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Review

# New antimicrobial therapies used against fungi present in subgingival sites—A brief review

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ABSTRACT

Although the main reservoir of *Candida* spp. is believed to be the buccal mucosa, these microorganisms can coaggregate with bacteria in subgingival biofilm and adhere to epithelial cells. The treatment of periodontal disease includes scaling and root planning (SRP) associated with proper oral hygiene. However, some patients may have negative responses to different therapeutic procedures, with a continuous loss of insertion, so the use of antimicrobials is needed as an adjuvant to SRP treatment. The use of a broad-spectrum antibiotic, such as tetracycline and metronidazole, as an aid in periodontal treatment has also been a factor for the development of superinfections by resistant bacteria and *Candida* species, even in patients with HIV. In the dental practice, the most commonly used antifungals are nystatin and fluconazole. However, the introduction of new drugs like the next generation of azoles is essential before the onset of emergent species in periodontal disease. Plants are good options for obtaining a wide variety of drugs. This alternative could benefit a large population that uses plants as a first treatment option. Plants have been used in medicine for a long time and are extensively used in folk medicine, because they represent an economic alternative, are easily accessible and are applicable to various diseases. Herein, we briefly review the literature pertaining the presence of *Candida* sp. in periodontal pockets, the conventional antifungal resistance and new therapies that include natural antifungal agents are reviewed.



## Contents



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## 1. Introduction

*Candida* species are commensal yeasts in healthy humans and may cause systemic infection under immunocompromised situations due to its high adaptability to different host miches. It was suggested that when *C. albicans* accessed the periodontal tissues, they may be harmed by the production of metabolites by these yeasts.[1]

The periodontal disease is a chronic infection that affects the gingiva and bone that supports the teeth. This chronic inflammatory disease results from the response to microrganisms in dental biofilm and may remain confined to the gingival tissues with minimal tissue alterations; alternatively, this disease may progress to extreme periodontal destruction with the loss of attachment and alveolar bone. In addition to the presence of periodontal pathogens; such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*; genetic and environmental factors seem to increase the susceptibility of some individuals in developing this severe inflammatory disease.[2] Therefore, there is general support for this concept of periodontal disease. It is also well recognized that the presence of just pathogenic bacteria is insufficient to cause periodontitis. Progression of this disease occurs due to a combination of factors, including the presence of periodontopathogenic microorganisms, high levels of pro-inflammatory cytokines, matrix metalloproteinases (MMPs), prostaglandin E2 (PGE2), low levels of anti-inflammatory cytokines including interleukin-10 (IL-10), transforming growth factor (TGF-β) and tissue inhibitors of MMPs (TIMPs).[3,4] However most microorganisms found in subgingival biofilm is commensal, or also occurs in individuals with a healthy periodontium that is in equilibrium with the host. Thus, episodes of disease resulting from deficiencies in the ability of the host defence to fight the bacterial biofilm, changes the quantitative or qualitative subgingival microbiota.[5,6]

Periodontal diseases are classified as either gingivitis or periodontitis. Gingivitis is an infection associated with biofilm, characterized by an occurrence of reversible inflammatory phenomena limited to the gingival tissues and not affecting the supporting periodontal tissues. Clinical signs of gingivitis range from oedema, abnormal staining of normal gums, unusual redness, and loss of normal contour of gingival.[7] Periodontitis is defined as an inflammatory disease that involves the tissues of dental support, leading to irreversible alveolar bone resorption and destruction of the collagen fibres of the periodontal ligament. The severity and progression of this disease is influenced by local or systemic conditions or from both a combination of both.[8] Local factors are associated with poor oral hygiene, the presence of cavities, and the presence of dentures that can cause plaque accumulation. Systemic factors are related to the metabolism of the host, immunosuppressive therapy, malnutrition, diabetes mellitus and HIV infection (Tables 1 and 2).[7]

