수의학석사학위논문

Antimicrobial effect of *Chamaecyparis obtusa* and *Thuja orientalis* essential oils on *Staphylococcus pseudintermedius* isolated from canine skin and ears

개의 피부와 귀에서 분리한
*Staphylococcus psuedintermedius*에 대한
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Antimicrobial effect of *Chamaecyparis obtusa* and *Thuja orientalis* essential oils on *Staphylococcus pseudintermedius* isolated from canine skin and ears

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Abstract

Staphylococcus pseudintermedius is the most commonly isolated opportunistic pathogen from skin and ear in dogs. The prevalence of methicillin-resistant or multi-drug resistant *S. pseudintermedius* is increasing. This has resulted in limited selection

of antibiotics, which has become an important therapeutic challenge for veterinarians. The aim of this study is to evaluate the *in vitro* antimicrobial effect of *Chamaecyparis obtusa* and *Thuja orientalis* essential oils against bacteria and biofilm produced by *S. pseudintermedius*. Antimicrobial effect of two essential oils was assessed using 30 isolates of *S. psuedintermedius* form dogs with pyoderma and otitis externa. To evaluate the antimicrobial effect on bacteria, minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were measured and the results were compared to those of cephalexin. The MICs and MBCs of cephalexin were determined in the range of 2~256 μg/ml and 64~>256 μg/ml, respectively. The MICs of both essential oils were confirmed in the range of 2^{-4}~2^{-5} % (v/v) and the MBCs were confirmed in the range of 2^{-1}~2^{-4} % (v/v) and 2^{-2} ~2^{-4} % (v/v) for *C. obtusa* and *T. orientalis*, respectively. Although 70% of isolates are resistant to cephalexin, the MICs and MBCs of both essential oils were determined in constant range on all isolates including cephlexin resistant isolates. To evaluate the antimicrobial effect on established biofilm, biofilm inhibitory concentrations (BICs) and biofilm eradication concentrations (BECs) were determined. BICs of both essential oils were identified in the range of twofold~sixfold of MICs, %, v/v). BECs of *C. obtusa* and *T. orientalis* were identified in the range of fourfold~ninefold of MICs (%, v/v) and twofold~ninefold of MIC (%, v/v), respectively. Also, significant dose-dependent

decrement of biofilm formation by both essential oils was determined by colorimetric microtiter assay for most isolates when comparing with the positive controls.

This study demonstrates a significant *in vitro* antimicrobial effect of both essential oils on bacteria as well as biofilm produced by *S. pseudintermedius*. These results suggest the potential value of essential oils of *C. obtusa* and *T. orientalis* as alternative treatment options for skin and ear infections by *S. pseudintermedius*.

Key words: *Chamaecyparis obtusa*, *Thuja orientalis*, essential oils, antimicrobial effect, *Staphylococcus pseudintermedius*, dog

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CONTENTS

Introduction

Staphylococcus pseudintermedius is the most commonly isolated opportunistic pathogen from skin and ear in dogs (Duijkeren *et al*., 2011; Yoon *et al*., 2010; Stegmann *et al*., 2010). Over the last few years, concerns about considerably increasing prevalence of methicillin-resistant *S. pseudintermedius* (MRSP) have been raised in many countries (Beck *et al*., 2012; Feng *et al*., 2012, Song *et al*., 2013; Duijkeren *et al*., 2011). A relatively high proportion of MRSP was found among the *S. pseudintermedius* isolated from dogs with pyoderma in Japan (66%) and Korea (78%) (Onuma *et al*., 2012; Song *et al*., 2013). Furthermore, resistance not only to beta-lactam antibiotics but also to other commonly used antibiotics meaning multi-drug resistance has been confirmed by recent studies (Song *et al*., 2013; Duijkeren *et al*., 2011; Onuma *et al*., 2012; Ruscher *et al*., 2010; Yoon *et al*., 2010). For this reason, limited selection of antibiotics is becoming an important therapeutic challenge for veterinarians.

Essential oils as a solution for the increasing resistance to antibiotics have been drawing attention in various fields. Antimicrobial effect of diverse essential oils such as tea tree oil, geranium oil, lavender oil, grapefruit seed extract and manuka honey to multiple bacteria including MRSP has been examined for decades (Song *et al*., 2013; Lang and Buchbauer, 2011; NCC and Junior A, 2010; Burt, 2004; Edris, 2007).

