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Botanical Control of Parasites in Veterinary Medicine

AUTHORS DETAIL

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INTRODUCTION

Phytotherapy may be defined as the use of plants for the treatment of ailments and those represent a practice that dates since ancient times (Borges and Borges 2016). It refers to the use of whole plants, their parts such as flowers, leaves, roots and seeds as well as substances extracted from them (plant extracts and essential oils) for treating various diseases (Stanković et al. 2020). It also may imply their use to support traditional treatment with commercial drugs (Russo et al. 2009). Plants and their extracts are an important part of pharmacopoeia in less developed parts of the world, but more recently in the advancement societies (Russo et al. 2009; Borges and Borges 2016). However, plant-based products may also be used for the treatment of diseases in animals (Ul Abidin et al. 2021), prevalently in livestock (Calzetta et al. 2020). Ethnopharmacology may be implied in veterinary medicine due to the potential therapeutic efficacy, reduced susceptibility to microbial and parasitic resistance, as well as lowered risk of adverse effects and decreased residues in animal products and environment in comparison with chemotherapeutic agents (Calzetta et al. 2020). Moreover, botanical control of various diseases in animals can also be sustainable from the financial point of view (Prakash et al. 2021).

Therefore, medicinal plants are a valuable part of the field of drug discovery and represent an important source of new drugs and drug leads (Liu et al. 2020). In this regard, antiparasitic properties are a common point of focus in studies aimed to validate the pharmacological effects of herbal products. A huge number of such plants and their products are considered suitable for the treatment of almost every parasitic disease in livestock (Athanasiadou et al. 2007). In pets, there are also an increasing number of such studies in dogs and cats, whereby plants product were proven to be effective against various parasites (Štrbac et al., 2021a).

Resistance in Parasites as the Main Problem and Novel Strategies

The resistance in different parasitic species nowadays represents a worldwide problem due to decreasing efficacy of commercial drugs and consequent economic losses. Antiparasitic resistance (AR) may be defined as the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage (Geary et al. 2012). Although it is considered that AR is a natural and heritable process which will develop anyway for a certain time, the role of humans in its occurrence refers to the rate and speed of its development (Shalaby 2013). The main factors that may promote AR development are treatment frequency (especially overfrequent treatments), miss-dosing (especially underdosing), prophylactic mass treatments, continuous use of a single drug without combination or rotation and poor pasture management in the case of livestock (Shalaby 2013; Mphahehlele et al. 2019).

In the case of protozoan parasites, it was reported that the effectiveness of antiprotozoal drugs is being decreased (Capela et al. 2019). The especial problem represents protozoan infections for which usually very few treatment options exist such as trypanosomiasis (including durina), babesiosis, theileriosis and leishmaniosis, whereby continuous use of these drugs predictably leads to drug resistance (de Koning 2017). In terms of liver flukes, the main concern is with triclabendazole whereby its success in the treatment of these infections has led to over-reliance on this drug and the emergence of resistance, although

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resistance in *Fasciola hepatica* to albendazole is also reported (Fiarweather et al. 2020). The problem of AR of cestodes such as *Echinoccocus* (*E.*) *granulosus* to synthetic drugs is also present (Pensel et al. 2014). However, it is said that the problem of AR is far more severe in small ruminants, which requests dramatic changes in approaches to nematode control for decades (Kaplan 2004). In the case of gastrointestinal nematodes such as *Haemonchus* (*H.*) *contortus*, single and multi-resistant strains of various species to all groups of anthelmintic drugs that are used in practice (benzimidazoles, macrocyclic lactones, imidazothiazoles and even to newly developed drugs such as monepantel) are already reported, whereby the estimated time of the development of AR to a new drug is now less than 10 years after introduction to the market (Fissiha and Kinde 2021). The annual cost of anthelmintic resistance only in Europe is recently estimated at €38 million with a tendency to increase in the future, which in turn endangers the sustainability of livestock (Vinner et al. 2020) and world's food supply. In the end, AR is already present in many ectoparasites, whereby a range of pesticide drugs such as organophosphates, organochlorides and synthetic pyrethroids are no longer that effective due to their intensive use, which makes the control of ectoparasites very difficult (McNair 2015).

