Conventional therapy and new antifungal drugs against *Malassezia* infections

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Abstract

Malassezia yeasts are commensal microorganisms occurring on the skin of humans and animals causing dermatological disorders or systemic infections in severely immune-compromised hosts.

Despite attempts to control such yeast infections with topical and systemic antifungals, recurrence of clinical signs of skin infections as well as treatment failure in preventing or treating *Malassezia furfur* fungemia suggest the occurrence of resistant phenomena. Standardized methods for *in vitro* antifungal susceptibility tests of these yeasts are lacking, thus resulting in variable susceptibility profiles to azoles among *Malassezia* spp. and lack of clinical breakpoints. The inherent limitations to the current pharmacological treatments for *Malassezia* infections both in humans and animals stimulated the interest of the scientific community to discover new, effective antifungal drugs to treat these infections. In this review, data about the *in vivo* and *in vitro* antifungal activity of the most commonly employed drugs (i.e., azoles, polyenes, allylamines and echinocandins) against *Malassezia* yeasts, mainly those causing human bloodstream infections, are summarized and their clinical implications discussed. In addition, the usefulness of alternative drugs is presented.

Keywords: *Malassezia,* therapy, fungemia, skin diseases, essential oils, plant extracts, peptide, antifungal susceptibility test, antifungal resistance.

Introduction

Malassezia yeasts are commensal organisms that occur on the skin of human and animals, but they may become pathogenic under the influence of predisposing factors, such as gene-induced variation, environmental conditions, lifestyle, hygiene, and the immune system (**Theelen et al., 2018**).

Since the designation of the genus *Malassezia* by Baillon in 1889, its taxonomy has been updated. Presently the genus comprises 18 liphophilic species, with *Malassezia furfur*, *Malassezia sympodialis*, *Malassezia globosa* and *Malassezia restricta* as the main agents of human dermatological disorders in immunocompetent patients, *Malassezia pachydermatis* as the mains agent of dermatitis or otitis of pet animals, and *M. furfur*, *M. pachydermatis* and *M sympodialis* as the hitherto reported agents of systemic infections in severely immunocompromised hosts (**Prohic et al, 2016; Ilahi et al., 2017; Pedrosa et al., 2018; Guillot and Bond, 2020; Rhimi et al., submitted for publication**). Clinical manifestations include various forms of dermatitis in humans and animals, i.e. pityriasis versicolor (PV), seborrheic dermatitis (SD), atopic dermatitis (AD) and *Malassezia* folliculitis (MF) in humans, dermatitis or otitis in animals, and nosocomial bloodstream infections in humans (BSI, **Velegraki et al., 2015; Theelen et al., 2018; Saunte et al., 2020; Rhimi et al., submitted for publication**). Despite attempts to control such yeast infections with topical and systemic antifungals, recurrent infections are often noticed, especially in human and animal patients with dermatitis (**Negre et al., 2009; Hald et al., 2015; Bajwa, 2017; El-Housiny et al., 2018; Saunte et al., 2020)**. Moreover, the induction of *in vitro* fluconazole (FLZ) resistance in *M. pachydermatis* (**Jesus et al., 2011; Cafarchia et al., 2012**) as well as the clinical evidence of treatment failure with itraconazole (ITZ) in dogs, terbinafine (TER) in PV patients, FLZ and flucytosin in human BSI (**Gupta et al. 2004**; **Iatta et al., 2014; Choudhury et al., 2014; Al-Sweih at al., 2014; Angileri et al., 2019; Chen et al., 2019; Bond et al., 2020**), suggest the probable occurrence of resistance phenomena in these species. In particular, mis-sense mutations in the ERG11 as well as overexpression of ERG4 and ERG11 genes were proposed to be associated with ketoconazole

(KTZ) resistance in *M. pachydermatis* (**Kim et al., 2018; Kano et al., 2019b**). Mutations in the same gene were described in field isolates with tolerance to ravuconazole (**Kano et al., 2019a**) and in miconazole-resistant clones of CBS1879 (the neotype culture of *M. pachydermatis*) selected by serial passage on miconazole supplemented media (**Kano and Kamata, 2019**).

In addition, resistance phenomena were also considered after a synergic effect of the combination of FLZ or voriconazole (VOR) observed with modulators or inhibitors of drug efflux pumps (i.e., haloperidol and promethazine) in *M. furfur* strains (**Iatta et al., 2017**). Thus far, *in vitro* susceptibility testing for *Malassezia* spp. has not yet been standardized, neither by the Clinical and Laboratory Standards Institute (CLSI) nor by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (**Arendrup et al., 2014**), likely because of slow growth, lipid dependency and a tendency to form cell clusters, thus resulting in the unavailability of clinical breakpoints (CBPs). Many published data describe a variable susceptibility to azoles among *Malassezia* spp. most likely due to different test conditions (e.g. culture medium, inoculum size and end-point determination). In addition, reports that describe clinical failure of treatment of *Malassezia* infection are starting to appear, but the studies comparing *in vivo,* and *in vitr*o antifungal susceptibility are still scant. The limitations inherent to the current pharmacological treatments of *Malassezia* infections both in humans and animals supported the interest of the scientific community to discover new, effective antifungal drugs to treat *Malassezia* related infections. Thus, different essential oils or plant extracts, chelating agents, and peptides have been found useful *in vitro* and *in vivo* against these organisms and their infections (**Sharma et al., 2012; Nardoni et al, 2014; 2016; Cafarchia et al., 2014; Ryu et al., 2011; Polonelli et al., 2012**; **Hensel et al., 2009; Dessinioti et al., 2013; Cavana et al., 2015; Mahboubi, et al., 2017; Chan et al., 2019**). In this review, data about the *in vivo* and *in vitro* antifungal activity of the most commonly employed drugs (i.e., azoles, polyenes, allylamines and echinocandins) against *Malassezia* yeasts are summarized and their clinical implication discussed. In addition, the usefulness of alternative drugs is presented. Specific focus will be on the three *Malassezia*

species relevant to human bloodstream infections: *M. pachydermatis*, *M. furfur* and *M. sympodialis*.

Material and Methods

A Medline (PubMed) search of articles published in English from 2000 till to date was performed using the keywords "*Malassezia*", "skin diseases", "pytiriasis versicolor", "Seborheic dermatitis", "fungemia", "therapy", "azole resistance" "chelating agents", "peptide", "essential oil" and "plant extracts". The articles were reviewed concerning *Malassezi*a conventional or alternative therapies in immunocompetent and immunosuppressed patients**.**

Conventional therapy of *Malassezia* infection

Three classes of antifungals (i.e., azoles, polyenes and echinocandins) are mainly used to manage all types of fungal infections. Azoles and polyenes, which includes amphotericin B (AmB), are frequently employed to treat *Malassezia* infections both in humans and animals. Echinocandins, such as caspofungin or micafungin, were launched in the early 2000's but are not frequently used against *Malassezia* species, In particular, the low sensitivity of basidiomycetous yeasts to echinocandins should be considered mainly because they are used for initial therapy of fungal infections for patients with a recent history of exposure to azoles and who suffer from moderately severe or severe illness (**Chen et al., 2014**). In this respect, their use in preventing fungal infection in hospitalized patients could maybe be selective for *Malassezia*, leading to *Malassezia* bloodstream infections.

