Scientific Research Publishing

Open Journal of Veterinary Medicine, 2017, 7, 63-75
http://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Effectiveness of *Thymus vulgaris* Essential Oil in the Treatment of Skin Infections in Dogs

Vincenzo Naccari[1], Bianca Maria Orlandella[2], Vittorio Fisichella[2], Francesco Naccari[2*], Santo Caracappa[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy
[2]Department of Veterinary Sciences, University of Messina, Messina, Italy
Email: *fnaccari@unime.it

How to cite this paper: Naccari, V., Orlandella, B.M., Fisichella, V., Naccari, F. and Caracappa, S. (2017) Effectiveness of *Thymus vulgaris* Essential Oil in the Treatment of Skin Infections in Dogs. *Open Journal of Veterinary Medicine*, **7**, 63-75.
https://doi.org/10.4236/ojvm.2017.76007

Received: April 27, 2017
Accepted: June 19, 2017
Published: June 22, 2017

Abstract

Currently, there are no data in the literature on the topical treatment with *Thymus vulgaris* essential oil (EO) in bacterial skin infections of dogs. This study evaluates the effectiveness of *Thymus vulgaris* EO for topical use in dogs with skin 18 half-breed dogs, affected by skin infections housed in a rescue shelter were studied. The bacteria isolated from these dogs were identified by MALDI-TOF-MS. The susceptibility of the isolated microorganisms to *Thymus vulgaris* L. EO was estimated *in vitro* by bacteriological test (CLSI 2015), in comparison to some antimicrobials drugs (*amoxicillin-clavulanic acid*, *doxycycline*, *thiamphenicol and marbofloxacin*) and to *Citrus bergamia* EO. The dogs, divided in two groups at *random*, were treated topically for 7 days with *Thymus vulgaris* L. EO (Group 1: n. 10 animals) and *Citrus bergamia* Risso e Poiteau EO (Group 2: n. 8 animals) respectively. The bacteria isolated were: *Staphylococcus pseudintermedius* (7 samples), *Staphylococcus sciuri* (4), ESBL *Escherichia coli* (3) and *Proteus mirabilis* (4). In all animals treated with *Thymus vulgaris* EO, the clinical signs decreased rapidly within 5 days from the administration, with complete remission 7 days after the treatment. No bacterial growth was observed from skin swabs after 7 days of treatment. None of the treated animals showed local or general side effects. The use of *Thymus vulgaris* EO could be a possible alternative or additional treatment to antibiotics in dermatological infections, particularly in cases refractory to conventional therapy.

Keywords

Bacteriological Skin Infections, Dog, *Thymus vulgaris* Essential Oil

1. Introduction

The indiscriminate use of antibiotics in human and veterinary medicine has

greatly contributed to the spread of microbial resistance and the increase of unexpected adverse reactions and allergies [1] [2]. Recently, in order to counteract the development and spread of bacterial resistance, promising prospects are essential oils (EOs), of which we already know the potential validity in the prevention and treatment of various infectious diseases [3] [4] [5].

EOs are a complex mixture of organic substances, aromatic oily liquids, biodegradable and with low toxicity, synthesized from plants and characterized by their aroma, generally produced from spices, aromatic herbs, fruits, flowers and plant material (buds, seeds, leaves, twigs, bark, wood, roots). These EOs can be obtained by expression, fermentation and extraction but the method of steam distillation is the most commonly used for their commercial production [6].

At present more than 3000 EOs are known, of which about 300 are commercially significant. The greatest use of EOs in the European Union (EU) is in food (as flavourings), perfumes (fragrances and aftershaves) and pharmaceuticals (for their functional properties).

Various studies report that EOs of many aromatic plants, in particular *Lamiaceae* (*Zea*, *Thymus*, *Melaleuca alternifolia*, etc.), possess antibacterial activity against many multi-resistant microbial species, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa*, *Klebsiella pmeumoniae*, ESBL *E. coli*, *Acinetobacter* spp, *Campylobacter jejuni*, *Listeria monocytogenes*, *Bacillus cereus*, etc. [7]-[12]. Furthermore, other studies show that these EOs also have antifungal activity [13] [14] [15]. Finally, it has been found that the EOs or their components possess antiviral [16], antioxidant [17] [18], antiparasitic [19] and anticancer activity [20] [21]. The mechanism of action of these EOs is due to the presence of terpenoid components, monoterpenoid (C10) and sesquiterpenoid (C15) metabolites, responsible also for anti-inflammatory effects [22] [23]. The most effective EOs seem to be those with higher content of alcohols and phenols, but also of aldehydes, therefore an accurate knowledge of the dosage and caution in the administration mode is necessary, to avoid sensitivity phenomena, accumulation and toxicity [24].

