POSSIBILITIES AND LIMITATIONS OF THE USE OF ESSENTIAL OILS IN DOGS AND CATS

Filip ŠTRBAC¹*, Kosta PETROVIĆ², Dragica STOJANOVIC³, Radomir RATAJAC³

¹University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Novi Sad, Serbia
²Agricultural school with accommodation for students in Futog, Novi Sad, Serbia
³Scientific Veterinary Institute Novi Sad, Novi Sad, Serbia
*Corresponding author: Filip Štrbac, strbac.filip@gmail.com

Summary

Essential oils have numerous medicinal properties which include antiseptic, anti-inflammatory, anticancer but also antiviral, antimicrobial and antiparasitic effects. Although researches have highlighted different possible application of these oils, little is known about their use in animals including dogs and cats. Specifically, essential oils showed effects against various bacterial (Staphylococcus spp., Pseudomonas aeruginosa, Enterococcus spp.), fungal (Malassezia pachydermatis, Microsporum canis, Trichophyton mentagrophytes), endoparasitic (Giardia duodenalis, Echinococcus granulosus, Toxocara canis) and ectoparasitic (Otodectes cynotis, Demodex canis, Ctenocephalides felis, some ticks etc) pathogens in dogs and cats. In addition, essential oils exhibit numerous positive properties such as complex chemical composition (high efficacy, less susceptibility to resistance), their natural origin etc. Therefore, certain essential oils based formulations are already in use, such as different shampoos. However, the main limitation for the use of these plant products is the insufficiently tested safety of use. Thus, some essential oils are considered toxic to pets, especially when used on cats. The main reason for this is most likely the deficiency of some liver enzymes that are involved in metabolic processes (as UDP-glucuronosyltransferase in cats), which leads to the accumulation of some active ingredients in the organism and their toxicity. Therefore, caution and rational application are needed in terms of adequate selection of plant species, dosage, concentration and the methods of use. In addition, since essential oils are prone to evaporation and instability, the encapsulation technique can further increase their in vivo efficacy as well as enable controlled release. That can reduce the required dose and additionally increase safety of their application.

Keywords: phytotherapy, pets, resistance, encapsulation
INTRODUCTION
Ether extracts or essential oils are concentrated, aromatic and hydrophobic volatile liquids of complex chemical composition and characteristic odor. They are synthesized in different parts of plants, most often in leaves and flowers, but also in stems, seeds, buds, rhizomes and roots, and are secondary metabolites of the plants (Fotsing and Kezetas, 2020). It is considered that their role in the plants is primarily protective, but they are also useful in attracting pollinators (Butnariu and Sarac, 2018). Today, essential oils have found wide application in various areas, so they are used in the cosmetics industry, food industry, but also for the treatment of various diseases, which is known as aromatherapy (Dhifi et al. 2016).

The pharmacological potential of essential oils comes from a number of compounds from different chemical groups that are part of their composition. This primarily includes terpenes and sesquiterpenes, then terpenoids (oxidized derivatives such as phenols, alcohols, aldehydes, ketones, oxides and esters), as well as phenylpropanoid compounds (Dhifi et al. 2016). It is important to mention that the chemical composition and thus the biological properties of essential oils differ depending on various endogenous and exogenous factors such as plant species and hemotype, age, part of the plant from which it is extracted, geographic region, climate, presence and number of microorganisms, insects and others (Maes et al., 2019). Essential oils are extracted from the plant by various methods such as hydrodistillation, solvent extraction, cold pressing, steam distillation, microwave extraction, supercritical fluid extraction and others (Fotsing and Kezetas 2020).

Until now, numerous medical effects of essential oils are known and proven, such as antioxidant, antiinflammatory, anticancer, but also antiviral, antibacterial, antifungal, antiparasitic, etc (Dhifi et al., 2016). This is used in traditional medicine of many cultures all around the world for the treatment of various diseases, which is still used today. However, despite its enormous potential, little is known about the use of essential oils in veterinary medicine, including a small practice (Ratajac et al., 2011). Therefore, the aim of this study is to consider the possibilities and limitations of the use of essential oils in dogs and cats through a review of various studies conducted so far.