In vivo studies for current therapy of *Malassezia* infection

Specific guidelines for the treatment of *Malassezia* dermatitis/otitis have been published both for animals (**European Scientific Counsel Companion Animal Parasites ESCAAP guideline, 2011**) and humans (**Hald et al., 2015**) and they were extensively reviewed until now (**Negre et al., 2009; Hald et al., 2015; Clark et al., 2015; Saunte et al., 2020)**. Recently, the World Association of Veterinary Dermatology

commissioned the development of clinical consensus guidelines for the diagnosis and treatment of *Malassezia* dermatitis in dogs and cats (**Bond et al., 2020**). Concerning *Malassezia* dermatitis in dogs, there was strong evidence for twice-weekly use of a 2% miconazole/2% chlorhexidine shampoo to treat these infections. Moderate evidence was available for a 3% chlorhexidine shampoo (**Bond et al., 2020**). The employment of oral KTZ (5-10 mg/kg, once or twice daily) and oral ITZ (5 mg/kg, once daily or two consecutive days per week) for the duration of 3 weeks was also indicated for treatment of severe infections with moderate evidence (**Bond et al., 2020**). Different studies point out the efficacy of pulse administration of 5 mg/kg of ITZ or 30 mg /kg of TER for a minimum of 3 weeks for treating *M. pachydermatis* dermatitis both in cats and dogs (**Bond et al., 2020**). For the treatment of *Malassezia*-related human skin diseases, studies recommended that PV and SD patients might be sufficiently treated with topical agents (e.g. KTZ, Ciclopirox olamine, Miconazole, Clotrimazole, TER, Selenium sulphide, Zinc pyrithione, Propylene glycol) but to prevent relapses maintenance therapy is normally suggested (**Hald et al., 2015; Saunte et al., 2020)**. Topical application of KTZ shampoo (twice weekly) or miconazole cream (twice daily) are useful treatment options for PV and SD in humans (**Hald et al., 2015; Saunte et al., 2020)**. Terbinafine 1% cream, but not oral terbinafine, is also effective IN PV patients (**Saunte et al., 2020**). For treatment of widespread PV lesions and when topical treatment seems insufficiently effective, systemic therapy with FLZ (400 mg/day for at least 2 weeks) or ITZ (200 mg/day up to 3 weeks) may be considered. The effect of these two agents seems to be similar but FLZ is generally preferred for PV and MF, and ITZ for SD (**Theelen et al., 2018)**. Oral TER seems not effective for treatment of PV possibly because of a more uneven distribution at the skin surface (**Gupta et al., 2004a; 2004**). Recently, a review study on SD suggested that topical antifungal and anti-inflammatory agents are useful to ameliorate SD symptoms. Systemic therapy with ITZ (200 mg; once daily × 7 days, then once daily × 2 days/month for maintenance) or TER (250mg

once daily × 4–6 weeks or 12 days monthly × 3 months) is needed only in cases with widespread lesions and in cases that do not respond to topical treatment (**Borda et al., 2019**).

Finally, systemic catheter-related *Malassezia* infections are usually treated with catheter removal, administration of systemic antifungal therapy, and in some cases by discontinuation of the lipid infusion (**Table 2, Arendrup et al., 2014).** Intravenous AmB proved to be useful to control blood stream infections in preterm infants and adults (see **Table 1**). Therapy with azoles, mainly FLZ and POS, may represent an alternative option, but clinical studies suggest their failure to prevent or treat *Malassezia* fungemia (see **Table 1**). There are no reports yet about treatment of *Malassezia* infections, of any kind, with echinocandins likely because they are not active against basidiomycetes yeasts.

Antimicrobial susceptibility profiles of *Malassezia* spp.

The susceptibility of *Malassezia* to antifungal compounds has been tested using a modified Clinical and Laboratory Standards Institute (CLSI) broth micro-dilution protocol and Agar-based diffusion methods, such as Disk Diffusion (DD) and the E-test (ET). The agreement analysis between agar-based diffusion methods and modified CLSI standard reference procedures suggested that DD might not represent a valid alternative to determine azoles and AmB antifungal susceptibility of *Malassezia* yeasts, with the exception of *M. pachydermatis* (**Rojas et al., 2016; Watanabe et al 2014; Pasquetti et al., 2011; Yurayart et al., 2013**). In contrast, concordance between ET and CLSI BMD methods for azoles and AmB was reported in different studies for *M. pachydermatis*, but only when specific media or a longer reading time (i.e., 72h) was employed (**Velegraki et al., 2004; Cafarchia et al., 2012; Álvarez-Pérez et al., 2014)**. Concerning antifungal susceptibility profiles of *Malassezia* yeasts, mainly species causing *Malassezia* fungemia, and *Malassezia globosa*, were evaluated in this review, comparing data on minimum inhibitory concentration **(**MIC) of drugs most commonly employed to treat dermatitis and /or fungemia

obtained by using a modified CLSI broth micro-dilution test. As is well known, the culture medium, inoculum size, incubation time, and criteria used to determine MIC endpoints differ widely among studies, since no reference method has been developed for these yeast species (**see Tables 2, 3, 4, 5**). Regardless of the media or other conditions employed, the *Malassezia* antifungal susceptibility profiles against azoles AmB and TER vary between species but also a substantial amount of intraspecies variation was observed. Some of the higher MIC-ranges were specifically observed for *M. furfur* and *M. globosa* (**Rojas et al., 2016; Carrillo-Munoz et al., 2013; Iatta et al., 2014; Cafarchia et al., 2012 - Tables 3, 4, 5,6**). ITZ, POS and KTZ generally were most active against all tested *Malassezia* species, and FLZ, and AmB were found to be the less active (**Tables 2, 3, 4, 5**). In particular, wide MIC ranges and more intra-species variation to FLZ, and AmB were registered for *M. furfur*, *M. sympodialis*, and *M. globosa* (**Velegraki et al., 2004, Rojas et al., 2014**). In addition, the MIC values for FLZ, ITZ of *M. furfur* isolates from blood stream infected (BSI) patients were generally higher than those obtained from human skin diseases (**Yurayart et al., 2013; Carrillo-Munoz et al., 2013; Rincon et al.,2006; Velegraki et al., 2004; Miranda et al, 2007; Cafarchia et al., 2015**), thus suggesting that the clinical source of the *Malassezia* isolates might be pivotal in strain susceptibility (**Yurayart et al., 2013; Carrillo-Munoz et al., 2013; Rincon et al.,2006; Cafarchia et al., 2015)**. As all BSI isolates originated in Italy and were tested in one laboratory, geographical and laboratory-dependant methodology factors could potentially also play a role in the observed differences. The susceptibility for VOR is highly variable within the *M. furfur* strains and sometimes the MIC values are higher than those previously reported for other fungi (i.e., *Candida* spp. and/or *Aspergillus* spp.), thus suggesting a lower efficacy of this drug for this yeast species (**Cafarchia et al., 2015; Miranda et al, 2007; Arendrup et al., 2014; Rodriguez-Tudela et al., 2008; Pfaller et al., 2012**). With few exceptions (**Brilhante et al., 2018**), AmB is very active against *M. pachydermatis* (**Table 5**), whereas it is less active against some *M. furfur* isolates that were obtained both from skin infected and BSI patients (**Velegraki et al., 2004; Rojas et al., 2014; Iatta et al,**