In vitro and preclinical studies report that the most valid EOs in bacterial infections, with or without fungal overlap, are *Thymus vulgaris* (red thyme), *Melaleuca alternifolia* (TTO), *Corydothymus capitatus* (common oregano), *Cinnamomum zeylanicum* (cinnamon) and *Citrus bergamia* (bergamot) [25].

Thymus EOs have always been considered rich in beneficial properties for human and animal health and the Egyptians also used it for embalming. Today, there is a strong interest in the biological properties of *Thymus* Eos for its evident effectiveness in several dermatological, uro-gynecological and gastrointestinal diseases [26] [27]. The main constituents of *Thymus vulgaris* EO, responsible for many therapeutic properties, are phenols and, in particular, *thymol* (30% - 70%), recognized as an antiseptic, antispasmodic and vermicide, used in the preparation of products based on thyme for internal and external use, and *car-*

vacrol (3% - 15%), an antiseptic widely used in perfumery; other constituents are linalool, cymene, timene, alpha-pinene and luteolin [28] [29]. Therefore, this EO possesses antimicrobial, antifungal, anti-inflammatory, expectorant and balsamic activity and is used against inflammation of the airways, such as asthma and cough. Finally, for its antiseptic properties it is also used for acne and for the treatment and disinfection of small wounds [30] [31].

In recent years, there has arisen particular interest in the use of some EOs in various human diseases, however there are few studies investigating effects on animal pathogens. In our previous studies, the antimicrobial activity of some EOs (*Thymus vulgaris* and *Eucalypsus globus*) was tested in the treatment of ovine mammary pustular dermatitis and livestock mastitis documenting the effectiveness of the *Thymus vulgaris* EO in this disease [32] [33] [34].

The aim of this investigation was to study *in vivo* the therapeutic effects of *Thymus vulgaris* EO for topical use against pathogen strains in skin infections of dogs housed in a rescue shelter.

2. Materials and Methods

2.1. Animals

The study was carried out during the summer 2015 on 18 half-breed dogs (3 - 10 *years old and body weight* 15 - 20 kg), suffering from skin diseases, housed in a rescue shelter located in Messina (Southern Italy). The animals, fed with a commercial dry food and free of antibiotics, were selected based on evident skin lesions among 120 examined dogs.

2.2. Clinical Signs

The dogs affected by skin infections showed clinical signs such aspruritus, alopecia and/or erythema, presence of pustules or crusts, ulcers and/or erosions. The lesions were observed at the level of armpit, abdomen, groin and thigh. General symptoms included slightly raised body temperature, anorexia and depression. Diagnosis of skin infection in dogs was performed by clinical signs, cytological, bacteriological and mycological investigations and skin scrapings for parasites on cutaneous samples.

2.3. Sampling Collection

Dog skin samples were obtained from pustules and/or epidermal collarettes, using sterile swabs and plastic brushes, respectively for bacteriological and mycological surveys, and skin scrapings using sterile scalpels for cytological investigation and scrapings for parasites. The samples were transferred under sterile conditions and examined at the laboratory of infectious diseases of the University of Messina.

2.4. Cytological Examination

Samples of skin scrapings from affected dogs, taken with sterile disposable scal-

pels, were streaked onto slides, dried, stained with May Grunwald-Giemsa and microscopic examination was performed at high power under oil immersion (1000×).

2.5. Bacteriological Investigations

Within 1 hour from collection, the skin swab samples were transferred into test tubes of *Selenite-broth* (Biolifes. r. l., Milano, Italy) and incubated to 37˚C for 24 hours. The next day, from each tube was removed the bacterial inoculum and swiped into the plates containing the following selective and differential media, *SS-Salmonella-Shigella agar* (Oxoid, Basingstoke, Hampshire, UK) e *SM-Staphylococci* 110 *medium* (Biolifes. r. l., Milano, Italy), and then incubated to 37˚C for 24 hours, for the isolation of Gram-negative and Gram-positive bacteria respectively. Afterwards, the isolated colonies were transferred into tubes containing *BHI-Brain Heart Infusion broth* (Biolifes. r. l., Milano, Italy) for the identification and into tube slant containing *Mueller Hinton II agar* (Biolifes. r. l., Milano, Italy) for the conservation of the strains and incubated at 37˚C for 24 h. The isolated colonies were submitted to rapid bacterial identification by Mass Spectrometry VITEK MS (bioMérieux SA, Marcy l'Etoile, France). The samples for bacteriological investigations were performed before and 3, 5, 7 and 15 days after the treatment.