Advantages and possibilities of the use of essential oils
Essential oils have certain properties that make them suitable for use in veterinary practice, including dogs and cats. This primarily refers to the mentioned complex chemical composition, which usually means a large number of different active compounds. Due to that, there is a synergistic effect between them and high activity against various pathogens. On the other hand, a large number of different compounds can contribute to lower susceptibility to resistance compared to commercial preparations (Ferreira et al., 2018), which is very important given the huge problems caused by antimicrobial and antiparasitic resistance in modern veterinary and human medicine. Finally, the natural origin of essential oils certainly contributes to better environmental friendliness compared to synthetic substances, and according to some, less toxicity to
animals themselves. In addition to all the above, the number of plant species from which they can be isolated is large, which, with easy availability and reasonable price, makes these preparations suitable for use in countries with developed biodiversity such as Serbia.

In various studies conducted so far, essential oils have been tested for different indications in farm animals. Research has often been based on the effectiveness against various endoparasites. Thus, for example, the effectiveness of oregano - *Origanum vulgare*, thyme - *Thymus vulgaris*, mint - *Mentha x piperita*, savory - *Satureja hortensis* (Strbac et al., 2021), juniper - *Juniperus communis* (Strbac et al., 2020a), hajduk herbs - *Achillea millefolium* (Strbac et al., 2020b), lavender - *Lavandula officinalis* (Ferreira et al., 2018), rosemary - *Rosmarinus officinalis* (Pinto et al., 2019), various species of eucalyptus (*Eucalyptus spp.*), lemongrass (*Cymbopogon spp.*) and many others (Andre et al., 2018) against gastrointestinal nematodes in sheep was proven. Other indicate the importance of using essential oils as an alternative to antibiotics in pig (Omonijo et al., 2018) and broiler production (Krishan and Narang, 2014), and numerous studies have been conducted related to the possibility of their use to control various ectoparasites (Abbas et al., 2018). In addition, similar oils or their isolated ingredients are mentioned in various *in vitro* and *in vivo* studies in dogs and cats, which indicates their multiple potential for use in veterinary medicine.

**Antimicrobial activity**

Research has shown that there are a number of indications that essential oils may have when it comes to antimicrobial use in dogs and cats. This includes dermatological diseases which can be caused by various pathogens and which are very common in small veterinary practice. Ebani et al. (2020) examined the possibility of using several essential oils against staphylococcal skin pathogens in dogs. The results showed high *in vitro* efficacy, with the most effective being essential oils of oregano, mountain savory (*Satureja montana*) and thyme with a minimum inhibitory concentration (MIC) of 0.29-0.58 mg/mL, 0.56-1.12 mg/mL and 0.58-1.16 mg/mL, respectively. Several essential oils showed high activity even against methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical cases of pyoderma in dogs, with cinnamon (*Cinnamomum verum*) and lemon balm (*Melissa officinalis*) essential oils showing the best bacteriostatic and bactericidal effect. The effect was also shown by mountain beetle and manuka (*Leptospermum scoparium*) (Nocera et al., 2020).

The causes of skin diseases in dogs and cats are often various dermatophytes that are dangerous because of their zoonotic potential. Nardoni et al. (2015) examined the *in vitro* activity of 20 commercially available essential oils against selected dermatophytes. The highest activity was shown by essential oils of thyme, *Thymus serpyllum* (MIC for *Microsporum canis* 0.025%, *M. gypseum* 0.25%, *Trichophyton mentagrophytes* 0.1%), oregano (*M. canis* and *M. gypseum* 0.025%), and aromatic litsee, *Litsea cubeba* (*M. canis* 0.025%, *M. gypseum* and *T. mentagrophytes* 0.25%). The essential oils of thyme (2%) and oregano (5%), along with rosemary oil (5%), were part of a formulation tested *in vivo* on seven cats with symptoms of *M. canis* infection, and various oils were tested *in vitro*. 

