

In Vitro Antifungal Activity of Epigallocatechin 3-*O*-Gallate against Clinical Isolates of Dermatophytes

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Previously, we reported that epigallocatechin 3-*O*-gallate (EGCg) has growth-inhibitory effect on clinical isolates of *Candida* species. In this study, we investigated the antifungal activity of EGCg and antifungal agents against thirty-five of dermatophytes clinically isolated by the international guidelines (M38-A2). All isolates exhibited good susceptibility to EGCg (MIC₅₀, 2-4 µg/mL, MIC₉₀, 4-8 µg/mL, and geometric mean (GM) MICs, 3.36-4 µg/mL) than those of fluconazole (MIC₅₀, 2-16 µg/mL, MIC₉₀, 4-32 µg/mL, and GM MICs, 3.45-25.8 µg/mL) and flucytosin (MIC₅₀, MIC₉₀, and GM MICs, >64 µg/mL), although they were less susceptible to other antifungal agents, such as amphotericin B, itraconazole, and miconazole. These activities of EGCg were approximately 4-fold higher than those of fluconazole, and were 4 to 16-fold higher than flucytosin. This result indicates that EGCg can inhibit pathogenic dermatophyte species. Therefore, we suggest that EGCg may be effectively used solely as a possible agent or combined with other antifungal agents for antifungal therapy in dermatophytosis.

Key Words: Epigallocatechin 3-*O*-gallate; Dermatophytes; *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, Susceptibility

Dermatophytosis, mycotic infections, is one of among the most common and widespread worldwide infectious diseases and represent an important public health problem yet unresolved. It can be caused by keratinophilic and keratinolytic dermatophytes, particularly *Microsporum canis* (*M. canis*), *Trichophyton mentagrophytes* (*T. mentagrophytes*), and *Trichophyton rubrum* (*T. rubrum*).¹⁻⁸ Although many antifungal agents have been developed during the last decades and have become available for dermatophytosis, they are confined to a relatively few chemical groups. In addition, the occurrence of resistance or side effects in clinically isolated strains leads to failure in the treatment of mycosis.⁹ Thus, effective antifungal agents, which are highly effective and safe, are necessary and important for the extermination of antibiotic-susceptible and -resistant strains.

In recent years, there are several reports on antifungal activity of natural prod-

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ucts.¹⁰⁻¹⁴ Green tea polyphenols have been reported to have an antimicrobial effect against oral, intestinal, and food-borne bacteria, antitoxicity against various bacterial haemolysins, and antiviral activity.¹⁵⁻²⁰ The main polyphenol component of green tea, epigallocatechin 3-*O*-gallate (EGCg), with direct antibacterial properties, can decrease bacterial invasion by inhibition of bacterial gelatinase activities.^{21,22} Okubo, et al.²³ reported that black tea extract inhibited the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum*, but it was not by EGCg only. Recently, we showed that EGCg has growth-inhibitory effect on clinical isolates of *Candida* species, but not on dermatophytes.²⁴ In this study, therefore, we investigated the antifungal activity of EGCg and five antifungal agents, such as amphotericin B, flucytosin, fluconazole, itraconazole, and miconazole against clinical isolates of dermatophyte species by determination of minimum inhibitory concentration (MIC).