A complex microbiota can be found in the periodontal pocket affected by the disease, where approximately 500 species of bacteria can occur, amongst them are *A. actinomycetemcomitans*, and other microorganisms such as *Entamoeba* sp., virus, some Enterobacteriaceae, *Pseudomonas* sp., and *Candida* species.[9–12] This fact has led to an extensive study of microbiological samples from periodontal lesions, especially in cases where there is a poor response to conventional treatment. In many of these cases, fungi have been found colonizing the periodontal pockets. There are several reports associating the occurrence of severe periodontitis with the isolation of *Candida* species from periodontal lesions.[13,14] However, the clinical significance of these observations and the role of microorganisms in the pathogenesis of periodontal diseases are not well understood. Many scientific investigations have been performed in order to extend the knowledge in this area.[15] However, the presence of fungi in periodontal pockets has not yet received the necessary focus to understand its role as periodontal pathogens, although they have been recognized for their ability to adhere to the epithelium, express virulence factors and induce inflammatory reactions.[16]

The treatment of periodontal disease includes scaling and root planning (SRP) associated with proper oral hygiene. However, some patients may have negative responses to different therapeutic procedures, with a attachment loss, so the use of antimicrobials is needed as an adjuvant to SRP treatment.[17] The use of a broad-spectrum antibiotic, such as tetracycline and metronidazole, as an aid in periodontal treatment has also been a factor for the development of

**Table 1 – Data on resistance of *Candida* spp. isolated from subgingival sites.**

| Isolates | % Azoles resistance | % Amphotericin resistance | References |
|---|---|---|---|
| *C. albicans* | 4.2 | 0 | Waltimo et al.[64] |
| Non-*C. albicans* | 6.25 | 6.25 | Ito et al.[62] |
| *C. albicans* | 3.3 | 0 | Ito et al.[62] |
| Non-*C. albicans* | 17 | No studied | Jewtuchowicz et al.[56] |
| *C. albicans* | 0 | No studied | Jewtuchowicz et al.[56] |
| Non-*C. albicans* | 42.8 | 71.4 | Furletti et al.[61] |
| *C. albicans* | 3.6 | 72.9 | Furletti et al.[61] |

**Table 2 – *In vitro* antifungal activity of natural extracts on *Candida* species (μg/mL), according Höfling et al.[94]**

| | AC | MP | SC | TA | RO | PG | Fluconazole |
|---|---|---|---|---|---|---|---|
| *C. albicans* | 15 | 7 | 1 | 3 | 1 | 3 | 32 |
| Non-*C. albicans* | 15–30 | 1–7 | 1 | 1–15 | 1–7 | 1–3 | 32–64 |

AC, *Arrabidae chica*; MP, *Mentha piperita*; SC, *Syzygium cumini*; TA, *Tabebuia avellanedae*; RO, *Rosmarinus officinalis*; PG, *Punica granatum*.

superinfections by resistant bacteria and *Candida* species.[18,19] The use of a broad-spectrum antibiotic, such as tetracycline and metronidazole, as an aid in periodontal treatment associated with SRP has been recommended for the treatment of periodontal disease.[18,19]

Metronidazole and amoxicillin antibiotics to be seem indicated for the treatment of periodontal infections. Lotufo et al.,[20] performed antimicrobial susceptibility tests *in vitro* for 105 strains of anaerobic bacteria isolated from patients with periodontitis. According to the results, the antimicrobial metronidazole was more action on the organism studied. None of the isolates showed resistance to metronidazole. Amoxicillin also showed good results, with approximately 94% of strains sensitive to this drug.

In the dental practice, the most commonly used antifungals are nystatin and fluconazole. It is believed that the presence of *C. albicans* in subgingival sites is in the form of biofilms, which could explain the resistance to antifungal therapy.