2015; Rojas et al., 2016; Sharma et al., 2017). The antifungal activity is also linked to the AmB formulations used in the tests (**Iatta et al., 2014**). The higher antifungal activity of liposomal AmB (l-AmB) compared to the deoxycholate AmB (d-AmB) might be due to the lipophilic nature of this yeast (**Iatta et al., 2015**). A higher efficacy of AmB was recorded for *M. furfur* strains coming from patients pre-treated with FLZ, which most likely was the result of a synergic effect of azoles with AmB, suggesting that the combination of FLZ plus AmB might be more effective towards faster clearance of the BSI **(Iatta et al., 2015)**. The variations in susceptibility among the *Malassezia* species to TER were greater if compared to the azole drugs (see **Tables 2, 3, 4, 5**), with *M. furfur* being less susceptible than *M. sympodialis* and *M. pachydermatis* (see **Tables 2, 3, 4**). The observed *in vitro* susceptibility values of *Malassezia* to echinocandins suggest that representatives of this genus are intrinsically resistant to these antifungals. Indeed, MIC values >32mg/l were often recorded for *M. pachydermatis* and *M. furfur* regardless of the employed CLSI protocol (**Prado et al., 2008, Yurayart et al., 2013; Al-Sweih et al., 2014**).

***In vitro* susceptibility of *Malassezia* species and clinical outcome**

The correlation of antifungal susceptibility with clinical outcome for patients infected by *Malassezia* yeasts is rarely reported and deserves further investigation. Preliminary results show that FLZ resistance (i.e., high MIC values, usually MIC >64 mg/L) correlates well with a poor clinical response. It has been found that *M. furfur* strains from human patients developing *M. furfur*-related BSI after receiving FLZ prophylaxis (3mg/kg) presented high MIC values regardless of the applied media for testing the in vitro susceptibility (**Iatta et al., 2014**). On the contrary, the highest MIC values for AmB detected in *M. furfur* strains derived from patients with a positive clinical outcome with AmB therapy alone or followed by FLZ, suggest that the protocols employed (i.e., media, reading time as well inoculum concentration) to test the AmB *in vitro* susceptibility may have been inappropriate (**Iatta et al., 2015**). However, the evidence of high in *vitro* activity of AmB against *M. pachydermatis* by using the same methodology suggests that *M. furfur* might express low sensitivity to this drug and that

the AmB susceptibility may be related to the yeast species (**Iatta et al., 2015**). Similar results (i.e., AmB MICs> 2mg/l) were obtained by other authors using the same *Malassezia* species but different methods (**Valegrakiet al., 2004; Rojas et al., 2014**). Variations in quantity or type of sterols in cell membranes, as well as inhibition of the oxidative action of AmB (i.e., high activity of fungal intracellular catalase and/or superoxide dismutase) might explain the poor AmB susceptibility of *M. furfur* isolates (**Iatta et al., 2014; Valegrakiet al., 2004; Rojas et al., 2014**). Incongruences observed between clinical outcome and *in vitro* results need to be further investigated. In part, these findings might be attributed to the employment of additional drugs such as FLZ that act synergistically with AmB in resolving fungemia (**Iatta et al., 2015**). Even when further collaborative studies are urgently needed to correlate the *in vitro* results with clinical outcomes, the collection of data herein presented, suggests that the high FLZ and VOR MICs might indicate that they are not a good treatment option for *M. furfur* skin or bloodstream infections and that the ITZ is the preferred drug to treat skin infections or BSI.

Alternative approaches for antifungal therapies of *Malassezia* infections

Because of the alarming increase of fungal infections and the appearance of drug resistance phenomena in pathogenic fungi, the scientific community started investigating alternative antifungal strategies. The most promising alternative approaches are the employment of chelating agents, peptides, "drugs" that target fungal virulence factors, and plant-derived products (**Bernal et al., 2013**; **Mehra et al., 2012**). A number of these alternative agents have shown to be active both *in vivo* and *in vitro* against *Malassezia* yeasts and their infections.

Antimicrobial adjuvants *against Malassezia* spp.

Antimicrobial adjuvants can act in different ways, namely as inhibitors of enzymes that inactivate drugs, efflux pump inhibitors, bacterial membrane permeabilizers, biofilm dispersers, inhibitors of antibiotic resistance elements, and inhibitors of bacterial cell physiological pathways (**Bernal et al., 2013**). In particular, the *in vitro* antimicrobial activity of N-acetylcysteine, Tris-EDTA and disodium EDTA against

M. pachydermatis strains isolated from cases of canine otitis externa has been recently demonstrated (**Chan et al., 2019**). N-acetylcysteine was never tested against *Malassezia* yeasts before, but it is a known mucolytic agent with bactericidal activity inhibiting the uptake of amino acids, such as cysteine by bacterial cells, or it reacts with the sulfhydryl group of bacterial cell proteins. N-acetylcysteine is used as a flushing solution to physically remove biofilm as part of the management of canine otitis externa (**Chan et al., 2019**) and thus should be considered in the management of otitis caused by *M. pachydermatis*.

Chelating agents, such as Tricide or Tris EDTA, alter the permeability of the cell wall of microorganisms by removing divalent cations, and have shown an excellent antifungal activity against *M. pachydermatis* as well as synergistic activity with drugs (i.e., miconazole, chlorhexidine, climbazole) commonly employed to treat *Malassezia* infection in dogs (**Hensel et al., 2009; Cavana et al., 2015; Chan et al., 2019; Bond et al., 2000**). However, the small number of animals tested, and the absence of control groups makes these results very preliminary and call for more detailed studies. In addition, these drugs were never tested against other *Malassezia* species and also the potential use in human *Malassezia* diseases should be further explored and validated in clinical trials.