2.6. Identification of Bacterial Isolates by MALDI-TOF-MS

The bacterial isolates were identified by MALDI-TOF MS (*Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry*), designed to provide an accurate and reliable bacterial identification rapidly. This procedure allows the measurement in an extremely accurate molecular weight of biological macromolecules and to determine their identity according to the mass/charge ratio. In particular, MALDI-TOF-MS is valid for the detection of high-abundance soluble proteins, including ribosomal and other structural proteins, directly from microbial cells, resulting in spectra that are analyzed with the VITEK MS system (bioMérieux SA, Marcy l'Etoile, France), using for reading the software Axima (Shimadzu Kyoto, Japan)-SARAMIS database (Spectral ARchive And Microbial Identification System) (AnagnosTec, Berlin, Germany). The spectra were acquired in positive linear mode in the range of *m/z* 2000 to 20,000. The isolated colonies were seeded in a 48-well metal plate with disposable loops, using as a reference strain *Escherichia coli* ATCC 8739.

2.7. *In Vitro* Antimicrobial Activity

The susceptibility of the isolated microorganisms to *Thymus vulgaris* EO was estimated by the *in vitro* bacteriological test using the disk diffusion method (Kirby-Bauer) in *Mueller Hinton II agar* (Biolifes. r. l., Milano, Italy) and measuring the diameter of the growth inhibition area, as recommended by *CLSI* (Clinical and Laboratory Standards Institute 2015) [34]. In particular, *in vitro*

activity of *Thymus vulgaris* EO on the bacterial strains isolated from skin samples of dogs was evaluated in comparison to *Citrus bergamia* EO and some antimicrobial drugs widely used in veterinary medicine (*amoxicillin-clavulanic acid*, *doxycycline*, *thiamphenicol and marbofloxacin*) (Oxoid). Specifically, paper disks (6 mm diameter) were loaded with 20 µl (18 µg) of antimicrobial drugs, mixed with Tween 80 (Sigma Tau) (1:1 v/v) and then placed on the surface of the *Muller Hinton II agar*, inoculated with a suspension of the various isolated microorganisms (0.1 ml of 10^8 CFU/ml). The plates were incubated at 37˚C for 24 h, subsequently the diameter (mm) of the growth inhibition areas were measured. All the assays were performed in duplicate. The microorganisms were considered very susceptible, susceptible, poorly susceptible and resistant when inhibited *in vitro* by concentrations of 0.56 µg/ml or less, 0.57 - 6.25 µg/ml, 6.25 - 12.5 µg/ml and greater than 12.5 µg/ml respectively.

2.8. Research for Mites and Mycological Investigations

Samples of skin scrapings and hairs were taken from the dogs using a sterile scalpel, then streaked on a specimen slide with the addition of a drop of KOH (10%) and sealed with coverslip and then observed microscopically (40× - 60×).

Afterwards, various cutaneous samples were collected from the dogs using sterile plastic brushes and were sealed in individual sterile bags. Subsequently, the brushes were impressed on Petri dishes containing Potato Dextrose agar (Biolife). The growth of fungal colonies was kept under observation for 7, 15, 21 and 30 days. Suspect colonies which developed (white, smooth, raised, not mucous) only in samples n. 3, 7 and 8 were seeded in Dermatophyte Selective Medium (Biolife).

2.9. Drugs

The EOs tested (*Thymus vulgaris* L. and *Citrus bergamia* Risso & Poiteau) were purchased directly from the market (KOS srl, Lab. di Erboristeria, Prato, Italy).

2.10. Pharmacological Treatment

The present investigation with an extra-label use of *Thymus vulgaris* essential oil in dogs was approved by Commission on Drug Experimentation of the University of Messina and carried out in accordance with local ethical regulations for veterinary practice [35] [36] [37] and with the prior informed consent of the kennel veterinarian.

The use of topical treatments on dogs were reported in the register of the rescue shelter "Millemusi" (Messina).

The dosage of *T. vulgaris* EO (20 ml/1000ml H_2O) was prepared in accordance with the manufacturer's instructions. In particular, for this trial the use of a positive control group (treated with *Citrus bergamia* EO (20 ml/1000ml H_2O) rather than an untreated group was applied according to regulations for veterinary practice. Dogs were blocked in pairs and each animal assigned to one or

other group *at random*. The two groups of animals were subjected to topical treatment using a sprayer.

In particular 4 ml of *T. vulgaris* EO (Group 1: n. 10 animals) and *C. bergamia*EO (Group 2: n. 8 animals) respectively were sprayed in the areas affected by lesions for 7 days consecutively. Dogs with complete disappearance of clinical signs of skin infection were subjected to further preventive treatment for 10 days with *T. vulgaris* EO (Group 1). To prevent licking a collar was applied to each dog.

2.11. Clinical Score

Effectiveness of essential oils was evaluated on the basis of the changes in the animal's clinical signs, observed before treatment (T0) and 3, 5, 7 and 15 days after the drugs administrations, using a scores system [38] as follows:

- pruritus (0 *absent*; 1 *slight*; 2 *moderate*; 3 *marked*).
- alopecia and/or erythema (0 *absent*; 1 *slight*; 2 *moderate*; 3 *marked*).
- pustules and/or crusts (0 *absent*; 1 *slight*; 2 *moderate*; 3 *marked*).
- ulcers and/or erosion (0 *absent*; 1 *slight*; 2 *moderate*; 3 *marked*).
- general health (0 good, 1 quite good, 2 fair, 3 poor).