Materials used in this study, *Chamaecyparis obtusa* and *Thuja orientalis*, are evergreen coniferous trees distributed over Asia. Antimicrobial effects of *C. obtusa* against various bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* have been demonstrated, and the essential oil of *C. obtusa* has been commercially used in soap, toothpaste and cosmetics (Hong *et al*., 2004; Yang *et al*., 2007; Park *et al*., 2010; Lee *et al*., 2009). Although not well known as *C. obtusa*, antimicrobial effect of *T. orientalis* also has been confirmed against several bacteria. (Jain and Garg, 1997; Ezzat, 2001) However, antimicrobial effect on *S. pseudintermeius* of two essential oils has not been studied.

Recently, biofilm formation as an important virulence factor of most bacteria is growing concern for both human and veterinary medicine, because of the relation between biofilm producing ability of bacteria and increased resistance to antibiotics (Nostro *et al*., 2007; Singh *et al*., 2013; Jacques *et al*., 2010; DiCicco *et al*., 2012). Therefore, the antimicrobial effect on biofilm has become an important factor of antimicrobial agent. Although, the biofilm producing ability of *S. pseudintermedius* has not completely known, one recent report confirmed the biofilm producing ability in a high proportion of *S. psuedintermeius* isolates obtained from dogs (Singh *et al*., 2013).

The aim of this study is to assess the potential of *C. obtusa* and *T. orientalis* essential oils as alternatives to antibiotics by demonstrating the *in vitro* antimicrobial effect against bacteria and biofilm produced by *S. pseudintermedius*.

Materials and method

1. Essential oil

Pure 100% essential oils of *C. obtusa* and *T. orientalis* were provided by Innohai Co., Seoul, Republic of Korea. The aerial parts of *C. obtusa* and *T. orientalis* were collected and essential oils were extracted by steam distillation. The collecting location, year, and extracting institutions are shown in Table 1.

2. Bacterial isolates

The bacteria used were *S. pseudintermedius* (n=30) isolated aseptically from dogs suffering from pyoderma (n=25) or otitis externa (n=5) submitted to the veterinary teaching hospital, Seoul National University (Seoul, Korea) over the period of 2009 to 2013. Signalment of patients is shown in Table 2. All isolates were initially identified as *S. intermedius* group (SIG) using standard biochemical tests and the Vitek 2 system (bioMérieux, Hazelwood, MO, USA). These isolates were further identified as *S.*

pseudintermedius by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assay. The assay detected the *MboI* restriction pattern of the 320 bp *pta* gene fragment using the oligonucleotide primer (forward, 5′-AAA GAC AAA CTT TCA GGT AA-3′; reverse, 5′-GCA TAA ACA AGC ATT GTA CCG-3′), as described previously. (Bannoehr *et al*., 2009)

3. Effect on bacteria

Antimicrobial effect of two essential oils on bacteria was evaluated by measuring minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2011). These results were compared to those of cephalexin.

(1) Measuring MICs

The MICs were determined using an agar dilution method. Briefly, twofold serial dilution of each essential oil (ranging from 2^{-1} to 2^{-10}%, v/v) and cephalexin (ranging from 256 to 0.125 μg/ml) was prepared in Muller-Hinton agar (MHA; BD Diagnostic Systems) with 1% (v/v) Tween-80. MHA with 1% (v/v) Tween-80 without essential oil and cephalexin, was used as a positive growth control. The plates were incubated with a final inoculum of $1X10^7$ CFU/ml of each isolate and incubated at 37℃ for 16 h. The MICs were determined as the lowest concentration of test agent inhibiting visible growth of each bacterial isolate on the agar plate. The results of both intermediate and resistant isolates according to CLSI guidline are interpreted as resistant.

(2) Measuring MBCs

The MBCs were determined using a broth dilution method. Briefly, 100$\mu\ell$ of twofold serial dilution of each essential oil (ranging from 2^{-1} to 2^{-5} %, v/v) and

cephalexin (ranging from 256 to 2 ㎍/ml) was prepared in Muller Hinton broth (MHB; BD Diagnostic Systems) with 1% (v/v) Tween-80, and added in 96-well polystyrene microtiter plates (SPL Life Science, Seoul, Korea). MHB with 1% (v/v) Tween-80 without essential oil and cephalexin was used as a positive growth control. Bacterial cultures were grown in MHB at 37 ℃ overnight. An aliquot of 100 ㎕ was inoculated into each well with a final inoculum of $5X10^5$ CFU/ml. After 16 h of incubation at 37℃, 20㎕ of each culture medium from the microtiter plate wells onto MHA plates. After 16 h of incubation at 37℃, MBCs were determined as the lowest concentration of each compound where no bacterial colonies appeared on the MHA. All MICs and MBCs measurements were performed in duplicate.