Not only from the aspect of resistance, but the use of only commercial antiparasitics is no longer sustainable because of the price of these drugs that continues to rise (Prakash et al. 2021). For example, the mean wholesale price of multiantiparasitic drugs albendazole and mebendazole increased between 2010. and 2019. from $3.16 to $582 and from $32 to $2853, respectively (Junsoo Lee et al. 2021). Finally, adverse effects on the host animals, residues in different animal products such as meat and milk as well as in the ecosystem and biodiversity represent a serious problem with many chemotherapeutic drugs which are currently available (Veerakumari 2015). All of this requires searching for novel strategies for the control of parasites in veterinary medicine, which according to Hoste et al. (2014) should be based on (i) developing new concepts of the use of chemical antiparasitic drugs (eg. target selective treatments); (ii) rational management of pastures; (iii) stimulating the host immune response (eg. development of vaccines) and (iv) investigating the efficacy of new drugs (phytotherapy, homeopathy and nutraceuticals). Some other alternatives are also suggested such as genetic selection of naturally resistant animals to parasites, biological control (the use of fungi, bacteria and even other parasites) (Maqbool et al. 2017), and in the case of ectoparasites, insect growth regulators (McNair 2015).

Plant Formulations and Advantages of their use

Plant products that are exhibiting pharmacological properties are often called plant secondary metabolites (PSM) and they make plants competitive in their own environment (Teoh et al. 2015). One of the most commonly examined plant products against parasites of veterinary importance are essential oils (EOs). They may be defined as aromatic, concentrated and complex mixtures of volatile nonpolar compounds extracted from plant material (Nehme et al. 2021; Štrbac et al. 2021b; Štrbac et al. 2022a) and represent a part of a plant immune system (Butnariu and Sarac 2018). EOs possess a wide number, varying from 20-80, of bioactive compounds that made up their composition, Ellse and Wall 2014). These compounds are belonging to various chemical groups including terpenes, terpenoids and phenylpropanoid derivates (Morsy 2017). However, the efficacy of EOs is often attributed to their major component(s), although the presence of other compounds may be important for synergistic effects (Ellse and Wall 2014). The other form of herbal products is plant extracts that are also complex mixtures containing a wide variety of secondary metabolites in different concentration ranges (Borges and Borges 2016), whereby the main difference between them and EOs is by obtaining process. Namely, while EOs are usually obtained for various commercial uses by various forms of distillation (mostly steam), plant extracts are mostly obtained by various forms of solvent extraction such as maceration and enfleurage (George et al. 2014). The most commonly examined types of plant extracts are aqueous and alcoholic (ethanolic and methanolic). Both EOs and extracts may be obtained from different parts of the plants such as leaves, flowers, seeds, wood and bark and even roots (Butnariu and Sarac 2018). Anyhow, plant extracts and EOs are extensively used in the control of parasitic, as well as bacterial and fungal diseases in animals (Abbas et al. 2018).

Interestingly, the principle of self-medication in animals is also well-known, whereby the infected grazing animals with various endoparasites on the field are searching for plants with higher amounts of bioactive compounds (Torres-Acosta et al. 2012), suggesting that the whole plants may also be used. In most cases, plant products with proven effects against endoparasites were applied perorally, for example, in animal feed or as a supplement, although there are some notes that rectal or injectional applications may also be used (Katiki et al. 2019). Oral application may be single, or consecutively during a couple of days, whereby single or a combination of plant products may be used. In the case of ectoparasites, usually different formulations of a few products or their bioactive ingredients are examined, which were applied topically or orally. In those cases, plant-based products especially EOs may also be used as repellents to protect animals against various ticks or insects such as flies (Lachance and Grange 2014).

Plant-based drugs have already shown the effect against various parasites (and their different life stages) of veterinary and medical importance (Bauri et al. 2015). Their pharmacological effects including antiparasitic derive from numerous types of bioactive compounds belonging to various chemical groups with a possible different mechanism of action (Butnariu and Sarac 2018), as noted earlier, whereby some of them exhibit strong activity against various parasites. The presence of various compounds and their synergism is often associated with lower susceptibility to the development of resistance in comparison with synthetic drugs (Bauri et al. 2015; Borges and Borges 2016), consisting mainly of one active substance. Also, it is important to note that in the cases where the efficacy of plants is not enough to control parasites alone, they can be used along with other alternatives and rationally used commercial drugs in a form of integrated control. Next, plant formulations offer a possibility to reduce a problem with side effects and residual amounts in animal products, given to their natural origin which is often associated with lower toxicity to host animals and their raise free from chemical inputs. Environmental aspects including soil properties also favor plant drugs due to their biodegradability (Veerakumari 2015). In the end, due to mentioned financial aspect of the use of only commercial drugs and their increased price, the incorporation of botanical drugs and formulations into veterinary medicine is justified since these are much cheaper (Ul Abidin et al. 2021).