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**References**

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Four of the seven animals fully recovered, both clinically and culturally, while in the *in vitro* test, among others, the highest antifungal activity was shown by thyme and oregano oils (Mugnaini et al., 2012).

Inflammation of the external ear canal (*otitis externa*) is one of the most common diseases in dogs and cats, especially in breeds with predisposing factors, and can be caused by numerous factors. Ebani et al. (2017) also examined the efficacy of nine essential oils against various pathogens isolated from clinical cases of this disease. When it comes to bacteria, the most effective were again the essential oil of oregano with MIC values of 2.36 µg/µL (*Staphylococcus aureus*) and 1.18 µg/µL (*S. pseudintermedius*), as well as the essential oil of nutmeg, *Salvia sclarea* with 2.23 µg/µL (*S. pseudintermedius*) and 17.86 µg/µL (*Pseudomonas aeruginosa*). A similar result was obtained when it comes to different fungi, where oregano (MIC values in *Candida albicans, Aspergillus niger* and *A. fumigatus* were 0.19 µg/µL) was most effective. Similar to this research, Sim et al. (2019) tested essential oils of thyme, oregano as well as their isolated main constituents thymol and carvacrol against various causes of *otitis externa* in dogs. The tested oils and ingredients showed an antimicrobial effect against all bacterial and fungal isolates, with MIC values ranging from 100–292 µg/mL for gram-positive bacteria and *Proteus mirabilis*, while a slightly weaker effect was against *P. aeruginosa* and *Malassezia pachydermatis* with a MIC of 400–2 292 µg/mL.

*Mentioned Malassezia pachydermatis* is a common cause of inflammation of the external ear canal and dermatological complications in dogs. When it comes to the use of essential oils in dogs, this yeast is one of the most commonly tested pathogens. Thus, many essential oils, including oregano, thyme, thyme, mint, cinnamon and many others, have shown *in vitro* efficacy against malasesia. In a study by Pistelli et al. (2012), where more than 10 essential oils were tested, oregano, thyme and basil (*Ocium basilicum*) showed the best efficiency with 0.8% each, v/v MIC values. Various formulations of essential oils were tested *in vivo* against *M. pachydermatis*, including a formulation consisting of six oils in different concentrations (bitter orange, *Citrus aurantium* 1%, lavender 1%, oregano 0.5%, marjoram, *Origanum marjoram* 0, 5%, mint 0.5% and Mediterranean immortelle, *Helichrysum italicum var. italicum* 0.5%). The animals received topical treatment twice a day for a month, and after that period the clinical status of the treated dogs was significantly improved (Nardoni et al., 2014). In another study, 25 atopic dogs were treated with several different formulations based on essential oils, with the best effect shown by a combination of lemon, *Citrus x lemon* (1%), nutmeg (0.5%) and rosemary (1%) which led to a significant improvement in all treated dogs (Nardoni et al., 2017).

Urinary tract infections in dogs and cats are also common, often caused by various bacteria such as *Escherichia coli* and *Enterococcus spp.*, and fungi such as various types of candida. The efficacy of various essential oils against these pathogens isolated from clinical cases was examined by Ebani et al. (2018). The most effective antibacterial action against *E. coli* showed essential oils of thyme and oregano with MIK values of 0.146-0.585 and 0.293-1.183 mg/mL, and the same oils were also the most effective against...
Enterococcus spp. (MIK 1.171-2.342 and 1.183 mg/mL, respectively). Basil also showed good antibacterial activity. On the other hand, the high antifungal activity against Candida albicans and C. famata was shown by most of the tested essential oils, i.e. in addition to the mentioned also star anise oil (Illicium verum).

**Antiparasitic activity**

Essential oils have shown efficacy against various endoparasites in various studies. When it comes to protozoa, one of the most important species is the gardia (Giardia duodenalis), the cause of intestinal infections in dogs, cats and humans. In the study of Popruk et al. (2017), more than 10 essential oils were tested against this endoparasite, with the best effect being shown by the essential oil of lime, Citrus × aurantiifolia with IC$_{50} = 6.96 ± 0.13$ µg/mL with the already mentioned aromatic litsea with IC$_{50} = 60.67 ± 0.82$ µg/mL, and both oils showed a dose-dependent effect. In a study by Moon et al. (2006), in which were tested two types of lavender (classic and hybrid) against gardenia, both showed effect even at very low concentrations (<1%). Namely, their application led to the complete in vitro elimination of gardia and other tested protozoa, with a slightly better result of classical lavender.