Peptides against *Malassezia* spp.

Antifungal peptides (AFPs) are small, predominantly positively charged protein fragments, which exert direct and indirect antifungal activities through different modes of action, many causing the permeabilization of the fungal membrane. Many different categories of AFPs with various modes of action have been described so far and are the ongoing subject of further research. They include lysozyme, defensins, cathelicidins, lactoferrin and novel synthetic peptides (**Mehra et al., 2012**). Lysozyme is an enzyme classically known for its bactericidal activity. Lysozyme is active against yeasts such as *Candida* spp. and *Aspergillus* spp. but preliminary studies on *Malassezia* spp. revealed its inefficacy to control population of these yeasts most likely due to the *Malassezia* membrane or cell walls composition (**Murai et al.,**

2004; Hoq and Ibrahim 2011). Beta-defensins (BDs) and cathelicidin (Cath) are antimicrobial skin peptides produced by cutaneous and mucosal epithelial cells as well as mast cells (**Mehra et al., 2012**). They attach to microorganisms based upon a high affinity for anionic molecules present on the microbes surface leading to membrane damage, disruption of the ion gradient, and subsequent death of the micro-organism (**Mehra et al., 2012**). It has been shown that both *M. furfur* and *M. pachydermatis* are able to stimulate the production of BDs and Cath by human keratinocytes, indicating that the innate immune response of keratinocytes might have a role in controlling such infections (**Lopez-Garcia et al., 2006**; **Brasch and al., 2014**; **Angrisano et al., 2013; Buommino et al., 2013**). Similarly, *M. sympodialis* stimulates Cath (LL-37) secretion from the monocyte-derived dendritic cells in patients with atopic eczema, thus causing a more severe inflammatory response (**Agerberth et al., 2006**). It has been shown that BDs and Cath have a high *in vitro* fungicidal activity against *M. pachydermatis* within 2 h of exposure (**Santoro and Maddox, 2014**), thus confirming their important role as potential new antifungal agents. One of the most studied antimicrobial peptides is lactoferrin, a component of the innate immune system, which can be purified from milk or produced recombinantly. Multiple studies have examined the efficacy of various derivatives of this peptide, showing promise for treatment of *Malassezia*-related diseases. A clinical study assessing the efficacy of Lactoferricin in the treatment of canine otitis showed that the peptide was a rather successful treatment option, although *Malassezia* spp. involvement was only established based on cytological examination (**Cornegliani et al., 2017**). Fifty *Malassezia* spp. isolates, derived from 45 dogs and five cats, were examined for their lactoferricin MICs, all showing growth inhibition at various MIC values (**Biasibetti et al., 2017**). A lactoferricin B like peptide derived from the centipede *Scolopendra subspinipis* showed promising results for *M. furfur* with a MIC of 10-20 µM though only one strain was studied (**Choi et al., 2013**). Bruni and coll. observed antifungal activity of b-Lactoferricin against *Malassezia* species isolated from cats and dogs with otitis (unpublished data, **Bruni et al., 2016**) and initial results from an ongoing *in vitro* study in one of the author's labs

showed that peptides constructed from modified variants of human Lactoferrin, result in inhibitory effects for *M. furfur*, *M. pachydermatis*, *M. globosa* and *M. restricta* (**unpublished data**).

Recently, synthetic peptides with sequences identical to fragments of the constant region of different classes (IgG, IgM, IgA) of antibodies (Fc-peptides) proved to exert an *in vitro* fungicidal effect against *M. furfur* and *M. pachydermatis* (**Polonelli et al., 2012; Cafarchia et al. 2014**). One of these peptides, namely the killer decapeptide, engineered from the variable region of a single-chain recombinant anti-idiotypic antibody was also effective in reducing clinical symptoms and the population size of *M. pachydermatis* in the ear canal of dogs with *Malassezia* otitis, thus indicating its usefulness in controlling *Malassezia* related otitis externa in dogs (**Cafarchia et al., 2014**). In a study by Holm et al., 21 different antimicrobial peptides and cell-penetrating peptides were tested for their potential to inhibit the growth of *M. sympodialis*. Six peptides with antifungal activity towards *M. sympodialis* were identified, showing potential for further exploration in treatment approaches (**Holm et al., 2011**). A recent review evaluated the potential of peptides against fungal biofilms, a potential virulence factor for *Malassezia* yeasts. Though *Malassezia* yeasts were not considered, various types of peptides showed anti-biofilm activity for other clinically relevant fungi and it would be useful to explore this also for *Malassezia*, especially in the context of parenteral nutrition where catheters may be subject to *Malassezia* biofilm formation, potentially causing *Malassezia* bloodstream infections (**Oshiro et al., 2019**). Although many initial studies point towards the potential of peptides for treatment of *Malassezia*-related diseases, more research is required with a strong need for standardization and inclusion of more strains tested per species.

Essential oil and plant extracts against *Malassezia* infections

In recent years, the interest in selecting sustainable antifungal products from land plants has increased. Some data are available for *Malassezi*a species of both human and veterinary interest, indicating that extracts of several plants show *in vitro* and *in vivo* activity against

Malassezia spp and their infections. Publications on essential oils between 2013 and 2018 were usefully reviewed by **Donato et al. (2019**). However comparisons between studies are hampered by an absence of agreed standard testing methods that are not yet optimized, arbitrary assignment of interpretative criteria, and likely batch variation in activities of essential oils prepared by different methods (**Bismark et al., 2019; Guillot and Bond 2020;** see **Tables 6, 7**). Initially, essential oil of *Melaleuca alternifolia* (i.e., tea tree oil) showed good *in vitro* activity against *M. furfur*, *M. sympodialis*, *M. slooffiae*, *M. globosa*, *M. obtusa* and *M. pachydermatis* with concentrations lower than that commonly present in commercially available products, thus showing promising efficacy to control *Malassezia* related infections *in vivo* (**Hammer et al., 2000; Bond et al., 2020**). Successively, other essential oils from different plants, alone or formulated as a mixture (i.e., Malacalm®), showed strong fungicidal activities against different *Malassezia* species, mainly *M. furfur* and *M. pachydermatis* (**Table 6).** The 50% lethal dose of these compounds usually ranged from 1.4 gr/kg to 9gr/kg which is lower than their MIC values against *Malassezia* species (**Table 6**), thus showing the potential of these compounds in the control of *Malassezia* infections**.** The potent antifungal activity of the essential oils was most likely attributed to monoterpene phenol derivatives, mainly geraniol, linalool, limonene and eugenol (**Table 6**). Recently, Oregano oil and thyme oil and their major phenolic components (carvacrol, thymol) have demonstrated their fungicidal activity against *M. pachydermatis* when tested using agar dilution (**Sim et al., 2019**). Interestingly, limonene disrupt the proton motive force electron flow and might damage the cytoplasmic membrane of microorganisms (**Thakre et al. 2018**). Geraniol modulates the efflux pump activity in *Candida* spp. (**Singh et al., 2018**). Eugenol, thymol and carvacrol might reduce ergosterol biosynthesis and block drug transporter pumps or could intercalate between the fatty acid chains of the membrane lipid bilayer, disturbing its fluidity and permeability (**Teodoro et al., 2015, Marchese et al., 2017**). The alkene α-pinene acts on *C. albicans* cells by inhibiting respiration and ion transport processes *(***Rivera-Yañez et al., 2017**). Other plant metabolite extracts have also drawn attention as they showed antifungal properties against *Malassezia*