As described in our previous study,²⁴ all tests performed in RPMI-1640 medium (Sigma, St. Louis, MO, USA) with L-glutamine and low glucose (2 mg/mL), without phenol red and sodium bicarbonate, buffered with 0.165 M 3-(*N*-morpholino) propanesulfonic acid, pH adjusted to 7.0 with NaOH and sterilized. EGCg was also kindly supplied by Pharma Foods International Co. Ltd. (Kyoto, Japan), and the purity of EGCg exceeded 97%, and the concentration of EGCg from 0.06 to 32 µg/mL was used. Dry plates including five antifungal agents such as amphotericin B (AMPH; 0.03-16 µg/mL), flucytosin (5FC; 0.125-64 µg/mL), fluconazole (FLCZ; 0.125-64 µg/mL), itraconazole (ITCZ; 0.015-8 µg/mL), and miconazole (MCZ; 0.06-32 µg/mL), were purchased from Eiken Chemical Co., LTD. (Tokyo, Japan). Thirty-five clinical isolates of three dermatophyte species (thirteen *M. canis* strains; IFM 41048, 41054, 41061, 41115, 46053, 53789, 53817, 54153, 54155, 54156, 54157, 54158, 54199, eleven *T. mentagrophytes* strains; IFM 46600, 46609, 46634, 47174, 47176, 53815, 53931, 55190, 55191, 55192, 55193, and eleven *T. rubrum* strains; IFM 45623, 45625, 47162, 47163, 47164, 47165, 47166, 47167, 47170, 55188, 55189), and three *Candida* strains, *Candida albicans* (ATCC 90028), *C. parapsilosis* (ATCC 90018), and *C. krusei* (ATCC 6258) for quality control were obtained from the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University in Japan. All dermatophytes were cultured on potato dextrose agar (PDA) slant at 35°C for 10 to 14 days, whereas three *Candida* strains were cultured on PDA slant at 35°C for 24 hours and passaged twice at a 48 hours interval before use.

All standard antifungal susceptibility testing was performed according to the document M38-A2, published by the Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards, or NCCLS).²⁵ As described in our previous study,²⁴ fungal cell suspensions were spectrophotometrically standardized to a turbidity equivalent to that of a 0.5 McFarland standard. The suspensions were prepared from 10 to 14 day cultures grown on PDA, and the density was 2×10^5 colony forming unit (cfu)/mL. The final inoculum was adjusted to approximately 2×10^4 cfu/mL. All tests were performed in 96-wells plate. Aliquots of one hundred microliters of suspension were inoculated into each well containing diluted EGCg or five antifungal agents. Drug-free controls and growth controls were included for each assay. The plates were incubated at 35°C and read visually after 4 to 7 days. The MICs of EGCg and five antifungal agents were defined as the lowest drug concentrations that resulted in a 50% and 90% reduction in growth compared with that of the drug-free growth control, as recommended by the CLSI.²⁵ Geometric mean (GM) MICs were determined to facilitate comparisons of the activities of EGCg and five antifungal agents. Data shown are from three separate tests and were analyzed statistically by calculating means and S.D of the means.

MICs were determined after 7 days of culture because the growth of some strains for 4 days was insufficient. MIC₅₀ and MIC₉₀, the MIC values that inhibited 50 and 90% of isolate growth, as well as the MIC range of EGCg and antifungal agents, AMPH, FLCZ, 5FC, ITCZ, and MCZ, against thirty-five clinical isolates of three dermatophyte species are summarized in Table 1. Although, the susceptibilities were different depending on the types of strains and species, the dermatophytes used in this study were in general susceptible to EGCg. The MIC ranged from 0.5 to 16 µg/mL with all isolates, and the GM MICs were 3.69 µg/mL in *M. canis*, 3.80 µg/mL in *T. mentagrophytes*, and 3.36 µg/mL in *T. rubrum* species. Moreover, all isolates exhibited better susceptibilities to EGCg (MIC₅₀, 2-4 µg/mL, MIC₉₀, 4-8 µg/mL, and GM MICs, 3.36-4 µg/mL) than to FLCZ (MIC₅₀, 2-16 µg/mL, MIC₉₀, 4-32 µg/mL, and GM MICs, 3.45-25.8 µg/mL) and 5FC (MIC₅₀, MIC₉₀, and GM MICs, >64 µg/mL). As shown in Table 1, however, they were slightly less susceptible to other antifungal agents. The GM MICs of EGCg were approximately 7- to 17-fold in *M. canis*, 3- to 17-fold in *T. mentagrophytes*, and 1- to 19-fold in *T. rubrum* higher than those of FLCZ and 5FC. Among EGCg and five antifungal agents tested in this study, only the GM

Table 1. Antifungal Activities of EGCg and Antifungal Agents Against Clinical Isolates of Dermatophytes

