Review

Composition, Bioactivities, Safety Concerns, and Impact of Essential Oil on Pets' and Animals' Health

Natarajan Sisubalan , Bhagavathi Sundaram Sivamaruthi * , Periyanaina Kesika , Chaiyavat Chaiyasut *

Posted Date: 30 November 2023

doi: 10.20944/preprints202311.2008.v1

Keywords: essential oils; chemical constituents; toxicity; pets; safety considerations; bioactivity.

Review

Composition, Bioactivities, Safety Concerns, and Impact of Essential Oil on Pets' and Animals' Health

Natarajan Sisubalan [1,2], Bhagavathi Sundaram Sivamaruthi [1,2,*], Periyanaina Kesika [1,2] and Chaiyavat Chaiyasut [2,*]

[1] Office of Research Administration, Chiang Mai University, Chiang Mai 50200, Thailand. Sisubalan.n@cmu.ac.th (N.S.); sivamaruthi.b@cmu.ac.th (B.S.S.); kesika.p@cmu.ac.th (P.K.)

[2] Innovation Center for Holistic Health, Nutraceuticals, and Cosmeceuticals, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand. chaiyavat@gmail.com (C.C.)

* Correspondence: sivamaruthi.b@cmu.ac.th (B.S.S.); chaiyavat@gmail.com (C.C.).

Featured Application: Essential oils (EOs) are concentrated volatile mixtures obtained from aromatic plant parts and are rich in terpenes, terpenoids and phenylpropanoids. EOs have distinct biological and pharmacological properties. EOs composition, properties, benefits, safety considerations, and effects of EOs on pets and animals are summarized. The applications of EOs range from antimicrobial effects to antioxidant, anti-inflammatory, and anticancer activities etc. Safety considerations, with potential toxicity, are essential when integrating EOs into animal care practices. The paper also described the regulatory concerns and future perspectives on applying EOs in veterinary medicine and health care.

Abstract: Essential oils (EOs) are highly concentrated and volatile blends of nonpolar substances, are derived from aromatic plant components and comprise terpenes, terpenoids and phenylpropanoids, exhibiting diverse biological and pharmacological properties. The burgeoning pet industry is interested in EOs as a potential solution for common health issues in domestic animals, particularly in addressing antimicrobial resistance. The present study summarizes the composition, properties, benefits, safety considerations, and effects of EOs on pets and animals. The applications of EOs range from antimicrobial effects to antioxidant, anti-inflammatory, and anticancer activities etc. Furthermore, EOs are used extensively in various industries, including beauty care products, detergents, and fragrances. The chemical constituents of EOs, exemplified by eucalyptus EO and rosemary EO, highlight their distinct aromatic profiles and potential benefits. Nevertheless, understanding the chemical makeup of EOs is fundamental in assessing their potential impacts on biological systems. Safety considerations, including potential toxicity, are essential when incorporating EOs into animal care routines. The feed additives incorporating EOs have shown promise in influencing gut microbiota balance, reducing inflammation, and acting as antioxidants. However, cautious application is paramount, considering the potential risks associated with high doses or multiple administrations. Preliminary studies suggest low toxicity levels, but further research is required to evaluate the safety of EOs. Though studies reported the beneficial effects of EOs on pets and animals, further research is needed to validate the findings in real-world conditions. The paper also discussed the regulatory considerations and future perspectives on applying EOs in veterinary medicine.

Keywords: essential oils; chemical constituents; toxicity; pets; safety considerations; bioactivity.

1. Introduction

Essential oils (EOs) are being used in pet food as a new way to address pet health-associated issues [1]. Recently, EOs have become popular because of their significant biological [2] and pharmacological properties [3]. EOs have demonstrated antioxidant, low-haemolytic, antibacterial, and anticancer effects [4,5]. Moreover, EOs are used in various industries, including cosmetic

 doi:10.20944/preprints202311.2008.v1

products, detergents, soaps, cleansing gels, and fragrances, which hold substantial economic importance. Unlike esters of fatty acids [6], EOs are highly concentrated and volatile. They are obtained from the end products of primary metabolites found in various aromatic plant parts, including stems, leaves, bark, resin, flowers, fruit, seeds, and roots [7].

EOs are intricate and highly concentrated blends of volatile, nonpolar substances. They fall under the category of secondary metabolites in plants, playing a crucial role in enabling plants to thrive in their respective environments [8–10]. These blends encompass various compounds, including terpenes, terpenoids, and phenylpropanoids, with potential applications in various pharmacological contexts [11–14].

Feed additives like probiotics, prebiotics and EOs can be incorporated into pet food, individually or in combination. These supplements can offer significant benefits to the animal, including acting as antioxidants, reducing inflammation, regulating the immune system, and influencing the balance of gut microbiota [15].

The demand in EOs for pets could be attributed to several key factors. Firstly, pet owners have a growing preference for natural and holistic approaches to pet care. EOs, derived from plants, are seen as a more organic alternative to conventional pet products [16]. Secondly, EOs are known for their potential therapeutic benefits. They can address a range of common pet issues [16]. The natural approach [17,18] aligns with the desire of many pet owners to minimize the use of synthetic chemicals in their pets' care. The availability and accessibility of EOs have increased significantly in recent years, with a wide array of products tailored specifically for pets, which made it more convenient for pet owners to incorporate EOs into their wellness routines.

The present review summarizes the composition, properties, safety concerns, and beneficial effects of EOs on pets. Also, advantages and limitations of EOs for the pet's healthcare.

2. Chemical Constituents of Essential Oils

EOs are extremely concentrated aromatic constituents obtained from various plants, renowned for their aromatic and therapeutic properties. Understanding the chemical constituents of EOs is crucial for comprehending their potential effects on biological systems. Recently, researchers have been interested in exploring the constituents of EOs due to the market demand worldwide. The chemical compositions of EOs have been reported previously. The chemical structure of major compounds present in EOs is represented in Figures 1 and 2.

Eucalyptus EO is characterized by its high content of 1,8-cineole, making up about 63.1% of its composition. Additionally, it contains various monoterpene hydrocarbons, including p-cymene, α-pinene, α-limonene, γ-terpinene, β-pinene, and β-myrcene [29]. *Lavandula angustifolia* EO (*L. angustifolia*) is rich in linalyl acetate (27.5%) and linalool (24.1%), constituting 51.6% of the oil's composition. Other significant compounds include E-β-ocimene, terpinen-4-ol, caryophyllene, carvacrol, lavandulyl acetate, (Z)-β-farnesene, and (Z)-β-ocimene. These constituents play a crucial role in determining the quality of the EO [30]. Rosemary EO is characterized by its key compounds like eucalyptol (9.48% to 12.58%). Lavender EO also contains significant amounts of eucalyptol (25.9% to 22.7%), borneol (12.43% to 14.09%), and camphor (9.61% to 5.76%). *Ferulago contracta* EO (*F. contracta*) is made-up of bornyl ester (13.42% to 14.32%), trans-β-Ocimene (11.52% to 6.78%), and limonene (8.93% to 3.34%). These components are crucial in defining the aromatic profile of the respective oils [31].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

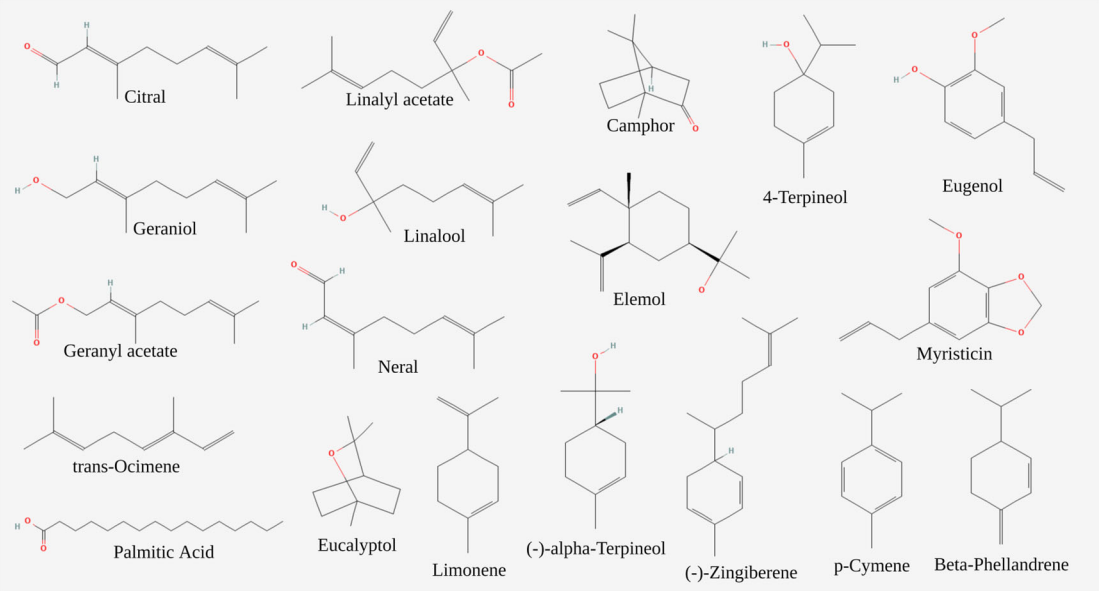

Figure 1. The chemical structure of major compounds of representative essential oils (Set 1). Retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