Studies that Examined the Antiparasitic Efficacy of Plant Products

Due to the urgent need for new drug sources against parasites in animals, the number of such studies is increasing in the last two decades, especially in recent times. Scientific validation of the sustainable use of plant products is needed prior to their approval for parasites control. Within that perspective, the potent efficacy and selectivity of many new plant products against various groups of parasites (protozoa, helminths and arthropods) have been revealed (Abo-El-Saboud et al. 2018; Calzetta et al. 2020). Some examples are given bellow.

Protozoa - *Babesia spp.*, a tick born protozoan parasites, are one of the major pathogens that infect erythrocytes in a wide range of animals and may cause several clinical signs. Nerolidol, a sesquiterpene compound present in EOs of many plants and approved by the U.S. FDA as a food flavoring agent, caused the significant *in vitro* growth inhibition of four *Babesia* species with IC_{50} values of 21, 23.1, 26.9 and 29.6 µM for *Babesia* (*B.*). *bovis*, *B. caballi*, *B. ovis* and *B. bigemina*, respectively at growth inhibition assay (Aboulaila et al. 2010). In a study of Aboulaila et al. (2018), *in vitro* inhibition assay of several plant-based decoctions including green tea, hibiscus, cinnamon and peppermint against *Babesia* and *Theileria* species was examined. The most successfully were green tea and cinnamon with IC_{50} values of 3.83, 6.25, 2,2 and 5,3% (v/v) as well as 7.83, 19, 5.9, 12.1, and 6% (v/v) against *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *T. equi*, respectively. In a study of Guz et al. (2020), EOs of *Achillea millefolium*, *Eugenia caryophyllus* and *Citrus grandis* were the most active against *B. canis* on anti-babesial assay with IC_{50} values of 51.0, 60.3 and 61.3 µg/mL, respectively.

Several trypanosomes may cause diseases that affect humans or animals, mostly in the region of Africa. EOs of *Cymbopogon citratus*, *Eucalyptus citriodora*, *Eucalyptus camaldulensis*, and *Citrus sinensis* were found to possess *in vitro* dose-dependent activity against *Trypanosoma* (*T.*) *brucei brucei* and *T. evansi*, whereby all oils decreased the number of parasites over time at doses of 0.4, 0.2 and 0.1 g/mL (Habila et al. 2010). On the other hand, crude extract of *Cymbopogon citratus* leafs and *Lepidium sativum* seeds, administrated to mice at different doses ranged 100-400 mg/kG, significantly reduced the parasite load of *T. congolense*, but at the same time decreased lymphocytosis and increased neutrophil counts and, in the case of *Cymbopogon citratus*, significantly improved bodyweight of tested animals (Emiru et al. 2021). In a study of Azeredo et al. (2014), EOs of several plants were *in vitro* evaluated for inhibition activity against *T. cruzi*, the causative agent of Chagas disease, whereby *Cinnamomum verum* was the most effective against epimastigote form of parasites (IC_{50}/24h was 24.13 µg/mL).

Plant-based formulations, given in the food or water to the poultry, are examined in several studies for their effects against *Eimeria* spp., and are often considered very promising anticoccidial agents. Thus, broiler chicks supplemented with a premix (1 g/kG feed) containing the oregano (50 g/kG premix) and garlic (5 g/kg premix) EOs had improved final body weight, feed conversion ratio and reduced faecal oocyst excretion of *Eimeria tenella* (Sidiropoulou et al. 2020). Also, extract of several plants given to broilers in diets contained 30 mg/kG of extract, especially *Nectaroscordum tripedale*, were effective in the control of the same agent by reducing oocyst count per gram of faeces and improving previously mentioned parameters of performance in tested animals (Habibi et al. 2016). Natural formulations based on encapsulated thymol and carvacrol (active compounds of some EOs) at doses of 60 and 120 mg/kG given to the broilers in a corn or soybean meal-based diet have led to the reduction of side effects in broilers vaccinated (in doses 25 times higher than recommended) against coccidiosis (Lee et al. 2020).

