Brief note

Determination of antimicrobial activity of plant-derived polyphenols against *Malassezia pachydermatis* and *Staphylococcus intermedius*

Malassezia pachydermatis と *Staphylococcus intermedius* に対する
植物由来ポリフェノールの抗菌活性の調査

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Abstract: The emergence of antimicrobial-resistant pathogens has significantly affected treatment outcomes for malasseziosis and pyoderma in dogs. Here, we determined the antimicrobial activity of plant-derived polyphenols against *Malassezia pachydermatis* and *S. intermedius*, the causative agents of malasseziosis and SIG infection, respectively. The polyphenols tested were castalagin, (+)catechin, (–)–epigallocatechin, (–)–epicatechin gallate, (–)–epigallocatechin-3-O-gallate, myricitrin, prodelphinidin, procyanidin, resveratrol, rutin, theaflavin, and thearubigin. Minimum inhibitory concentration (MIC) values of these polyphenols against two yeast species, *M. pachydermatis* and *Candida albicans*, and two bacterial species, *S. intermedius* and *Achromobacter xylosoxidans*, were determined using an agar plate assay. Castalagin had the lowest MIC values against *M. pachydermatis* (100 µg/ml) and *S. intermedius* (50 µg/ml), suggesting its therapeutic potential for skin diseases caused by these microbes in dogs.

Key words: *malassezia pachydermatis*, polyphenols, *staphylococcus intermedius*

要約: *Malassezia pachydermatis* と *Staphylococcus intermedius* に対する植物ポリフェノールの抗菌活性を調査した。ポリフェノールはエピガロカテキン、エピカテキンガレート、没食子酸エピガロカテキン、カスタラギン、カテキン、テアフラビン、テアルビジン、プロシアニジン、プロデルフィニジン、ミリシトリン、ルチンそしてレスベラトロールを用いた。これらポリフェノールの最小発育阻止濃度 (MIC) は寒天平板培地法により求めた。*M. pachydermatis* と *S. intermedius* に対する MIC が最も低値であったのはカスタラギン (それぞれ 100 µg/ml と 50 µg/ml) であった。以上の結果から、カスタラギンは *M. pachydermatis* と *S. intermedius* グループが原因となるイヌの皮膚疾患の治療薬として効果的である可能性が示された。

キーワード: ポリフェノール, *Malassezia pachydermatis*, *Staphylococcus intermedius*

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Fig. 1. The structural formulas of the polyphenols used in this study. EGC: (–)-epigallocatechin; EGg: (–)-epicatechin gallate; EGCg: (–)-epigallocatechin-3-O-gallate.

Malasseziosis, a common skin disease in dogs, is caused by *Malassezia* spp., which are part of the skin and mucosal microbiota⁶⁾. The *Staphylococcus intermedius* group (SIG) of bacteria comprises opportunistic pathogens that cause superficial pyoderma in dogs^{1, 7)}. Long-term use of antimicrobials is often required for treating chronic skin diseases caused by *M. pachydermatis* or SIG in dogs. Multiple antimicrobials may also be used to treat diseases caused by a combination of these microbes. Excessive use of antimicrobials may lead to the emergence of antimicrobial-resistant pathogens and alterations in the cutaneous or intestinal flora, that may preclude the use of appropriate antimicrobial therapies^{7, 10, 11)}. The emergence of antimicrobial-resistant pathogens also significantly affects treatment outcomes^{2, 4)}, leading to the need for alternative treatment strategies.

Polyphenols are a subset of phenols bearing multiple hydroxyl groups on their aromatic components. Various polyphenol-containing veterinary dietary supplements are commercially available. These include plant-derived licorice glabra polyphenol (GlavonoidTM, Kyoritsu Seiyaku, Tokyo, Japan) and lychee polyphenol (Oligonol[®], Meiji Seika Pharma, Tokyo, Japan) which