Plants are good options for obtaining a wide variety of drugs.[21] This alternative could benefit a large population that uses plants as a first treatment option.[22] Plants have been used in medicine for a long time and are extensively used in folk medicine, because they represent an economic alternative, are easily accessible and are applicable to various diseases.[23] Therefore, these constitute an excellent alternative in the search for substances that can be used to develop new antifungal drugs.[24] It is necessary to seek new antifungal agents that are fungicides, which cause disruption or destruction of biofilms, which are effective in isolates that express resistance using several molecular mechanisms and which are not toxic.

In the present report, the literature on the presence of *Candida* spp. in periodontal pockets, the conventional antifungal resistance and new therapies that include natural antifungal agents are reviewed.

## 2. Colonization of the oral cavity by *Candida* spp.

Based on their prevalence in healthy and asymptomatic populations, the isolation of *Candida* spp. from the oral cavity does not necessarily imply an infection.[25] Many studies have shown that approximately half of the healthy adult population carries yeasts in the oral mucosa, however, the prevalence has been found to vary amongst different population groups.[25,26] Several different groups present levels of oral colonization by yeasts larger than the average population in general, with these groups being known at-risk populations.[27] Studies report a higher prevalence of *Candida* species in patients with Down's syndrome, in individuals with salivary gland hypofunction, decreased flow or salivary pH and diabetes mellitus. These conditions seem to alter the oral environment and promote colonization by these and other species of opportunistic pathogens.[28] The occurrence of these fungi has also been reported in HIV-positive patients, with rates of infection that are higher than in other at-risk populations.[29] The increasing proportion of these fungi suggests a deficient immune response associated with the progression of viral infection in HIV-infected individuals, which could be a predictive factor for the development of candidiasis.[28] The occurrence of yeast is also common in patients with advanced cancer with oral candidiasis a serious medical condition amongst these patients. Epidemiological studies are therefore needed on the distribution and virulence potential of these yeasts in different population groups, addressing risk factors and developing strategies for the control and prevention of infections.[27,30,31] Yeasts are found colonizing various sites in the oral cavity (lingual, palate, tonsils, mucosa of the lips and cheeks, caries, periodontic and endodontic lesions).[32–35] Siqueira and Rôças[35] found *C. albicans* species associated with bacteria in teeth with periodontal pockets around areas of root exposure. For those authors, resistance to intra canal drugs, and the ability of these yeasts to colonize and invade the dentine tubules, may explain the presence of yeast in persistent endodontic infections.

The use of a prosthesis is another factor that may favour colonization of the oral cavity by *Candida* spp.[36] with a report indicating that the microbiota between a prosthesis and palate mucosa has a composition similar to dental biofilm, except for a greater proportion of *Candida* species, a fact related to the development of candidiasis on the mucosa of the palate.

Kleinegger et al.[37] concluded that a number of natural barriers existed in the mucosal surfaces and body fluids; preventing the colonization in healthy individuals. These barriers are more or less effective, depending on factors related to age, gender, smoking, diet, drugs and the host immune status. This explains the fact that not all individuals harbour *Candida* spp.

Saliva helps maintain oral health, provides a buffering capacity and provides lubrication of the mucous membranes; therefore, qualitative and quantitative changes in saliva inevitably affect the physiology, defence mechanisms and microbial ecology of the mouth.[38] Lactoferrin and lysozyme are two proteins in the innate immune response present in saliva and exert an antifungal modulating effect on the implantation of species of *Candida* in the oral cavity.[39] Other important proteins in human saliva that have a cytotoxic action on bacteria and fungi are the histatins, estaterins, lactoperoxidase and calprotectin.[37] According to Lin et al.,[40] when there is a decrease when the concentration of salivary histatins, dysfunction of these proteins occurs and candidiasis tends to manifest. HIV-infected individuals show a reduction in salivary flow and an anti *Candida* activity of saliva and are often suffering from oropharyngeal candidiasis. For those authors, the saliva contained mucins and aggregated IgA, histatin, lactoferrin and lysozyme, which remained focused on mucosal surfaces and exerted an antimicrobial effect.[41] *C albicans* is able to connect to several species of streptococci (*S. oralis*, *S. sanguinis*, *S. gordonii*, and *Fusobacterium*) through recognition receptor polysaccharides in the bacterial cell surface. *F. nucleatum* is an anaerobic gram-negative bacillus commonly associated with periodontal lesions and isolated from subgingival biofilm.[41,42] Biofilm formation has been considered an important strategy for microbial survival and proliferation in the oral environment. The complex structure of a biofilm allows microorganisms to offer protection against the antimicrobial mechanisms of saliva and hinder the action of antimicrobial agents.[43] It is believed that most of the