Giardia (*G.*) *duodenalis* is the most prevalent flagellate protozoan infecting humans worldwide, but also dogs. On the other hand, other animals may act as reservoirs for this parasite and be related to zoonotic transmission. EO of *Citrus × aurantifolia* exhibited *in vitro* activity against *G. duodenalis* at antigiardial assay with an IC_{50} value of 6.96 µg/mL, whereby for example EOs from other plants were less or not effective in the same study (Popruk et al. 2017). The study of Moon et al. (2006) demonstrated that low concentrations (<1%) of EOs of *Lavandula angustifolia* and *L. x intermedia* during *in vitro* trial may completely eliminate *G. duodenalis*, *Trichomonas* (*T.*) *vaginalis* and *Hexamita* (*H.*) *inflate*. Hydroalcoholic extract of *Tanacetum vulgare*, applied *in vivo* to mice at a dose of 0.2 mL per day, significantly reduced giardia trophozoites count in the small intestine of animals 5 days after treatment (Muresan et al. 2021).

Trematodes and cestodes - Fasciolosis caused by *Fasciola* (*F.*) *hepatica* is considered as the most important hepatic disease in veterinary medicine that causes major economic losses in the livestock industry. *In vitro* anthelmintic effect of fifteen tropical plant extracts on the activity of excysted flukes of *F. hepatica* was evaluated by Alvarez-Mercado et al. (2015), whereby the most potent was *Artemisia Mexicana* with an IC_{50} value of 92.85 mg/L, and which along with *Bocconia frutescens*, had a 100% efficacy at the lowest dose tested. Abbas et al (2020) evaluated the *in vivo* anthelmintic effect of the herbal mixture that includes 17 plants against *F. hepatica* in goats, which were administrated at dose rates of 1400, 1200 and 1000 mg/kg at an interval of 7 days for four weeks, whereby it reduced the number of eggs per gram in faeces for 25-52.94% and 29.55-82.35% depending on the dose, on Day 15 and 30, respectively.

The other herbs that showed promising effects against *F. hepatica* and *F. gigantica* were *Allium sativum*, *Lawsonia inermis*, *Opuntia ficus*, *Lantana camara*, *Bocconia frutescens*, *Piper auritum*, *Artemisia mexicana* and *Cajanus cajan*. The effect of these plants was on the inhibition of adult fluke motility as well as the induction of the rupturing of internal organs such as the uterus and caeca (Nwofor et al. 2019). The activity of six natural compounds (quercetin, silymarin, naringenin, flavone, resveratrol and betamide) were evaluated against *Opistorchis* (*O.*) *felineus* by using motility and mortality assays, whereby the most effective substance on the motility of adult flukes was quercetin with an IC_{50} value of 5.1 μM. On the other hand, a concentration of 10 μM flavone led to a mortality of 22-35% by day 15, which was significantly higher than that of untreated worms (Mordvinov et al. 2021).

Echinococcosis represents a tapeworm cosmopolitan zoonotic disease where dogs are definitive hosts of the parasite (adult worms), while livestock and humans are intermediate hosts (larval, cystic form). The *in vitro* effect of *Thymus vulgaris* and *Origanum vulgare* EOs against *Echinococcus* (*E.*) *granulosus* protoscoleces and cysts was evaluated by Pensel et al. (2014). The effect was based on the loss of protoscolex viability and loss of cyst mass, which was also confirmed at the ultrastructural level. Interestingly, isolated thymol, the main compound of *T. vulgaris* EO, had a considerably greater effect than that observed with EOs, which was explained by the antagonistic effect between components of EOs. In a similar study, thymol at a concentration of 5 µg/mL, as well as EOs of *Rosmarinus officinalis*, *Mentha piperita* and *Mentha pulegium* at 10 µg/mL showed *in vitro* effect on the proliferation of *E. granulosus* larval cells with a reduction of protoscolex viability as follows: *M. pulegium* 82%, *M. piperita* 77%, *R. officinalis* 71% and thymol 63%. (Albani et al. 2014). The *in vitro* effect of thymol was also demonstrated against *Mesocestoides* (*M.*) *corti* adult worms as well as on tetrathyridia, for which mainly changes were observed in its morphology (lower concentrations) and surface alterations and damage (higher concentrations) (Maggiore and Elissondo, 2014).