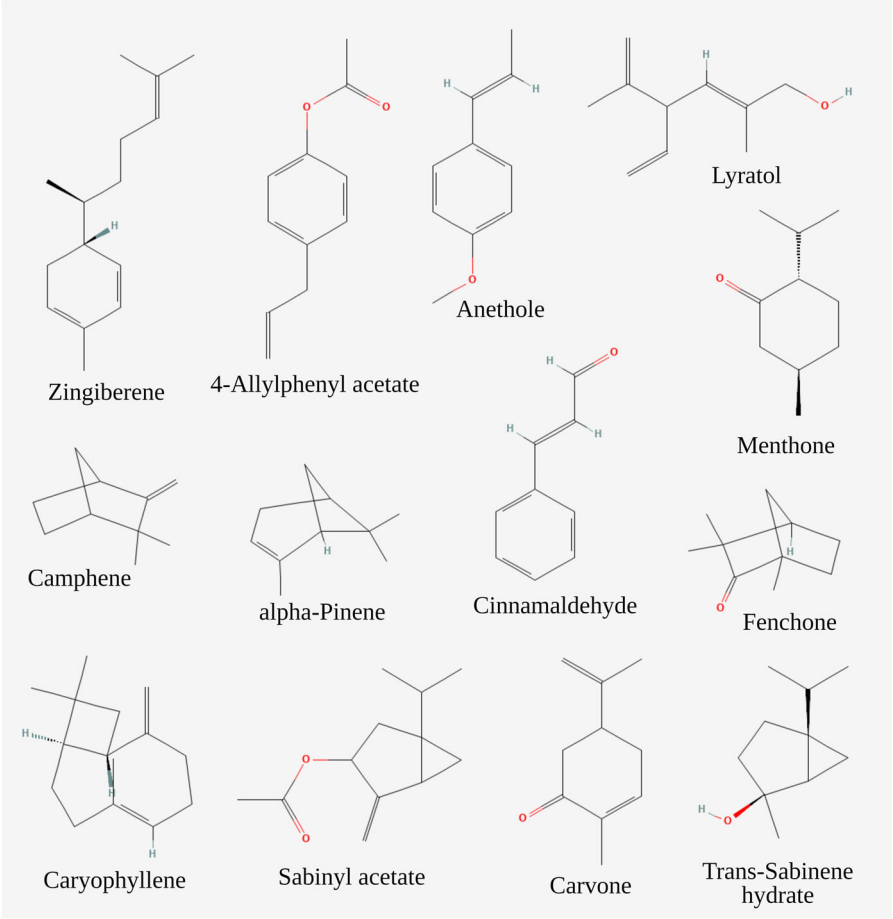

Figure 2. The chemical structure of major compounds of representative essential oils (Set 2). (Retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

Peppermint EO contains major components like menthol, menthone, 1,8-cineole, menthofuran, and isomenthyl acetate, making up a significant portion (72.4% of the entire EO) [32]. *Cymbopogon* spp. EO has α-elemol (ranging from 29.5% to 53.1%), geraniol (37.1%), and citral (90.4%) in different

 doi:10.20944/preprints202311.2008.v1

parts such as roots, root hair with stalk, and leaves [20]. *Cymbopogon martinii* EO (*C. martinii*) varies in composition between leaves and roots. In leaves, the major compounds are neral (36.1%) and geranial (53.1%), while in roots, it comprises α-elemol (31.5%), neral (16.6%) and geranial (25.0%) [33]. Lemongrass EO also shows a distinction between leaves and roots. In leaves, geraniol dominates (76.6%), followed by geranyl acetate (15.2%). In roots, geraniol is the major component (87.9%), with geranyl acetate constituting 4.4% [33].

The representative constituents were given in Table 1, and a list of the constituents of the EOs was given in supplementary Table S1.

Table 1. Major chemical compounds of the representative essential oils (EOs).

Phytocompounds (Concentration in %)	Source of EOs	Ref.
Geranial (35.07%)	*Lippia alba*	[1]
Neral (27.8%)		
Trans-caryophyllene (6.72%)		
Geranial/α-citral (42.88%)	*Cymbopogon citratus*	[1]
β-Citral (32.15%)		
Myrcene (9.82%)		
Carvacrol (18.97%)	*Origanum vulgare*	[1]
Trans-sabinene hydrate (17.75%)		
Terpinen-4-ol (7.57%)		
Thymol (48.9%)	*Thymus vulgaris*	[19]
p-Cymene (19.0%)		
γ-Terpinene (4.1%)		
Carvacrol (3.5 %)		
β-Caryophyllene (3.5 %)		
Carvacrol (12.8%)	*Thymus tosevii*	
α-Terpinyl acetate (12.3%)		
cis-Myrtanol (11.2%)		
Thymol (10.4%)		
Menthol (37.4%)	*Mentha piperita*	[19]
Menthyl acetate (17.4%)		
Menthone (12.7%)		
Limonene (6.9 %)		
Carvone (49.5%)	*Mentha spicata*	
Menthone (21.9%)		
Limonene (5.8 %)		
1,8-Cineole (26.54%)	*Rosmarinus officinalis* L.	[20]
α-Pinene (20.14%)		
Camphor (12.88%)		
Camphene (11.38%)		
β-Pinene (6.95%)		
cis-α-Santalol (39%)	*Santalum album*	[21]
cis-β-Santalol (17.38%)		
β-Curcumen-12-ol (9.71%)		

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

n-Hexadecanoic acid (78.25%) Tetradecanoic acid (7. 24%) Silane, triethylfluoro- (3.96%)	*Azadirachta indica*	[22]
1,6-Octadien-3-ol, 3,7-dimethyl- (41.74%) Silane, triethylfluoro- (36.71%) Bicyclo [2.2.1] heptan-2-one, 1,7,7-trimethyl-, (+)- (6.91%)	*Lavandula angustifolia*	[22]
a-Zingiberene (36.78%) b-Sesquiphellandrene (10.25%) ar-Curcumene (9.51%) a-Farnesene (6.84%) Camphene (3.80%) b-Bisabolene (3.65%)	*Zingiber officinale*	[23]
1,8-Sineol (24.38%) cis-β-Farnesene (12.19%) β-Pinene (8.48%) Phenol, 4-(2-propenyl)-, acetate (6.01%) (S)-4-(1-Acetoxyallyl) phenyl acetate (5.66%)	*Alpinia galanga*	[24]
Trans-Anethole* γ-Himachalene*	*Pimpinella anisum*	[25]
Linalool* 1,8-Cineole* Methyl eugenol*	*Ocimum basilicum*	
Limonene* Linalyl acetate* γ-Terpinene* Linalool*	*Citrus bergamia*	[25]
Eugenol* Cinnamyl acetate*	*Cinnamomum zeylanicum*	
Terpinen-4-ol* α-Terpineol* 1,8-Cineole* α-Terpinene* γ-Terpinene*	*Malaleuca alternifolia*	
Eugenol* β-Caryophyllene* 1,8-Cineole* α-Pinene*	*Syzygium aromaticum*	[25]
Anethole* Fenchone*	*Foeniculum vulgare*	
Geranial* Neral*	*Zingiber officinale*	
β-Caryophyllene*	*Hypericum perforatum*	

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

α-Pinene*		
Linalyl acetate*	*Lavandula angustifolia*	[25]
Linalool*		
Terpinen-4-ol*		
Ocimene*		
Geranial*	*Cymbopogon citratus*	
Neral*		
1,8-Cineole*	*Thymus mastichina*	
Linalool*		
Menthol*	*Mentha piperita*	[25]
Menthone*		
α-Thuyone*	*Rosmarinus officinalis*	
α-Pinene*		
Camphene*		
Camphor*		
Lyratol*	*Artemisia vulgaris*	[25]
1,8-cineole*		
α-Thuyone*	*Salvia officinalis*	
Camphor*		
1,8-Cineole*		
α-Humulene*		
Carvacrol*	*Satureja montana*	[25]
p-Cymene*		
1,8-Cineole*	*Thymus vulgaris*	
β-Phellandrene*		
Camphor*		
Terpinene (52.24%)	*Melaleuca alternifolia*	[26]
Dihydro-α-terpineol (5.97)		
Diterpene (2.87%)		
(L)-alpha-terpineol (18.32%)	*Citrus limon*	
Alpha-terpinol (13.43%)		
Trans-4-thujanol (9.64%)		
α- Terpinolene (5.81%)		
Citral propylene glycol acetal (5.73%)		
Geranial propylene glycol acetal (4.00%)		
α-Terpineol acetate (3.60%)		
(E)-Cinnamaldehyde (69.0 %)	*Cinnamomum verum* J. Presl	[27]
Eugenol (6.43%)	(Bark oil)	
b-Caryophyllene (6.33%)		
Linalool (5.02%)		
Eugenol (79.0%)	*Cinnamomum verum* J. Presl	
Eugenyl acetate (2.71%)	(Leaf oil)	

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

Benzyl benzoate (3.54%)		
(E)-Cinnamaldehyde (0.86%)		
Sabinene*	*Myristica fragrans* Houtt.	[28]
a-Pinene (pin-2(3)- ene) *		
Myristicin*		
b-Pinene (pin-2(10) ene) *		
4-Terpineno*		
Limonene*		
c-Terpinene*		

*Major compounds of the plant and percentage are not available.