yeasts (**Table 7**). These natural compounds lead to novel structural entities, which can be used as a precursor for the development of better and more effective molecules. The 50% lethal dose of these compounds usually ranged from 0.8 gr/kg to 60 gr/kg, which is generally higher than for essential oils. In contrast, the MIC values against *Malassezia* yeasts are generally lower than those registered for essential oils (**Tables 6, 7**), thus suggesting they are potentially useful in the control of *Malassezia* infections. In particular, the phenolics flavonoids and phenolic acids, saponins and fatty acids, were the most frequently retrieved compounds effective against *Malassezia* cells (**Table 7**). Their antifungal activities varied depending on the solvent used for their extraction and the *Malassezia* species concerned (**Rhimi et al., 2017**). Usually, the highest amount of phenol compounds in the extracts caused the highest antifungal activities against different species of *Malassezia* spp. (**Rhimi et al., 2017**). The activities of single compounds against *Malassezia* yeasts were unfortunately never studied, but the efficacy of flavonoids or fatty acids is well known against *Candida* spp.. Particularly, the mechanism of action of phenolic compounds might be due to perturbation of the membrane lipid. Phenolic acids cause disruption of ion transport whereas the hydroxyl and/or carboxylic acid groups of phenolic compounds play an important role in destabilizing the fungal cytoplasmic membrane (**Rhimi et al., 2017**). Fatty acids might act by disintegrating the cytoplasmic membrane and interfering with the fatty acid acylation, or with fungal sphingolipid biosynthesis by N-myristoyltransferase (NMT) inhibition, but their toxicity remains a serious issue for their general acceptance (**Carballeira, 2008**). Since MICs values of plant extract derived phenolic acids against fungi are highly variable, some studies have explored the possibility of synergistic activity with existing antifungal agents. Recently, synergistic antifungal action between chlorogenic and gallic acids with FLZ or KTZ was demonstrated for *C. albicans, C. bovina* and *C. parapsilosis*, but not for *M. furfur* or *C. krusei* strains, thus suggesting that the synergistic activities of phenol compounds with azoles might be yeast species dependents, most likely due to different membrane compositions of the species involved (**Rhimi et al., 2020**). Synergistic interactions were also reported between carvacrol and

miconazole or nystatin, thymol and nystatin, and between clotrimazole and essential oils of Melaleuca alternifolia, Mentha piperita, and Origanum vulgare) agains *M. pachydermatis* . (**Schlemmer et al., 2019b; Bohmova et al., 2019**). However, Most of the studies with essential oil and plant extracts have been conducted in vitro and their utility in clinical practice remains largely untested. The *in vitro* activities of these components are only predictive of their *in vivo* efficacy and the *in vivo* efficacy should be proven with well-conducted and controlled blinded trials. Until now, only six *in vivo* studies were performed with essential oils applied to *Malassezia* infected hosts (**Table 8**). Two studies focused on animals with *M. pachydermatis* otitis and/or dermatitis and four on humans with PV mainly caused by *M.. sympodialis* or *M. furfur* and/or *M globosa* (**Table 8)**. In the first two studies, an oil mixture composed of *Citrus limon* 1%, *Salvia sclarea* 0.5%, *Rosmarinus officinalis* 1%, *Artocarpus nobilis* 0.5%, and a commercially available product (Malacalm®) applied twice daily for 2 weeks and 1 month respectively, achieved a good clinical outcome for dogs with no recurrence of otitis or skin disorders (**Nardoni et al., 2014, 2016**). In the study on PV patients, a shampoo, a cream, a topical lotion containing the essential oils from *Cymbopogon citratus* or *Artemisi sieberi* applied three times a week (shampoo) or twice a day (cream or lotion) for 40 days or 15 days, respectively, archieved a good clinical outcome (**Table 8**). The results showed that the essential oils were safe and efficacious in treating PV with an efficacy comparable to KTZ products (**Mansouri et al., 2004; Rad et al., 2008; Khosravi et al., 2009; Carmo et al., 2013**). Although the MIC_{90} values of essential oils were usually high when compared to those of conventional antifungal drugs (i.e., azoles), the *in vivo* results were of interest suggesting that essential oils might be promising candidates to control *Malassezia* infections requiring long-term therapy both in humans and animals. Future studies enrolling a large number of patients and blinded control groups should be useful to confirm the data.

Conclusion

In summary, we reviewed the available data concerning both the currently used conventional therapy options of *Malassezia* infections and

in vitro antifungal profiles of *Malassezia* yeasts obtained using modified CLSI protocols. Guidelines for the treatment of *Malassezia* spp. skin disorders have been assessed for both pet animals and humans, but those related to systemic mycoses still need to be developed. Clinical evidences indicated the efficacy of azole drugs for the control of the skin infections and of AmB for systemic ones. However, the common recurrence of skin disorders as well as the severity of the infections suggests the usefulness of the employment of high doses of antifungal agents for prolonged times for the control of *Malassezia* infections. Even if the MIC data of *Malassezia* species vary according to the protocol used to test the susceptibility, there are evidences of a very low susceptibility of these yeasts to some azoles (e.g. FLZ, VOR) and echinocandins. Although *Malassezia* species show differences in their antifungal susceptibility *in vitr*o, the *in vivo* efficacy of these antifungal agents needs to be further evaluated by correlating *in vitro* MICs with clinical outcomes. Only very few studies showed high azole MIC values with unsuccessful treatment and these results need to be validated with multicentre studies in order to promptly develop therapeutic guidelines. The genus *Malassezia* consists of a heterogeneous group of species and genotypes, able to cause similar pathologies, yet with potentially varying susceptibility to different antifungal agents. For this reason, efficacy testing of new drugs should preferably include multiple *Malassezia* species and multiple strains per species. The low susceptibility to FLZ and VOR should be considered when long term or prophylactic therapy is expected. As maintenance therapy is crucial for successful management of relapsing infections, the employment of alternative drugs should be encouraged. Both plant based compounds (i.e., essential oils and phenolic compounds) and peptides achieved a good clinical and cytological outcome and offer a promising alternative to the currently available therapeutic or prophylactic protocols for recurrent cases of *Malassezia* infections. Moreover, these natural compounds could be also administrated in combination with conventional antifungal agents, synergistically enhancing their antifungal activity and acting as chemo-sensitizers to overcome antifungal resistance. Studies targeting drugs against fungal virulence factors (i.e., secreted proteins such as lipases,

phospholipases, and oxidoreductases) should be encouraged in order to design new therapeutic regimens in the future.