Nematodes - The widest number of research aimed to evaluate the anthelmintic effect of plants was conducted against gastrointestinal nematodes in ruminants, especially sheep, which is understandable due to the emergence of resistance of these parasites. In most cases, the effect was proved against blood-sucking nematode *Haemonchus* (*H.*) *contortus*, whereby different *in vitro* (egg hatch test, larval development test, larval and adult motility tests etc.) as well as *in vivo* tests (faecal egg count reduction test, the controlled efficacy test) were used. The EOs so far proven for efficacy against GINs in sheep were listed by André et al. (2018) and Štrbac et al. (2022b), whereby the effect of EOs such as *Origanum vulgare*, *Thymus vulgars*, *Coriander sativum*, *Lavandula officinalis*, *Citrus sinensis*, *Cinnamomum verum*, different *Mentha* spp., *Cymbopogon* spp., *Eucalyptus* spp. as well as their isolated bioactive compounds such as carvacrol, thymol, anethole, cinnamaldehyde, eugenol, carvone, eucalyptol should be emphasized. These compounds may affect the nematode reproductive system causing their lower fertility or may induce different neurological and structural changes leading to nematode paralysis and death (Štrbac et al. 2022b).

In some cases such as in the study of Katiki et al. (2019), toxicity studies were also performed, where the safety of the application of EO formulations was proved, at least from the aspect of physical examination, blood count and the function of liver and kidney. The main problem with most of these studies was the *in vivo* efficacy of these formulations, which was usually lower in comparison with their *in vitro* activity, and the efficacy of commercial drugs as well, due to anatomical-physiological specificities of the ruminant gastrointestinal tract and the instability nature of EOs on the other hand. However, this problem may be overcome by the use of the encapsulation technique in the preparation of these formulations or with a possible different way of use instead of peroral administration (Štrbac et al. 2022b; Štrbac et al., 2022c).

In the case of extracts, the list is also wide and in most of these studies a wider number of plants or the mix of different extracts was examined, whereby their effect was usually attributed to larval inhibition and increased adult mortality of sheep GINs (Jayanegara et al. 2022). On the other hand, the *in vivo* effect of herbal-based dewormer containing 17 plants was also demonstrated against *H. contortus* in goats, with a total reduction of EPGs in animal faeces from 33.33-61.76% and 40-91.18% on days 15 and 30, respectively, depending on the dose used. An increase in erythrocyte count, packed cell volume and haemoglobin concentration was also recorded, suggesting the role of examined herbal dewormer in reducing the signs of anaemia caused by blood-sucking *H. contortus* and *F. hepatica* as well (Abbas et al. 2020). In the end, several EOs were tested for activity against the mix of different GINs isolated from faecal samples of cattle, whereby *Cymbopogon citratus* had the lowest larval and migration inhibition concentration (IC_{50}) values of 3.89 and 7.19 mg/ml, respectively (Saha and Lachance 2019).

Ascaris (*A.*) *suum* represents one of the most prevalent nematode parasites in pigs that also causes significant economic losses. A wide range of condensed tannins from diverse plant sources showed effect against this nematode, related to the reduced migratory ability of newly hatched L_3, as well as the reduced motility and survival of L_4 recovered from pigs. On an ultrastructure level, it was shown that tannins cause significant damage to the cuticle and digestive apparatus of the larvae (Wiliams et al. 2014). Microencapsulated, plant-based mixed functional food composed of several compounds isolated from EOs, given perorally to the pigs daily in a dose of 1.0 mg/kg after fourteen days significantly reduced worm counts (76.8%), female worm counts (75.5%), FEC (68.6%), and worm volume (62.9%) (Kaplan et al. 2014). In a study of Rakhshandehroo et al. (2017), methanolic extracts of *Artemisia dracunculus* and *Mentha pulegium* at all tested concentrations (50, 75, 100 and 125 mg/mL) had significant lethal effects on larvae of *Parascaris (P.) equorum*, which is common causative agent of disease in equids, especially young horses.

