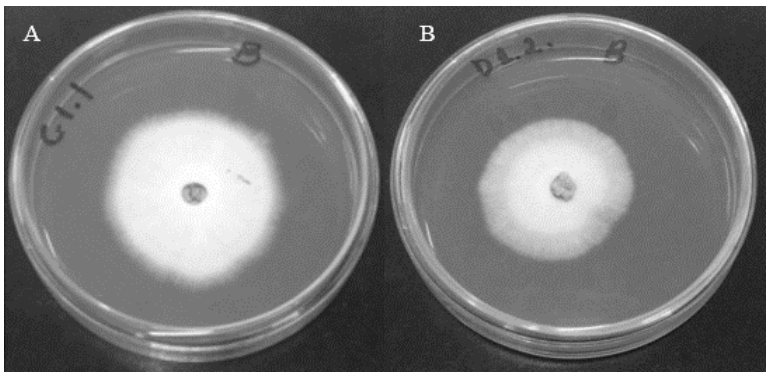

Figure 2. Action of aqueous extracts against *G. multiplicatum*. (A. action of only distilled water; B. action of the *Mimosa caesalpiniifolia* aqueous extract).
Figura 2. Ação dos extratos aquosos contra *G. multiplicatum.* (A. ação da água; B. ação do extrato aquoso de *Mimosa caesalpiniifolia*).

Both ethanol and the ethanol-based extracts did not show antifungal activities against the two fungi. This behaviour is shown in Figure 3 with the action of ethanol extracts against *G. lobatum*.

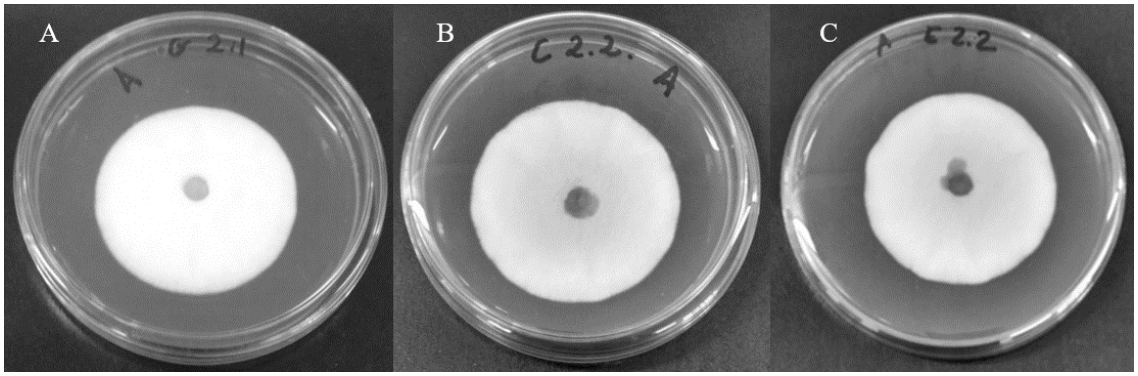

Figure 3. Action of ethanol extracts against *G. lobatum*. (A. action of ethanol only; B. action of *Anacardium occidentale* ethanol extract; C. action of *Mimosa caesalpiniifolia* ethanol extract).
Figura 3. Ação dos extratos etanólicos contra *G. lobatum.* (A. ação do etanol; B. ação do extrato etanólico de *Anacardium occidentale*; C. ação do extrato etanólico de *Mimosa caesalpiniifolia*).

The hexane-soluble extractives potentiated the solvent action and there was a greater mycelial inhibition of *G. multiplicatum* (Figure 4) than *G. lobatum* (Figure 5).