manifestations of candidiasis are associated with biofilm formation, and recognition of the biofilm features may help in developing therapeutic strategies for these infections.[44] For the current author, the biofilms formed by *C. albicans* and *C. dubliniensis* have several features in common with bacterial biofilms, including the structural heterogeneity and reduced susceptibility to antimicrobial agents when mature. These biofilms consist of a mixture of yeast and filamentous cells embedded in a matrix of exopolymers, which serves as a reservoir for the release of infective organisms in the oral cavity. This can allow the survival of yeast in their ecological niches during infectious episodes, which, according to Ramage et al.,[44] has important clinical, treatment and prevention implications. Thus, the biofilms containing mostly *C. albicans* could be implicated, not only in mucosal candidosis, but also in the development of caries[45] and in the pathogenesis of periodontal disease.[46,47]

### 2.1. *Candida spp. in subgingival sites*

*Candida* species possess virulence factors relevant in the pathogenesis of periodontal disease, such as the ability to adhere to the epithelium and invade the gingival connective tissue, the ability to inhibit the function of polymorphonuclear neutrophils, and produce enzymes such as collagenases and proteinases which degrade immunoglobulins.[32,47–49] According Hägewald et al.,[33] microorganisms that are capable of degrading IgA may acquire a selective advantage in the colonization of oral surfaces. Those authors believe that the proteolysis of immunoglobulins facilitates the penetration and spread of potentially toxic substances or antigens released by the subgingival microbiota. That process could perpetuate inflammatory changes associated with destructive periodontal diseases. The periodontal alterations have been considered a result of an exacerbated immune response against the host tissues, with changes in cellular and humoral immune responses that allow different species, such as *Candida*, to colonize the subgingival environment.[50] The detection of fungi in the subgingival region has been suggested to contribute to the pathogenesis of periodontal disease and to increase the possibility of candidiasis, mainly in cases of immune depression.[32,46] However, the role of yeasts, mainly *Candida albicans*, in chronic periodontitis is yet unclear.[51]

Isolation of subgingival *Candida* species has been described by many authors in different manifestations of periodontal disease, for example, in cases of aggressive periodontitis,[33] chronic periodontitis,[9,49] in periodontal pockets of smokers,[52] diabetics,[9,53] myelosuppressed patients[54] and in perimplantitis. Certain *Candida* species are considered to be commensal organisms within the oral cavity. Indeed, the prevalence of oral yeast in the general population is about 34%.[54] In 24 patients with acute periodontal infection and chemotherapy-induced myelosuppression, microorganisms were detected in high concentrations in subgingival pockets with a predominance of *Staphylococcus epidermidis*, *C. albicans*, *S. aureus*, and *Pseudomonas aeruginosa*, with combinations of these detected in some patients.[54]

Raber-Durlacher et al.,[55] addressed the pathogenesis of periodontal disease and the possibility of transmission of systemic subgingival microorganisms in patients with cancer treated with chemotherapy. Those authors reported that oral infections are larger problems, mainly because there is a higher risk of infections spread from microorganisms of the mouth during the neutropenia occurring after chemotherapy. Thus, the inflamed periodontal tissues may act as a focus of infection, bringing significant morbidity and, in some cases can become life-threatening. Still, there is evidence that gingivitis and periodontitis are associated with fever and sepsis in these patients, because the ulcerated epithelium of periodontal pockets may serve as a route of entry of microorganisms into the bloodstream, and the propagation of systemic endotoxins and other inflammatory mediators.