4. Effect on biofilm formation

The effect of essential oils on biofilm formation was tested using a colorimetric microtiter plate assay (Cramton *et al*., 1999; Nostro *et al*., 2007; Sandasi *et al*., 2008).

Briefly, bacterial cultures were grown overnight in Tryptic Soy broth (TSB; BD Diagnostic Systems) with 1% glucose. One hundred microliters of an inoculum of $1X10^6$ CFU/ml in TSB was inoculated into each well of 96-well polystyrene microtiter plates in the presence of 100㎕ of essential oils at different concentrations (0.25 MIC, 0.5MIC, MIC, %, v/v). After incubating plates at 37℃ for 24 h, each well was washed three times with Phosphate-buffered saline (PBS) and stained with crystal violet solution for 15 min at room temperature. To eliminate unbound crystal violet dye, the plates were washed three times with PBS. The plates were air-dried and 150 ㎕ of 95% ethanol were added to each well to destain biofilm-attached crystal violet. One hundred microliters from each well was then transferred to a new microtiter plate and the optical density was measured at 450 nm by an enzyme-linked immunosorbent assay reader (BioRad, Munich, Germany). Wells containing the tested bacteria without essential oil were used as positive control and wells containing growth medium without bacterial inoculation were used as negative controls. All tests were performed in duplicate. For convenience of comparison, relative biofilm formation, which means

the percentage of optical density relative to the values of positive controls which were set as 100%, was used. Relative biofilm formation = (mean OD_{450} of treated well/mean OD_{450} of control well) X 100

5. Effect on established biofilm

The inhibitory effect of essential oils on established biofilm was evaluated as described previously with some modifications (Johnson *et al*., 2002, Nostro *et al*., 2007). Bacterial cultures were grown overnight in TSB with 1% glucose. An inoculum of $1X10^6$ CFU/ml in TSB was added to 96-well polystyrene microtiter plates to form biofilm. After 24 h of incubation at 37 ℃, the supernatant containing bacteria was gently removed and the wells were washed three times with PBS. Each well was filled with 100 ㎕ of essential oils with 1% (v/v) Tween-80, ranging from the MIC to ninefold of the MIC (%, v/v). The OD_{450} was measured at time 0. After the plates were incubated for 24 h at 37℃, the OD_{450} was measured again. The biofilm inhibitory

concentrations (BICs) were determined as the lowest concentration where no increase in OD_{450} at time 24 h compared to OD_{450} at time 0. After taking biofilm from the bottom of wells above BIC using a sterilized polypropylene loop and needle (SPL Life Science, Seoul, Korea), it was streaked on Tryptic Soy agar (TSA; BD Diagnostic Systems) plates, and incubated for 24 h at 37 ℃. The biofilm eradication concentrations (BECs) were determined as the lowest concentration where no bacterial growth occurred on the TSA plates. All BICs and BECs measurements were performed at least in duplicate.

6. Statistical analysis

Values of inhibitory effect on biofilm formation of both essential oils at different concentrations were compared with the values of negative controls using student's *t*-test (Excel, Microsoft). *P*-values < 0.05 were considered significant.

Results

1. Determination of MICs and MBCs

The MICs and MBCs of two essential oils against *S. pseudintermedius* were determined and the results were compared with those of cephalexin which is one of the most commonly used antibiotics for canine pyoderma patients. While the MICs of both essential oils were obtained in the rage $2^{-4} \sim 2^{-5}$%, v/v, that of cephalexin were obtained in the range of 2 ~ 256 μg/ml. These results indicate that 70 % of isolates are resistant to cephalexin. The results of the MBCs of essential oils of *C. obtusa* and *T. orientalis* were obtained in the range of $2^{-1} \sim 2^{-4}$ %, v/v and of $2^{-2} \sim 2^{-4}$, %, v/v, respectively. The results of MICs and MBCs indicate the bactericidal effect of *C. obtusa* and *T. orientalis* essential oils on 83 % and 93 % of isolates, respectively. The MBCs of cephalexin were obtained in the range of 64~>256 μg/ml (Figure 1 and Table 3).

