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In Vitro Activities of Ketoconazole, Econazole, Miconazole, and *Melaleuca alternifolia* (Tea Tree) Oil against *Malassezia* Species

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The in vitro activities of ketoconazole, econazole, miconazole, and tea tree oil against 54 *Malassezia* isolates were determined by agar and broth dilution methods. Ketoconazole was more active than both econazole and miconazole, which showed very similar activities. *M. furfur* was the least susceptible species. *M. sympodialis*, *M. slooffiae*, *M. globosa*, and *M. obtusa* showed similar susceptibilities to the four agents.

Lipid-dependent *Malassezia* yeasts are commonly found on human skin, in particular, on the upper body, where sebum excretion is highest (10, 13). Although usually saprophytic, *Malassezia* spp. are also considered to be etiological agents in superficial skin diseases, such as pityriasis versicolor, seborrhoeic dermatitis, and *Malassezia* folliculitis, and infrequently cause systemic disease associated with lipid-rich hyperalimentation fluids (13).

Recently, several new *Malassezia* species have been described, resulting in seven species now being included in the genus (3, 4). Despite these major taxonomic revisions, little work has subsequently been published about the in vitro susceptibilities of these species to various antifungal agents. Therefore, the aim of this study was to determine the comparative activities of ketoconazole, econazole, miconazole, and the topical agent tea tree oil against *Malassezia* species.

The following reference strains were obtained from the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands: *M. furfur* CBS 1878, *M. globosa* CBS 7966, *M. obtusa* CBS 7876, *M. slooffiae* CBS 7956, and *M. sympodialis* CBS 7222. The following isolates were obtained in our laboratory as described previously (6); *M. furfur* ($n = 10$), *M. globosa* ($n = 4$), *M. obtusa* ($n = 1$), *M. slooffiae* ($n = 2$), *and M. sympodialis* ($n = 30$). These were identified according to previously published methods (3, 5, 11, 14). In addition, one isolate of *M. sympodialis* was kindly provided by Chris Heath at the Department of Microbiology and Infectious Diseases, Royal Perth Hospital, and one isolate of *M. furfur* was kindly provided by The Western Australian Centre for Pathology and Medical Research. All organisms were maintained on Dixon's agar (18), and all incubations, including susceptibility tests, were at 32°C.

Tea tree oil (batch 971) was kindly supplied by Australian Plantations Pty. Ltd., Wyrallah, New South Wales, Australia, and complied with the International Standard ISO 4730 (7, 8). Stock solutions of econazole (Sigma Chemical Co., St. Louis, Mo.), miconazole (Sigma), and ketoconazole (Janssen Biotech, Olen, Belgium) powders were prepared in dimethyl sulfoxide and stored at −20°C.

For broth and agar dilution assays, inocula were prepared by growing organisms on Dixon's agar for 72 h. Colonies were suspended in saline, and suspensions were adjusted to approximately 5×10^6 CFU/ml, as determined by viable counts. For the agar dilution assay, a series of twofold dilutions of each agent were prepared in medium A agar (10). Final concentration ranges were as follows: tea tree oil, 0.008 to 1.0% (vol/vol); ketoconazole, 0.001 to 0.5 μg/ml; miconazole, 0.015 to 32 μg/ml; and econazole, 0.03 to 32 μg/ml. For dilutions with tea tree oil, a final concentration of 0.5% (vol/vol) Tween 20 was incorporated into the agar to enhance oil solubility (7). After drying for 30 min, plates were inoculated with 1-μl spots containing approximately 10^3 CFU per spot by using a multipoint replicator (Mast Laboratories, Ltd., Liverpool, United Kingdom). Plates were incubated for 7 days. MICs were then determined as the lowest concentration of the agent preventing the growth of the isolate, disregarding one or two colonies. The broth dilution assay was based on that recommended by the National Committee for Clinical Laboratory Standards (16). A series of twofold dilutions of each agent were prepared in medium A broth in a 96-well microdilution tray. In tests with tea tree oil, a final concentration of 0.001% (vol/vol) Tween 80 was added to medium A broth to enhance oil solubility (7). Each well was inoculated with a final concentration of approximately 1.5×10^3 to 3.0×10^3 CFU/ml, as confirmed by viable counts. Microdilution trays were incubated for 48 h, and then 5-μl aliquots from each tray well were spot inoculated onto Dixon's agar. The surfactant components of both medium A broth and Dixon's agar meant that larger subculture aliquots were not feasible. Subcultures were incubated until colonies were visible—usually 2 to 7 days. MICs were determined as the lowest concentration of the agent resulting in the maintenance or reduction of the inoculum. Minimum fungicidal concentrations (MFCs) were determined as the lowest concentration of the agent resulting in no growth. Each isolate was tested at least twice on separate occasions, and if results differed, isolates were retested and modal MICs or MFCs were selected.

The MICs shown in Table 1 demonstrate that ketoconazole was the most active of the imidazoles, followed by miconazole and econazole, which were similar in activity. *M. furfur* was the species least susceptible to imidazoles: the remaining species were similar. Tea tree oil was active against all *Malassezia* species, for which the MICs were similar.