The effectiveness of *Thymus vulgaris* EO (Group 1) and *Citrus bergamia* EO (Group 2) was evaluated by comparing the clinical signs in dogs and the microorganisms isolated from skin swab samples before and to 3, 5, 7 and 15 days after the treatment. All animals were kept under observation during the experiment to check for possible side effects.

2.12. Statistical Analysis

The data were expressed as mean ± S.D. elaborated using an analysis of variance (ANOVA) InStat 3.0 software (GraphPad Software San Diego, CA, USA) was applied to assess the significance of the difference between the scores recorded before and 3, 5, 7, and 15 days after the treatment with *Thymus vulgaris* and *Citrus bergamia* EOs respectively. Difference with $p < 0.05$ were considered significant.

3. Results

3.1. Cytological Examination

The cytological investigation of skin samples performed on 18 dogs with skin diseases highlighted the presence of inflammatory cells (neutrophils, lymphocytes, plasma cells and bacteria) (**Figure 1**). This cytological pattern, found in all the dogs, is indicative of the presence of a skin inflammation process.

3.2. Bacteriological Test

The bacteria isolated by microbiological test from the skin swab samples of 18 dogs affected by bacterial skin disease were: *Staphylococcus pseudintermedius* (n. 7 samples), *Staphylococcus sciuri* (n. 4), ESBL *Escherichia coli* (n. 3) and

Proteus mirabilis (n. 4).

3.3. *In Vitro* Antimicrobial Activity

The *in vitro* susceptibility of the isolated microorganisms in dogs to *Thymus vulgaris* and *Citrus bergamia* EOs and other antimicrobials widely used in veterinary medicine *amoxicillin-clavulanic acid*, *doxycycline*, *thiamphenicol and marbofloxacin*) is reported in Table 1. Antimicrobial activity test *in vitro* showed that *Thymus vulgaris* EO is the most active antibacterial drug in the comparative analysis.

3.4. Bacteria Findings in Skin Swabs Samples during Pharmacological Treatment

The bacteriological findings in the skin samples of dogs before (T0 baseline) and

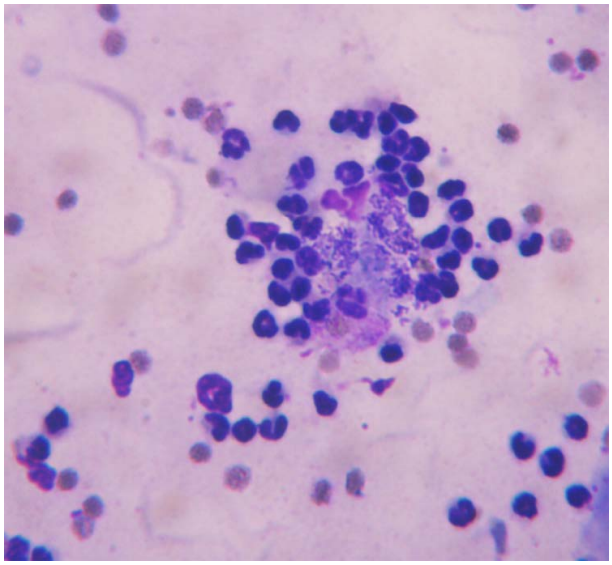


Figure 1. Cytological appearance of inflammatory cells (neutrophils, lymphocytes, plasma cells and bacteria) in skin samples of dog with skin diseases.

Table 1. Susceptibility to various antibacterial drugs of Gram-positive and -negative microorganisms isolated from skin samples collected from 18 dogs with skin infections.

DRUGS	Bacterial strains			
	S. pseudintermedius (*n.* 7)	*S. sciuri* (*n.* 4)	ESBL *E. coli* (*n.* 3)	*P. mirabilis* (*n.* 4)
Thymus vulgaris	++++	++++	+++	+++
Citrus bergamia	++	++	+	+
Amoxicillin-ac. clavulanic	++	+	+	+
Doxycycline	++	+	-	-
Thiamphenicol	-	-	+	+
Marbofloxacin	++	+	-	+

+++ *Highly susceptible* (*MIC* ≤ 0.56 μg/ml); ++ *Susceptible* (*MIC* 0.57 - 6.25 μg/ml); + *Poorly susceptible* (*MIC* 6.26 - 12.5 μg/ml); - *Resistant* (*MIC* > 12.5 μg/ml).

