Essential Oils as a Treatment Possibility Alternative in Dogs with Skin Infections

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Abstract*. The aim of this study was to investigate the susceptibility of bacteria to essential oils and to evaluate the possibility of using essential oils in treatment of skin infections in dogs. For the study, samples were taken from 6 dogs with skin infection, from which 11 bacterial species were identified and their susceptibility to antibiotics was determined. It was found that 70% of the isolates were multi-resistant. Bacterial susceptibility to essential oils was studied by the serial dilution method. Thyme, oregano and cinnamon leaf oils had broader-spectrum antibacterial activity (inhibited 90.9% of bacterial species) than other essential oils studied. Predominantly essential oils were effective in a concentration < 2.0%. An qual part mixture of 4 best acting essential oils – thyme, cinnamon leaf, oregano and geranium – showed synergistic properties and was effective even at a concentration of 0.1%. The mixture, however, did not have a bactericidal effect on Pseudomonas aeruginosa even at higher concentrations. Consequently, essential oils are effective against a wide range of bacterial species at low concentrations, well below the safe concentration recommendations, making them an effective alternative to antibiotics for the treatment of canine skin infections.*

Introduction

Skin diseases in dogs are among the most common reasons why owners consult a veterinarian (Soedarmanto et al., 2011; Tresch et al., 2019). When skin defence mechanisms are weakened, a transient or persistent skin microbiota can become pathogenic and cause a bacterial infection that often requires treatment with antibiotics (Miller et al., 2012). Carriers of multidrug-resistant bacteria are at particular risk because of a very limited choice of antibiotics (Nazarali et al., 2015). Due to the rapid antibacterial resistance development and multidrug-resistant bacteria spread, alternative antibacterial therapies are being actively investigated and the use of antibiotics in clinical practice is promoted to be minimized (Beever et al., 2015; Tresch et al., 2019).

There is currently a growing scientific interest in essential oils because of their great antibacterial properties found in a big number of *in vitro* studies, which suggest that essential oils could replace traditional therapies with antibiotics and antiseptics (Ruzauskas et al., 2020; Tresch et al., 2019). Essential oils are very complex mixtures of terpenoid and non-terpenoid substances, the amount and species of which depend on the plant species and growing conditions (Wynn & Fougère, 2007). It is terpenoids that have a broad spectrum of antibacterial properties – they act by breaking down bacterial membrane structures, causing lysis and intracellular fluid leakage (Khalil et al., 2017; Mann et al., 2000). The synergistic antibacterial effect of combining essential oils is often mentioned (Al-Bayati, 2008; Wynn & Fougère, 2007).

Multiple researches have not shown any risk of spontaneous bacterial resistance to essential oils development. The multicomponent nature of essential oils suggests that the likelihood of bacterial adaptation to multiple substances at a time is very low (Davis et al., 2005; Hammer et al., 2008). It is also thought that the antibacterial mechanisms of action of essential oils by rapid membrane damage limit the resistance development (Yap et al., 2014).

Pure essential oil is toxic to cells reaching 50% mortality in 24 hours even at low concentrations (0.1%), so it is necessary to dilute it with base oils which reduce the cytotoxicity by at least twice (Orchard et al., 2019). When not diluted, essential oils cause side effects such as contact dermatitis, sensitization, exacerbation of inflammation and pain (Tisserand, 2014), so it is necessary to follow the precautions of use: storage, dosing and dilution recommendations (Wynn & Fougère, 2007). Dilution recommendations in the literature are for humans only and have wide limits (Kerr, 2002; Tisserand, 2014; Wynn & Fougère, 2007). Few veterinarian clinical trials indicate that better treatment outcomes are achieved with lower concentrations of essential oils (up to 10%) by inhibition of inflammation, reduction in healing time, and reduction or absence of side effects (Costa et al., 2019; Dursun et al., 2003; Gunal et al., 2014; Kerr, 2002).