Botanical anthelmintics have also shown an effect against various nematodes in dogs and cats. In a study of Sinott et al. (2019), a concentration of 0.6 mg/ml of EO of Brazilian red propolis (the source of plant *Dalbergia ecastophyllum*) showed 100% larvicidal activity against *Toxocara* (*T.*) *cati* after exposure for 48 h, while 300 μg/mL represented the IC_{50}. *Ancylostoma* (*A.*) *caninum*, one of the most important hookworms in dogs, is the most tested parasites in such studies, with 12 plants showed *in vitro* anthelmintic effect on eggs, larvae and adult worms, and 6 plants showed *in vivo* efficacy (Ekawardhani et al. 2021). For example, 500 mg/ml of extract of *Euphorbia hirta* obtained from the leaf of the plant, given three days in a row in 2 stages per 2 weeks intramuscularly and perorally to dogs, reduced 100% FEC at the second stage (Adedapo et al. 2005). In another study, the combination of plant extract obtained from the seed of *Citrus aurantiifolia* (40 mg/kg) given with mebendazole (50 mg/kg) per day for two weeks to dogs also reduced 100% FEC at the end of the experiment (Hassanain et al. 2015).

Plants were effective not only against gastrointestinal nematodes, but also against heartworms (*Dirofillaria* (*D.*) *immitis*) and lungworms (*Dictyocaulus* (*D.*) *viviparous*). Thus, several plant extracts showed microfilaricidal effects against *D. immitis* in a study of Merawin et al. (2010), whereby *Zingiber officinale* exhibited the strongest activity given that its concentrations of 100 μg/ml μg/ml, 10 μg/ml and 1 μg/ml effectively reduce the relative movability to 93.72, 88.12 and 87.95%, respectively after 24 h. Using the larval migration inhibition assay, the effect of condensed tannins, as well as an extract containing crude sesquiterpene lactones, from *Cichorium intybus* on the motility of L_1 and L_3 larvae of *D. viviparous* was demonstrated (Molan et al. 2003).

Ectoparasites - Plant extracts and especially EOs are increasingly used in the controlling of diseases caused by ectoparasites in animals. Their effect is often related to a harmmful effect on the nervous system of ectoparasites, which may be due to the inhibition of releasing of acetylcholinesterase important for their activity and synaptic transmission, or due to the act on Octopamine whose disruption results in complete breakdown of the nervous system (Abbas et al. 2018). Botanical antiectoparasitic agents may be used for acaricidal and insecticidal purposes or for repelling them (Adenubi et al. 2018).

Among ectoparasites, tickborne infections are considered the most devastating due to causing major economic losses and their role in transmission of many serious pathogens (protozoa and bacteria). Thus, EO of *Tagetes minuta* showed dose-dependent efficacy against four species of ticks (*Rhipicephalus* (*Boophilus*) *microplus*, *Rhipicephalus* (*R.*) *sanguineus*, *Amblyomma cajennense* and *Argas miniatus*) on an adult immersion test (AIT) and the larval packet test (LPT), with a more than 95% efficacy at the concentration of 20% (Garcia et al. 2012). The same EO used at the same concentration also promoted the significant effects on all biological indicators analyzed for *R. microplus* (number of ticks, the average weight of the ticks, the average egg weight per engorged female and larval viability), since it showed 99.98% efficacy compared to the control group (Andreotti et al. 2013). EO of *Ocimum gratissimum* exhibited great larvicidal activity against different ticks (*R. microplus*, *Amblyomma sculptum* and *R. sanguineus*) with a IC_{50} values of 2.0 mg/mL, 5.5 mg/mL and 6.0 mg/mL, respectively (Ferreira et al. 2019).

The EOs of *Rosmarinus officinalis*, *Mentha spicata* and *Origanum majorana* showed strong repellency of 100, 93.2 and 84.3%, respectively against the tick *Ixodes* (*I.*) *ricinius* nymphs (El-Seedi et al. 2012). On the other hand, EOs of *Syzygium aromaticum*, *Thymus serpyllum* and *Thymus vulgaris* were the most effective in a study of Štefanidesová et al. (2017), since they repelled 83, 82 and 68% of tick *Dermacentor* (*D.*) *reticulatus*, respectively, at a concentration of 3%. However, the mixture of *Thymus serpyllum* (1.5%) and *Cymbopogon winterianus* (1.5%), showed higher repellency (91%) than individual oils. An orally applied formulation consisting of garlic oil (2.5%), allicin (0.05%) and rapeseed oil (8%) to the dogs infested by various ticks (*Ixodes* spp. and *R. sanguineus*) at the dose of 0.25 ml/kg for 3 successive days has led to the decrease in a tick number for 100% starting from 12 hours from the 3rd dose up to 28 days. Moreover, treatment with this mixture improved the health condition of tested animals since all haematological and biochemical parameters returned to normal values after the treatment (Amer and Amer 2020).

