SCIENTIA NATURALIS
Scientia Naturalis, v. 3, n. 5, p. 2250-2259, 2021
Home page: http://revistas.ufac.br/revista/index.php/SciNat
ISSN 2596-1640

Chemical profile, antifungal and modulatory activity of the aqueous and ethanolic extracts of *Libidibia ferrea* (Mart.) L.P. Queiroz (Pau-Ferro)

Roberta Oliveira de Sousa[1], Marta Maria de França Fonteles[2], Tania Maria Sarmento da Silva[3]; Henrique Douglas Melo Coutinho[4], Izadora Alencar Nogueira[5], Fernando Gomes Figueredo[6*], Cícero Roberto Nascimento Saraiva[7]

[1]Discente do Centro Universitário Doutor Leão Sampaio, Curso de Biomedicina, Juazeiro do Norte, CE, Brasil, [2]Docente da Universidade Federal do Ceará, Fortaleza, CE, Brasil, [3]Docente do Departamento de Química, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil, [4]Docente do Departamento de Química Biológica, Universidade Regional do Cariri, Crato, CE, Brasil, [5]Discente da Faculdade de Medicina Estácio de Juazeiro do Norte, Curso de Medicina, Juazeiro do Norte, CE, Brasil, [6]Docente da Faculdade de Medicina Estácio de Juazeiro do Norte, Centro de Ciências da Saúde, Juazeiro do Norte, CE, Brasil, [7]Docente do Centro Universitário Doutor Leão Sampaio, Curso de Biomedicina, Juazeiro do Norte, CE, Brasil. *fgfigueredo@gmail.com

Recebido em: 26/07/2021 **Aceito em: 15/11/2021** **Publicado em: 30/12/2021**

ABSTRACT

The study aimed to evaluate the antifungal and modulatory activity of aqueous and ethanolic extracts of *Libidibia ferrea* (Mart.) L.P. Queiroz. The ripe fruits of the species Libidibia ferrea were collected at Sítio Riacho, in the municipality of Vieirópolis in the state of Paraíba. Then, the aqueous and ethanolic extracts were obtained to perform the tests. The chemical constituents were analyzed in High Efficiency Liquid Chromatography and the extracts were tested against *Candida albicans* species (CA 40006 and CA 4387) by microdilution method and for modulation, the chosen drug was Fluconazole. In the analysis of chemical constituents, hydrolysable tannins were detected. The IC50 of the aqueous extract presented a much lower value than the control with Fluconazole in front of *C. albicans*, while in front of *C. tropicalis* the products tested presented a value of IC50 higher than that of the control. In modulation, the tested products potentiated the action of Fluconazole against *C. albicans* and only the aqueous extract potentiated the antifungal effect against the *C. tropicalis* strain. With this, we have shown promise to make clinical research, in the search for compounds that serve as therapeutic alternatives to conventional antifungals, in an attempt to combat the growing fungal resistance to these drugs.
Keywords: Fungi. Medicinal plants. Antifungal resistance. *Candida albicans*. *Candida tropicalis*.

Perfil químico, atividade antifúngica e modulatória dos extratos aquoso e etanólico de *Libidibia ferrea* (Mart.) L.P. Queiroz (Pau-ferro)

RESUMO

O estudo teve como objetivo avaliar a atividade antifúngica e modulatória dos extratos aquoso e etanólico de *Libidibia ferrea* (Mart.) L.P. Queiroz. Os frutos maduros da espécie *Libidibia ferrea* foram coletados no Sítio Riacho, no município de Vieirópolis no Estado da Paraíba, após foram obtidos os extratos aquoso e etanólico para a realização dos testes. Os constituintes químicos foram analisados em Cromatografia Líquida de Alta Eficiência e os extratos foram testados frente as espécies de *Candida albicans* (CA 40006