Most essential oils have an LD50 1–20 mL/kg, so dosing and dilution have to be especially careful for smaller animals (Wynn & Fougère, 2007) and those with concomitant diseases or conditions (Poppenga,

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2002; Vanhaelen et al., 2002). It is important to thoroughly examine other effects of an essential oil intended to use to ensure safety in patients with contraindications, pregnancy, drug interactions possibility or coagulation issues (Khalil et al., 2017; Poppenga, 2002).

The aim of this study was to investigate the antibacterial effect of essential oils by determining their minimum inhibitory concentrations and to evaluate whether safe concentrations of essential oils can be sufficiently effective in the treatment of dog skin bacterial infections based on cytotoxicity studies and dilution recommendations provided in the scientific literature.

Materials and methods

Isolation and identification of bacteria

For the studies, samples were collected from the skin of 6 dogs with infection in Amies transport media with a swab (Transwab, MWE, UK). The clinical material was inoculated on universal soy-tryptone agar media with 7% sheep blood (Liofilchem, Italy) and selective media: Cetrimide Agar (Liofilchem, Italy), Slanetz-Bartley Agar (Liofilchem, Italy), Mannitol-Salt Agar (Liofilchem, Italy) and Endo Agar (Biolife, Italy). The media were incubated under aerobic conditions at +35°C and the blood agar under anaerobic conditions for up to 5 days, with daily media review and collection of grown colonies to identify the species.

Pure cultures were determined by morphological properties, growth pattern in universal (colony size, haemolysis, pigments) and selective media. Some bacteria were identified by oxidase, catalase, urease enzyme production, Gram-staining, agglutination reaction with specific sera, bacterial motility and gas (indole and hydrogen sulfide) production. In addition, biochemical studies were performed using Microgen (United Kingdom) identification systems (Staph-ID, Strep-ID, Bacillus-ID system, GN-A + B-ID system) according to the manufacturer's instructions. In cases when it was not possible to identify bacterial isolates by classical biochemical assays, the analysis of 16S rRNA sequences using primers 27F and 515R was performed as described previously (Ruzauskas et al., 2018).

Determination of bacterial susceptibility to antibiotics

After identification of the bacteria, their susceptibility to antibiotics was investigated by the disk diffusion method according to Kirby-Bauer. Susceptibility was assessed and interpretation of results was performed according to EUCAST recommendations (EUCAST, 2021) with updated clinical breakpoints. Antibiotics were cascaded by distinguishing the antibiotics of first, second, and third choice (Beco et al., 2013).

Selection of essential oils

Essential oils were selected according to dilution recommendations for humans, which are classified according to the skin irritation properties of the main substance (Table 1) (Tisserand, 2014). The selected substances belonged to moderately and mildly irritating substances, and the maximum concentration of 2.0% was chosen for the research.

Table 1. Classification of essential oils according to the degree of skin irritation and recommendations for their dilution for skin application

Skin irritation degree	Essential oil and its main substance		Maximum concentration recommendation
Severely irritating essential oils	Horseradish – sinigrin		Use on the skin is not recommended
	Mustard – allyl isothiocyanate		
	Garlic leaves – diallyl trisulfide		
Very irritating essential oils	Over 50%	Cinnamon bark, cassia – cinnamaldehyde	Dilute to 0.1%
	Sandalwood – santol		
	Saffron – safranal		
Moderately irritating essential oils	About 50%	Essential oils with cinnamaldehyde	Dilute to 1%
		Essential oils with eugenol	
		Essential oils with citral	
		Essential oils with carvacrol (> 50%)	
Mildly irritating essential oils	Essential oils with carvacrol (< 50%)		Dilute to 20%
	Essential oils with benzoic acid		
	Essential oils with citral (< 50%)		
	Essential oils with citronellol		
	Essential oils with thymol		
	Essential oils with geraniol		
	Essential oils with linalool		