Jewtuchowicz et al.[56] identified different species of yeasts using conventional mycological methods and specific polymerase chain reaction (PCR) assays from samples at sites of periodontal disease isolated from immunocompromised patients, such as those with advanced HIV infection. Amongst 76 fungal organisms isolated, *C. dubliniensis* comprised 10.5% of total, which corresponded to 4.4% of patients studied. *C. albicans* was the most frequently isolated species of yeast.

However, Sardi et al.[9] detected some species of *Candida*, using the PCR method, in higher quantities in diabetic patients when compared with non-diabetic patients with chronic periodontal disease. *C. albicans* were found in 57.3%, *C. dubliniensis* in 75.6%, *C. tropicalis* in 15.85% and *C. glabrata* in 4.87% of the periodontal pockets of diabetic patients. For non-diabetic patients, 19.17% and 13.69% of the periodontal sites presented *C. albicans* and *C. dubliniensis*, respectively. *C. tropicalis* and *C. glabrata* were not found in the periodontal pocket of non-diabetic patients. Urzúa et al.[57] analysed the composition of the yeast microbiota present in the mucosal and subgingival sites of healthy individuals and patients with aggressive and chronic periodontitis, using phenotypic and genotypic methods. Despite the varied profiles of the species present in the mucosa of the three groups analysed, only *C. albicans* and *C. dubliniensis* were capable of colonizing the periodontal pockets in patients with chronic periodontitis, whilst only *C. albicans* was identified in the subgingival sites of healthy individuals and patients with aggressive periodontitis.

Periodontal conditions were studied in two cross-sectional studies of adult, insulin-dependent diabetics and age- and sex-matched controls. In one study, 154 diabetics and 77 control patients participated. In the other study, 82 diabetics and 99 control patients took part. The number of individuals exhibiting severe periodontal disease was superior in the diabetic group than in the control group.[58] However, a relationship between diabetes mellitus, periodontal disease and the presence of *Candida* spp was not found. Additionally, the moderately increased glucose content of diabetic patients did not result in higher mean numbers of *C. albicans*. Similar results were obtained by Yuan et al.,[53] who verified that there were no significant differences in the prevalence of the some microorganisms, including *C. albicans*, between the diabetic and the non-diabetic groups.

Järvensivu et al.[47] investigated the occurrence and extent of penetration of *C. albicans* in periodontal tissues of patients with chronic periodontitis in gingival tissue specimens collected during periodontal surgery. These specimens were examined by immunohistochemistry using specific antibodies to *C. albicans*; the presence of hyphae penetrating the

periodontal tissue was observed. Those authors suggested that an environmental change may have promoted the germination of hyphae that have a greater capacity to adhere to host tissues, and that the crevicular fluid and periodontal pockets formed a favourable environment for germination of these morphological structures. *C. albicans* could then play a role in the infrastructure of the subgingival biofilm, and their adherence to the periodontal tissues, since they are more resistant to immune mechanisms that most microorganisms present at that location.

Barros et al.[49] studied *Candida* species in the periodontal pockets of chronic periodontitis patients without systemic changes; the most prevalent species was *albicans* with only one isolate of *C. dubliniensis*.

Cuesta et al.[59] analysed patients with periodontal disease and found 25.6% of *Candida* species with *C. albicans* as the most prevalent at 76.2%.