3. Some of the Bioactive Properties of Essential Oils

The distinct elements of EOs have been applied independently and demonstrated various biological effects, including antimicrobial, antioxidant, anti-inflammatory, and insecticidal properties [34] (Figure 3).

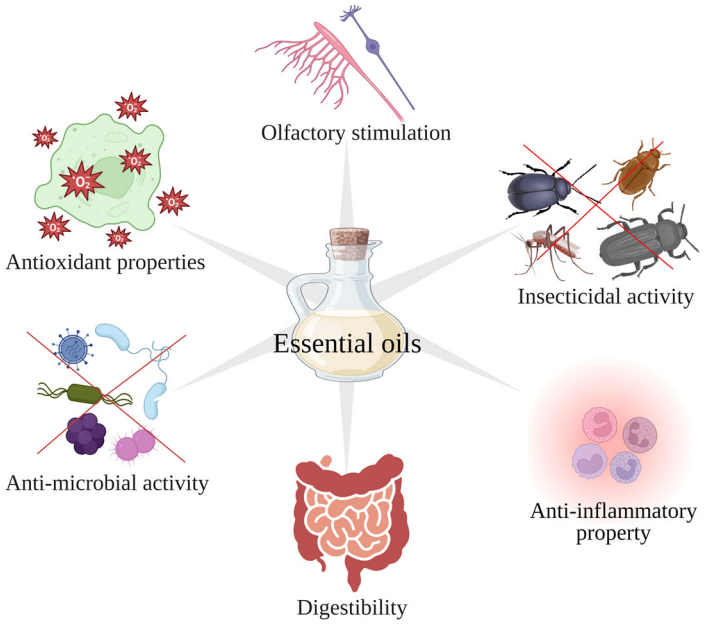

Figure 3. The representative bioactive properties of essential oils.

3.1. Antimicrobial Activity

Cinnamon EO exhibited the most promising minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against the strains obtained from clinical endometritis having dairy cows, *Escherichia coli* (*E. coli*) (MIC: 2048 mg/mL; MBC: 32768 mg/mL) and *Trueperella pyogenes* (*T. pyogenes*) (MIC: 512 mg/mL; MBC: 16384 mg/mL). Cinnamon EO and other EOs also showed potent synergistic inhibitory activity against *T. pyogenes* and *E. coli* [35].

EOs from Cinnamomum zeylanicum (C. zeylanicum) (MIC: 2.52 mg/mL), Cymbopogon citratus (C. citratus) (MIC: 1.118 mg/mL), Litsea cubeba (L. cubeba) (MIC: 1.106 mg/mL), Mentha piperita (M. piperita) (MIC: 1.14 mg/mL), Syzygium aromaticum (S. aromaticum) (MIC: 1.318 mg/mL), Ocimum basilicum (O. basilicum) (MIC: 9.15 mg/mL), and Pelargonium graveolens (P. graveolens) (MIC: 17.8 mg/mL) showed inhibitory activity against E. coli [36]. C. zeylanicum EO (MIC: 1.26 mg/mL to 0.63 mg/mL), S. aromaticum EO (MIC: 2.637 mg/mL to 0.164 mg/mL), and their combination (MIC: 1.289 mg/mL to 0.322 mg/mL) exhibited significant inhibitory activity against Salmonella strains [37]. In

addressing pathogens accountable for otitis externa in dogs and cats, the EO from Salvia sclarea (S. sclarea) showed inhibitory activity against Staphylococcus pseudointermedius (S. pseudointermedius), with an MIC value of 2.23 µg/µL. Origanum vulgare EO inhibited the growth of both Staphylococcus aureus (S. aureus) and S. pseudointermedius, with MIC values of 2.36 µg/µL and 1.18 µg/µL, respectively. Conversely, no discernible activity was observed against Pseudomonas aeruginosa (P. aeruginosa) [38]. Thymus vulgaris (T. vulgaris) and O. vulgare (O. vulgare) EOs showed antimicrobial activity against Candida albicans (C. albicans), Candida famata (C. famata), E. coli and Enterococcus spp. [39]. Similarly, the EOs from O. vulgare and Rosmarinus officinalis (R. officinalis) exhibited the lowest MIC values when combating fungi. S. sclarea, R. officinalis, and T. vulgaris demonstrated MIC values below <0.3 µg/µL against Candida tropicalis (C. tropicalis), while O. vulgare displayed a potential anti-mycological effect against aspergilli. Trichosporon sp. showed sensitivity exclusively to R. officinalis, whereas both R. officinalis and O. vulgare inhibited Rhodotorula sp. [38].

The antidermatophytic activity of EOs from M. piperita, Cinnamonum vernum (C. vernum), and Jasminum officinale (J. officinale) was investigated against Trichophyton equinum (T. equinum) and Microsporum canis (M. canis). Pure M. piperita and C. vernum EOs demonstrated robust antifungal effects against T. equinum and M. canis, comparable to standard antifungal drugs' efficacy [40].

The effects of *O. vulgare* EO (OVEO) with its phenolic constituents, thymol and carvacrol against four clinical strains of *Candida*, including *C. albicans*, *Candida glabrata (C. glabrata)*, *Candida krusei (C. krusei)*, and *Candida tropicalis (C. tropicalis)* had been demonstrated. The MIC and minimum fungicidal concentration (MFC) values were determined for each strain. For all *Candida* species, the MIC values of OVEO ranged from 780 to 1560 µg/mL, and the MFC values ranged from 780 to 3120 µg/mL. Carvacrol exhibited MIC and MFC values between 97.5 and 195 µg/mL, respectively. Thymol demonstrated MIC values between 195 and 390 µg/mL, and MFC values ranged from 390 to 780 µg/mL. In contrast, *C. albicans* displayed susceptibility to fluconazole, with MIC values of 0.5 µg/mL. The MFC for fluconazole in all tested strains fell within 64 to >128 µg/mL [41].

Similarly, the mycelial growth and spore germination of *Botrytis cinerea (B. cinerea)* was significantly inhibited by the EO of *O. vulgare* and its primary components, thymol and carvacrol, when tested *in vitro*. *In vivo* experiments, EO, at a concentration of 250 mg/L, exhibited strong antifungal effects, reducing the decay in cherry tomatoes by 96.39%. Additionally, thymol and carvacrol completely inhibited gray mold. *In postharvest scenarios, O. vulgare EO could be an environmentally friendly and non-toxic botanical fungicide for controlling gray mold* [42].

EO of lemongrass was active against the clinical isolates of *Malassezia pachydermatis (M. pachydermatis)*. Also, diluted oregano, palmarosa, winter savory, cinnamon leaf, clove, Indian melissa, and rose geranium EOs also exhibited bioactivity against *M. pachydermatis* [43].

Loizzo et al. [44] demonstrated that *Laurus nobilis (L. nobilis)* berries EO had an IC_{50} value of 120 mg/mL against severe acute respiratory syndrome coronavirus (SARS-CoV), with a selectivity index (SI; TC_{50}/IC_{50}) of 4.2. The oil also showed noteworthy activity against herpes simplex virus (HSV)-1, with an IC_{50} value of 60 mg/mL [44]. Kumar et al. [45] reported the angiotensin-converting enzyme 2 (ACE2) inhibitory effects of ten EOs. Geranium and lemon EOs exhibited significant ACE2 inhibitory effects *in vitro*. Immunoblotting and qPCR analysis further confirmed the potent ACE2 inhibitory effects of geranium and lemon EOs. The major compounds of geranium EO were citronellol, geraniol, and neryl acetate, while limonene was the predominant compound in lemon EO. The findings suggest that geranium and lemon essential EOs could serve as valuable natural anti-viral agents, potentially contributing to preventing SARS-CoV-2/COVID-19 invasion into the human body [45]. Four EOs [Lemon- *Citrus limon* (*C. limon*), sweet orange - *Citrus sinensis* (*C. sinensis*), grapefruit - *Citrus paradisi* (*C. paradisi*), and rosemary cineole (*R. officinalis* chemotype 1.8 cineole)] effectively reduced the viral loads of hepatitis A virus (HAV) [46].

The in vitro study on the anthelmintic activities of Lavandula officinalis (L. officinalis), Anthemis nobile (A. nobile) and Citrus aurantifolia (C. aurantifolia) EOs against Haemonchus contortus (H. contortus) showed significant inhibitory effects on egg hatching in the egg hatch test. The dose-dependent response profile was observed for all oils in inhibiting egg hatching. Additionally, the

 doi:10.20944/preprints202311.2008.v1

anthelmintic activity of these oils was confirmed in the larval development test. C. aurantifolia, A. nobile and L. officinalis EOs exhibited a larval development inhibition rate greater than 85%, with IC_{50} values of 0.187, 0.375 and 1.5 mg/mL, respectively [47].