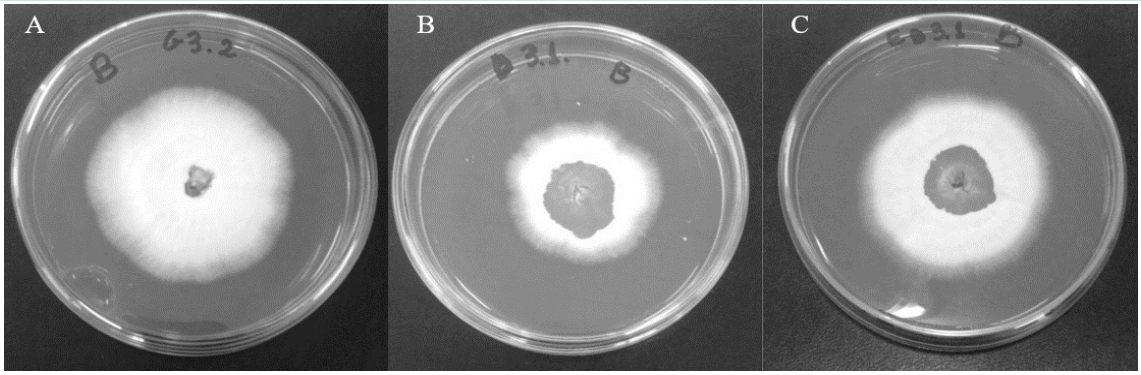

Figure 4. Antifungal activity of hexane and hexane-based extracts by the formation of inhibition halos on *G. multiplicatum*. (A. action of hexane; B. action of *Ziziphus joazeiro* hexane extract; C. action of *Mimosa caesalpiniifolia* hexane-based extract).

Figura 4. Atividade antifúngica do hexano e extratos hexanólicos pela formação dos halos de inibição sobre *G. multiplicatum*. (A. ação do hexano; B. ação do extrato hexanólico de *Ziziphus joazeiro*; C. ação do extrato hexanólico de *Mimosa caesalpiniifolia*).

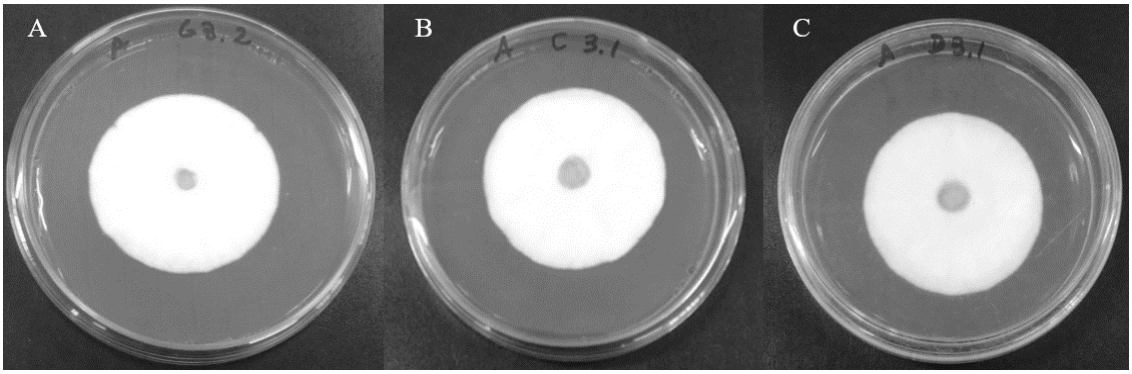

Figure 5. Antifungal activity of hexane and hexane-based extracts by the formation of inhibition halos on *G. lobatum*. (A. action of pure hexane; B. action of *Anacardium occidentale* hexane-based extract; C. action of *Ziziphus joazeiro* hexane-based extract).

Figura 5. Atividade antifúngica do hexano e extratos hexanólicos pela formação dos halos de inibição sobre *G. lobatum*. (A. ação do hexano; B. ação do extrato hexanólico de *Anacardium occidentale*; C. ação do extrato hexanólico de *Ziziphus joazeiro*).

DISCUSSION

Antifungal action against both fungi species, something that also may have occurred, essentially, due to their chemical composition. The lack of antifungal activity from ethanol-based extracts may have occurred due to the low concentration of compounds that may have antifungal properties—such as alcohols, aldehydes, ketones, ethers, among other organic compounds. Furthermore, the possible presence of chelates with nutritional value could have generate favorable conditions for fungi development (OLIVEIRA, 2008; SILVA *et al*., 2021).

The largest halo in this study was registered immediately after the application of pure hexane and hexane-based extracts on the first day of assessment. On average, the halos caused by the hexane-based extracts were about 50% larger than that of pure hexane, and this higher percentage of action was attributed to hexane-soluble compounds present in the barks of the studied species. As determined in the chemical characterization of the extracts (SILVA *et al*., 2021), the presence of tannins and saponin can have provided a certain level of antifungal activity against both fungi.

In many tests, tannins and other plant extracts are used as inhibitors of microbial development, but the vast majority of tests make use of pure essential oils for bacterial control, seeking natural antibiotics as alternatives for the food industry processes. Only a few studies have been carried out on the effect of plant extracts on mycelial growth, , being noteworthy the recent researches of Liu *et al.* (2021), Naim *et al*. (2022) and Karami-Osboo *et al*. (2023).