Several essential oils have shown effects against Echinococcus granulosus in several different studies, whose transient forms of cysts can parasitize domestic mammals and humans, causing a serious clinical signs. Thus, Pensel et al. (2014) have proved the in vitro effect of oregano and thyme against the protoscolex and cysts of this tapeworm. The effect was based on the lost of protoskolex viability and loss of cyst mass, which was confirmed at the ultrastructural level. In vitro efficacy of thymol at a concentration of 5 µg/mL, as well as essential oils of rosemary, mint and mint, Mentha pulegium (10 µg/mL, respectively) against cell proliferation of E. granulosus was examined by Albani et al. (2014). The results showed a significant effect in the form of reduction of protoscolex viability, where the effect was as follows: thymol 63%, mint 77%, mint, Mentha pulegium 82% and rosemary 71%.

Essential oils have also shown an effect against various nematodes that parasitize dogs and cats. Thus, the effect of the essential oil of cinnamon, citronella, lemongrass, aromatic litsee and vetiver and their ingredients against Toxocara canis has been known for many years, since Nakamura et al. (1990) showed strong nematicidal activity with minimum lethal concentrations less than 0.1 mg/mL. The essential oil of Brazilian red propollis showed a effect against T. cati, with a concentration of 600µg/mL showing 100% larval activity after 48 hours of exposure (Sinott et al., 2019). Essential oil and ethanolic extract of Epazote (Mexican tea, Dysphania ambrosioides) were tested in vitro and in vivo against a significant dog nematode, Ancylostoma caninum (Monteiro et al., 2017). In in vitro testing, the extract proved ineffective against the L3 stage, while the essential oil was effective at a concentration of 150 µL/mL. However, a significant result
is that the oil proved to be effective in the treatment of naturally infected dogs, when it led to a reduction in the number of nematode eggs in the feces of 82.14%.

Many researchers have proven the effectiveness of essential oils on various ectoparasites of dogs and cats, including mites, fleas and ticks. The effect of essential oils of garlic (*Allium sativum*), marjoram and ozonated olive oil against clinical cases of infection with ear mite *Otodectes cynotis*, a significant ear parasite in cats, was investigated by Yipel et al. (2016). In a study obtained on 28 animals, 10 days after treatment the best effect was shown by garlic essential oil together with control (permethrin), and 30 days after treatment the order of effectiveness was as follows: permethrin, garlic, marjoram and olive oil. However, practically all oils led to the elimination of parasites, since the average value of parasites per animal in all tested oils was less than 1. Tea tree essential oil (*Melaleuca alternifolia*) was tested against *Demodex canis*, a dog mite with zoonotic potential, and showed a faster and stronger effect compared to amitraz (Neves et al., 2020). Namely, the average time required to eliminate the last parasite at all concentrations (100-3.13%) of tea tree was less compared to amitraz (8.00-100.67 minutes compared to amitraz which took an average of 333.33 minutes). In a study done by Sedzikowska et al. (2015), who examined the effect of several essential oils on the survival rate of demodex, the best effect was shown by the essential oils of tea tree and sage (*Salvia officinalis*) with an average survival rate of parasites of only 7 minutes, as well as mint oil of 11 minutes.

The effectiveness of the essential oil of red pepper (*Schinus molle*) against cat flea, *Ctenocephalides felis felis* was examined by Batista et al. (2015). The results were interesting considering that the oils from the fruit and leaves of the plant were tested, and showed different efficiency. Namely, the oil obtained from the leaves showed 100% efficiency against adult forms at a concentration of 50 µg/cm², and the one obtained from the fruit only at a concentration of 800 µg/cm², which indicates the influence of the plant part from which the essential oil is extracted on its pharmacological properties. Clove oil (*Syzygium aromaticum*) was tested against cat flea together with the main ingredient eugenol. Interestingly, eugenol itself has shown better pulicidal and better efficacy against egg development in adult forms compared to whole essential oils, at different time intervals (24 and 48 hours after incubation). However, the authors concluded that both eugenol and whole oil have the potential to be used against cat fleas (Lambert et al., 2020).