The reduction in bacterial levels in the feces of dogs in the natural antioxidant feed (NAT) group may be attributed to the antimicrobial properties of EOs [48]. The finding aligns with Silva et al. [49] report, which investigated the antimicrobial effects of EOs (specifically cloves and lemongrass) in swine, cattle, and poultry feces. They observed that these oils demonstrated effectiveness against both Gram-negative and Gram-positive bacteria. Xie et al. [50] noted that eugenol, a key component of clove oil, plays a central role in its antimicrobial activity. The authors explained that eugenol hinders the bacteria' production of amylase and proteases, leading to their weakening and breakdown. Eugenol is commonly utilized in dentistry for its sealing and antiseptic properties. Combining various antioxidant compounds is an effective defense mechanism against oxidative stress. Each component's distinct mechanisms work together, reducing the total bacterial count in the feces of dogs that consume a diet containing natural antioxidants. This decrease is linked to a reduction in *E. coli* and Salmonella in the feces, which can be attributed to the well-established antimicrobial effects of rosemary and cloves [51].

3.2. Antioxidant Properties

Zhang et al. [52] demonstrated the impact of natural oregano EO (NOEO) on antioxidant properties in the serum of 21-day-old broilers. In comparison to the control group, broilers receiving antibiotics, NOEO, and synthetic oregano EO (SOEO) demonstrated significantly higher concentrations of serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Furthermore, dietary supplementation with NOEO and SOEO increased serum glutathione reductase (GR) concentration. The malondialdehyde (MDA) level decreased with dietary antibiotics and NOEO supplementation, but it was not affected by dietary SOEO supplementation on day 21. On the 42^{th} day, the serum MDA proportion did not vary amongst the four therapies. Though, SOEO and NOEO supplementation raised serum proportions of GR and SOD compared to the control. Additionally, birds nourished beside antibiotics, SOEO, and NOEO exhibited elevated serum concentrations of GSH-Px, and total antioxidant capacity (TAC) compared to those fed with a control diet [52].

The laying hens in the experimental group exhibited significantly elevated levels of TAC (9.8 ± 0.8 mmol/L) compared to the basal diet feed control group (CON). Conversely, MDA levels (10.7 ± 1.4 nmol/mg) were notably lower in the experimental group than in the CON group. Birds supplemented with oregano EO showed consistently higher concentrations of GSH-Px. Additionally, the experimental group exhibited significantly lower alanine and aspartate transferase concentrations [53].

Broilers that were provided with thyme EO (TEO) and rosemary EO (REO) in their diets exhibited numerically lower levels of total antioxidant status (TAS) and total oxidant status (TOS). In contrast, all groups (3-day-old male broiler chicks (Ross 308) were arbitrarily separated into five groups of 80 animals per treatment in a total of four hundred chicks) receiving TEO and REO demonstrated significantly elevated SOD activity and glutathione (GSH) levels, along with reduced MDA in the breast muscle. Conversely, catalase (CAT) activity in the breast muscle of the broilers was statistically similar across all groups ($p > 0.05$). Furthermore, it determined that dietetical supplementation of REO and TEO elevated the oxidative stress index (OSI) and TOS levels while decreasing TAS levels ($p < 0.05$). The investigated doses (Control: Basal diet alone, Thyme-1: Basal diet + 0.15 g kg-1 of thyme EO (TEO), Thyme-2: Basal diet+0.30 g kg-1 of TEO, Rosemary-1: Basal diet + 0.10 g kg-1 of REO, and Rosemary-2: Basal diet + 0.20 g kg-1 of REO) of dietary TEO and REO enhanced intestinal morphology and boosted antioxidant metabolism, particularly in the breast, drumstick, and liver [54].

The EO from *Thymus algeriensis* (*T. algeriensis*) (TAEO) has reduced 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS •+) radicals, with a mean IC_{50} value of 2.7 mg/mL. TAEO also exhibited a scavenging capacity of hydrogen peroxide (H2O2), with an IC_{50} value of 512 mg/mL. The involvement of electron transfer

 doi:10.20944/preprints202311.2008.v1

mechanisms was identified in TAEO, supported by its reducing power in the TAC assay (313 mg ascorbic acid equivalent (AAE)/g of EO) and ferrous iron reduction (25.4 mg AAE/g TAEO). The lipophilic nature of TAEO components significantly inhibited oxidation reactions in lipidic systems, as evidenced by the b-carotene bleaching assay (55.5% at 5 mg/mL). However, within the tested concentration level (1-10 mg/mL), TAEO could not bind with ferrous ions [55].

The observed rise in SOD activity following EO blend (EOB) (100 mg EOB/kg diet (EO100); 200 mg EOB/kg diet (EO200); 400 mg EOB/kg diet (EO400)) supplementation to pigs is considered advantageous, as GSH levels showed no decline. EOB effectively triggers the antioxidant defense system without elevating the reactive oxygen species (ROS) levels. If ROS had increased, GSH levels would have decreased due to its involvement in neutralizing H_2O_2, underscoring the significance of investigating these compounds to comprehend their efficacy in the diet of weaned pigs. The addition of 200 and 400 mg EOB/kg diet led to a decrease in oxidative stress. It proved beneficial for blood biochemical and hematological profiles and the liver status of antioxidants in nursery pigs. EO supplementation did not adversely affect the pigs' growth performance or gastrointestinal health [56].

3.3. Anti-Inflammatory Activity

A combination of *Eucalyptus globulus (E. globulus)* EO with a drug (flurbiprofen) showed significantly better membrane stabilization effects than groups treated with only *E. globulus* EO or drug. When comparing the EO and drug combination-treated group with the drug-treated group, the former demonstrated significantly better anti-inflammatory and antipyretic effects, with non-significant differences in the analgesic model [57].

The EOs derived from *Eucalyptus citriodora* (*E. citriodora*) (EC), *Eucalyptus tereticornis* (*E. tereticornis*) (ET), and *E. globulus* (EG) at a dose of 100 mg/kg, but not at 10 mg/kg, significantly reduced swelling in rat hind paws caused by carrageenan and dextran within 1-4 h after administration compared to control. While there wasn't a clear distinction in the effectiveness of the three EOs in most experiments (Control, EC, ET, EG for 1h, 2h, 3h and 4h), ET oil extracts showed greater efficacy against dextran-induced paw swelling at the 100 mg/kg dose. Compared to the positive control, dexamethasone (1 mg/kg), which nearly completely inhibited neutrophil migration, the EOs demonstrated 70-80% inhibitions compared to the control [58].

The EO from TAEO effectively suppressed acute inflammation induced by xylene application on the ears of mice. The reduced ear inflammation showed a pattern of dose dependence in the examined groups, ranging from 11.6 mg at 200 mg of TAEO/kg body weight to 9.2 mg at 600 mg of TAEO/kg body weight. Compared to the control group, these values were considerably lesser. The excessive dose, 600 mg of TAEO/kg body weight, showed a 58% decrease in xylene-mediated ear inflammation [55].

The repeated inhalation of peppermint EO significantly decreased heightened levels of MDA in the skeletal muscles induced by a two-week exhaustive swimming regimen in rats. MDA is a marker for lipid peroxidation, a process triggered by free radicals damaging cell membranes and generating ROS. Inflammation is also believed to contribute to ROS formation and lipid peroxidation. Peppermint EO, known for its free radical-scavenging and anti-inflammatory properties, could potentially counteract ROS, reduce inflammation-mediated ROS generation, and consequently lower MDA levels [59].

3.4. Insecticidal Activity

According to the findings of Medeiros et al. [60], both *T. vulgaris* (commonly known as thyme) and thymol exhibited significant insecticidal effects against *Cochliomyia hominivorax* (*C. hominivorax*), specifically to third-stage larvae. It suggests the potential for enhancing novel formulations to control the myiasis. *C. hominivorax* is prevalent in tropical and subtropical regions of the Caribbean and South America, excluding Chile, and is accountable for 64.3% of reported myiasis occurrences in domesticated populations of animals [61]. *T. vulgaris* EO's positive effects mainly rely on the key component thymol. It has been reported as a potential insecticide, insect repellent, acaricide and

 doi:10.20944/preprints202311.2008.v1

fungicide [62]. The EO of *O. vulgare*, where thymol is the major component, demonstrated the LC_{50}, which is 3.07 times lower than that of *T. vulgaris* EO after 48 h of exposure [60]. However, validating *in vitro* findings with *in vivo* experiments is crucial, considering factors like quantity and persistence and assuring the interaction with larvae beneath real-world environments. Additionally, the topical application of EO must be thoroughly evaluated for safety and concern [60].