2. Effect on biofilm formation

The results of effect on biofilm formation of two essential oils were converted to relative biofilm formation. Although biofilm formation of 10 % and 23 % of isolates were not inhibited at 0.25 MIC by *C. obtusa* and *T. orientalis* eseential oils respectively, significant dose-dependent decrement of biofilm formation by both essential oils was observed for most isolates compared to positive control. The mean values of relative biofilm formation of *C. obutsa* were 74.1 ± 26.1 (p-value < 0.001) at 0.25 MIC (%, v/v), 63.2 ± 29.1 (p-value < 0.001) at 0.5 MIC (%, v/v), 55.0 ± 26.1 (p-value < 0.001) at MIC (%, v/v) and *T. orientalis* was 80.3 ± 22.9 (p-value < 0.001) at 0.25 MIC (%, v/v), 57.7 ± 21.7 (p-value < 0.001) at 0.5 MIC (%, v/v), 50.0 ± 26.8 (p-value < 0.001) at MIC (%, v/v) (Figure 2).

3. Effect on established biofilm

The effect on established biofilm of all isolates was identified in the presence of both

essential oils at higher concentration than MICs. BICs of both essential oils were identified in the range of twofold ~ sixfold of MIC (%, v/v). BECs of *C.obtusa* and *T. orientalis* essential oils were identified in the rage of fourfold ~ ninefold of MICs (%, v/v) and twofold ~ ninefold of MICs (%, v/v) respectively (Figure 3 and table 3).

Discussion

S. pseudintermedius is a leading causative agent of pyoderma and otitis externa in dogs which has become a growing concern of resistance to various antibiotics (Yoon *et al*., 2010; Song *et al*., 2013; Duijkeren *et al*., 2011; Ruscher *et al*., 2010). In order to find a solution for the limited option of antibiotics, this study demonstrated the antimicrobial effect of *C. obtusa* and *T. orientalis* essential oils against *S. pseudintermedius in vitro*, identifying a possibility of these agents to be used as an alternative therapeutic agent.

In present study, the MICs and MBCs of cephalexin were determined in broad

range, suggesting 70 % of isolates were resistant. In contrast, the MICs and MBCs of both essential oils were measured in a constant range for all isolates including cephalexin resistant isolates. These results indicate that all isolates were susceptible to both essential oils irrespective of the status of cephalexin resistance.

Studies have shown that *C. obtusa* essential oil retains significant antimicrobial effect on diverse microorganisms including bacteria and fungi. Compared to the most effective MIC (0.156, %, v/v) for *S. aureus* (Arima et al., 2002), lower MIC (0.051, %, v/v) was determined for *S. pseudintermedius* in this study. Although studies about *T. orientalis* essential oil are limited, a study revealed dose-dependent antimicrobial effect on *S. aureus* using disk diffusion test. In this study, the MIC of *T. orientalis* evaluated was 0.054, %, v/v on *S. pseudintermedius*, which is comparable to the MIC of *C. obtusa* (Jain and Garg, 1997). Taken together, this study demonstrates that both essential oils have a significant antimicrobial effect on *S. pseudintermedius* as on *S. aureus*.

A bacterial biofilm is characterized by a sessile composite of bacteria surrounded by self-produced matrix consists of carbohydrates, proteins and DNA attached to biologic or non-biologic surfaces. Increased resistance to antibiotics of biofilm-producing bacteria may be due to the protective nature of biofilm against host immune responses, shear forces and antimicrobial penetration (Jacques *et al*., 2010; Singh *et al*., 2013; Nostro *et al*., 2007; DiCicco *et al*., 2012). Therefore, the importance of inhibitory effect of antimicrobial agent on bacteria as well as on biofilm is increasing.

Inhibitory effect of two essential oils on biofilm formation as well as established biofilm was demonstrated in this study. Although different inhibitory effect was identified depending on essential oils and bacterial isolates, both essential oils showed significant inhibitory effect in a dose-dependent manner on biofilm formation of most isolates. The mean values of relative biofilm formation of both essential oils at MICs were less than 60%. The BICs and BECs, which were evaluated to identify inhibitory effect on established biofilm, were determined to be higher concentration than MICs. Also, the BICs and BECs were identified in broad ranges, which suggest that each

isolate produces biofilm with diverse characteristics causing difference in susceptibility to antimicrobial agents. Despite of this characteristic, both essential oils showed inhibitory effect on established biofilm produced by all isolate within the set ranges.