Table 1. The polyphenol compounds used in this study

Polyphenols	Host plants
Castalagin	<i>Castanea crenata</i>
(+)-Catechin	<i>Camellia sinensis</i>
EGC	<i>Camellia sinensis</i>
EGg	<i>Camellia sinensis</i>
EGCg	<i>Camellia sinensis</i>
Myricitrin	<i>Myrica rubra</i>
Procyanidin	<i>Eriobotrya japonica</i>
Prodelphinidin	<i>Elaeocarpus sylvestris</i> var. <i>ellipticus</i>
Resveratro	<i>Polygonum cuspidatum</i>
Rutin	<i>Fagopyrum esculentum</i>
Theaflavin	<i>Camellia sinensis</i>
Thearubigin	<i>Camellia sinensis</i>
Hinokitiol	<i>Polygonum cuspidatum</i>

EGC: (–)-epigallocatechin; EGg: (–)-epicatechin gallate; EGCg: (–)-epigallocatechin-3-O-gallate.

are mainly used as antioxidants. Polyphenols are also known to have antimicrobial activity against various bacterial species^{3, 8, 9)}.

For the present study, it was hypothesized that plant-derived polyphenols would have antimicrobial activity

Table 2. The microorganisms used in this study

Microorganisms		Source	
Yeast	<i>Candida albicans</i>	clinical bronchomycosis	NBRC1594
	<i>Malassezia pachydermatis</i>	ear of dog with otitis externa	JCM10131
Bacteria	<i>Achromobacter xylosoxidans</i>	ear discharge	NBRC15126
	<i>Staphylococcus intermedius</i>	pigeon nares	JCM2422

NBRC: National Institute of Technology and Evaluation Biotechnology Center, RIKEN BioResource Research Center.

Table 3. The minimum inhibitory concentration (MIC) values of the polyphenols against the yeast species

Polyphenols	<i>Candida albicans</i>	<i>Malassezia pachydermatis</i>
Castalagin	>1,600	100
(+) Catechin	>3,200	>3,200
EGC	>3,200	3,200
ECg	>3,200	3,200
EGCg	>3,200	800
Myricitrin	>3,200	>3,200
Prodelphinidin	>3,200	800
Procyanidins	>3,200	>3,200
Resveratrol	>3,200	>3,200
Rutin	>3,200	>3,200
Theaflavin	1,600	800
Thearubigin	>3,200	1,600
Hinokitiol	≤50	≤50

EGC: (–)-epigallocatechin; ECg: (–)-epicatechin gallate; EGCg: (–)-epigallocatechin-3-O-gallate. Minimum inhibitory concentration: µg/ml.

Table 4. The minimum inhibitory concentration (MIC) values of the polyphenols against the bacterial species

Polyphenols	<i>Achromobacter xylosoxidans</i>	<i>Staphylococcus intermedius</i>
Castalagin	50	50
(+) Catechin	>3,200	>3,200
EGC	100	200
ECg	800	800
EGCg	100	50
Myricitrin	3,200	1,600
Prodelphinidin	800	800
Procyanidins	200	100
Resveratrol	3,200	3,200
Rutin	>3,200	>3,200
Theaflavin	100	100
Thearubigin	200	200
Hinokitiol	≤50	≤50

EGC: (–)-epigallocatechin; ECg: (–)-epicatechin gallate; EGCg: (–)-epigallocatechin-3-O-gallate. Minimum inhibitory concentration: µg/ml.

against *M. pachydermatis* and *S. intermedius*. The aim of this study was to test this hypothesis by measuring the minimum inhibitory concentration (MIC) values.

Twelve kinds of polyphenols were used in this study (Fig. 1). These polyphenols were kindly provided by the Nagasaki University Graduate School of Biomedical Sciences (Table 1). The polyphenols were extracted from plants using the HPLC-based technique reported by Taguri *et al.*^{8,9)}. They were tested on two yeast species, *M. pachydermatis* and *Candida albicans*, and two bacterial species, *S. intermedius* and *Achromobacter xylosoxidans*. The yeasts and bacteria were obtained from the National Institute of Technology and Evaluation and the RIKEN BioResource Research Center (Table 2). Hinokiol, an essential oil derived from *Fallopia japonica* was used as

a control.