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Table 7. Plant extracts with *in vitro* antifungal activities against *Malassezia* species.

ant Family and spp.	Extract source/ Solvent	Main components (supposed activities)	Methods for antifungal determination	*Malassezia* spp.	Inhibition zone /MIC values /Lethal dose	References
maryllidaceae, *llium sativum* *lium cepa*	Fresh product /Water	Sulfur-based compounds	AD	*M. furfur*	AD (0.0039–16 µg /mL) LD_{50} 3 g/Kg -32 g/kg	Shams-Ghahfarokhi et al., 2006 Shenoy et al., 2009 Gatsing al., 2005
piaceae, *priandrum sativum*	Leaves /Water and silver nanoparticles	Flavonoids	CLSI BMD with Leeming Notman	*M. furfur*	MIC (25 µg /mL) LD_{50} up 5 g/kg	Sathishkumar et al., 2016 Patel et al., 2012
quifoliaceae, *ex paraguariensis*	Leaves/water	Phenolic acid (Chlorogenic acids and dicafeoylquinic acids)	AD	*M. furfur*	AD (18.5 mm with 1000 mg/mL) LD_{50} 4.2 g/kg	Filip et al., 2010 Ash, 2004
sparagaceae, *sparagus racemosus*	Roots /Ethanol and saponin extract	Saponin (Shatavarin I, IV, V, VIII, VI, VII, IX, X) Immunoside, Asparanin, Racemoside (A, B,C)	DD and CLSI BMD with lipid medium	*M. furfur*, *M. globosa*	DD (6.73-12.5mm with 1 mg/disc) MIC (0.40-25 mg/mL) LD_{50} NI Not toxic up 64 g/kg	Onlom et al., 2014 Narendranath et al., 1986
steraceae, *rtemisia abrotanum* *rtemisia vulgaris* *ittrichia viscosa* ` *uchea carolinensis*	Leaves or Crude extract /Methanol or Ethanol or water or Ethanol/water	Terpenes (1,8-cineole and, coumarins), flavonoid (quercetin), phenolic acids (chlorogenic acids and dicafeoylquinic acids), fatty acids (linoleic and the linolenic acids),	DD and CLSI-BMD with lipid medium.	*M. pachydermatis*, *M. furfur*, *M. slooffiae*, *M. globosa*, *M. sympodialis* *M. restricta* *Malassezia* spp	DD (8-17.5 mm with 50 mg/mL) MIC (0.125 -5 mg /mL) LD_{50} 0.83 g/kg - up 3 g/kg	Brodin et al., 2007; Rhimi et al., 2017. 2018 Biabiany et al., 2013 Han et al., 2017 Parra et al., 2001 Abbas et al., 2017 Vaghasiya, 2011

Table 8. *In vivo* antifungal activities of essential oils against *Malassezia* infections.

	Host	Etiology	Country	Family Plant Tested	Formulation concentration	Protocol/ Length of Treatment	RCT	Outcome: Improvement in clinical signs and mycology	R
	25 dogs	*M. pachydermatis*	Italy	Lamiaceae (*Salvia sclarea Rosmarinus officinalis.* Rutaceae (*Citrus limon)* Asteraceae (*Anthemis nobilis*)	Mixture composed by *C. limon* 1%, *S. sclarea* 0,5%, *R. officinalis* 1%, *A. nobilis* 0,5% in sweet almond oil	Once a day/ 15 days	yes	84 % of dogs	N al
itis	20 dogs	*M. pachydermatis*	Italy	Rutaceae (*Citrus aurantium*) Lamiaceae (*Lavandula officinalis Origanum vulgare, Origanum majorana Mentha piperita*), Asteraceae (*Helichrysum italicum var.* italicum)	Mixture of *C. aurantium* 1% *L. officinalis* 1%, *O. vulgare* 0.5%, *O. majorana* 0.5% *M. piperita* 0.5%, *H. italicum* 0.5%	Twice a day/ 30 days	yes	90% of dogs	N al
sis lor	96 humans	*M. sympodialis, M. furfur, M. obtusa*	Brazil	Poaceae (*Cymbopogon citratus*)	essential oil formulations at 1.25 µL/mL	Shampoo: three times a week Cream: twice a day/40 days	yes	60% of dogs	C al
sis lor	100 humans	*M. furfur*	Iran	Compositae (*Artemisia sieberi Besser*)	Topical lotion 5%	Twice a day/ 15 days	yes	92.2% after four weeks	R 20
sis lor	68 humans	*Malassezia* spp.	Iran	Compositae (*Artemisia sieberi Besser*)	Mycoderm lotion 10%	Twice a day/ 15 days	No	91.9% after two weeks and 100% after four weeks	K al
sis lor	120 humans	*Malassezia* spp.	Iran	Compositae (*Artemisia sieberi Besser*)	Cream3% Lotion5%	Twice a day/ 15 days	No	46.7% with cream 3 % and 100% with lotion 5%	M al

Table 1. *In vivo* prospective studies on the treatment of *Malassezia* spp. fungemia reporting clinical and mycological outcome.

References	Yeasts species	Agent Tested	Protocol	Length of Treatment	Hosts /Outcome
Chen et al., 2019	*M. furfur*	Amphotericin B	1 mg/kg/day/CVC removal	NR	1 Preterm infant/alive
Lee et al., 2019	*M. pachydermatis*	Amphotericin B	NR	2 days	1 adult 62-year-old/died
Pedrosa et al., 2018	*M. furfur*	Fluconazole and Liposomal Amphotericin B	10 mg/kg /day and/or (3 or 5 mg/kg /day) CVC removal	50 days	1 Preterm infant and 2 Adults with fluconazole prophylaxis/ Adults died and infant alive
Aguirre et al., 2015	*M. sympodialis*	Amphotericin B deoxycholate	1mg/kg/day (accumulate dosage 20 mg/kg)/ CVC removal	21 days	1 Preterm infant/ Alive
Roman, 2016	*M. pachydermatis* + mycobacteria	Liposomal Amphotericin B + nafcillin	5 mg/kg/day IV/CVC removal	7 days	1 Adult/Alive
Choudhury and Marte, 2014	*M. pachydermatis*	Liposomal Amphotericin B	1 mg/kg/day//CVC removal	NR	1 Adult with oral Posaconazole prophylaxis/Alive
Al-Sweih, 2014	*M. pachydermatis*	Liposomal Amphotericin B	NR	7 days	1 Preterm infant /Alive
Iatta et al., 2014	*M. furfur*	Liposomal Amphotericin B	From 2.5 to 5mg/kg/day/CVC removal	6-20 days	6 preterm infants 3 with fluconazole prophylaxis /Alive
Oliveri et al., 2011	*M. furfur*	Liposomal Amphotericin B	4 mg/kg/day/CVC removal	45 days	1 Preterm infants
Rosales et al., 2004	*M. furfur*	Amphotericin B	NR	26 days	1 preterm infant/Died