The higher mycelia density of *G. lobatum* prevented the displacement of the extracts, acting as a physical barrier and hindering the formation of inhibition halos. Therefore, there was no expressive antifungal

action of the extracts against *G.*, although there was little mycelial inhibition when the hexane-based extracts were applied. On the other hand, the thin mycelial structure formed by *G. multiplicatum* facilitated the action of the extracts. Thus, the difference in the action of the extracts between the two species of fungi is attributed to the distinct formation of mycelial structures.

Although some extracted compounds are toxic to plants, the use of these substances can be studied as an alternative for the treatment of diseases in plants. This fact has been observed in several studies using extracts for the treatment of fungal diseases in plants (OLIVEIRA *et al*., 2008; ROZWALKA *et al*., 2008; MAHLO *et al*., 2010; ALI *et al*., 2017; TIAIBA *et al*., 2018). In addition, these compounds are part of a group of wood preserving substances, so tests analysing them as well as the applicability of their industrial use in potential to as preservative product can be carried to aiming to minimize the employment of chemical substances to prevent the deterioration of wood products.

We recommend testing extracts of these tree species at greater concentrations, especially hexane-based extracts, in addition to the use of other tree parts as material source, such as wood, roots, leaves, fruits and seeds in order to increase the possibility of finding new active antibiotics and antifungal principles. In this study, a temperature close to 90ºC was used, which may have degraded some compounds, reducing the antifungal potential of the extracts. Thus, we also recommend testing the extraction at different temperatures to analyse the effect of this parameter on the extract efficiency to mycelial inhibition. Finally, it is necessary to test the extract's inhibition potential against other types of microorganisms, such as bacteria.

CONCLUSIONS

From the context and results presented, it can be concluded that:

- Among the evaluated extracts, only the hexane-based extracts of the bark of the three species (*Anacardium occidentale*; *Ziziphus joazeiro*; and *Mimosa caesalpiniifolia*) had antifungal activity against *Ganoderma multiplicatum*; the hexane-based extracts had halos about 50% larger than the pure hexane ones(control), showing that was indeed influence of the bark composition on the antifungal effect.
- No satisfactory action of the extracts against *Ganordema lobatum* was observed, something that could be influenced by the low concentration of the extracts, and consequently a possible low concentration of potential antifungal compounds.

REFERENCES

ALBERTIN, R. M. Domínios morfoclimáticos. In: ALBERTIN, R. M.; GUIMARÃES, D. V.; RIFFEL, E.; YEGHIAIAN, L.; SILVA, F. P.; FORNAZIEIRO, M. P. A. **Geografia física do Brasil**. Porto Alegre: SAGAH, 2021. p. 93-106.

ALI, M. A.; ALHAG, A. F. M.; ABDEL-RAHIM, A. M. Sensitivity of *Candida albicans* to aqueous and alcohol extracts of four medicinal plants. **Journal of Ayurvedic and Herbal Medicine**, Lynnfield, v. 3, n. 3, p. 133 - 138, 2017.

ALMEIDA, J. R. G. S.; OLIVEIRA, A. P.; SILVA, J. C.; SILVA, M. G.; OLIVEIRA, R. G.; LORENZO, V. P. Medicinal plants and natural products from Caatinga Biome with anti-inflammatory activity. In: THANGARAJ, P.; QUINTANS JR., L. J.; PONPANDIAN, N. **Nanophytomedicine: An emerging platform for drug delivery.** Boca Raton: CRC Press, p. 177 - 186, 2023.

BARROS, D. B.; NASCIMENTO, N. S.; SOUSA, A. P.; BARROS, A. V.; BORGES, Y. W. B.; SILVA, W. M. N.; MOTTA, A. B. S.; PINTO, J. E. L.; SAMPAIO, M. G. V.; BARBOSA, M. F. S.; FONSECA, M. C.; SILVA, L. A.; LIMA, L. O.; BORGES, M. G. S. A.; OLIVEIRA, M. B. M.; CORREIA, M. T. S.; CORREIA, M. T. S.; CASTELLANO, L. R. C.; GUERRA, F. Q. S.; SILVA, M. V. Antifungal activity of terpenes isolated from the Brazilian Caatinga: a review. **Brazilian Journal of Biology**, v. 83, e270966, 2023.