Finally, various formulations of essential oils have been tested against ticks in dogs. Thus, the formulation consisting of 2.5% garlic essential oil, 0.05% allicin (active ingredient from garlic) and 8% rapeseed oil in a dose of 0.25 mL/kg together with water was successively tested for three days in natural infested dogs with various ticks (*Ixodes spp.* and *Rhipicephalus sanguineus*). The formulation was highly effective as after the first dose the number of ticks decreased by 75%, after the second 92.85% and 99.42% after the third, while on the 7th, 14th and 21st day after treatment a complete absence of ticks was observed. In addition, all hematological and biochemical parameters after the treatment of infected dogs were within the reference values, so the authors concluded that the formulation is safe to use (Amer and Amer, 2020). An essential oil of a velvet species
that originate from South America, *Tagetes minutes*, was tested against *R. sanguineus*. The tested oil showed 100% *in vitro* efficacy against larvae, nymphs and adults of these ticks isolated from infested dogs (da Silva et al., 2016).

**Obstacles and restrictions on the use of essential oils**

One of the limitations for the use of essential oils in dogs and cats, as well as in other animals, are the unknowns related to various aspects of their use, which is contributed by the still insufficient amount of research and scientific confirmation of their effects. In many cases, the effectiveness of essential oils is attributed to anecdotal information and various attempts in practice, which is certainly not enough for widespread use, and can be dangerous for animals or veterinarians. Also, as could be seen in the previous section, a large number of studies relate to *in vitro* efficacy testing. Laboratory tests certainly have a number of advantages compared to field tests in the context of simplicity and short time needed to obtain results, as well as lower prices. They are useful primarily for the initial evaluation of new active substances, as well as the selection of those with the greatest potential for further testing (Ferreira et al., 2018). However, a complete picture of the possibility of using a substance requires confirmation of their effectiveness after application to the animals.

Another obstacle to the wider use of essential oils is some of their negative properties, such as instability. Namely, essential oils are composed of many lipophilic and volatile compounds, which are therefore subject to conversion and degradation processes. This leads to inactivation of active ingredients and loss of pharmacological properties of essential oils (Turek and Stintzing, 2013). In practical terms, this means that, in some cases, essential oils are quickly inactivated after applications, and the active ingredients do not reach, or reach to a lesser extent the target sites of action. Such an effect has been proven in various studies of oral use of essential oils in farm animals against endoparasites in the distal parts of the digestive tract, although the same oils have shown high efficacy *in vitro* (Hoste et al., 2008). However, most of such research was related to the application in ruminants, and the question is whether and to what extent these results can be interpreted for both dogs and cats. Also, the instability of essential oils probably does not affect their antiectoparasitic application to such an extent due to the direct application of certain formulations on the skin, as shown in the previously mentioned study against ticks in dogs (Amer and Amer, 2020).

However, currently the biggest limitation for the wider use of essential oils is the insufficiently tested safety of use. In human medicine, most essential oils are considered "generally safe" to use, which is why there are more than 300 different essential oils on the market that are successfully used for various purposes, and cases of intoxication are very rarely reported (Vostinara et al., 2020). When it comes to pets, some essential oils are considered toxic or potentially toxic, although most of this information comes from informal sources. Some of the essential oils most often mentioned in this context are cinnamon, mint, mint *Mentha pulegium*, tea tree oil, pine oil, oils of some citrus plants, sweet birch (*Betula lenta*) canaga (*Cananga odorata*), eucalyptus, lavender, evergreen oil, and sometimes oregano. A clinical case of tea tree essential oil intoxication with
symptoms of weakness, incoordination and muscle tremor has been reported in dogs and cats topically treated with high doses for dermatological diseases, but the animals recovered after supportive therapy (Villar et al., 1994). In another case, tea tree oil applied directly to the skin of three Angora cats in a dose of 20 milliliters per animal caused intoxication with symptoms of hypothermia, incoordination, dehydration, shivering, etc. After supportive therapy, two animals recovered within 24–48 hours and one died (Bischoff and Guale, 1998). When it comes to other essential oils, data are very limited.