e CA 4387) pelo método de microdiluição e para a modulação, a droga de escolha foi o Fluconazol. Na análise de constituintes químicos foram detectados taninos hidrolisáveis. A IC50 do extrato aquoso apresentou um valor bastante inferior ao do controle com Fluconazol frente a *C. albicans*, já frente *a C. tropicalis* os produtos testados apresentaram um valor da IC50 maior que a do controle. Na modulação, os produtos testados potencializaram a ação do fluconazol frente a *C. albicans* e apenas o extrato aquoso potencializou o efeito antifúngico frente a cepa de *C. tropicalis.* Com isso, foram demonstrados promissores para que sejam feitas pesquisas clínicas, na busca de compostos que sirvam como alternativas terapêuticas aos antifúngicos convencionais, numa tentativa de combater a crescente resistência fúngica a esses fármacos.
Palavras-chave: Fungos. Plantas medicinais. Resistência antifúngica. *Candida albicans. Candida tropicalis.*

INTRODUCTION

The use of medicinal plants as a therapeutic option, known as phytotherapy, is a millenary practice in the world, a rich medicinal tradition descended from the experience the population acquires with the surrounding botanical environment. The transfer of this knowledge from generation to generation encourages researchers through this empirical knowledge to an intense study for health promotion (FIRMO et al., 2011).

The secondary metabolites present in these plants give it a therapeutic power that has been confirmed with several studies in an attempt to seek effective new treatment measures for infections and bring less adverse effects to the community. Among the medicinal plants used in the popular clinic is *Libidibia ferrea* (Mart.) L.P. Queiroz belonging to the family Leguminosae found mainly in the North and Northeast of the country and popularly known as Pau-Ferro or Jucá (FRANCINEYDE et al., 2014).

Pau ferro is a large tree native of Brazil and, therefore, the Ministry of Health included the species in the National List of Medicinal Plants important for the Health System (KOBAYASHI et al., 2015). The literature describes it as presenting numerous properties such as anti-inflammatory, analgesic, antimicrobial, antiulcerogenic and, due to its wide use by the population, it seeks to carry out more and more studies to prove its therapeutic effects (DI STASI; HIRUMA LIMA, 2002; BORRÁS, 2003; CAVALCANTE, 2008). Compounds such as tannins, flavonoids, phenols, triterpenes, saponins are reported as present in the plant extract and are responsible for providing the therapeutic activity observed in the case of tannins as an action against bacterial infections (FIGUEIREDO et al., 2017).

Opportunistic fungal infections have become a challenge in the clinical reality, as they have increased greatly in recent decades and are allied to the increase in the

mortality rate due to the resistance they have been acquiring, even with the amount of antimycotics available commercially. (ABÍLIO et al., 2014; ZHANG et al., 2020)

The emergence of multidrug-resistant antifungal strains already available in the market is becoming one of the important causes of mortality in the world (MARTINI et al., 2020; CHOWDHARY et al., 2020). The genus *Candida* is the most responsible for the functions caused mainly in intensive care units (ICUs). This is what has motivated the search among the plant kingdom of substances with fungicide action that are effective and bring less adverse effects to the community (MÍMICA et al., 2009).

In view of the problem of the emergence of multidrug-resistant strains already available, it is relevant to search for natural products that can present good antifungal activity. The aim of this study is to identify the chemical profile and evaluate the antifungal and modulatory activity of aqueous and methanolic extracts of *Libidibia ferrea* (Mart.) L.P. Queiroz.

MATERIAL AND METHODS

Plant material

Mature or ripe fruits of *Libidibia ferrea* were collected in Brazil, in State of Paraíba, municipality of Vieirópolis at the *Sítio* Riacho, which is an area of the Caatinga, a Brazilian biome, in April of 2015. The species was identified by Maria de Fátima Agra, Biotechnology Department of the Federal University of Paraiba (UFPB) and deposited at the Herbarium Prof. Lauro Pires Xavier (JPB), UFPB.

Extraction

The fruits (33.53 g) of *Libidibia ferrea* were powdered and extracted with ethanol and then with water. The extracts were filtered and concentrated using a rotary evaporator to provide ethanolic (8.85 g) and aqueous (8.33 g) extracts.

Ensaios antifúngicos: preparo da solução inicial e de teste

The preparation of the initial solution of the samples was carried out by diluting 0.150g of the extracts in 1mL of dimethylsulxioxide (DMSO – Merck, Darmstadt, Germany) to obtain an initial concentration. From this concentration, dilution will be performed in sterile distilled water in order to reach the concentration of 16 384 μg/mL (test solution).