Infestation with *Dermanyssus* (*D.*) *gallinae*, a blood-feeding mite, represents a major problem in the poultry industry in recent years. EO of *Coriander sativum* at a concentration of 0.4 mg/cm^2 as well as *Ocimum basilicum*, *Mentha x piperita* and *Satureja hortensis* at concentration of 0.6 mg/cm^2 have led to >90% mortality of the mites after 24h of exposure using the *in vitro* direct contact method (Magdaş et al. 2010). The impact of the spraying of surfaces of hennery with the garlic extract on the number of *D. gallinae* was evaluated by Gorji et al. (2014), whereby two successive sprays 8 days apart reduced their number by 96%. In a study of Andriantsoanirina et al. (2022) several tens of EOs were *in vitro* evaluated against *Sarcoptes* (*S.*) *scabiei*, causative agent of sarcoptic mange in animals, whereby *Cinnamomum zeylanicum* and *Ocimum sanctum* oils were the most active in contact and fumigation bioassays, as well as in ovicidal activity. In this study, all mites were killed within one hour with these oils diluted at 1%. The ethanolic extract of *Ligularia virgaurea* at a concentration of 2 g/ml also exhibited strong acaricidal activity against *S. scabiei* since it killed all mites within 2 h (Luo et al. 2015).

Rabbits infested with *Psoroptes* (*P.*) *cuniculi* were topically treated two times at seven days interval with two ml of the EO of *Cinnamomum zeylanicun* leaves, whereby concentrations between 0.16 and 10% were effective as a drug and cured all animals (Fichi et al. 2007). The *in vivo* effect of EOs of *Allium sativum*, *Origanum majorana* and ozonated olive oil against the important ear ectoparasite *Otodectes* (*O.*) *cynotis* in cats was evaluated by Yipel et al. (2016), whereby practically all oils led to the elimination of parasites 30 days after treatment. The best results were shown by garlic EO along with permethrin 10 days after treatment. Several plant EOs were tested against *Demodex* (*D.*) *canis*, a dog mite with zoonotic potential, whereby *Melaleuca alternifolia* oil showed a faster and stronger effect compared to amitraz since it required less time to eliminate the parasites (8.100-100.67 minutes in comparison with 333.33 minutes) (Neves et al. 2020).

Finally, aqueous extract of *Azadirachta indica* was tested against sheep bot fly larvae (*Oestrus* (*O.*) *ovis*), whereby at different concentrations showed a significant, dose-dependent effect on time to L_1 mortality in an *in vitro* test, and interfered with larval development in an *in vivo* test (Cepeda-Palacios et al. 2014). Neem extract (A. indica) is also known for its wideuse (Ascher et al., 2000). *Lavandula officinalis* EO and camphor at 32% concentration were found to have a larvicidal effect against sheep blowfly, *Lucilia* (*L.*) *serrata*, since they cased the mortality of larva by 100 and 93.33%, respectively (Shalaby et al. 2016). EOs and extracts have also showed efficacy against many other ectoparasites including *Ctenocephalides* (*C.*) *felis* (cat flea), *Bovicola* (*B.*) *ocellatus* (chewing louse), *Haematopinus* (*H.*) *tuberculatus* and *Hippobosca* (*H.*) *equina* etc. (Abbas et al. 2018).

Conclusion

Antiparasitic resistance represents an urgent problem in veterinary medicine due to economic losses. In addition to the problem of residues in animal products and the environment, as well as the problem of rising drug prices, this interferes with the use of commercial chemotherapeutic agents. As a source of a wide number of bioactive compounds of natural origin, herbal medicines are marked as a promising alternative. The effect of plant products shown against various parasites may be utilized to reduce the use of commercial drugs, which may lead to slowing down the spread of resistance and solving other mentioned problems. Therefore, along with other alternatives and strategies for rational use of drugs, botanical anthelmintics offers a possibility for sustainable, integrated control of parasites of veterinary importance in future treatment approaches.

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