However, one of the factors related to a lack of response to periodontal therapy is the failure to eliminate the reservoirs of infectious organisms, or the appearance of superinfecting pathogens such as *Enterobacteriaceae*, *Pseudomonas* sp., *Staphylococcus* sp. and *Candida* species.[60] The treatment of periodontal disease includes SRP associated with proper oral hygiene. It has been shown that these procedures are essential for successful periodontal therapy, reducing pocket depth and eliminating periodontal microbiota.[60] However, some patients may have negative responses to different therapeutic procedures, so the use of antimicrobials is needed as an adjuvant treatment SRP.[17] The use of a broad-spectrum antibiotic, such as tetracycline or metronidazole, as an aid in periodontal treatment has also been a factor for the development of superinfection by resistant bacteria and *Candida* ecies.[18,19] The use of an antifungal is needed but many of these *Candida* spp. present in periodontal pockets are resistant to existing drugs, necessitating the search for natural alternatives.[56,61–64]

### 2.2. *Resistance of yeasts to synthetic antifungals*

In the treatment of fungal infections, oral antifungal drugs are administered. The most common antifungal medications are the azoles. However, this treatment becomes quite limited due to resistance problems and significant efficacy of drugs. Currently, the therapeutic practice covers a limited number of antifungals such as amphotericin B, fluconazole, itraconazole and more recently, voriconazole, although others also show promising results, such as posaconazole, ravuconazole, caspofungin and micafungin.[65]

The conventional treatment of periodontal disease is usually effective, except in cases of proven resistance to isolates. Some studies show the inefficiency of therapy depending on the selection of populations of the genus *Candida*. In these cases, the isolate of *Candida* spp. reports of resistance or treatment failure are attributed to the difficult access of antifungal drugs in these sites.[60]

In the dental practice, the most commonly used antifungals are nystatin and fluconazole.[63,65,66] Antifungal agents such as amphotericin B, 5-fluorocytosine, voriconazole and terbinafine are not usually employed in the treatment of oral candidiasis; however, they also deserve attention. Although these antifungals are available only for systemic use and are recommended for the treatment of disseminated infections, the determination of a minimum inhibitory concentration with respect to isolates from the oral cavity of patients with immunosuppression is important, especially in cases of periodontitis, for obtaining epidemiological data and the possibility of the oral cavity being the original focus of disseminated fungal infections.[62,67]

Waltimo et al.,[64] whilst evaluating the antifungal susceptibility amongst isolates of *C. albicans* in periodontal pockets, showed that 100% of these isolates were sensitive to amphotericin B and 5-flucytosine.

However, sensitivity to azole antifungals was shown to be variable. This fact corroborates with recent data that indicates an increasing azole resistance amongst *Candida* species, suggesting that the oral cavity, seems to be a major factor in the increased frequency of *C. albicans* and other non-albicans.[68–70] Dumitru et al.[71] studied isolates of *C. albicans* under conditions of hypoxia and found strains resistant to amphotericin B and four azole antifungal classes, thus concluding that these anaerobic yeasts were more resistant to antifungal drugs; thus explaining the resistance of biofilms of *C. albicans* to several antifungal drugs. Perkholfer et al.[72] evaluated anidulafungin and voriconazole alone and in combination against conidia and hyphae under conditions of hypoxia in isolates of *Aspergillus* and observed that anidulafungin exhibited excellent activity against conidia and hyphae of *Aspergillus* sp. The reading of the MIC for anidulafungin was optimal under hypoxic conditions. Results presented by Furletti et al.[61] showed that *C. albicans* isolated from periodontal pockets were resistant to amphotericin B and sensitive to fluconazole. This result shows that there may be difficulty in the infusion of the drug in the periodontal space or resistant strains may be due to overexpression of efflux genes.

Jewtuchowicz et al.[56] studied isolates of *C. albicans* and *C. dubliniensis* in patients with and without periodontitis and consistently found that only one isolate was resistant to fluconazole and voriconazole. *C. albicans* appears to be contributing to bacterial biofilm formation on these structures and hindering the penetration of certain antimicrobial drugs.[47] Still, for these studies, *C. albicans* was found typically in the outer layers of the biofilm, and seemed to act according as expected, as a barrier protecting the microorganisms of the deep interior from the action of immune mechanisms, aiding the resistance of the subgingival microbiota in the face of host defences, and contributing to the persistence of inflammation in adjacent tissues. Biofilms of *C. albicans* were highly resistant to the clinical action of antifungal and antimicrobial agents, including amphotericin B, chlorhexidine, nystatin and fluconazole.[72] In that work, it was demonstrated that as the *C. albicans* biofilms matured, there was concurrent acquisition and increased resistance of yeast cells in relation to the antimicrobials. The prophylactic use of fluconazole in low doses has been recommended for preventing fungal infections in immunocompromised patients. However, this has led to the selection of yeast microbes that are resistant to this antifungal agent, causing this resistance to appear in non-albicans species such as *C. glabrata* and *C. krusei*, in addition to having the sensitive *C albicans* be replaced by another of the same species that is fluconazole-resistant and to become resistant