Table 2. ***Malassezia furfur*** **from skin lesions (SL) and Blood Stream Infection (BSI): Range of MIC values (µg/ml) and MIC_{90} or MIC_{50} * in brackets of Fluconazole (FLZ), ketoconazole (KTZ), itraconazole (ITZ), voriconazole (VOR), posaconazole (POS), terbinafine (TER) and amphotericin B (AmB) obtained with modified CLSI protocols.**

References	N. isolates/host	FLZ	KTZ	ITZ	VOR	POS	TER	AmB	MEDIA	T°C/rea time
Sharma, 2017	241/ H SL	≤0.12->64 (16)	≤0.03-8 (1)	≤0.03-16 (1)	≤0.03-16 (1)	ND	ND	≤0.03 -16 (1)	Christensen's urea broth	32°C/96h
Rojas et al., 2016	30/Human SL	0.5-128 (32)	0.03-0.125 (0.03)	0.03 0.125 (0.03)	0.03-0.5 (0.25)	ND	0.125-32 (16)	0.25-16 (8)	Modified RPMI	32°C/48h
Cafarchia et al., 2015	60/Human BSI	8-128 (128)	ND	0.032-8 (1)	0.064-8 (2)	0.016-8 (0.5)	ND	ND	SAB+1% tween 80	32°C/48h
Cafarchia et al., 2015	18/Human SL	8-128 (>128)	ND	0.064-16 (0.5)	0.064-8 (2)	0.032-0.25 (0.25)	ND	ND	SAB+1% tween 80	32°C/48h
Iatta et al., 2014	16/Humans BSI	16– >128 (128)	ND	0.03–8 (0.5)	0.06–2 (1)	0.016–0.25 (1)	ND	16	SAB+ 1% tween 80	32°C/ 48
Iatta et al., 2014	16/Humans BSI	16– >128 >128	ND	0.125–1 (1)	0.125–4 (4)	0.125–0.5 (0.5)	ND	16	Modified RPMI	32°C/48-
Rojas et al., 2014	39/ Human SL	≤0.125–>64 (16)	≤0.03–0.25 (0.06)	≤0.03–0.125 (0.06)	≤0.03–0.5 (0.25)	ND	ND	0.25-4 (2)	RPMI	32°C/72h
Carrillo-Munoz et al., 2013	20/Human SL	38.5*	0.01*	0.01*	0.03*	0.02*	1*	Nd	RPMI	32°C/27h
Miranda et al., 2007	74 /Human SL	8	0.25	0.25	0.5	ND	ND	ND	Modified Leeming–Notman	32°C/72h
Rincon et al., 2006	52/human SL	ND	0.03-1 (0.5)	0.03-0.5 (0.25)	0.03-1 (0.5)	ND	ND	ND	Modified RPMI	32°C/72h
Sugita et al., 2005	12/human SL	ND	0.016-0.125 (.0.03)	0.016-0.125 (0.03)	ND	ND	ND	ND	Leeming–Notman	32°C/7da
Velegraki et al., 2004	12 /Human SL	0.5-32 (8)	0.3-1 (0.25)	0.03-0.06 (0.06)	0.03-16 (1)	0.03-32(2)	0.03-50 (12)	0.12-16 (1)	Modified RPMI	32°C/ 48

Table 3. ***Malassezia sympodialis*** **from skin lesions (SL): Range of MIC values (μg/ml) and MIC_{90} or MIC_{50}* in brackets of fluconazole (FLZ), ketoconazole (KTZ), itraconazole (ITZ), voriconazole (VOR) posaconazole (POS), terbinafine (TER) and amphotericin B (AmB) obtained with modified CLSI protocols.**

References	N. isolates/hosts	FLZ	KTZ	ITZ	VOR	POS	TER	AmB	MEDIA	T°C/Rea Time
Rojas et al., 2016	10 /Human SL	0.5-4 (2)	0.03-0.06 (0.03)	0.03-0.06 (0.06)	0.03-0.06 (0.06)	ND	0.125-0.5 (0.5)	0.5-2 (2)	Modified RPMI	32°C/72h
Rojas et al., 2014	20/ Humans SL	≤0.125–4 (2)	≤0.03–0.06 (0.03)	≤0.03–0.06 (0.06)	≤0.03–0.06 (0.06)	ND	ND	0.125–4 (2)	Modified RPMI	32°C/72h
Rincón et al., 2006	50 / Human SL	ND	0.015–4 (0.5)	0.015–2 (1)	0.015–1 (0.25)	ND	ND	ND	Modified RPMI	32°C/72h
Velegraki et al., 2004	8/Human SL	0.5–16 (8)	0.03–0.06 (0.03)	0.03–0.06 (0.06)	0.03–0.06 (0.04)	0.03–0.06 (0.03)	0.03–0.06 (0.06)	0.06–0.5 (0.25)	Modified RPMI	32°C/72h
Garau et al., 2003	21/Human SL	0.25-4 (1)	≤0.03	≤0.03	≤0.03	ND	ND	ND	Modified RPMI	32°C/72h
Nakamura et al., 2000	7/Human SL	ND	ND	0.025–0.1 (0.05*)	ND	ND	0.05–0.8 (0.2*)	ND	UREA	30°C/96h
Gupta et al., 2000	10/Human SL	ND	≤0.03 (100)	ND	≤0.03-0.125 (≤0.03)	ND	≤0.03-0.25 (≤0.03)	ND	Leeming–Notman	32°C/48h

Table 4. *Malassezia pachydermatis*: Range of MIC values and MIC_{90} or MIC_{50}* in brackets of fluconazole (FLZ), ketoconazole (KTZ), itraconazole (ITZ), voriconazole (VOR), posaconazole (POS), terbinafine (TER) and amphotericin B (AmB) obtained with modified CLSI protocols