Species (no. of isolates)	Antifungal drug	MIC data ($\mu\text{g/mL}$)			
		MIC range	MIC ₅₀	MIC ₉₀	Geometric mean MIC
<i>Microsporum canis</i> (13)	EGCG	0.5-8	2	8	3.69
	AMPH	0.5-4	0.5	2	1
	5FC	>64	>64	>64	>64
	FLCZ	16-32	16	32	25.84
	ITCZ	0.5-1	0.5	1	0.58
	MCZ	1-2	1	2	1.38
<i>Trichophyton mentagrophytes</i> (11)	EGCG	2-8	4	4	3.80
	AMPH	0.5-1	1	1	0.80
	5FC	>64	>64	>64	>64
	FLCZ	2-16	8	16	11
	ITCZ	0.06-0.25	0.125	0.25	0.14
	MCZ	0.125-0.1	0.5	1	0.57
<i>Trichophyton rubrum</i> (11)	EGCG	1-16	2	4	3.36
	AMPH	0.5-1	0.5	1	0.64
	5FC	>64	>64	>64	>64
	FLCZ	1-16	2	4	3.45
	ITCZ	0.125-0.5	0.125	0.25	0.18
	MCZ	0.06-1	0.25	0.5	0.38

EGCG, Epigallocatechin 3-O-gallate; AMPH, amphotericin B; 5FC, flucytosine; FLCZ, fluconazole; ITCZ, itraconazole; MCZ, miconazole.

MICs of FLCZ were different among the dermatophyte species tested; As shown in Table 1, *M. canis* (25.84 $\mu\text{g/mL}$), *T. mentagrophytes* (11 $\mu\text{g/mL}$), and *T. rubrum* (3.45 $\mu\text{g/mL}$). Among the antifungal agents tested, ITCZ (MIC₅₀, <0.125-0.5 $\mu\text{g/mL}$, MIC₉₀, <0.25-1 $\mu\text{g/mL}$, and GM MICs, 0.14-0.58 $\mu\text{g/mL}$) had the strongest antifungal activities regardless of the strain.

Recently, Weitzman and Summerbell¹ reported that proliferation of new classes of drugs, such as terbinafine and itraconazole, represents the most remarkable trend in dermatophytosis therapy. Itraconazole have been used effectively.²⁶ However, treatment with these agents for prolonged period requires periodic monitoring of liver activity.²⁷ Moreover, these agents may have drug interactions with other medications.²⁸ In this context, therefore, new antifungal agents from natural products could be useful alternatives for the treatment of dermatophytosis, because they have some advantages, such as reduced risk of side-effects and lower cost, and there has recently been growing interest in the use of medicinally important plants and their compounds to cure some diseases.

EGCg, a main component of tea catechins present in green tea, is known to possess antibacterial activity and the effects of certain antibiotics.²⁹⁻³¹ Hirasawa and Takada³² reported the susceptibility of *Candida albicans* to catechins including EGCg. Recently, we examined anticandidal effect

of EGCg under an *in vitro* condition, and we demonstrated in this study, potent antifungal activity of EGCg against clinical isolates of pathogenic dermatophytes *in vitro* compared with the other antifungal agents tested, FLCZ and 5FC. Among the dermatophyte species tested, *T. rubrum* was the most susceptible to EGCg: they were more susceptible than to 5FC and were similar to those of FLCZ, although they were less susceptible than to ITCZ, MCZ, and AMPH. GM MICs of EGCg towards *M. canis* and *T. mentagrophytes* were lower and they were more susceptible to EGCg than to FLCZ and 5FC. These results suggest that EGCg can be used as an antifungal agent or adjuvant with antifungal agents in dermatophytosis and can be applied in the field of antifungal therapy, although the dermatophytes were slightly less susceptible to it than to other antifungal agents, such as ITCZ, MCZ, and AMPH. Furthermore, EGCg does not develop resistances and it may avoid the side effects.^{21,24,30,31}

In conclusion, it would seem that EGCg may be used as an antifungal agent solely or in combination with other antifungal agents as previously reported^{24,32} or as an adjuvant. Although, higher concentration of EGCg than that used in the present study may be needed to treat human dermatophytosis patients, the mechanism of antifungal effects in dermatophytes has not yet been defined, and more studies such as *in vivo* or *ex vivo* experiments are needed to verify these possibilities. Nevertheless, the present study showed

that EGCg can be applied as an alternate antifungal agent to overcome resistance to other reported antifungal agents, and it may be effectively used as a possible agent or adjuvant for the treatment of dermatophytosis.

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