Ketoconazole was also the most active of the imidazoles in the broth dilution assay (Table 2). Miconazole and econazole showed similar activities against each species, but demonstrated differences in activity between species. *M. sympodialis* was more susceptible than *M. furfur* with all MICs at which 90% of isolates tested are inhibited (MIC_{90}s) and MFCs at which 90% of isolates tested are inhibited (MFC_{90}s) lower

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TABLE 1. In vitro susceptibilities of *Malassezia* spp. as determined by the agar dilution assay

Organism (no. of isolates tested)	Agent	MIC[a]		
		Range	50%	90%
M. furfur (012)	Tea tree oil	0.12–0.25	0.25	0.25
	Ketoconazole	0.03–0.25	0.06	0.25
	Miconazole	4–16	8	16
	Econazole	4–16	16	16
M. sympodialis (32)	Tea tree oil	0.016–0.25	0.25	0.25
	Ketoconazole	0.008–0.03	0.016	0.03
	Miconazole	0.12–0.5	0.5	0.5
	Econazole	0.12–1	0.5	0.5
M. slooffiae (3)	Tea tree oil	0.12–0.25		
	Ketoconazole	0.008–0.03		
	Miconazole	2–4		
	Econazole	0.25–0.5		
M. globosa (5)	Tea tree oil	0.03–0.12		
	Ketoconazole	0.008–0.016		
	Miconazole	0.12–2		
	Econazole	0.25–0.5		
M. obtusa (2)	Tea tree oil	0.12		
	Ketoconazole	0.008–0.016		
	Miconazole	0.5–2		
	Econazole	0.5–4		

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively. MICs were measured as percent volume per volume for tea tree oil and micrograms per milliliter for ketoconazole, miconazole, and econazole.

than those obtained for *M. furfur*. The MICs of tea tree oil were similar for *M. furfur* and *M. sympodialis*, but the MFCs were several dilutions lower for *M. furfur*. With ketoconazole, MICs and MFCs were equivalent or 1 dilution apart. For miconazole and econazole, MICs and MFCs differed by several dilutions for both species.

Ketoconazole was the most active of the three imidazoles tested—findings similar to those of previous studies (12, 20). A relatively small difference between inhibitory and fungicidal values was seen for ketoconazole, but not for econazole or miconazole. Van Cutsem et al. (19) showed a similar effect for ketoconazole against 10 strains of *Pityrosporum ovale*. In our study, *M. furfur* was the least susceptible species. Similarly, Mayser et al. (15) found that *M. furfur* isolates were least susceptible to polidocanol, with the MICs for them more than 10-fold higher than those for the remaining *Malassezia* species. In contrast, Leeming et al. (11) reported that *M. sympodialis* was the species most susceptible to terbinafine, and the remaining species, including *M. furfur*, showed very similar susceptibilities. Methodological differences between these studies, such as the test method, the medium used in the assay, and the criteria or method used for determining inhibitory and fungicidal concentrations, limit further comparison of results.

Tea tree oil and products containing the oil have been evaluated in vivo for the treatment of superficial fungal infections such as onychomycosis and oral candidiasis, with some favorable clinical outcomes (1, 9). Reports have been published previously describing the in vitro susceptibility of *Malassezia* species to tea tree oil (6, 17), and the present study confirms and extends these findings. However, there are no reports on the use of tea tree oil specifically for the treatment of *Malassezia* skin infections. Most tea tree oil products contain 5 to 10% tea tree oil, and this is likely to be adequate for clinical use. Different commercially available 100% tea tree oils vary little in their antimicrobial activity (2), however, the activity of tea tree oil can be antagonized by various excipients used in the formulation of products (7). In addition, as with many topical agents, there is a low risk of allergic reactions to 100% tea tree

TABLE 2. In vitro susceptibilities of *M. furfur* and *M. sympodialis* as determined by the broth dilution assay

Organism (no. of isolates tested)	Agent	MIC[a]		MFC[a]	
		Range	90%	Range	90%
M. furfur (10)	Tea tree oil	0.03–0.12	0.12	0.5–1.0	1.0
	Ketoconazole	0.06–0.25	0.25	0.06–0.5	0.25
	Miconazole	8–128	128	16–>256	>256
	Econazole	8–128	64	16–>256	>256
M. sympodialis (10)	Tea tree oil	0.016–0.12	0.06	0.06–0.12	0.12
	Ketoconazole	0.008–0.03	0.016	0.008–0.06	0.03
	Miconazole	0.25–4	1	0.5–>8	>8
	Econazole	0.25–4	1	1–32	16

[a] Percent volume per volume for tea tree oil and micrograms per milliliter for ketoconazole, miconazole, and econazole. 90%, MIC_{90} or MFC_{90}.

oil. We have recently shown the prevalence of such allergy to be approximately 5% (Greig et al., unpublished data).

In conclusion, this work has shown that individual *Malassezia* species vary in their susceptibility to several antifungal agents, with *M. furfur* being the least susceptible of the species tested. Tea tree oil may be a suitable alternative topical agent. In view of the apparent emergence of *Malassezia* as opportunistic pathogens, these data may have clinical significance.

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