Strains and cultivation medium

The standard strain of Candida albicans INCQS 40006 and another resistant CA 4387 were obtained from the Institute of Quality Control in Health (INCQS, FIOCRUZ, RJ) and incubated in Agar Sabouraud Dextrose (SDA, KASVI) at 37^{o}C for 24h. Then, a sample of each colony was transferred to test tubes containing 3mL of sterile saline solution, and the concentration was determined using a value of 0.5 on the McFarland scale (NCCLS, 2002). Fluconazole (capsule, Prati Donaduzzi) was used as a reference drug in the commercial drug modulation test.

Inoculum

All strains were initially maintained in test tubes containing sabouraud dextrose agar (SDA) inclined, under refrigeration (8ºC), in the Microbiology Laboratory of the Leão Sampaio University Center (Juazeiro do Norte - CE, Brazil). For the tests of the Minimum Inhibition Concentration (MIC) and Minimum Fungicide Concentration (MFC), in the preparation of the fungi inoculum, initially the isolates will be cultured in SDA medium def. 37°C for 24 hours (overnight). From these, suspensions of microorganisms will be prepared in tubes containing 3mL of sterile solution (NaCl to 0.9%). Then, these suspensions will be shaken with the aid of the vortex apparatus and turbidity will be compared and adjusted to that presented by the suspension of barry sulfate of the 0.5 tube of the McFarland scale, which corresponds to an inoculum of approximately 105 colony-forming units/mL – UFC/mL (SOUZA et al., 2007).

Determination of the minimum inhibitory concentration (MIC)

The determination of MIC was performed by the microdilution technique, using plates containing 96 cavities with flat bottom and in triplicate (ELLOF, 1998; SOUZA et al., 2007). In each plate hole, a medium with 100µL of the double concentrated CSD (Cold Solvent Degreaser) liquid was added. For distribution in the microdilution plate, eppendorf ® tubes containing 1.5 mL of solution containing 1350µL of double concentrated CSD and 150µL of fungal suspension were prepared. The plate was filled in the numerical direction by adding 100µL of this solution in each well (96-well plate) and then serialmicrodilution was carried out with the 100 µL solution of the natural product (JAVADPOUR et al., 1996).

For distribution in the microdilution plate, eppendorf ® tubes containing 1.5mL of solution containing 1350µL of double concentrated CSD and 150µL of fungal suspension were prepared. The plate was filled in the numerical direction by adding 100µL of this solution in each well (96-well plate) and then serialmicrodilution was carried out with the 100µL solution of the natural product (JAVADPOUR et al., 1996).

Tefluconazol modulation test

The solution containing the essential oil was tested in sub-inhibition concentration (MIC/16). The volume of 100µL of a solution containing CSD, 10% of the inoculum and natural product were distributed in each well in the alphabetic direction of the plate. Soon after, 100µL of each antifungal, individually, was mixed to the first well, proceeding to microdilution in series, in a ratio of 1:1 to the penultimate cavity, the last cavity will be used for fungal growth control. Antifungal concentrations gradually ranged from 512 to 0.5µg/mL (COUTINHO et al., 2008). Dilution controls of natural products were used, where the inoculum was replaced by saline, and the sterility control of the medium. The reading was done in spectrophotometer, with wavelength of 630 nm, ELISA Termoplate®, at the Natural Products Research Laboratory (LPPN in Portuguese) of the Regional University of Cariri (URCA).

Determination of the minimum fungicide concentration (MFC)

In each well of the MIC test plate, a sterile rod was added, which after homogenizing the medium contained in the cavity, was subcultured in a Petri dish containing SDA, through the transfer of a small aliquot of the test solution (medium + inoculum + natural product) to verify cell viability. The plates were incubated at 37 °C for 24 hours, and were verified for growth or non-growth of *Candida* colonies. The MFC was defined as the lowest concentration capable of inhibiting the growth of fungal colony against the natural product (ERNST et al., 1999).