The *in vitro* effectiveness, LC_{50} and harmfulness of EOs derived from *Alpinia zerumbet* (*A. zerumbet*) (Pers.) B. L. Burtt & R. M. Sm, *Laurus nobilis* L. (*L. nobilis*), *Cinnamomum* spp., *Mentha spicata* L. (*M. spicata*), *Cymbopogon nardus* (L.) Rendle (*C. nardus*) and *Ocimum gratissimum* L. (*O. gratissimum*) were reported against infantile stages and adult forms of *Ctenocephalides felis* (*C. felis*) (cat fleas). *O. gratissimum* EO demonstrated the highest efficacy in the *in vitro* assays against all the stages of the flea, exhibiting adulticidal (LC_{50} = 5.85 μg cm^{-2}), ovicidal (LC_{50} = 1.79 μg cm^{-2}), and larvicidal (LC_{50} = 1.21 μg cm^{-2}) activity at low doses. The findings could enhance toxic-free products to control flea cats and dogs [63].

The toxicity of each component in Ajwain (*Carum copticum*, L. – *C. copticum*) EO (AEO) and their combined effects on *Chilo suppressalis* (*C. suppressalis*) larvae. AEO and thymol exhibited significant insecticidal activity with LD_{50} of 13.10 and 17.11 μg/larvae. The effectiveness of insecticidal activity was further enhanced by combining thymol with γ-terpinene or p-cymene. Among these compounds, thymol appeared as a promising biopesticide for controlling *C. suppressalis* populations, demonstrating insecticidal properties and compatibility with other components possessing high acetylcholinesterase inhibition capabilities [64].

The *R. officinalis* (REO) EO induces mortality, diminishes geotaxis (climbing ability), and has a repellent effect comparable to conventional repellents against the *Drosophila melanogaster* (*D. melanogaster*) model. The study revealed that the EO can induce oxidative damage and disrupt the antioxidant defenses in adult fruit flies, leading to significant larvicidal effects that result in mortality and hindered larval development. Furthermore, when the EO (at 3.2 μg/mL) is combined with paraquat, there is a synergistic impact on survival and geotaxis. The study suggested the prooxidant mechanism of REO, linked to oxidative damage and impairment of enzymatic and nonenzymatic systems. REO demonstrates dual potential as a bio-insecticide and larvicide against adult and third-instar larvae of *D. melanogaster*, with approximate LC_{50} values of 6.9 μg/mL and 1.81%, respectively [65].

4. Effects of EO on the Health Status of Pets and Animals

In treating *Malassezia otitis* (*M. otitis*) externa in atopic dogs, a combination of EOs was used. *T. vulgaris* exhibited the lowest MIC at 0.05%, whereas *R. officinalis*, *S. sclarea*, and *Lavandula hybrida (L. hybrida)* indicated higher MIC levels (>2%). The MIC for ketoconazole was less than 0.03 μg/ml. The EOs combinations 1 (0.5 % *C. paradisi*, 0.5 % *S. sclarea*, 0.5 % *O. basilicum*, and 1% *R. officinalis*) and 3 (1% *S. sclarea*, 1% *R. officinalis* and 1% *L. hybrida*) showed the MIC at 50% concentration. In contrast, mixtures 4 (1% *C. limon*, 0.5% *R. officinalis*, 1% *C. paradisi*, and 0.5% *Anthemis nobilis (A. nobilis*) and 5 (1% *A. nobilis*, 0.5% *C. paradisi*, 0.5% *T. vulgaris*, and 1% *L. hybrida* 1%) inhibited fungal growth at a 75% dilution. The Mixture 2 (1% *C. limon*, 0.5% *S. sclarea*, 1% *R. officinalis*, and 0.5% *A. nobilis*) showed MIC at 25% concentration. The synergic activity of EOs showed significant results *in vivo* studies, even at sub-MIC concentrations. Mixture 2 showed better results *in vivo* studies, whereas Mixture 5 showed insignificant results. Some dogs showed adverse effects like swollen erythema against mixture 4 [66].

A recent study investigated the combined impact of yeast cell wall and oregano EO on dog's digestion, taste preference, intestinal fermentation byproducts, and gut microbiota. Including this blend in the dogs' diet decreased the apparent total tract digestibility of dry matter and intake ratio compared to the control diet. It also positively influenced beneficial microorganisms in the fecal matter, resulting in greater [67].

Graham et al. [68] reported the olfactory stimulating properties of EOs (lavender, chamomile, rosemary, and peppermint EOs) in dogs housed in a rescue shelter. The dogs were exposed to each olfactory stimulant, facilitated by the diffusion of EOs, for 4 h per day over 5 days, with a 2-day

 doi:10.20944/preprints202311.2008.v1

interval between each exposure. The study revealed that prolonged exposure to EOs had a noticeable effect on the dog's behavior, without any clear signs of habituation to the smells. Interestingly, stimulating odors (Rosemary and peppermint EOs) increased activity levels among the sheltered dogs. In contrast, stress-reducing odorants (Lavender and chamomile EOs) did not produce a similar effect despite introducing all odors for the same duration. Notably, the dogs did not acclimate to any odors, even those known for stress reduction, such as lavender and chamomile. However, the possibility of eventual habituation with prolonged exposure remained uncertain. The study suggested that the observed behavioral changes were likely attributable to the introduced odors rather than other environmental influences. The findings underscored the importance of carefully selecting odors, as introducing scents that induce agitation or stress could have detrimental effects. It was highlighted that stimulating odors might not be suitable for dogs already displaying hyperactivity or abnormal behavior. In contrast, calming scents could contribute to the improved well-being of dogs [68].

A topical formulation containing polyunsaturated fatty acids (PUFAs) and EOs impacts the itching and skin lesions associated with canine atopic dermatitis (CAD). The dogs were treated with a test formula or placebo on the upper part of the neck once a week. Degree of CAD and severity, measured by the Canine Atopic Dermatitis Extent and Severity Index-03 (CADESI-03), as well as pruritus scores, were assessed by veterinarians and owners before and after the study. The results showed a significant improvement in CADESI-03 and pruritus scores in the treatment group compared to the placebo group. Furthermore, more dogs in the treatment group exhibited at least a 50% improvement in CADESI-03 and pruritus scores compared to the placebo group. The topical preparation containing PUFAs and EOs proved to be a safe and beneficial treatment for alleviating the clinical signs of CAD [69].

In a blinded crossover clinical trial, dogs underwent a dental cleaning and examination, which included a periodontal assessment involving probing and evaluations of plaque, calculus, and gingivitis. Later, pet owners administered a gel (either active or a placebo) to the soft tissues inside their dogs' mouths twice daily for 4 weeks. Subsequently, the dogs' teeth underwent another cleaning, and the owners then applied the alternative gel for an additional 4 weeks. Clinicians evaluated bad breath immediately after the initial cleaning and at the 4th and 8th weeks, while owners provided weekly scores for bad breath. In the group that switched from placebo to active gel, bad breath reduced during the placebo application and continued to decrease during the application of the active gel. Seven out of 9 owners reported reduced bad breath when using the active gel. Applying the topical gel containing menthol, thymol EO, and polyphenolic antioxidants (phloretin and ferulic acid) reduced oral malodor in dogs [70].

Goode et al. [71] aimed to assess the impact of various EOs or active ingredients in commercially available repellents on Ixodes ricinus (tick) attachment to dogs. Turmeric EO prevented the climbing response by a tick and had a longer residual activity than other EOs. A subsequent blanket drags field assay compared tick attachment on blankets treated with turmeric EO, orange EO, N, N-Diethyl-3-methylbenzamide (positive control), or 1% coco glucoside excipient solution (negative control). The results, based on the counting of 899 ticks, showed an average of 23.3 ± 21.3, 26.9 ± 28.6, 2.6 ± 2.0, and 3.4 ± 3.7 ticks per blanket were found in negative control, orange EO treatment, turmeric EO treatment, and positive control groups, respectively. Dogs sprayed with 2.5% turmeric EO (n = 24) and 2.5% orange EO (n = 16), control (n = 15) were allowed to walk in known tick-infested areas. Dogs sprayed with turmeric EO had a significantly lower percentage of ticks attached to their legs or belly than dogs sprayed with orange EO and control dogs. These findings suggest that turmeric EO may be useful in a tick management program for domestic dogs [71].

In the quest for effective and safe control of *Rhipicephalus sanguineus* Sensu Lato (*R. sanguineus*) (Brown dog tick) on dogs, a microemulsion formulation containing thymol and eugenol was developed and assessed for its safety and physical characteristics. Microemulsions demonstrate strong physical stability. Treatment with microemulsion led to a significant decrease in larvae infesting the dogs on the first day, while the numbers of nymphs and adults did not show a notable reduction. When assessing the reproductive biology of engorged females, there was a notable impact

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

on larval hatchability, resulting in an impressive control rate of 85.5%. In conclusion, the microemulsion effectively reduces the number of larvae and affects the reproductive parameters of engorged female brown dog ticks. Still, it is also safe for dogs and maintains physical stability over two years [72].

The dog feed enriched with natural antioxidants (a blend of EOs and vitamin E) was assessed for its impact on food preservation and canine health. The experimental group receiving the natural antioxidant feed showed a notable reduction in ROS levels, signifying a mitigation of oxidative stress. Also, an increase in non-protein thiol and glutathione S-transferase levels was noted, potentially elucidating the observed decline in ROS levels and concluding that incorporating natural antioxidants into dog feed not only enhances food preservation but also elevates systemic antioxidant levels, effectively minimizing the adverse effects induced by free radicals in the dogs [73].