Each polyphenol's MIC value was determined using agar plate assays⁵⁾. Agar plates were prepared with polyphenol concentrations of 50–3,200 µg/ml in 60-mm sterile dishes. YM agar (formulated with 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1.5% agar, and pure water) and Mueller-Hinton agar (formulated with 30.0% peptone, 1.75% casein, 0.15% starch, 1.7% agar, and pure water) were used for the yeast and bacterial cultures, respectively. Each inoculum was prepared at a concentration of 1.0×10^6 CFU/ml and streaked in a 20-mm line on the agar plates. The cultures were incubated for 48 h at 28°C for yeast or 37°C for bacteria. The MIC of a polyphenol was defined as the minimum concentration at which microbial

Table 5. The relationships between inoculum size and the minimum inhibitory concentration of castalagin

Microorganisms	Inocula	Concentration in Castalagin (µg/ml)							MIC
		1,600	800	400	200	100	50	0	
<i>Malassezia pachydermatis</i>	1×10 ⁴	–	–	–	–	+	+	+	200
	1×10 ³	–	–	–	–	–	–	+	
	100	–	–	–	–	–	–	+	
<i>Candida albicans</i>	1,400	±	±	±	±	+	+	+	>1600
	140	±	±	±	±	+	+	+	
	14	±	±	±	±	+	+	+	
<i>Achromobacter xylosoxidans</i>	4.0×10 ⁴	–	–	–	–	–	+	+	100
<i>Staphylococcus intermedius</i>	8.8×10 ³	–	–	–	–	–	–	+	50

±: Colony size was reduced with no change in bacterial count.

growth was inhibited. Growth inhibition was confirmed at the end of the 48-h culture period. Two experiments were performed for each microbial species and the lower MIC value (indicating stronger activity) was selected as the representative value. The polyphenol with the lowest MIC value among the 12 tested was then used to determine the MIC values for different inoculum sizes.

Of all the polyphenols used in this study, seven showed MIC values of <3,200 µg/ml against *M. pachydermatis*. Of these, castalagin, a polyphenol derived mainly from *Castanea crenata* and *Quercus acutissima*, had the lowest MIC value, 100 µg/ml. When tested against *C. albicans*, only theaflavin (1,600 µg/ml) showed a real-number MIC value (Table 3). These results suggest that castalagin may be effective against skin diseases caused by infection with *M. pachydermatis*, even if used as a monotherapy. Our results also show that (–)-epigallocatechin-3-O-gallate had a MIC value (50 µg/ml) comparable to that of castalagin against *S. intermedius*. All polyphenols had MIC values between 100 and 3,200 µg/ml against this bacterium except for (+)catechin and rutin, whose MIC values were >3,200 µg/ml. Castalagin had a low MIC value of 50 µg/ml against *A. xylosoxidans*, a non-glucose fermenting gram-negative bacterium. Except (+)catechin and rutin, the other polyphenols had values between 100 and 3,200 µg/ml against *A. xylosoxidans* and *S. intermedius* (Table 4). This suggests a wide antimicrobial activity spectrum of polyphenols against bacteria. The MIC values of castalagin against yeast and bacterial species of different inoculum sizes are shown in Table 5. The MIC value was 200 µg/ml against 1 × 10⁴ CFU/ml of *M. pachydermatis* and 50 µg/ml against 8.8 × 10³ CFU/

ml of *S. intermedius* (Table 5). These results suggest that 200 µg/ml of castalagin may be effective against different types of pathogens.

This study had some limitations. One of these was that the only type of bacteria tested among the SIG was *S. intermedius*. Therefore, it will be necessary to determine the MIC values of the tested polyphenols against *S. delphini* and *S. pseudintermedius*. Also, the method of testing used for the yeast species in this study has not yet been formally accepted. Furthermore, while the aim of this study was to assess *Malassezia* spp., only one strain of one species of this pathogen was actually tested. Therefore, further investigations will need to be conducted using more strains.

In conclusion, among the 12 polyphenols tested in this study, castalagin appeared to exert the highest antimicrobial activity, suggesting it could be used in the treatment of canine chronic skin diseases associated with infections with *M. pachydermatis* and SIG. The potential use of castalagin for these purposes should be investigated further.

Conflict of interest

None of the authors has any conflict of interest to declare.

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