Statistical analysis

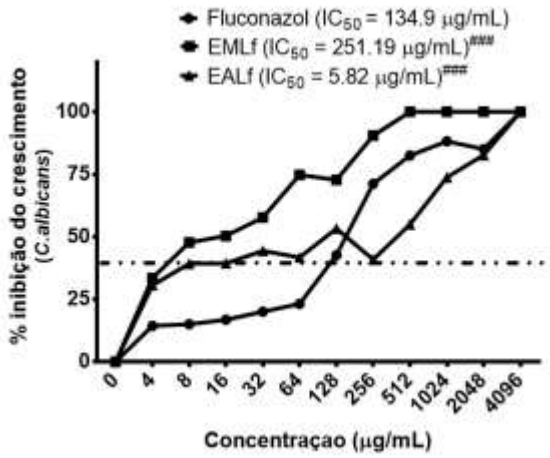
The tests were performed in triplicate and the results were expressed in geometric mean. For statistical analysis, two-way ANOVA was applied followed by the Bonferroni test, considering significance of $p \leq 0.05$.

RESULTS AND DISCUSSION

The values of minimum injunction concentrations (MIC) of aqueous and methanolic extracts of *Libidibia ferrea* (Mart.) L.P. Queiroz and the commercial antifungal Fluconazole were 4 096μg/mL, compared to the species of *Candida albicans* and *Candida tropicalis*, the same result obtained for Minimum Fungal Concentration (MFC).

Figure 1 shows the graph of the Minimum Injunction Concentrations of EELf, AELf and antifungal Fluconazole (Fluc), in front of *Candida albicans* species (CA INCQS 40006), respectively. IC50 AELf presented a much lower value than the control with Fluconazole against *C. albicans*.

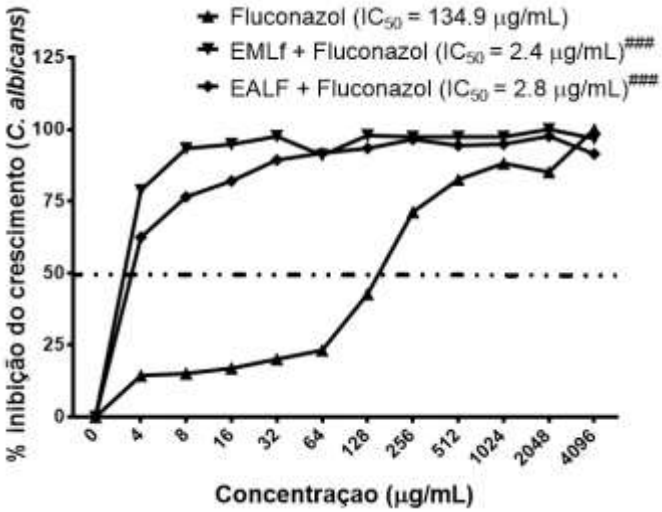
Figure 1- Minimum Inhibitory Concentration of the aqueous extracts and *Libidibia ferrea* (Mart.) L.P. Queiroz and Fluconazole against *C. albicans*



IC50 means the concentration that has managed to inhibit at least 50% of microoganisms. In literature, Rocha et al., (2019) tested the species *Hyptis martiusii* Benth (Lamiaceae) using the same methodology, and results were found to inhibit fungi similar to the present study, where the natural product was able to have greater inhibiting activity compared to *Candida albicans*. In research developed by Ferreira (2012), he observed that the aqueous extract of *Libidibia ferrea* is inhibited when being tested against *Candida albicans*.

Regarding modulation tests, the results are expressed in Figure 2, represented below. In Figure 2, the tested products enhanced the action of Fluconazole against *C. albicans*.

Figure 2- Modulatory potential of the aqueous and methanolic extracts of *Libidibia ferrea* (Mart.) L.P. Queiroz.



The uses of extracts associated with Fluconazole can potentiate the effect of Fluconazole. It is an alternative way to reduce the dose of antifungal for treatment, causing less side effects to the patient (JÚNIOR et al., 2015).