Spain thyme essential oil (*Thymus zygis* thymol) with thymol (35.06%), sage essential oil (*Salvia sclarea*) with linalyl acetate (68.24%), geranium essential oil (*Pelargonium graveolens*) with citronellol (23.86%), radiant eucalyptus essential oil (*Eucalyptus radiata*) with eucalyptol (72.93%), ginger lemongrass essential oil (*Cymbopogon martinii* var. motia) with geraniol (73.08%), oregano essential oil (*Origanum vulgare*) with carvacrol (57.36 %), and cinnamon leaf essential oil (*Cinnamomum verum*) with eugenol (67.94%) were used in this study. The percentage of the main substances for each essential oil was indicated in GS/MS analysis provided by the manufacturer.

Determination of bacterial susceptibility to essential oils

The study was performed by the serial dilution method. Pure bacterial culture, cultured for 24 hours at 35°C under aerobic conditions taken from solid medium was diluted to 1.0 McFarland density with sterile saline. Then, 10.0 µL of culture solution was inoculated into 8 tubes with Mueller Hinton Broth (Liofilchem, Italy). The needed amount of essential oils was added to each tube. One tube was used for negative control without any oil. Then, the mixture was mixed for 20 seconds by an automatic shaker and incubated for 24 hours at +35°C. After incubation, 100 µL of the suspension was inoculated onto Tryptic soy agar to see the vitality of the bacteria. For this purpose, the plates were cultured for 24 hours at +35°C under aerobic conditions and bacteria growth was observed. In the absence of growth, the plates were incubated for up to 3 days. The process was performed with each of the 11 bacterial cultures tested with different concentrations of an essential oil – 2.0%, 1.5%, 1.0%, 0.5% and 0.2%.

Determination of synergistic antibacterial effects of essential oils

After evaluating four best acting essential oils – thyme, geranium, oregano and cinnamon leaf – the equal part mixture was made. All essential oils used for the mixture contained different main substances. The antibacterial effect of the mixture was tested by the serial dilution method, using the same technique as for the individual essential oils described in the section "Determination of bacterial susceptibility to essential oils". The antibacterial effect of the mixture at 0.1% concentration on isolated bacteria was studied. The susceptibility of *Pseudomonas aeruginosa* was further investigated for the mixture up to a concentration of 2.0%.

Statistical data analysis

The analysis of the results was performed using "Microsoft Office Excel 2017" and "SPSS/20" statistical programs. Crosstabulations were developed to examine the antibacterial activity of each essential oil, and the differences in the effect of the oils were determined according to the Pearson chi-square, the Kruskal–Wallis null hypothesis and the Fisher exact tests. The results of the calculations were considered statistically reliable if they reached more than 95% or $P < 0.05$.

Results

Pure bacterial cultures were isolated and identified from 6 dogs with skin infections (Table 2). Two different *S. pseudintermedius* isolates were isolated from the patient No. 1. All isolated bacteria species belonged to opportunistic pathogens and to transient or persistent microbial species.

The results of bacterial susceptibility to antibiotics showed that all bacteria were resistant to at least 2 antibacterial agents (Table 2). For most of the isolates, multidrug resistance was identified, especially for first- and second-line antibiotics. The resistance of *Citrobacter spp.*, *S. aureus*, *E. coli* and *P. aeruginosa* to the third-line antibiotics was found (erythromycin, imipenem, chloramphenicol and meropenem). *P. aeruginosa* was susceptible to only 2 of 7 antibiotics tested.

After studying the effect of individual essential oils, it was found that the effect of essential oils on bacteria differed depending on the type of an essential oil $P < 0.01$. Oregano, thyme and cinnamon leaf oils were found to inhibit 90.9% of bacterial species by a concentration of 1.0% (Table 3). Thus, these essential oils are established to have a broad-spectrum antibacterial effect. Other essential oils had a narrower bacterial species inhibition spectrum. All essential oils differed from each other in a statistically significant manner $P < 0.01$.