Batista et al. [74] revealed the chemical constituents of extracts and EO obtained from *Schinus molle* (*S. molle*). They evaluated their effectiveness against adults of *C. felis* and its eggs, a prevalent flea species infesting in Brazil on dogs and cats. The non-polar (n-hexane) extract exhibited 100% effectiveness (800 μg cm^{-2}; LD_{50} = 524·80 μg cm^{-2}). Lupenone was the major compound of the extract. EOs extracted from fruits and leaves demonstrated 100% efficacy against adult fleas at concentrations of 800 μg cm^{-2} (LD_{50} = 353·95 μg cm^{-2}) and 50 μg cm^{-2} (LD_{50} = 12·02 μg cm^{-2}), respectively [74].

A study was conducted on fourteen cats having symptoms of spontaneous dermatophytosis caused by *M. canis*. In group 1, cats were administered orally with itraconazole at a 5 mg/kg/day dose for one week, with at least 6 weeks of total treatment duration. Neutral shampoo, about 5 mL containing *Thymus serpyllum* (*T. serpyllum*) (2%), *O. vulgare* (5%), and *R. officinalis* (5%) EOs were used to wash twice a week. In group 2, cats received the same oral itraconazole dose and 2% miconazole/2% chlorhexidine shampoo twice a week during treatment. By week 3 after-treatment, two cats in group 1 were clinically healthy, and all others achieved clinical health by week 11. At the trial's conclusion, all cats in this group tested negative in fungal cultures. The mean time to clinical cure and mycological cure for group 1 cats was 6 weeks (median 4 weeks, range 3–11 weeks) and 15 weeks (median 14 weeks, range 7 to 42 weeks), respectively. In group 2, one cat was dermatologically normal by week 3, and all cats achieved clinical cure by week 10. By the end of the study, 6 out of 7 cats in this group were negative on fungal culture. The mean time to clinical cure and mycological cure for group 2 cats was 5.9 weeks (median 6 weeks, range 3–10 weeks) and 12.8 weeks (median 6 weeks, range 7–21 weeks), respectively. The study suggested that *T. serpyllum*, *O. vulgare*, and *R. officinalis* EOs containing shampoo could be an alternative to conventional cat dermal treatment [75].

The antifungal efficacy of EOs derived from *C. limon, T. serpillum*, *R.officinalis, O. vulgare* and *Illicium verum* (*I. verum*) was investigated against eleven clinical isolates of *M. canis*. Among the tested EOs, *T. serpillum* and *O. vulgare* exhibited the lowest MICs, followed by *I. verum*, *R. officinalis*, and *C. limon*. A formulated mixture comprising 5% *O. vulgare*, 5% *R. officinalis*, and 2% *T. serpillum* in sweet almond oil demonstrated enhanced antimycotic activity compared to individual components. Subsequently, this mixture was treated in seven symptomatic *M. canis*-infected cats. Clinical and cultural recovery was observed in four of the seven treated cats, highlighting the potent antifungal activity of *T. serpillum* and *O. vulgare* EOs [76].

One hundred and fifty cats were exposed to various odor conditions: control (no smell), biologically relevant odor (rabbit scent- prey scent), and biologically non-relevant odors (lavender and catnip scents). The cats were exposed to the odors for 3 h a day over five successive days. Cats' behaviors were recorded every 5 min on days one, three, and five. The cats showed limited interest in scented clothes, spending slightly over 6% of the observation time. Particularly, cats exposed to catnip-scented cloths displayed significantly more interest, spending an average of 11.14% of the observation time. Across all conditions, interest in clothes decreased in the second and third hours, indicating habituation. Olfactory stimulation influenced certain aspects of the cats' behavior. Both catnip and prey scents led to a higher frequency of behaviors associated with lowered activity. Catnip also triggered a specific play-like behavior called the 'catnip response' [77].

The anthelmintic efficacy of peppermint EO (*M. piperita* L.) has been reported. In the egg hatch test *in vitro*, the ovicidal activity ranged from 21.0 to 90.3%, depending on the concentration of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

EO. The *in vivo* fecal egg count reduction test was conducted using the mean dose of EO (150 mg/kg), which demonstrated a certain level of anthelmintic efficacy, with an average reduction in nematode eggs of 26.9 and 46.0% at days 7 and 14 after treatment, respectively [78].

Anti-toxic effects of *O. vulgare* EO (OEO) against aflatoxin B1 (AFB1) have been reported. Forty-eight New Zealand White growing rabbits aged four weeks were randomly divided into four groups with four replicates, each containing three animals: control group (only basal diet), AFB1 group (0.3 mg AFB1/kg diet), OEO group (1 g OEO/kg diet), and co-exposed group (1 g OEO/kg + 0.3 mg AFB1/kg diet). The results indicated that OEO significantly alleviated the toxic effects of AFB1 on rabbit kidneys by reducing cystatin C levels. Additionally, OEO mitigated oxidative stress and lipid peroxidation in the co-exposed group. Furthermore, OEO mitigated DNA damage and inflammatory responses, along with the downregulation of genes encoding stress and inflammatory cytokines. Moreover, OEO preserved the cytoarchitecture of rabbit kidneys treated with AFB1 [79] (Table 2).

Table 2. Summary of essential oils for pets and animals' healthcare.

Study subjects	Essential oil	Dose and duration	Results	Ref.
Dogs with otitis	Mixture 1: *Citrus paradisi* (0.5%), *Salvia sclarea* (0,5%), *Ocimum basilicum* (0.5%), *Rosmarinus officinalis* (1%). Mixture 2: *Citrus limon* (1%), *R. officinalis* (1 %), *Anthemis nobilis* (0.5 %), *S. sclarea* (0.5%). Mixture 3: *S. sclarea* (1%), *Lavandula hybrida* (1%), *R. officinalis* (1 %). Mixture 4: *C. limon* (1%), *R. officinalis* (0.5%), *C. paradisi* (1%), *A. nobilis* (0.5 %). Mixture 5: *Thymus vulgaris* (0.5 %), *A. nobilis* (1%), *C. paradisi* (0.5%), *L. hybrida* (1%).	200 µL of oil mix per ear once daily for 2 weeks	Mixture 2 showed better improvement in canine otitis.	[66]
Dogs	Diet 1: Control; Diet 2: 1.5 kg/ton of yeast cell wall and oregano EO (1.5 YCO); Diet 3: 3.0 kg/ton of yeast cell wall and oregano EO (3.0 YCO).	1.5 kg/ton YCO or 3.0 kg/ton YCO twice a day for 20 days	Dogs treated with the YCO blend showed signs of enhanced intestinal function. Beneficial bacterial diversity was increased. The concentrations of histamine, phenol, and ammonia were reduced.	[67]
Dogs	EOs of *Lavandula angustifolia*, *Anthennis*	*EO was diffused using an oil burner	*L. angustifolia* and *A. nobilis* EOs improved	[68]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

	nobilis, Cymbopogon citrates and *Mentha piperita.*	into the dogs' places for 4 h per day for five consecutive days. After 2 days of break, the next EO was used.	the behaviors and relaxation of dogs in the rescue shelter.	
Dogs with CAD	PUFAs: (6 mg/mL of a-linolenic and 30 mg/mL of linoleic acid); EOs (neem oil, rosemary extract, lavender oil, clove oil, tea tree oil, oregano extract, peppermint extract and cedar bark extract) *	Dogs <10, 10 to 20, and 20 to 40 kg received 0.6, 1.2, and 2.4 mL once a week for 8 weeks.	The topical preparation containing PUFAs and EOs ameliorates the clinical signs of CAD and is safe for dogs.	[69]
Dogs	Placebo or active gel** containing EO compounds (menthol and thymol) and polyphenolic antioxidants (phloretin and ferulic acid).	12 mm of gel/side of mouth. Twice daily for 4 weeks.	A daily application of tested formulation following an initial dental cleaning reduced halitosis in dogs.	[70]
Dogs	EOs of turmeric and orange (negative control)	2.5% (v/v) of turmeric and orange EOs diluted in water with a 1% coco glucoside excipient. Ten sprays per day for 28 days.	Dogs treated with turmeric EO showed a significantly reduced percentage of ticks attached to their legs or bellies compared to controls.	[71]
Dogs infested with ticks	Microemulsion containing 0.5 mg/ mL of thymol and 0.5 mg/ mL of eugenol.	Each dog was sprayed with 10 mL of microemulsion /kg daily for 3 days.	The microemulsion reduced the number of tick larvae in dogs and reduced the larval hatching. The microemulsion was stable and safe.	[72]
Dogs	A mixture of EOs of 6% clove, 2% rosemary, 1% oregano, 3.3% vitamin E, and 87.7% soybean oil (vehicle).	1% of test mixture in dry feed. The control and test groups had 380 g of feed daily for 28 days. Then, the animals were swapped with a 15-day washout period.	Improved the antioxidant status of the study subjects.	[73]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 November 2023 doi:10.20944/preprints202311.2008.v1