The mechanisms of antimicrobial activity of essential oils have not been clearly evaluated, but it has been suggested that the cytotoxic effect on cellular membrane is caused by components of essential oils such as phenol, aldehyde and alcohol (Bakkali *et al*., 2008; Cox *et al*., 2000). Terpinen-4-ol, one of the chemical components of essential oil of *C. obtusa*, was shown to have high antibacterial activity against various bacteria (Park *et al*., 2010). However, further study to confirm the mechanism of antimicrobial effect of both essential oils on bacteria is necessary.

Despite the antibacterial properties of both essential oils, toxicity and side effects when applied *in vivo* must be considered when applying essential oils as topical agent. Although the exact mechanism is unclear, a few studies suggested that the cytotoxic effect of components of essential oils on cellular membrane also affect the normal cells,

reporting the cytotoxic effect of essential oils on human epithelial and fibroblast cells *in vitro* and skin irritation in mice *in vivo* (Park *et al*., 2010; Soderberg *et al*., 1996). Therefore, further study to determine toxicity and side effects when applied *in vivo* is also necessary to apply these agents effectively and safely.

In conclusion, this study demonstrates a significant *in vitro* antimicrobial effect of both essential oils to bacteria as well as biofilm produced by *S. pseudintermedius*. These results suggest that *C. obtusa* and *T. orientalis* essential oils may be alternative treatment options for skin and ear infections caused by *S. pseudintermedius*.

Table 1. Detailed information of essential oils.

	C. obtusa	*T. orientalis*
Collecting location	Hwasoon, Jeonnam, Republic of Korea	Mt. Baekdu, Yanggang, Democratic People's Republic of Korea
Collecting year	2003	2011
Extracting institution	Enbita Co., Ltd, Republic of Korea	Pyongyang essential oils research centre, Democratic People's Republic of Korea

Table 2. Signalment of patients and sample isolated location.

Patient number	Age (years)	Sex	Breed	Sample isolated location
1	10	F	Maltese	Skin
2	10	MC	Poodle	Skin
3	8	FS	Shih Tzu	Skin
4	6	MC	Miniature Pinscher	Skin
5	14	MC	Maltese	Skin
6	11	MC	Shih Tzu	Skin
7	11	FS	Cocker Spaniel	Ear
8	9	FS	Maltese	Skin
9	9	FS	Cocker Spaniel	Skin
10	9	F	Poodle	Skin
11	11	MC	Cocker Spaniel	Skin
12	11	MC	Cocker Spaniel	Ear
13	9	FS	Maltese	Skin
14	10	FS	Maltese	Skin
15	10	MC	Pug	Skin
16	12	FS	Cocker Spaniel	Ear
17	12	FS	Cocker Spaniel	Ear

18	8	F	Cocker Spaniel	Skin
19	9	M	Cocker Spaniel	Skin
20	3	F	Golden Retriever	Skin
21	Unknown	M	German Shepherd	Skin
22	8	FS	Pug	Ear
23	6	MC	Shih Tzu	Skin
24	3	MC	Dachshunds	Skin
25	14	MC	Shih Tzu	Skin
26	12	MC	Pekinese	Skin
27	15	F	Shih Tzu	Skin
28	1	M	Beagle	Skin
29	1	M	Beagle	Skin
30	6	MC	Shih Tzu	Skin

Table 3. Effect of *C. obtusa* and *T. orientalis* essential oils on bacteria and established biofilm. Mean values of all isolates.

	MICs (%, v/v)	**MBCs (%, v/v)**	**BICs (%, v/v)**	**BECs (%, v/v)**
C. obtusa	0.051 ± 0.015	0.167 ± 0.090	0.158 ± 0.078	0.333 ± 0.083
T. orientalis	0.054 ± 0.013	0.138 ± 0.072	0.334 ± 0.081	0.292 ± 0.090

Data are expressed as the means ± standard deviation (SD).