3, 7 and 15 days after the end oftopical treatment for 7 days with *Thymus vulgaris* and *Citrus bergamia* EOs respectively are indicated in Table 2. The severity of the bacterial skin infection of dogs was expressed in the conventional manner (from + to ++++) in relation to the quantity of bacteria present (Table 1). No microorganisms were isolated in all skin samples of dogs at 7 and 15 days after the end of *Thymus vulgaris* EO treatment. No variation in the bacterial load, instead, was recorded in the animals treated with *Citrus bergamia* EO.

3.5. Skin Scrapings for Mites and Mycological Investigations

The skin scrapings for mites and mycological investigations for dermatophytes in all skin samples of dogs were negative.

3.6. Variations in Clinical Scores Recorded during Antibiotic Treatments

Table 3 and Table 4 report the mean scores for clinical signs (pruritus, alopecia and/or erythema of the skin, presence of pustules or scabs, ulcers and/or erosions)

Table 2. Bacteria isolated from skin samples before treatment (T0 baseline) and 3, 5, 7 and 15 days after topical sprayed for 7 days with *T. vulgaris* and *C. bergamia* EOs, respectively, in dogs affected by skin infection.

***Thymus vulgaris* EO** (10 *dogs*)					
Strains	**T0**	**3 days**	**5 days**	**7 days**	**15 days**
S. pseudepidermidis (*n.* 4)	++++	+++	+	-	-
S. sciuri (*n.* 2)	++++	++	+	-	-
ESBL E. coli (*n.* 2)	++++	++	+	-	-
P. mirabilis (*n.* 2)	+++	++	-	-	-
***Citrus bergamia* EO** (8 *dogs*)					
Strains	**T0**	**3 days**	**5 days**	**7 days**	**15 days**
S. pseudepidermidis (*n.* 3)	++++	+++	+++	++	++
S. sciuri (*n.* 2)	++++	++++	++	++	+
ESBL E. coli (*n.* 1)	++++	+++	+++	+++	+++
P. mirabilis (*n.* 2)	++++	+++	+++	++	++

++++ = 10^6 *colony forming units* (*cfu*); +++ = 10^5 *cfu*; ++ = 10^4 *cfu*; + = 10^3 *cfu*.

Table 3. Effects of topical treatment with *Thymus vulgaris* EO for 7 days on the mean (±S.D.) scores of clinical signs in 10 dogs with skin infections.

Clinicalsigns	Before treatment	after 3 days	after 5 days	after 7 days	after 15 days
Pruritus	2.67 ± 0.18	**1.75 ± 0.46	*0.37 ± 0.12	-	-
Alopecia and/or erythema	2.84 ± 0.58	**1.48 ± 0.35	*0.42 ± 0.12	-	-
Pustules or crusts	2.51 ± 0.60	**1.86 ± 0.35	*0.25 ± 0.17	-	-
Ulcers and/or erosions	2.37 ± 0.51	**1.37 ± 0.51	*035 ± 0.27	-	-
General health	2.84 ± 0.35	**1.81 ± 0.64	*0.43 ± 0.21	-	-

*$p < 0.01$; **$p < 0.05$.

in dogs affected with skin infection before and at various time after the treatment with *Thymus vulgaris* and *Citrus bergamia* EOs respectively.

In Figure 2 is reported the trend of visible topical effect before, during and after the treatment with Thymus vulgaris EO (Group 1). The dogs with skin infection (Figure 2(a)) showed a moderate decrease of dermic clinical signs after 3 days but significant after 5 days (Figure 2(b)); an evident remission of disease it was observed after 7 days (Figure 2(c)), with complete disappearance of ulcers and erosions and initial regrowth of hair, followed by skin normalization. The treatment with *Citrus bergamia* EO (Group 2), instead, showed no significant effects in dogs with bacterial diseases of the skin and the clinical signs persisted markedly for the duration of our investigations.

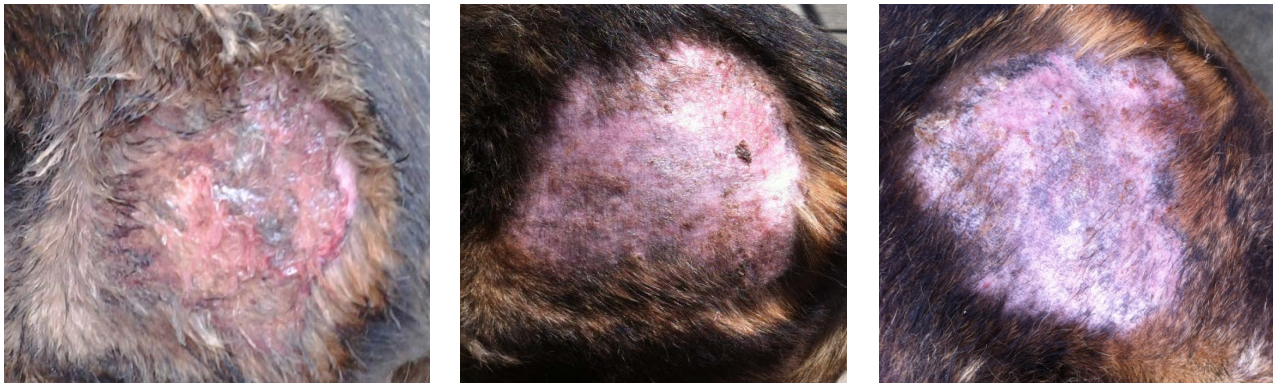
No local or general signs of *Thymus vulgaris* EO intolerance were recorded in any treated dogs and no relapses occurred in the month following treatment.