It was found that different bacterial species had different susceptibility depending on the type of an essential oil (Fig. 1). The figure shows the difference of an antibacterial effect on *E. coli* bacteria between sage and oregano essential oils: sage essential oil did not affect the bacterial growth even at 2.0% concentration, when oregano was effective at 0.5%. Data in the Table 3 show that the most sensitive bacteria to all essential oils were *P. multocida, Citrobacter spp., S. pseudintermedius-1, S. pseudintermedius-2* and *Streptococcus canis*, i.e., no specific essential oil was required to inhibit them. Other bacterial species required a specific essential oil for inhibition. *P. aeruginosa* bacteria was not suppressed by any of the 7 essential oils at a 1.0% concentration. This bacterium was affected only by thyme and oregano oil at 1.0% and 1.5%, respectively (Table 3).

Statistical analysis of the results to determine the relationship between bacterial Gram-staining (cell wall structure) and susceptibility to essential oils yielded statistically unreliable results ($P > 0.05$) meaning that essential oils may act equally on both gram-positive and gram-negative microorganisms.

The study of the made mixture of essential oils (thyme, geranium, oregano, cinnamon leaves) by serial dilutions resulted in a minimum inhibitory concentration of $\leqslant 0.1\%$ for all bacterial species (except

Table 2. Identified bacterial species and their susceptibility to antibiotics.

Patient number	Isolated bacteria species	Susceptibility to antibiotics														
		Penicillin	Gentamicin	Trimethoprim	Tetracycline	Cefalexin	Amoxicillin +CA	Cefoxitin	Enrofloxacin	Ciprofloxacin	Cefovecin	Erythromycin	Piperacine	Imipenem	Chloramphenicol	Meropenem
1	*Streptococcus canis*	R			R							S			S	
	Staphylococcus pseudintermedius-1	R	R	I	R			S	S			S				
	Staphylococcus pseudintermedius-2	R	R	S	R			S	I			S				
2	*Enterococcus faecalis*									R				I		
	Citrobacter spp.						R	R		S				S	R	S
	Staphylococcus chromogenes	R	R	S	S			R	S	R		S				
3	*Acinetobacter schindleri*		R							R				S		S
	Staphylococcus aureus	R	R	S	I			S	S			R				
4	*Escherichia coli*		R			S	R	R	S		R		S	R	R	S
5	*Pasteurella multocida*	R			R		R			S						
6	*Pseudomonas aeruginosa*		R						R		R		S	S	R	R

Note: R = resistant, I = intermediate, S = susceptible.

Table 3. Susceptibility of isolated bacteria to essential oils and their mixture.

Type of the essential oil	Main substance	Minimum inhibitory concentration of bacteria species (essential oil concentration, %)											Inhibition of bacteria species, n = 11, %
		E. coli	*S aureus*	*P. aeruginosa*	*Pasteurella multocida*	*Citrobacter spp.*	*Acinetobacter spp.*	*S. pseudintermedus-1*	*S. pseudintermedus-2*	*S. chromogenes*	*Enterococcus faecalis*	*Streptococcus canis*	
Oregano	Carvacrol 57.26%	0.5	0.2	1.5	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	90.9
Thyme	Thymol 35.06%	0.2	0.5	1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	90.9
Geranium	Citronellol 23.86%	> 2	0.2	> **2**	0.2	0.5	1	0.5	0.2	> 2	1	0.2	54.6
Ginger lemongrass	Geraniol 73.08%	0.5	1	> **2**	0.2	0.2	0.2	0.2	0.2	1	2	0.2	63.6
Eucalyptus	Eucalyptol 72.93%	0.2	0.5	> **2**	0.5	0.5	0.2	0.2	0.2	> 2	0.5	0.2	71.9
Cinnamon leaf	Eugenol 67.94%	0.2	0.2	> **2**	0.2	0.2	0.2	0.5	0.2	0.2	0.5	0.2	90.9
Sage	Linalyl acetate 68.24%	> 2	0.5	> **2**	0.5	0.5	0.5	0.5	0.5	> 2	> 2	0.5	63.6
Mixture T,G,O,C 1:1:1:1	Carvacrol thymol, eugenol, citronellol	⩽ 0.1	⩽ 0.1	> **2**	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	⩽ 0.1	90.9
Bacteria species susceptibility to essential oils, n = 7, %		71.4	85.7	–	100	100	85.7	100	100	42.8	57.1	100	–