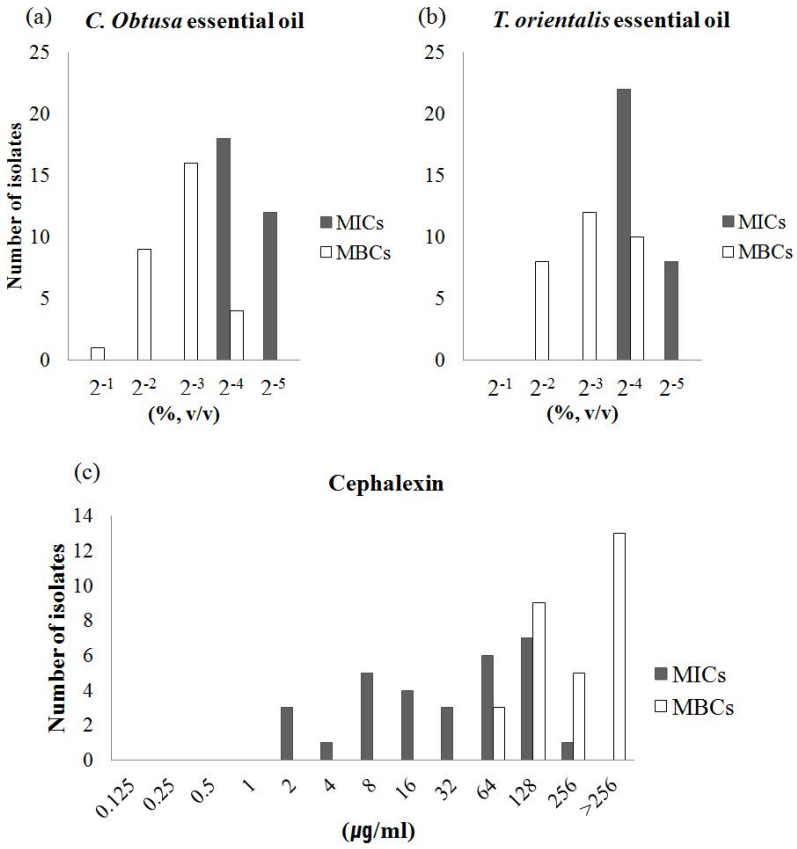

Figure 1. Minimal inhibitory concentrations (MICs) and minimal bactericidal concentratins (MBCs) of *C. obtusa* (a) and *T. orientalis* (b) essential oils and cephalexin (c) against *S. pseudintermedius*.

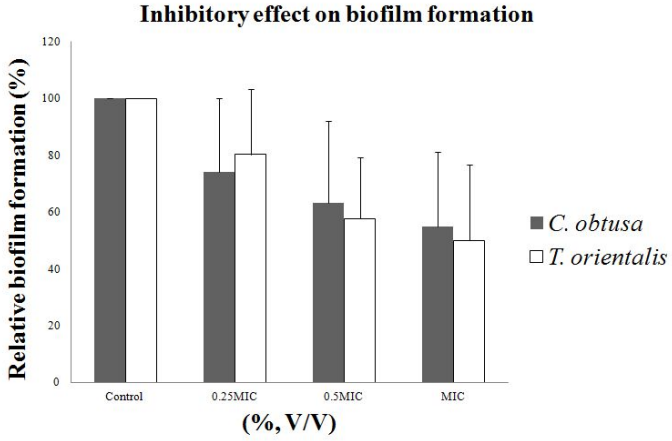

Figure 2. Effect of *C. obtusa* and *T. orientalis* essential oils on biofilm formation. Data are expressed as the means ± SD.

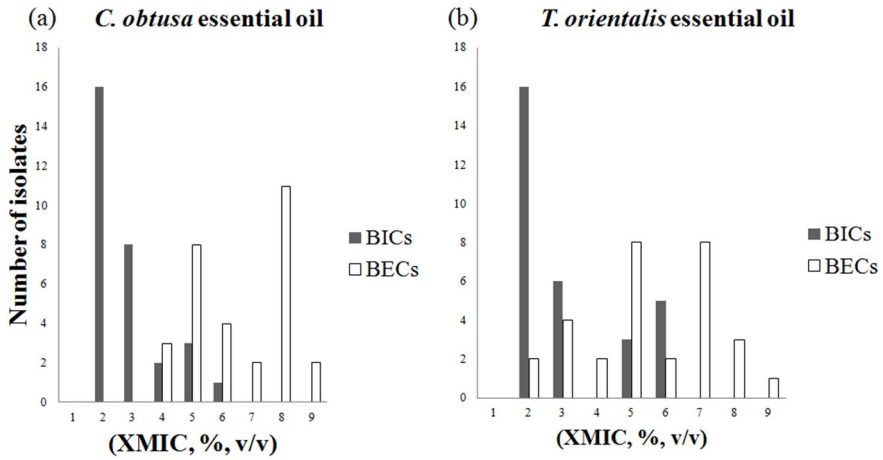

Figure 3. Biofilm inhibitory concentrations (BICs) and biofilm eradication concentrations (BECs) of *C. obtusa* (a) and *T. orientalis* (b) essential oils *against S. pseudintermedius*.