4. Discussion

Our study, original for the results obtained, documents the potential therapeutic efficacy of *Thymus vulgaris* EO in skin infections caused by Gram-positive and -negative bacteria of dogs. In particular, this study shows an improvement in the clinical symptoms after 3 days of treatment with complete healing after 7 days. Furthermore, no local or general adverse reactions were observed during the

Table 4. Effects of topical treatment with *Citrus bergamia* EO for 7 days on the mean (±S.D.) scores of clinical signs in 8 dogs with skin infections.

Clinicalsigns	Before treatment	after 3 days	after 5 days	after 7 days	after 15 days
Pruritus	2.57 ± 0.18	2.61 ± 0.96	2.25 ± 0.50	2.52 ± 0.32	2.35 ± 0.65
Alopecia and/or erythema	2.64 ± 0.58	2.50 ± 0.89	2.57 ± 0.51	2.43 ± 0.49	2.45 ± 0.51
Pustules or crusts	2.41 ± 0.46	2.36 ± 0.85	2.28 ± 0.37	2.23 ± 0.51	2.19 ± 0.62
Ulcers and/or erosions	2.28 ± 0.60	2.32 ± 0.37	2.18 ± 0.60	2.29 ± 0.37	2.21 ± 0.46
General health	2.74 ± 0.34	2.65 ± 0.99	2.52 ± 0.45	2.45 ± 0.60	2.50 ± 0.45



(a) (b) (c)

Figure 2. Trend of visible topical effect in dog with skin infection (Case N. 2, Group1): (a) Before of *Thymus vulgaris* EO treatment; (b) 5 days and (c) 7 days after.

various stages of therapeutic treatment. *Citrus bergamia* EO used, in comparison, as a positive control in dogs for skin infections caused by Gram-positive and -negative bacteria, instead, showed no therapeutic effects.

The mechanism of antimicrobial activity of *Thymus vulgaris* EO is quite complex and, to date, has not been well elucidated. It is very likely that the antibacterial action of this EO at the cellular level, for the qualitative and quantitative content of various organic compounds, is unrelated to a single mechanism [39]. No clinical studies are reported in literature on EOs use in animal deseases; the few data available related only to in vitro study are scarce and inadequate to a possible application in veterinary medicine and little is known about their safety after oral or parenteral administration. In our previous investigation, the therapeutic efficacy of Thymus vulgaris essential oils has been documented in ovine mammary postural dermatitis [34] but currently, there are no studies on the topical use in dog skin infections. Anyway, the topical use of EOs on the skin and mucous membranes appears to be the most promising strategy to prevent or treat skin infections.

The results of our investigations show that the topical use of *Thymus vulgaris* EO, also for its low cost, could constitute a possible alternative or a support to antibiotic-therapy in dermatological infections, in particular in cases refractory to conventional therapy. In fact, this EO, in addition to showing a good antimicrobial activity, has other biological and therapeutic properties (antifungal, anti-inflammatory, etc.). It is believed also extremely unlikely that bacteria can develop drug resistance to EOs, which consist of several molecular components, compared to an antibiotic consisting of a single active substance [28]. Moreover, it seems that EOs can effect synergistic activity with antibiotics, improving their therapeutic efficacy [4] [7].

5. Conclusion

In conclusion, this study documents for the first time the effectiveness of *Thymus vulgaris* EO for topical use in dogs with skin infections. It is important to note that the follow-up with *Thymus vulgaris* EO (7 days) is quite short as it is not an antibiotic and the speed of therapeutic effect is related to the high content of antioxidant substances, which possess antimicrobial, antifungal, anti-inflammatory effects, etc., as widely reported in literature. However, to evaluate the optimal use of this EO in skin infections of the dog, further studies are needed, in particular on a greater number of cases.

Conflict of Interest Statement

The authors declare no conflict of interest.

Sources of Funding

Funding was provided by the Istituto Zooprofilattico Sperimentale *"A. Mirri"* of Palermo (Italy).

Acknowledgements

The authors are grateful to Dr. Alessandro Taormina, veterinarian in charge of the rescue shelter of Messina (Southern Italy).