to fluconazole during treatment.[73,74] From a clinical standpoint, the most important feature of biofilm growth is the resistance to antimicrobial agents exhibited by organisms.[75,76] Studies have shown that biofilms formed by *Candida* species were more resistant to major antifungal agents used in the clinic, such as amphotericin B, fluconazole, itraconazole, and ketoconazole.[77] New azoles, like voriconazole and ravuconazol, were also ineffective against biofilms.[52,78–81] The intrinsic resistance of *Candida* species biofilms to fluconazole, an agent commonly used for antifungal treatment due to greater resistance to amphotericin B, has been reported.[81] However, therapeutic levels of echinocandins may inhibit the metabolic activities in *C. parapsilosis* biofilms,[82–84] whilst lipid formulations of amphotericin B also showed activity against the same biofilms.[77,81] The progression of drug resistance by *Candida* biofilms has been associated with the parallel increase of the maturation process.[85] Furthermore, some researchers have also shown that biofilms of *Candida* developed statically with the presence of minimal matrix and exhibited the same level of resistance to drugs (fluconazole and amphotericin B) as the cells grown in a lab and exhibiting large amounts of matrix.[86] Therefore, there are many controversies regarding the mechanisms of resistance to antifungal agents. In addition to the reduced sensitivity described by some authors in periodontal disease, it is believed that the presence of *C. albicans* in subgingival sites allows the formation of biofilms, which could explain the resistance to antifungal therapy. Several molecular mechanisms of resistance to antifungal agents in *C. albicans* have been described, highlighting in particular: the increased efflux of antifungal agents due to the over expression of efflux genes, CDR1, CDR2 (the family of ABC membrane transport proteins – ATP Binding Cassette)[87] and MDR1 (family protein Major Facilitator); the amino acid substitutions in Erg11p enzyme (lanosterol 14-α desmetilase), encoded by the gene ERG11. This gene in turn can be expressed in cells with super changes in several of the biosynthetic pathways for ergosterol, as no formation of the toxic metabolite 14-α metilergosta-8, 24-diene-3 β, α 6-diol metabolite from 14 α-metilfecosterol due to changes in the ERG3 gene.[87–89] Considering it essential to understand how genes are regulated, CDR1 and CDR2 and other genes are often co-regulated and are over-expressed simultaneously, therefore it is believed that there is a chance of mutations in genes regulating this expression.[90]