Cats infected with *Microsporum canis* (dermatophytosis)	Shampoo containing *Thymus serpyllum* (2%), *O. vulgare* (5%), and *R. officinalis* (5%), and itraconazole.	Oral itraconazole (5 mg/kg/day) for 1 week, every 2 weeks for at least 6 weeks. Washed twice a week using 5 mL of shampoo during treatment.	The treatment was effective, and shampoo could be an alternative cat dermatophytosis treatment.	[75]
Cats infected with *Microsporum canis* (dermatophytosis)	EO mixture containing 2% *Thymus serpillum,* 5% *O. vulgare,* and 5% *R. officinalis* in sweet almond oil	EO mixture was applied to the lesion for one month. Itrafungol® (5 mg/kg/day) for 1 week, washout period for 1 week (3 cycles)-served as efficacy control.	Five out of seven cats that received EO treatment showed recovery. No adverse effects were observed in any of the treated cats.	[76]
Sheep	EO of *Mentha piperita* diluted in sunflower oil at 1: 4.5 ratio.	Regular diet (barley and maize grains) with 150 mg/kg EO. Diet and 3.8 mg/kg of albendazole (Positive control). Diet and 50 mL of sunflower oil/ animal (negative control). For 14 days.	EO of *M. piperita* has potent anthelmintic efficacy. It could be used to control gastrointestinal nematodes in sheep.	[78]
Rabbit	*O. vulgare* EO*	Control, AFB1 group (0.3 mg AFB1/kg diet), OEO group (1 g OEO/kg diet), and Combination group (1 g OEO/kg + 0.3 mg AFB1/kg diet) for 8 weeks.	OEO supplementation improved the harmful effects of AFB1. Improved the antioxidant levels, Decreased the inflammation, and reversed oxidative DNA damage in rabbits.	[79]

*Concentration is not available; **PerioSciences LLC with exclusive technology licensing to Tooth To Tail Animal Inc., Dallas, USA; PUFAs: Polyunsaturated fatty acids; EOs: Essential oils; YCO: Yeast cell wall and oregano EO; CAD: Canine atopic dermatitis; AFB1: Aflatoxin B1; OEO: *O. vulgare* EO.

5. Safety Considerations of Essential Oils

While EOs have gained popularity, a major concern lies in the limited studies on their toxicity [80]. The potentially harmful effects of EOs and their components are typically assessed in lab animals

 doi:10.20944/preprints202311.2008.v1

like rodents. Initial tests on rats have shown that most EOs have low toxicity, with an LD_{50} range of 1-20 g/kg [81]. However, studies examining their effects on the host are scarce.

Preliminary toxicity tests on peppermint EO (*M. piperita*) in sheep demonstrated no adverse effects on the behavior, blood parameters, or kidney and liver functions. It indicates the safety of using the formulation on sheep, especially in the short term [78]. Similar results were observed for lemongrass EO *Cymbopogon schoenanthus* L. (*C. schoenanthus*) applied to sheep at doses of 180 and 360 mg/kg orally and for the encapsulated combination of anethole and carvone applied to lambs at doses of 20 and 50 mg/kg orally. In both cases, there were no toxic effects on animal behavior or liver and kidney functions, affirming their safety [82,83].

Studies demonstrate that pesticides classified as having minimal risk and not registered with the Environmental Protection Agency (EPA) can have notable detrimental effects on dogs and cats. Among the exposed animals, most (92%) exhibited symptoms after using naturally derived plant flea products. It's worth noting that even when labeled as "natural" and applied according to the instructions, these minimal-risk pesticides may still have adverse effects on animals. Furthermore, individual animals may display varying sensitivity to EOs due to their distinct host characteristics [84,85].

In a study, pets infested with fleas were thoroughly treated with an infusion derived from *Melissa officinalis* (*M. officinalis*) (at concentrations of 10% and 18% w/v) for 30 min. The animals' coats were allowed to air dry after this treatment. Additionally, alternative formulations, including a spray made from *Citrus* spp. (prepared as a decoction), *L. officinalis* (also prepared as a decoction) and EOs from *Thuja plicata* (*T. plicata*) and *Juniperus communis* (*J. communis*) were employed against the fleas [86]. In all trials, effective repellence was observed 24 h after treatment. However, the study did not provide information regarding the percentage of infestation post-treatment, which consequently hindered the determination of overall efficacy [84].

Utilizing botanical anthelmintics presents various benefits. These include their diverse chemical composition, comprising compounds from different chemical groups, which reduces the likelihood of resistance development. Additionally, their natural origin leads to fewer residues in animal products and the environment, making them an economically viable option [47,87–89]. Furthermore, employing an encapsulation technique can enhance the *in vivo* effectiveness of peppermint EO (*M. piperita*) by safeguarding its active components from degradation, thus increasing their availability [90,91].

Alternatively, better outcomes can be attained by augmenting the dosage or administering multiple doses over consecutive days instead of a single application. However, assessing the potential toxicity when employing higher doses or multiple administrations is imperative. Efficiency can also be enhanced and controlled release facilitated through alternative application methods like lick blocks containing plant-based compounds, allowing for prolonged usage [92].

6. Limitations in the Use of Essential Oils

- Comprehensive studies are scarce on the toxicity of EOs, particularly concerning pets and animals, leading to a lack of robust evidence on their potential risks and benefits.
- The effects of EOs can vary significantly among different species of animals. This variability introduces complexity in establishing standardized dosages and safety guidelines applicable across diverse animal groups.
- Determining the appropriate dosage and application methods of EOs for animals is challenging due to factors like body weight, metabolism, and individual sensitivities.
- The chemical composition of EOs could vary based on factors like plant source, extraction method, and storage conditions. This lack of standardization poses challenges in predicting their precise effects on animals and requires careful consideration of each oil's unique properties.
- Some EOs, even those derived from plants, can pose risks to animals. For instance, ingestion of tea tree oil has led to intoxication in both humans and animals, demonstrating the importance of informed usage.

7. Future Perspectives

The future of EO applications in veterinary care is poised for exciting advancements. Through cutting-edge research and personalized guidance, we anticipate a transformative approach to using EOs for animal health. Innovations in technology and controlled clinical trials will enhance the effectiveness and safety of these interventions. Additionally, a deeper understanding of the gut microbiome and regulatory enhancements will lead to a more refined and holistic approach to incorporating EOs in animal healthcare. These strides not only signify progress in veterinary medicine but also underscore a dedicated commitment to the well-being of our cherished animal companions.

8. Conclusions

The utilization of EOs in pet and animal care holds promise for addressing a range of health concerns. The diverse biological and pharmacological properties of EOs, encompassing antimicrobial, antioxidant, and anti-inflammatory effects, offer potential benefits for domestic and exotic species. However, caution must be exercised to ensure safe and effective application. Understanding the chemical composition of EOs is crucial in evaluating their potential impacts on biological systems, while preliminary toxicity studies provide important insights into their safety profiles. In insecticidal activity, thymol, and thyme EOs demonstrate notable efficacy against the larvae of *C. hominivorax*, suggesting potential applications in myiasis control. These findings present an important avenue for further research and development in combatting myiasis, particularly in regions where the parasite poses a significant threat to animal health.

While EOs offer promising therapeutic benefits, their mode of action and potential risks remain subjects of ongoing investigation. It is imperative to continue research in this area, focusing on understanding the mechanisms underlying their effects and conducting rigorous safety assessments. Regulatory frameworks warrant refinement for tailored veterinary guidelines. As research advances, personalized protocols and specialized formulations hold the potential to revolutionize animal healthcare. As the interest in natural and holistic approaches to pet care continues to grow, EOs represent a valuable area of exploration in veterinary medicine. With careful consideration and further study, EOs may emerge as valuable tools in enhancing the well-being of pets and animals.

Supplementary Materials: The following supporting information can be downloaded at www.mdpi.com/xxx/s1, Table S1: List of phytocompounds from selected essential oils.

Author Contributions: Conceptualization, C.C. and B.S.S.; methodology, N.S., B.S.S., and P.K.; software, N.S., C.C., and B.S.S.; validation, P.K., C.C., and B.S.S.; formal analysis, N.S., B.S.S., and P.K.; investigation, N.S., B.S.S., and P.K.; resources, C.C.; data curation, N.S., B.S.S., and P.K.; writing—original draft preparation, N.S., B.S.S., C.C., and P.K.; writing—review and editing, B.S.S., N.S., C.C., and P.K.; supervision, B.S.S., and C.C.; project administration, C.C.; funding acquisition, C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All the related data have been provided in the manuscript.

Acknowledgments: This study was partially supported by Chiang Mai University, Thailand. We thank the Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.