According to Figueredo et al., (2017), which characterized through UPLC/ XEVO-G2XSQTOF analysis the extracts tested in this work, a total of 22 polyphenolic compounds were identified from the ethanolextract of libidibia ferrea fruits. All of them were classified as elagitanines and galotaninos except for one, which belongs to quinic acid. The aqueous extract was analyzed 12 polyfelolics, among them elagitanines and gallotanines, except for a peak corresponding to syngic acid (FIGUEREDO et al., 2017). The mechanism of action of these compounds can be understood by their ability to precipitate proteins forming a protective layer and preventing the proliferation of microorganisms (PANSERA et al., 2003).

The combination of compounds can change the permeability of the membrane of fungal strains, favoring the intracellular passage of the antifungal, which acts by inhibiting the synthesis of ergosterol and there causing an increase in the death of the microorganism in lower concentration (JÚNIOR et al., 2015). Endo et al., (2010) reported that *Punica granatum* was able to present a synergistic action to Fluconazole, as it was also possible to observe in the present study.

Species such *Mimosa tenuiflora* and *Mimosa arenosa* are also described in the literature presenting tannins in their composition and managing to have a good antimicrobial activity (PEREIRA et al., 2015). In a phytochemical evaluation performed

by Kobayashi (2015), the presence of tannins in the fruits of the Pau-Ferro was also found, which may indicate that this compound is present in this species, regardless of the plant material used for chemical characterization tests.

According to Figueredo et al., (2017), the extracts tested were skilled and the presence of isolated substances derived from galoil quinic acid, galloyl shikimic acid, walloic acid, alagic acid and alagic acid compounds was observed.

Lagic acids have been highlighted in several studies for their potent antioxidant, anti-inflammatory, antimutagenic, antihyperglycemic, antimalarial, antimicrobial, antiplasmodic, antitumor, antibacterial and prebiotic activity. It can be used as a prevention of cardiovascular, neurodegenerative and anti-aging diseases (MAZZONE et al., 2013; SÁDECKÁ; TÓTHOVÁ, 2012; PRIYADARSINI et al., 2002; YÜCE et al., 2007; RATNAM et al., 2006; LI et al., 2015). It is also an efficient antifungal in the treatment of oral candidosis by *C. albicans* in mice, suggesting action on the cell wall (SAMPAIO, 2019).

Chichemical acid is an important intermediate compound in biochemical pathways of plants and microorganisms. Therefore, it is a pathway that originates protoalkaloids and alkaloids derived from aromatic aminoacids, hydrolysable tannins, coumarins and phenylpropanoids (ROWAN, 2011).

The walloon acid is a hydrolyzed tannin has been the target of several studies that have shown its biological activity as antihypertensive in rats (SHALABY, 2016) and efficient antifungal against *C. neoformans, C. tropicalis, C krusei, C. parapsilosis*, *T. mentagrophytes* and *T. rubrum* (SÁ et al., 2018). However, there are no reports in the literature on antimicrobial action.

CONCLUSION

With the results obtained, it is possible to conclude that the AELf and the MELF presented considerable antifungal potential against *C. albicans*, besides presenting a synergistic action when used with Fluconazole, making its action more efficient, and can be used in a concentration lower than that of the antifungal isolated. With this, the results are promising for further research to be done in the search for isolated compounds that serve as therapeutic alternatives to conventional antifungals, in an attempt to combat the growing fungal resistance to these drugs and reduce the side effects to the patient during treatment.

REFERENCES

CHOWDHARY, A.; TARAL. B.; SINGH, A.; SHARMA, A. **Multidrug-Resist**ant *Candida auris* Infections in Critically Ill Coronavirus Disease Patients, India, April–July 2020. **Emerging Infections Disseases,** v. 26, n. 11, p. 2694-2696, 2020.

BORRÁS M. R. L. **Plantas da Amazônia: Medicinais ou mágica? Plantas comercializadas no mercado Adolpho Lisboa**. Manaus-AM: Editora Valer 2003.