The causes of the potential toxicity of certain essential oils in dogs and cats could be related to some specifics of their metabolism. Namely, in cats, a deficiency of UDP-glucuronosyltransferase enzymes such as UGT1A6 and UGT1A9, which are important for glucuronidation processes, is known (Court, 2013). Therefore, active substances that are biotransformed and then eliminated in this way can accumulate in the body and cause symptoms of toxicity. This also applies to certain drugs such as paracetamol, propofol, carprofen or aspirin. Since phenolic compounds are metabolized in this way, essential oils containing them can also lead to symptoms of toxicity, so they should be avoided or used with great caution in cats. On the other hand, metabolism in dogs also has its specifics such as N-acetyltransferase enzyme deficiency (Gao et al., 2006), although cats are generally considered more sensitive to drug metabolism compared to dogs. In any case, any drug can be toxic if used incorrectly, which is also true for essential oils, which are very different from each other. In that sense, the correct selection of plant species with adequate use in terms of dosage, concentration and method of application can contribute to the efficient and safe use of essential oils.

**Encapsulation of essential oils**

Encapsulation techniques are more recent and interest for them is growing in various fields. Encapsulation is a process of protecting active components by physical or chemical processes, during which a protective coating is formed. In this way, the active substance is physically separated from the environment by creating a protective coating. The capsules formed can be of different sizes (macro, micro and nano). There are various encapsulation techniques, the choice of which mostly depends on the purpose, but emulsification and nanotechnology could be the most widely used in medical purposes (Maes et al., 2019; Lević et al., 2014).

The mentioned techniques could be of special importance when it comes to essential oils and their application in veterinary medicine. Namely, encapsulation reduces the interaction of active substances with various factors and reduces the rate of evaporation. This could reduce the inactivation of the active ingredients of essential oils in animals and increase their bioavailability (as antimicrobial and antiendoparasitic use). Furthermore, this technique allows controlled release of the active substance, which is important given that different applications often require increased retention of active ingredients and different release profiles (as antiectoparasitic application). The encapsulation of essential oils also reduces their strong odor, which can be significant considering the extremely sensitive sense of smell in dogs and cats. Finally, the encapsulation technique increases
the simplicity and precision of handling active substances (Maes et al., 2019; Radünz et al., 2018).

All the above speaks in favor of the fact that encapsulation can improve the efficiency of in vivo application of essential oils. In addition, increased in vivo efficacy can contribute to reducing the required dose and concentration of essential oils, which can further increase the safety of their use. In this way, the mentioned techniques could effectively remove or alleviate the previously mentioned obstacles to the use of essential oils.

CONCLUSION

The possibilities of using essential oils in dogs and cats are numerous and include antibacterial, antifungal, as well as use against various endo and ectoparasites. In this context, essential oils and active ingredients of oregano, thyme, thyme, cinnamon, mint, basil, lemon balm, mountain savory, eucalyptus, lavender, rosemary and many other domestic and exotic plants are most often tested. At the same time, the various advantages of the use of essential oils make this topic extremely relevant today in all areas of veterinary practice. This should be kept in mind especially due to the development of antimicrobial and antiparasitic resistance in an increasing number of pathogens. As with any medicine, the use of essential oils has some limitations, which is primarily due to the insufficient number of scientific studies to confirm the effectiveness and safety of use. However, the growing popularity of phytotherapy in veterinary medicine brings a growing number of scientific research, which offers the possibility of selecting the most suitable essential oils or formulations based on them for rational use in everyday practice. Also, the encapsulation technique offers the option of overcoming many barriers to the use of essential oils in dogs and cats, including increasing in vivo efficacy and safety.

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