References	N. isolates/host	FLZ	KT	ITZ	VOR	POS	TER	AmB	MEDIA	T° tin
Brilhante et al. 2018	22/Dogs	2–>64 (>64)	0.125->16 (4)	<0.03-8 (0.25)	<0.03-2 (1)	ND	0.03-0.25 (0.125)	1->16 (16)	SAB+1% tween 80	35
Álvarez-Pérez et al., 2016	216/ Dogs	4–>64 (32)	0.125–4 (0.5)	0.031-1 (0.63)	0.063–8 (1)	0.125-4 (0.5)	0.063–2 (1)	0.06-0.5 (0.25)	SAB+1% tween 80	32
Cafarchia et al., 2012, 2015	62/ Dogs	4–>64 (32)	<0.008 -0.06 (0.32)	0.008–0.125 (0.16)	0.016–0.50 (0.64)	0.008-0.03 (0.03)	0.250	ND	SAB+ 1% tween 80	32
Yurayart et al., 2013	45/Dogs	ND	<0.03-0.5 (0.03*)	<0.03-0.5 (0.04*)	ND	ND	ND	ND	Modified RPMI	35
Weiler et al., 2013	40/Dogs	0.01-1.0 (0.5)	0.01-0.5 (0.5)	0.01-0.5 (0.25)	0.01-0.25 (0.125	ND	ND	0.01-1.0 (0.5)	RPMI	37
Carrillo –Munoz et al., 2013	6/Dogs	1.2 *	0.02*	0.02*	0.01*	0.01*	0.01*	ND	RPMI	32
Brito et al., 2009	20/Dogs	8	<0.03	≤0.03-0.25 (0.03)	ND	ND	ND	0.25	RPMI	32
Prado et al., 2008	50/Dogs	4	0.03	<0.03	2	ND	ND	4	modified RPMI	32
Rincón et al., 2006	2/Dogs	ND	0.25	0.06	0.25	ND	ND	ND	modified RPMI	32
Velegraki et al., 2004	1/Dog	16	0.06	0.06	0.03	0.12	0.12	0.12	modified RPMI	32
Nascente et al., 2003	24/ Dogs, cats	0.5-64	0.03-8	0.03-4	ND	ND	ND	ND	RPMI	37
Garau et al., 2003	10 /Dogs	4	(≤0.03)	≤0.03-0.06 (≤0.03)	≤0.03-0.12 (0.06)	≤0.06	ND	ND	RPMI	32
Eichenberg et al., 2003	82/Dogs, cats	1-32	0.015-0.25	0.007-0.125	ND	ND	ND	ND	RPMI	35
Nakamura et al., 2000	12 Dogs	ND	ND	0.8-6.3	ND	ND	3.2-25	ND	UREA BROTH	30

Table 5. *Malassezia globosa* from skin lesions: Range of MIC Values and MIC_{90} or MIC_{50} *in bracket of Fluconazole (FLZ), ketoconazole (KTZ), itraconazole (ITZ) posaconazole (POS), terbinafine (TER) and amphotericin B (AmB) obtained with modified CLSI protocol

References	N. isolates/hosts	FLZ	KTZ	ITZ	VRZ	PSZ	TER	AMB	MEDIA	T°C/Rea Time
Rojas et al., 2017	10/ Human	1_64	0.03-0.'6	0.03	0.03-05	nd	0.25-32	nd	Modified RPMI	32°C/72h
Rojas et al., 2014	10 /Human	≤0.125–8	0.03	0.03-0.06	≤0.03–0.25	nd	nd	0.06–4	Modified RPMI	32°C/72h
Rincón et al., 2006	50 / Human	ND	0.015–>8	0.015–>8	0.03–>8	ND	ND	ND	Modified RPMI	32°C/72h
Velegraki et al. 2004	8/ Human	1-32	0.03–0.012	0.03–0.12	0.03–0.12	0.03–0.06 (0.03)	0.03–0.012 (0.06)	0.1–4	Modified RPMI	32°C/72h
Gupta et al., 2000	7/ Human	ND	≤0-03 - 125	≤0-03 - 125	0.03	ND	≤0.06-16	ND	Leeming–Notman Disk Diffusion	32°C/48h

Table 6. Plant extracts with *in vitro* antifungal activities against *Malassezia* species.

ant Family and spp.	Extract source/ Solvent	Main components (supposed activities)	Methods for antifungal determination	*Malassezia* spp.	Inhibition zone /MIC values /Lethal dose	References
maryllidaceae, *llium sativum* *lium cepa*	Fresh product /Water	Sulfur-based compounds	AD	*M. furfur*	AD (0.0039–16 µg /mL) LD_{50} 3 g/Kg -32 g/kg	Shams-Ghahfarokhi et al., 2006 Shenoy et al., 2009 Gatsing al., 2005
piaceae, *priandrum sativum*	Leaves /Water and silver nanoparticles	Flavonoids	CLSI BMD with Leeming Notman	*M. furfur*	MIC (25 µg /mL) LD_{50} up 5 g/kg	Sathishkumar et al., 2016 Patel et al., 2012
quifoliaceae, *ex paraguariensis*	Leaves/water	Phenolic acid (Chlorogenic acids and dicafeoylquinic acids)	AD	*M. furfur*	AD (18.5 mm with 1000 mg/mL) LD_{50} 4.2 g/kg	Filip et al., 2010 Ash, 2004
sparagaceae, *sparagus racemosus*	Roots /Ethanol and saponin extract	Saponin (Shatavarin I, IV, V, VIII, VI, VII, IX, X) Immunoside, Asparanin, Racemoside (A, B,C)	DD and CLSI BMD with lipid medium	*M. furfur, M. globosa*	DD (6.73-12.5mm with 1 mg/disc) MIC (0.40-25 mg/mL) LD_{50} NI Not toxic up 64 g/kg	Onlom et al., 2014 Narendranath et al., 1986
steraceae, *rtemisia abrotanum* *rtemisia vulgaris* *ittrichia viscosa* ` *uchea carolinensis*	Leaves or Crude extract /Methanol or Ethanol or water or Ethanol/water	Terpenes (1,8-cineole and, coumarins), flavonoid (quercetin), phenolic acids (chlorogenic acids and dicafeoylquinic acids), fatty acids (linoleic and the linolenic acids),	DD and CLSI-BMD with lipid medium.	*M. pachydermatis*, *M. furfur, M. slooffiae, M. globosa*, *M. sympodialis* *M. restricta* *Malassezia* spp	DD (8-17.5 mm with 50 mg/mL) MIC (0.125 -5 mg /mL) LD_{50} 0.83 g/kg - up 3 g/kg	Brodin et al., 2007; Rhimi et al., 2017. 2018 Biabiany et al., 2013 Han et al., 2017 Parra et al., 2001 Abbas et al., 2017 Vaghasiya, 2011