BRASILEIRO, B. G. PIZZIOLO, V. R.; RASLAN, D. S.; JAMAL, C. M.; SILVEIRA, D. Antimicrobial and cytotoxic activities screening of some Brazilian medicinal plants used in Governador Valadares district. **Revista Brasileira de Ciências Farmacêuticas,** v. 42, n. 2, p. 196-202, 2006.

CALIXTO JÚNIOR, J. T.; MORAIS, S. M.; MARTINS, C. G.; VIEIRA, L. G.; MORAIS-BRAGA, M. F. B.; CARNEIRO, J. N. P.; MACHADA, A. J. P.; MENEZES. I. R. A.; TINTINO, S. R.; COUTINHO, H. D. Phytochemical Analysis and Modulation of Antibiotic Activity by Luehea paniculate Mart. & Zucc. (Malvaceae) in Multiresistant Clinical Isolates of Candida Spp. **Biomed Research International**, v. 2015, p-1-12, 2015.

CAVALCANTE, R. **As plantas medicinais na Odontologia**: um Guia prático. Rio Branco-AC: Expressão Gráfica , 2008.

MARTINI, C.; TORELLI, GROOT, T.; CAROLIS, E. D.; MORANTOTTI, G. A.; ANGELIS, G. D.; POSTERARO, B.; MEIS, J. F.; SANGUINETTI, M. Prevalence and Clonal Distribution of Azole-Resistant *Candida parapsilosis* Isolates Causing Bloodstream Infections in a Large Italian Hospital. **Frontiers in Celular Infection Microbiology**, v. 10, n. 232, p. 1-11, 2020**.**

COUTINHO, H. D. M.; COSTA, J. G. N.; SIQUEIRA-JUNIOR, J. P.; LIMA, E. O. In vitro anti-staphylococcal actvity of *Hyptis martiusii* Benth against methicillin-resistant Staphylococcus aureus-MRSA strains. **Brazilian Journal of Pharmacognosy,** v. 18, (supl.), p. 670-675, 2008.

DI STASI, L. C.; HIRUMA-LIMA C. A. **Plantas medicinais na Amazônia e na Mata Atlântica**. São Paulo-SP: Unesp; 2002. 604 p.

ELLOF, J. N. A sensitive and quick microplate method to determined the minimal inhibitory concentration of plant extracts for bacteria. **Planta Medica,** v. 64, p, 711-713, 1998.

ENDO, E. H.; CORTEZ, D. A. G.; EUDA-NAKANURA, T.; NAKAMURA, C. V.; DIAS-FILHO, B. P. Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against *Candida albicans*. **Research in Microbiology**, v. 161, p. 534-540, 2010.

ERNST E. J.; KLEPSER, M. E.; ERNST, M. E.; MESSER, S. A.; PFALLER, M. A. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. **Diagnostic Microbiology and Infectious Disease,** v. 33, n. 2, p. 75-80, 1999.

FIGUEREDO, F. G.; SILVA, T. M. S.; AMORIM CAMARA, C.; SILVA, G. R.; FÁTIMA AGRA, M.; CAVALCANTE, M. R.; FRANÇA FONTELES, M. M. **Antimicrobial activities of natural products from *Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz var. *Ferrea*. Synthesis,** Properties and Biological Activities, Ed. Collins E**.** New York: Nova Science Publishers, p. 115, 2017.

FERREIRA, M. R. A. **Triagem antifúngica de extratos obtidos de espécies vegetais do Nordeste Brasileiro**. 2012, 190 f. Dissertação. (Mestrado em Ciências Farmacêuticas) - Universidade Federal do Rio Grande do Norte, Natal, 2012.

FRANCINEYDE, A. S; FBIO S. B. S; LEONOR, C. M. Biotechnical application of arbuscular mycorrhizal fungi used in the production of foliar biomolecules in ironwood seedlings [*Libidibia ferrea* (Mart. ex. Tul.) L.P. Queiroz var. *ferrea*]. **Journal Of Medicinal Plants Research**, v. 8, n. 22, 814-819, 2014.

JAVADPOUR, M. M., JUBAN, M. M., LO, W. C. J., BISHOP, S. M., ALBERTY, J. B., COWELL, S. M., MCLAUGHLIN, M. L. De novo antimicrobial peptides with low mammalian cell toxicity. **Journal of Medicinal Chemistry**, v. 39, n. 16, p. 3107-3113. 1996.