### 2.3. *Search of new natural antifungal drugs*

A search for new antifungal agents and the characterization of new targets which are more appropriate and efficient, including the emergence of resistant strains, has been proposed.[91] An ideal antifungal agent should have broad-spectrum antifungal activity and would not cause toxicity to the host.[62] Plants are good options for obtaining a wide variety of drugs.[21] Plants have been used in medicine for a long time and are extensively used in folk medicine because they represent an economic alternative, are easily accessible and would be applicable to various pathologies.[23] These constitute an excellent alternative for substances that can be used in the formulation of new antifungal agents.[24] The antifungal compounds of the plants assayed are not well known; however, the presence of flavonoids and terpenes and a certain degree of lipophilicity might determine toxicity by the interactions with the membrane constituents and their arrangement. Since plants produce a variety of compounds with antimicrobial properties, it is expected that screening programmes for some under-represented targets, such as antifungal activity, may yield candidate compounds for developing new antimicrobial drugs.[92] In addition, it is expected that plant compounds showing targets sites other than those currently used by antibiotics will be active against drug-resistant microbial pathogens.[93] Some extracts and essential oils of medicinal plants with antifungal activity were investigated by various researchers. Hofling et al.[94] observed activity against strains of *C. albicans* (CBS-562), *C. dubliniensis* (CBS-7987), *C. parapsilosis* (CBS-604), *C. tropicalis* (CBS-94), *C. guilliermondii* (CBS-566), *C. utilis* (CBS-5609), *C. krusei* (CBS-573), *C. lusitaniae* (B-060), *C. glabrata* (B-07), and *C. rugosa* (B-12) with the extracts of *Mentha piperita*, *Arrabidaea chica*, *Rosmarinus officinalis*, *Tabebuia avellanedae*, *Syzygium cumini* and *Punica granatum*.

The yeast *C. albicans*, frequently associated with infections in HIV (+) patients, was the most sensitive amongst all tested microorganisms. *Lippia sidoides* essential oil showed an appreciable amount of monoterpenes, a therapeutical potential that should not be ignored, and its phenolic compounds (thymol and carvacrol) showed activity against oral pathogens.[92] Duarte et al.[95] investigated the essential oils and ethanol extracts obtained from 35 medicinal plants for activity against *C. albicans* and found that 13 of them showed antifungal activity. The oil of *Achillea millefolium*, *Mikania glomerata* and *Stachys byzantina* all had a strong activity against *C. albicans*, whilst *Aloysia triphylla*, *Anthemis nobilis*, *Cymbopogon martini*, *Cyperus articulates*, *Cyperus rotundus*, *Lippia alba*, *Mentha arvensis* and *M. piperita* presented moderate activity. The essential oil obtained from the leaves of *Coriandrum sativum* showed antifungal activity against established biofilm and planktonic cells of *C. albicans* isolated from periodontal pockets.[96] More et al.[97] isolated *C. albicans* from periodontal pockets and found that six of these (*Annona senegalensis*, *Englerophytum magalismontanum*, *Dicerocarym senecioides*, *Euclea divinorum*, *Euclea natalensis*, *Solanum panduriforme* and *Parinari curatellifolia*), had an action on these organisms. Additionally, they also indicated that eight species of plants from South Africa had action against bacteria periodontipathogenic and cytotoxicity in Vero cell lines. Scorzoni et al.[22] indicated that there was contact of the crude extracts derived from EtOAc and EtOH *Kielmeyera rubriflora*, in addition to the commercial drug fluconazole, against yeast *C. krusei* and subsequent protein analysis by two dimensional electrophoresis. Several changes in protein expression were observed and both extracts were effective in inhibiting the expression of protein *C. krusei*, suggesting the existence of specific targets. In another study of Pterogyne nitens (Fabaceae), the antifungal activity of compounds from the plant and the substance purified pedalitina was able to inhibit the adhesion of *Cryptococcus neoformans* to lung epithelial cells with similar efficiency to conventional drugs. Additionally, changes were observed in protein expression after contact with this substance, showing a considerable activity in this yeast proteome.[98] Gregio et al.[99] evaluated the antimicrobial

properties of ginger extract against microorganisms found in the oral cavity, such as *Streptococcus mutans*, *Staphylococcus aureus*, *E. coli* and *C. albicans*, and observed action from the extracts glycol and hidroalccolicos; however, the ethanol extract and essential oil showed no antimicrobial activity effective under some experimental conditions.

## 3. Conclusion

The interest in natural medicine has increased remarkably in recent years as a result of side effects of conventional drugs, as well as the emergence of antimicrobial resistance to available drugs. Plants and their derivatives are therefore an important alternative in the search for new drugs. For the development of specific drugs for the treatment of periodontal disease caused by coinfection by *C. albicans*, further studies should be conducted on the proteins participating in biosynthetic pathways of vital components for this agent.

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