Note: T – thyme, G – geranium, O – oregano, C – cinnamon leaf essential oils

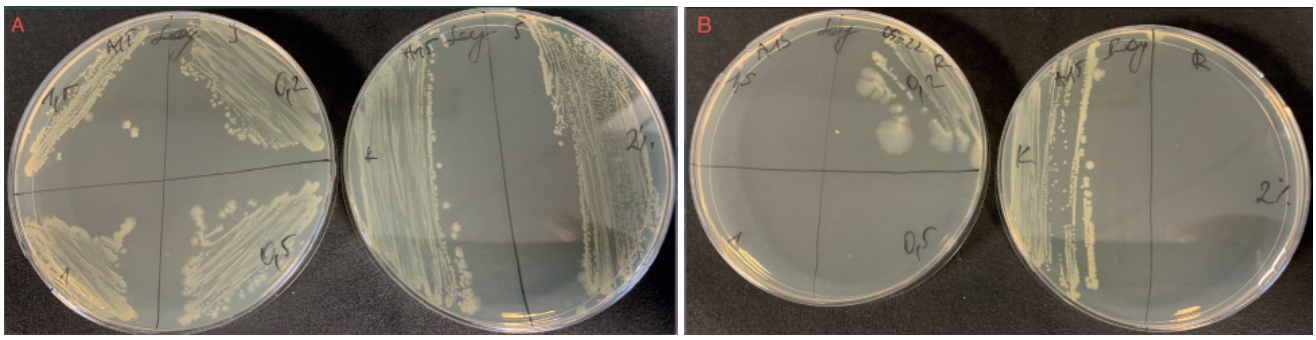

Fig. 1. Susceptibility comparison of *E. coli* (A15) to essential oils between two essential oils: A – sage essential oil had no effect (MIC > 2%), B – oregano essential oil – had a moderate effect (MIC = 0.5%)

P. aeruginosa). Synergy was found by lower MICs and a wider range of antibacterial effects. Statistical analysis of the results comparing the antibacterial effects of the individual oils and the mixture gave statistically significant results in all cases ($P < 0.01$).

Discussion

Bacterial multidrug resistance results confirm the problem of antibiotic resistance developing. The resistance found for the first, second and even third line antibiotics according to some authors could be greatly influenced by irresponsible use of antibacterial agents in clinical practice, not finishing the full course of antibiotics and contact transmission of multidrug resistant bacteria (Beever et al., 2015).

The studies have shown that the essential oils have a good effect on bacteria as mentioned in the literature. The antibacterial activity of essential oils has also been found to vary depending on the type of an essential oil (terpenoid type) (Oussalah et al., 2007; Tresch et al., 2019). The dependence of bacterial susceptibility to essential oils on the type of bacterium has been established as mentioned by other authors (Can Başer & Buchbauer, 2015; Orchard et al., 2019; Schnaubelt, 2012), but lower susceptibility of gram-negative bacteria to oils mentioned by Al-Bayati (2008) and Mann et al. (2000) was not statistically confirmed in this study.