BEZERRA NETO, E.; BARRETO, L. P. **Análises químicas e bioquímicas em plantas**. Recife: Editora Universitária da UFRPE, 2011, 267 p.

FERNÁNDEZ-COSTA, C.; PALANTI, S.; CHARPENTIER, J.-P.; SANROMÁN, M. A.; MOLDES, D. A sustainable treatment for wood preservation: enzymatic grafting of wood extractives. **ACS - Sustainable Chemistry & Engineering**, Washington DC, v. 5, p. 7557 - 7567, 2017.

KARAMI-OSBOO, R.; MAHBOUBIFAR, M.; MIRABOLFATHY, M.; HOSSEINIAN, L.; JASSBI, A. R. Encapsulated *Zataria multifora*'s essential oil inhibited the growth of *Aspergillus flavus* and reduced aflatoxins levels in contaminated pistachio nut. **Biocatalysis and Agricultural Biotechnology**, v. 51, 102796, 2023.

LIU, Y.; BENOHOUD, M.; YAMDEU, J. H. G.; GONG, Y. Y.; ORFILA, C. Green extraction of polyphenols from citrus peel by-products and their antifungal activity against *Aspergillus flavus*. **Food Chemistry: X**, v. 12, 100144, 2021.

MAHLO, S. M.; MCGAW, L. J.; ELOFF, J. N. Antifungal activity of leaf extracts from South African trees against plant pathogens. **Crop Protection**, Lincoln, v. 29, p. 1529 - 1533, 2010.

MEYER-VELTRUP, L.; BRISCHKE, C.; ALFREDSEN, G.; HUMAR, M.; FLAETE, P. O.; ISAKSSON, T.; BRELID, P. L.; WESTIN, M.; JERMER, J. The combined effect of wetting ability and durability on outdoor performance of wood: development and verification of a new prediction approach. **Wood Science and Technology**, Hamburg, v. 51, p. 615 - 637, 2017.

NAIM, N.; FAUCONNIER, M.-L.; ENNHALI, N.; TAHIRI, A.; BAALA, M.; MADANI, I.; ENNAHLI, S.; LAHLALI, R. Chemical composition profiling and antifungal activity of saffron petal extract. **Molecules**, v. 27, p. 8742 – 8757, 2022.

OLIVEIRA, G. A. P.; SILVA, M. S.; RABELLO, A. R.; ALVES, A. S.; ESPÍNDOLA, L. S.; PAULA, J. E.; VIEIRA, E. A.; LIMA, T. R.; ANJOS, J. R. N. **Tecido foliar de "murici" nativo do Cerrado (*Byrsonima* sp.; Família Malphighiaceae) possui princípio antimicrobiano contra crescimento micelial in vitro de fungos patogênicos de soja**. Brasília: ParlaMundi, 2008, 5 p.

R CORE TEAM. **R: a Language and Environment for Statistical Computing**. Vienna: R Foundation for Statistical Computing, 2021.

ROZWALKA, L. C.; LIMA, M. L. R. Z. C.; MIO, L. L. M.; NAKASHIMA, T. Extratos, decoctos e óleos essenciais de plantas medicinais e aromáticas na inibição de *Glomerella cingulata* e *Colletotrichum gloeosporioides* de frutos de goiaba. **Ciência Rural**, Santa Maria, v. 38, n. 2, p. 301 - 307, 2008.

SILVA, T. C.; ARAUJO, E. C. G.; LINS, T. R. S.; REIS, C. A.; SANQUETTA, C. R.; ROCHA, M. P. Non-timber forest products in Brazil: a bibliometric and a state of the art review. **Sustainability**, Basel, v. 12, n. 7151, p. 1 - 24, 2020.

SILVA, T. C.; BRAZ, R. L.; ARAUJO, E. C. G.; SILVA, G. A.; PALHA, M. L. A. P. F. Chemical characterization of bark compounds from Caatinga species. **Floresta**, Curitiba, v. 51, n. 4, p. 893 - 899, 2021.

TIAIBA, A.; DERBALI, M.; BENDAHMAN, B. S. *In vitro* effect of crude extracts from eight Algerian steppeplants on mycelial growth and sporulation of *Ascochyta pisi* Lib. **Anales de Biología**, Múrcia, v. 40, p. 115 - 120, 2018.

WASTOWSKI, A. D. **Química da madeira**. Rio de Janeiro: Editora Interciência, 2018. 566p.