References

[1] Riviere, J.E. and Papich, M.G. (2009) Veterinary Pharmacology and Therapeutics. 9th Edition, Wiley-Blackwell, Hoboken, 1431-1437.

[2] Giguère, S., Prescott, J.F. and Dowling, P.M. (2013) Antimicrobial Therapy in Veterinary Medicine. 5th Edition, John Wiley & Sons, Hoboken, 21-40.

[3] Kon, K.V. and Rai, M.K. (2012) Plant Essential Oils and Their Constituents in Copying with Multidrug-Resistant Bacteria. *Expert Review of Anti-Infective Therapy*, **10**, 775-790. https://doi.org/10.1586/eri.12.57

[4] Savoia, D. (2012) Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. *Future Microbiology*, **7**, 979-990. https://doi.org/10.2217/fmb.12.68

[5] Scozzoli, M. (2015) Possibili applicazioni degli Oli essenziali in Medicina Veterinaria. In: Mondello, F., Marella, A.M., Bellardi, M.G. and De Vito, M., Eds., *Essential Oils for Humans Health and the Enviroment*, Rapporti ISTISAN 15/6, Vol. 2, 56-59.

[6] Burt, S. (2004) Essential Oils: Their Antibacterial Properties and Potential Applications in Food. *International Journal of Food Microbiology*, **94**, 223-253.

[7] Bassolè, I.H. and Juliani, H.R. (2012) Essential Oils in Combination and Their Antimicrobial Properties. *Molecules*, **17**, 3989-4006. https://doi.org/10.3390/molecules17043989

[8] Carson, C.F., Hammer, K.A. and Riley, T.V. (2006) *Melaleuca alternifolia* (Tea Tree) Oil: A Review of Antimicrobial and Other Medicinal Properties. *Clinical Microbiology Reviews*, **19**, 50-62. https://doi.org/10.1128/CMR.19.1.50-62.2006

[9] Fisher, K. and Phillips, C.A. (2006) The Effect of Lemon, Orange and Bergamot Essential Oils and Their Components on the Survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus in Vitro* and in Food Systems. *Journal of Applied Microbiology*, **101**, 1232-1240. https://doi.org/10.1111/j.1365-2672.2006.03035.x

[10] Lang, G. and Buchbauer, G. (2012) A Review on Recent Research Results (2008/2010) on Essential Oils as Antimicrobials and Anti-Fungals. *Flavour and Fragrance Journal*, **27**, 13-19.

[11] Solorzano-Santos, F. and Miranda-Novales, M.G. (2012) Essential Oils from Aromatic Herbs as Antimicrobial Agents. *Current Opinion in Biotechnology*, **23**, 136-141.

[12] Warnke, P.H., Lott, A.J., Sherry, E., Wiltfang, J., *et al.* (2013) The Ongoing Battle against Multi-Resistant Strains: In Vitro Inhibition of Hospital-Acquired MRSA, VRE, Pseudomonas, ESBL *E. coli* and Klebsiella Species in the Presence of Plant-Derived Antiseptic Oils. *Journal of Cranio-Maxillo-Facial Surgery*, **41**, 321-326.

[13] Giordani, R., Hadef, Y. and Kaloustian, J. (2008) Composition and Antifungal Activities off Essential Oils off Some Algerian Aromatic Plants. *Fitoterapia*, **79**, 199-203.

[14] Martinez-de-Oliveira, J. (2006) Antifungal Activity of the Essential Oils of *Thymus pulegioides* on Candida, Aspergillus and Dermatophyte Species. *Journal of Medical Microbiology*, **55**, 1367-1373. https://doi.org/10.1099/jmm.0.46443-0

[15] Riberio Salguerio, L. (2006) Antifungal Activity of the Essential Oils of *Thymus capitellatus* against Candida, Aspergillus and Dermatophyte Strains. *Journal of Medical Microbiology*, **55**, 1367-1373. https://doi.org/10.1099/jmm.0.46443-0

[16] Martin, K. and Ernst, E. (2003) Antiviral Agents from Plants and Herbs: A Systematic Review. *Antiviral Therapy*, **8**, 77-90. https://doi.org/10.1111/j.2042-7166.2003.tb05815.x

[17] Grassman, J., Hippeli, S., Vollmann, R., *et al.* (2003) Antioxidative Properties of the Essential Oil from Pinusmugo. *Journal of Agricultural and Food Chemistry*, **51**, 7576-7582. https://doi.org/10.1021/jf030496e

[18] Ruberto, G., Barrata, M.T., Sari, M., *et al.* (2002) Chemical Composition and Antioxidant Activity of Essential Oils from Algerian Origanum Glandulosum Desf. *Flavour and Fragrance Journal*, **17**, 251-254. https://doi.org/10.1002/ffj.1101

[19] Pisseri, F., Bertoli, A. and Pistelli, L. (2008) Essential Oils in Medicine: Principles of Therapy. *Parassitologia*, **50**, 89-91.