The most resistant to essential oils was *Pseudomonas aeruginosa*, which was also mentioned by other authors as less sensitive than other bacteria. It requires higher concentrations of essential oils to inhibit. The resistance of this bacterium to essential oils is thought to be based on its inherent mechanisms of resistance to antibacterial agents (Arais et al., 2016; Chevalier et al., 2017; Smeriglio et al., 2017) and natural resistance to some natural antibiotics (Wynn & Fougère, 2007). In this study, only 2 essential oils were found to be effective against *Pseudomonas aeruginosa* – thyme and oregano with MIC 1.0% and 1.5%, respectively, although studies by different authors found significantly lower inhibitory concentrations of the same oils, such as 0.1% for thyme MIC. Such results may have been influenced by the reasons mentioned above. Although the literature mentions that *Pseudomonas aeruginosa* is more sensitive to a mixture of essential oils than to individual essential oils (Al-Bayati, 2008), the present study found the opposite: the mixture did not affect this bacterium even at higher concentrations. Data demonstrated that *P. aeruginosa* susceptibility may depend on the isolate.

The mixing of the 4 essential oils with different main substances achieved the synergistic effect mentioned in the literature, when the minimum inhibitory concentration for all bacterial species (except *P. aeruginosa*) was reduced at least twice (Al-Bayati, 2008; Wynn & Fougère, 2007). The synergy has also been identified for a wider range of bacteria species inhibition, making it possible not to look for a specific essential oil for the treatment. Research has also shown that mixing essential oils together increases their antibacterial activity and leaves cytotoxicity unchanged (Orchard et al., 2019; Ruzauskas et al., 2020). Thus, the blend was made from oils belonging to moderately and mildly irritating ones with dilution recommendations of up to 1% and up to 20%, and an effective concentration of 0.1% is more than 10 times lower than the safe one for dermal use (Beco et al., 2013; Tisserand, 2014). If such a mixture was prepared with *A. vera* or *S. chiensis* base oil, it is likely that the cytotoxicity would be reduced at least twice without attenuation of the antibacterial effect and it would have additional positive properties for wound healing (Edraki et al., 2014; Orchard et al., 2019).

Individual essential oils could be used to treat bacterial infections in dogs, but because bacteria species susceptibility to essential oils differs, the treatment should be started only after determining an effective essential oil type and its minimum inhibitory concentration to the infectious agent. A wider antibacterial spectrum and a lower effective concentration of the essential oil mixture would make it more convenient in clinical practice to use, thus achieving a positive effect and a minimal risk of side effects (Costa et al., 2019; Dursun et al., 2003; Gunal et al., 2014; Kerr, 2002). The treatment with essential oils is very promising, but until clinical trials of safety and effectiveness of their use in dogs are

made, such treatment can be used as an adjunct or as an alternative treatment when traditional treatment does not help.

Conclusions

In this study, 70% of dog skin isolates demonstrated multi-resistance to different classes of antibiotics.

The susceptibility of bacteria to essential oils was found to vary depending on the type of an essential oil and the species of bacterium: 6 of the 11 bacterial species required a specific essential oil to be inhibited. The most resistant to essential oils was *P. aeruginosa*, which was sensitive only to essential oils of thyme and oregano at concentrations $\geqslant$ 1.0%.

The essential oils of oregano, thyme and cinnamon leaf showed a broad antibacterial spectrum with 90.9% inhibition of bacterial species growth. It was found that the antibacterial potency of the essential oils did not depend on the Gram-staining of the bacteria ($P < 0.05$).

The essential oil mixture – thyme, geranium, oregano and cinnamon leaves – showed a synergistic effect by reduced minimum inhibitory concentration at least twice (to 0.1%) and by a broader spectrum of antibacterial effects than individual essential oils. The mixture did not affect *Pseudomonas aeruginosa*, so it may not be effective for treatment of the infection caused by *P. aeruginosa*.

The blend of essential oils was observed to be effective at very low concentrations against a wide range of bacteria and may therefore become an alternative to antibacterial therapy for the treatment of skin infections in dogs after further *in vivo* safety and efficacy clinical trials.

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