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국문 초록

개의 피부와 귀에서 분리한 *Staphylococcus pseudintermedius*에 대한 편백나무와 측백나무 에센셜 오일의 항균효과

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*Staphylococcus pseudintermedius*는 개의 피부와 귀에서 가장 흔히 분리되는 기회 감염균이다. 최근 methicillin 또는 대중 항생제에 내성을 보유한 *S. pseudintermedius*에 의한 감염이 증가하고 있으며, 이에 따라 항생제의 선택이 제한되는 경우가 많아 이는 수의사의 당면 과제가 되고 있다. 또한, 세균의 항생제에 대한 저항성을 증가시키는 것과 관련이 있는 것으로 생각되는 biofilm에 대한 관심이 증가하고 있다. 본 연구는 편백나무와 측백나무 에센셜 오일의 *S.pseudintermedius*의 부유세균과 biofilm에 대한 *in vitro*에서의 항균 효과를 평가하고자 수행되었다. 개의 농피증과 외이염 환자로부

터 분리된 30개의 *S. pseudintermedius* 균주에 대하여 에센셜 오일의 항균 효과를 평가하였다. 부유세균에 대한 항균력 평가를 위하여 에센셜 오일의 minimum inhibitory concentrations (MICs)과 minimum bactericidal concentrations (MBCs)를 측정하였으며 대조군으로 cephalexin을 사용하였다. Cephalexine의 MICs는 2~256 μg/ml, MBCs는 64~>256 μg/ml로 확인되었으며, 70%의 세균에서 cephalexine에 대한 저항성을 가지는 것으로 평가되었다. 에센셜 오일의 경우 cephalexin에 저항성을 나타낸 균주를 포함한 30개의 모둔 균주에 대하여 일정한 범위에서 MICs와 MBCs가 측정되었다. MICs는 두 에센셜 오일 모두 2^{-4}~2^{-5} % (v/v), MBCs는 편백나무의 경우 2^{-1}~2^{-4} % (v/v), 측백나무의 경우 2^{-2} ~2^{-4} % (v/v)에서 확인되었다. Biofilm에 대한 항균 효과를 확인하기 위하여 이미 형성된 biofilm에 대한 억제 효과를 biofilm inhibitory concentrations (BICs)와 biofilm eradication concentrations (BECs)를 통하여 평가하였다. 모든 세균 샘플에 대하여 에센셜 오일이 biofilm으로 부터의 세균 생성을 억제하고 biofilm을 제거하는 것이 확인되었다. BICs는 두 에센셜 오일 모두 MICs의 2배에서 6배로 확인되었으며, BECs는 편백나무의 경우 MIC의 4배에서 9배, 측백나무의 경우 MIC의 2배에서 9배로 확인되었다. Biofilm의 형성 과정에 대한 억제 효과를 colorimetric microtiter assay를 이용하여 평가하였다. 에센셜 오일이 포함되지 않은 양성 대조군과 비교했을 때, 대부분의 균주에 대하여 모든 설정 농도 범위 (0.25 MIC, 0.5 MIC, MIC)에서 유의적인 용량 의존성의 biofilm 형성 억제 효과가 확인되었다.

상기 결과들은 편백나무와 측백나무 에센셜 오일이 개의 피부와 귀의 감염을 유발하는 주된 원인균인 *S. pseudintermedius*에 대하여 탁월한 항균 효과를 가지고 있음을 나타낸다. 이는 본 제제 들이 개의 농피증과 외이염을 유발하는 원인체에 대한 항생제 대체물질로서의 활용 가능성이 높다는 것을 의미한다.

주요어: *Chamaecyparis obtusa*, *Thuja orientalis*, essential oils, antimicrobial effect, *Staphylococcus pseudintermedius*, dog

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