[20] Bhalla, Y., Gupta, V.K. and Jaitak, V. (2013) Anticancer Activity of Essential Oils: A Review. *Journal of the Science of Food and Agriculture*, **93**, 3643-3653.

[21] Lesgards, J.F.I., Baldovini, N., Vidal, N., *et al.* (2014) Anticancer Activities of Essential Oils Constituents and Synergy with Conventional Therapy: A Review. *Phytotherapy Research*, **28**, 1423-1446. https://doi.org/10.1002/ptr.5165

[22] Bakkali, F., Averbeck, S., Averbeck, D., *et al.* (2008) Biological Effects of Essential Oils—A Review. *Food and Chemical Toxicology*, **46**, 446-475.

[23] Peana, A.T., D'Aquila, P.S., Panin, F., *et al.* (2002) Anti-Inflammatory Activity of Linalool and Linalyl Acetate Constituents of Essential Oils. *Phytomedicine*, **9**, 721-726. https://doi.org/10.1078/094471102321621322

[24] Hamdan, D., El-Readi, M.Z., Nibret, E., *et al.* (2010) Chemical Composition of the Essential Oils of Two Citrus Species and Their Biological Activities. *Pharmazie*, **65**, 141-147.

[25] Mondello, F., Girolamo, A. and Di Vito, M. (2015) Potenzialità degli oli essenziali nelle malattie infettive. Essential Oils for Humans Health and the Environment. Rapporti ISTISAN 15/6, ii: 26-31.

[26] Bouzar, I.H., Mineccia, M., Giraldi, F. and Perotti, S. (2015) Oli essenziali in chirurgia ed oncologia. *TMA*, **2**, 27-28.

[27] Mugnaini, L., Nardoni, S., Pistelli, L., Leonardi, M., Giuliotti, L., Benvenuti, M.N., Pisseri, F. and Mancianti, F. (2013) A herbal antifungal formulation of Thimus serpillum, Origanum vulgare and Rosmarinus officinalis for treting ovine dermatofiphytosis due to Thricophitum mentagrofites. *Mycoses*, **56**, 333-337.

[28] Rota, M.C., Herrera, A., Martinez, R.M., *et al.* (2008) Antimicrobial Activity and Chemical Composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* Essential Oils. *Food Control*, **19**, 681-687.

[29] Soković, M.D, Vukojević, J., Marin, P.D., *et al.* (2009) Chemical Composition of Essential Oils of Thymus and Mentha Species and Their Antifungal Activities. *Molecules*, **14**, 238-249. https://doi.org/10.3390/molecules14010238

[30] Campagna, P. (2015) Oli Essenziali in Clinica Dermatologica. *TMA*, **2**, 32-34.

[31] Pisseri, F. (2015) Oli Essenziali in Dermatologia Veterinaria in Essential Oils for Humans Health and the Enviroment. Rapporti ISTISAN 15/6, ii: 59-62.

[32] Naccari, C., De Domenico, A., Previti, S., *et al.* (2006) Therapeutic Efficacy of Essential Oils in Ovine Mammary Pustular Dermatitis. 14 *Congreso Internacional de la Federacion Mediterranea de Sanidad y Produccion de Ruminantes*, La Coruña,

12-15 julio 2006.

[33] Fratini, F., Casella, S., Leonardi, M., *et al*, (2014) Antibacterial Activity of Essential Oils, Their Blends and Mixtures of Their Main Constituents against Some Strains Supporting Livestock Mastitis *Fitoterapia*, **96**, 1-7.

[34] Naccari, C., Niutta, P.P., Trombetta, D., De Domenico, A., Pizzimenti, F., Cagnardi, P., Carli, S. and Naccari, F. (2009) Pharmacokinetics and Efficacy of Teicoplanin against Intramammary Infections in Sheep. *Veterinary Record*, **165**, 19-22. https://doi.org/10.1136/vetrec.165.1.19

[35] Clinical and Laboratory Standards Institute (CLSI) (2015) Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fifth Informational Supplement (M100-S25), Wayne.

[36] Recommendation 2007/526/CE (2007) Recommendation of 18 June 2007 on Guidelines for the Accommodation and Care of Animals Used for Experimental and Other Scientific Purposes. *Official Journal of the European Union*, L197/1.

[37] Decreto Legislativo n. 116 (1992) Attuazione della direttiva n. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici. DL 27 gennaio 1992 n. 116, 1-18.

[38] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes. Official Journal of the European Union, 20.10.2010, L276/33.

[39] Di Vito, M., Girolamo, A. and Mondello, F. (2015) Gli oli essenziali antimicrobici promettenti per combattere le infezioni nosocomiali farmacoresistenti. *TMA*, **2**, 29-31.