KOBAYASHI, Y. T. S.; ALMEIDA, V. T.; BANDEIRA, T.; ALCÂNTARA, B. N.; SILVA, A. S. B.; BARBOSA, W. L. R.; ALMEIDA, M. B. Avaliação fitoquímica e potencial cicatrizante do extrato etanólico dos frutos de Jucá (*Libidibia ferrea*) em ratos Wistar. **Brazilian Journal of Veterinary Research and Animal Science**, v. 52, n. 1, p. 34-40. 2015.

MÍMICA, L. M. J.; UEDA, S. M. Y.; MARTINO, M. D. V.; NAVARINI, A.; MARTINI, I. J. Diagnóstico de infecção por Candida: avaliação de testes de identificação de espécies e caracterização do perfil de suscetibilidade. **Jornal Brasileiro de Patologia e Medicina Laboratorial,** v, 45, n. 1, p. 17-23, 2009.

PANSERA, M. R., SANTOS, A. C. A., PAESE, K., WASUM, R., ROSSATO, M., ROTA, L. D., SERAFINI, L. A. Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul. **Revista Brasileira de Farmacognosia**, v. 13, p. 17-22, 2003.

PEREIRA, A. V. AZEVEDO, T. K. B.; SANTANA, G. M.; TREVISAN, L. F. A.; HIGINO, S. S. S.; MACÊDO COSTA, M. R.; Azevedo, S. S. Analise da atividade antimicrobiana de taninos totais de plantas aromáticas do Nordeste brasileiro. **Revista Agropecuária Técnica**, v. 36, n. 1, p. 109-114, 2015.

REZENDE, H. A.; COCCO M. I. M. A utilização de fitoterapia no cotidiano de uma populacao rural, **Revista da Escola de Enfermagem da USP**, v. 36, n. 3, 2002.

ROCHA, J. E. COUTINHO, H. D. M.; SARAIVA, C. R. N.; DOS SANTOS, A. T. L.; MACHADO, A. J. T.; JÚNIOR, J. T. C.; COLARES, A. V. HPLC-DAD analysis and antifungal effect of Hyptis martiusii Benth (Lamiaceae) against Candida strains. **Asian Pacific Journal of Tropical Biomedicine**, v. 9, n. 3, p. 123, 2019.

ROWAN, D. D. Volatile metabolites. **Metabolites**, v. 1, n. 1, p. 41-63, 2011.

SAMPAIO, A. G.: **Avaliação do potencial de aplicação do ácido elágico/nanoencapsulado em ciclodextrina no tratamento de candidose bucal**. 2019, 100 f. Dissertação (Mestrado em Biotecnologia Bucal) - Universidade Estadual Paulista, São José dos Campos, 2019.

SOUZA, E. L.; STAMFORD, T. L. M.; LIMA, E. O.; TRAJANO, V. N. Effectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts. **Food Control**, v.18, n. 5, p. 409-413, 2007.

SÁ, S.; CHAUL, L. T.; ALVES, V. F.; FIUZA, T. S.; TRESVENZOL, L. M.; VAZ, B. G.; PAULA, J. R. Phytochemistry and antimicrobial activity of Campomanesia adamantium, **Revista Brasileira de Farmacognosia**, v. 28, p. 303-311, 2018.

FIRMO, W. C. A.; MENEZES, V. D. J. M.; CASTRO PASSOS, C. E.; DIAS, C. N.; ALVES, L. P. L.; DIAS, I. C. L.; OLEA, R. S. G. Contexto histórico, uso popular e concepção científica sobre plantas medicinais. **Caderno de Pesquisa,** v. 18, 2011.

MING-RUI, Z.; ZHAO, F.; WANG, S.; LV, S.; MOU, Y.; YAO, C. L.; LI, F. Q. Molecular mechanism of azoles resistant *Candida albicans* in a patient with chronic mucocutaneous candidiasis. **BMC Infectious Diseases,** v. 20, n, 1, p. 1-6, 2020.