Conflicts of Interest: The authors declare no conflict of interest.

 doi:10.20944/preprints202311.2008.v1

Abbreviations

EOs	Essential oils
TEO	Thyme EO
REO	Rosemary EO
NOEO	Natural oregano EO
SOEO	synthetic oregano EO
TAEO	*Thymus algeriensis* EO
T. algeriensis	*Thymus algeriensis*
L. angustifolia	*Lavandula angustifolia*
F. contracta	*Ferulago contracta*
T. plicata	*Thuja plicata*
J. communis	*Juniperus communis*
M. officinalis	*Melissa officinalis*
C. zeylanicum	*Cinnamomum zeylanicum*
C. citratus	*Cymbopogon citratus*
L. cubeba	*Litsea cubeba*
M. piperita	*Mentha piperita*
S. aromaticum	*Syzygium aromaticum*
O. basilicum	*Ocimum basilicum*
P. graveolens	*Pelargonium graveolens*
S. sclarea	*Salvia sclarea*
T. vulgaris	*Thymus vulgaris*
O. vulgare	*Origanum vulgare*
C. martini	*Cymbopogon martini*
R. officinalis	*Rosmarinus officinalis*
S. pseudointermedius	*Staphylococcus pseudointermedius*
S. aureus	*Staphylococcus aureus*
P. aeruginosa	*Pseudomonas aeruginosa*
B. cinerea	*Botrytis cinerea*
C. albicans	*Candida albicans*
C. famata	*Candida famata*
C. tropicalis	*Candida tropicalis*
T. equinum	*Trichophyton equinum*
M. canis	*Microsporum canis*
C. krusei	*Candida krusei*
C. glabrata	*Candida glabrata*
M. pachydermatis	*Malassezia pachydermatis*
M. otitis	*Malassezia otitis*
HSV–1	Herpes simplex virus 1
ACE2	Angiotensin-converting enzyme 2
HAV	Hepatitis A virus
SARS-CoV	Severe acute respiratory syndrome coronavirus
H. contortus	*Haemonchus contortus*
C. hominivorax	*Cochliomyia hominivorax*
C. suppressalis	*Chilo suppressalis*
C. felis	*Ctenocephalides felis*
D. melanogaster	*Drosophila melanogaster*
R. sanguineus	*Rhipicephalus sanguineus*
NAT	Natural antioxidant feed
SOD	Superoxide dismutase
GSH-Px	Glutathione peroxidase
GR	Glutathione reductase

 doi:10.20944/preprints202311.2008.v1

MDA	Malondialdehyde
TAC	Total antioxidant capacity
CON	Control group
TAS	Total antioxidant status
TOS	Total oxidant status
GSH	Glutathione
CAT	Catalase
OSI	Oxidative stress index
DPPH•	2,2-diphenyl-1-picrylhydrazyl
ABTS •+	2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
H_2O_2	Hydrogen peroxide
AAE	Ascorbic acid equivalent
ROS	Reactive Oxygen Species
PUFAs	Polyunsaturated fatty acids
CAD	Canine atopic dermatitis
CADESI-03	Canine Atopic Dermatitis Extent and Severity Index-03
AFB1	Aflatoxin B1
w/v	Weight/Volume
mg/mL	Milligram per milliliter
mmol/L	Millimoles per liter
nmol/mg	Nanomoles per milligram
g/kg	Grams per kilogram
µg/µL	Microgram per microliter
µg cm^{-2}	Micrograms per square centimeter
mg/kg	Milligrams per kilogram
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
SI	Selectivity index
LC_{50}	Half lethal concentration
LD_{50}	Half lethal dose
IC_{50}	Half maximal inhibitory concentration
TC_{50}	Half-maximal toxic concentration
EPA	Environmental Protection Agency

References

1. Possamai, M.C.; dos Santos, I.C.; Silva, E.S.; Gazim, Z.C.; Gonçalves, J.E.; Soares, A.A.; de Melo Germano, R.; Fanin, M.; de Sá, T.C.; Otutumi, L.K. *In vitro* bacteriostatic activity of *Origanum vulgare, Cymbopogon citratus*, and *Lippia alba* essential oils in cat food bacterial isolates. *Semin. Cienc. Agrar*. **2019,** *40*, 3107-3122**.**
2. Van Raamsdonk, L.W.; Ozinga, W.A.; Hoogenboom, L.A.; Mulder, P.P.; Mol, J.G.; Groot, M.J.; Van der Fels-Klerx, H.J.; De Nijs, M. Exposure assessment of cattle via roughages to plants producing compounds of concern. *Food Chem*. **2015,** *189*, 27-37.
3. Chaiyasut, C.; Sivamaruthi, B.S.; Wongwan, J.; Thiwan, K.; Rungseevijitprapa, W.; Klunklin, A.; Kunaviktikul, W. Effects of *Litsea cubeba* (Lour.) Persoon essential oil aromatherapy on mood states and salivary cortisol levels in healthy volunteers. *Evid. Based Complement. Alternat. Med*. **2020**, *2020*, 4389239.
4. Lans, C. Do recent research studies validate the medicinal plants used in British Columbia, Canada for pet diseases and wild animals taken into temporary care? *J. Ethnopharmacol*. **2019**, *236*, 366-392.
5. Gomes, H.I.; Dias-Ferreira, C.; Ribeiro, A.B. Overview of *in situ* and *ex situ* remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. *Sci. Total Environ*. **2013**, *445-446*, 237-260.
6. Zema, D.A.; Calabrò, P.S.; Folino, A.; Tamburino, V.; Zappia, G.; Zimbone, S.M. Valorisation of citrus processing waste: A review. *Waste Manag*. **2018**, *80*, 252-273.

7. Chirani, M.R.; Kowsari, E.; Teymourian, T.; Ramakrishna, S. Environmental impact of increased soap consumption during COVID-19 pandemic: Biodegradable soap production and sustainable packaging. *Sci. Total. Environ*. **2021** 796, 149013.
8. Teoh, E.S. Secondary metabolites of plants. *Med. Orchids Asia*. **2015**, *5*, 59-73.
9. Štrbac, F.; Krnjajić, S.; Stojanović, D.; Novakov, N.; Bosco, A.; Simin, N.; Ratajac, R.; Stanković, S.; Cringoli, G.; Rinaldi, L. Botanical control of parasites in veterinary medicine. *One Health Triad*. **2023**, *3*, 215-222.
10. Thangaleela, S.; Sivamaruthi, B.S.; Kesika, P.; Tiyajamorn, T.; Bharathi, M.; Chaiyasut, C. A narrative review on the bioactivity and health benefits of alpha-phellandrene. *Sci Pharm*. **2022**, *90*, 57.
11. Castagna, F.; Palma, E.; Cringoli, G.; Bosco, A.; Nisticò, N.; Caligiuri, G.; Britti, D.; Musella, V. Use of Complementary Natural Feed for Gastrointestinal Nematodes Control in Sheep: Effectiveness and Benefits for Animals. *Animals (Basel)* **2019**, *9*, 1037.
12. Bhatti, M.Z.; Ismail, H.; Kayani, W.K. Plant Secondary Metabolites: Therapeutic Potential and Pharmacological Properties. In: *Secondary Metabolites-Trends and Reviews*, 1st ed.; Vijayakumar R., Raja S., Eds.; IntechOpen: London, United Kingdom, 2022; Volume 1, doi: 10.5772/intechopen.103698.
13. Thangaleela, S.; Sivamaruthi, B.S.; Kesika, P.; Bharathi, M.; Kunaviktikul, W.; Klunklin, A.; Chanthapoon, C.; Chaiyasut, C. Essential oils, phytoncides, aromachology, and aromatherapy-a review. *Appl. Sci*. **2022**, *12*, 4495.
14. Sivamaruthi, B.S.; Kesika, P.; Chaiyasut, C. The composition, pharmacological and economic importance of essential oil of *Litsea cubeba* (Lour.) Pers. *Food Sci. Technol. (Campinas)* **2022**, *42*, e35720.
15. Sivamaruthi, B.S.; Kesika, P.; Chaiyasut, C. Influence of Probiotic Supplementation on Health Status of the Dogs: A Review. *Appl. Sci.* **2021**, *11*, 11384.
16. Genovese, A.G.; McLean, M.K.; Khan, S.A. Adverse reactions from essential oil-containing natural flea products exempted from Environmental Protection Agency regulations in dogs and cats. *J. Vet. Emerg. Crit. Care (San Antonio)* **2012**, *22*, 470-475.
17. Sardi, J.C.O.; Scorzoni, L.; Bernardi, T.; Fusco-Almeida, A.M.; Mendes Giannini, M.J.S. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J. Med. Microbiol*. **2013**, *62*, 10-24.
18. Spampinato, C.; Leonardi, D. Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. *Biomed. Res. Int*. **2013**, *2013*, 204237.
19. Soković, M.D.; Vukojević, J.; Marin, P.D.; Brkić, D.D.; Vajs, V.; Van Griensven, L.J.L.D. Chemical Composition of Essential Oils of *Thymus* and *Mentha* Species and Their Antifungal Activities. *Molecules* **2009**, *14*, 238-249.
20. Jiang, Y.; Wu, N.; Fu, Y.J.; Wang, W.; Luo, M.; Zhao, C.J.; Zu, Y.G.; Liu, X.L. Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environ. Toxicol. Pharmacol.* **2011**, *32*, 63-68.
21. Subasinghe, U.; Gamage, M.; Hettiarachchi, D.S. Essential oil content and composition of Indian sandalwood (*Santalum album*) in Sri Lanka. *J. For. Res*. **2013**, *24*, 127-130.
22. Hussein, K.A.; Joo, J.H. Chemical composition of neem and lavender essential oils and their antifungal activity against pathogenic fungi causing ginseng root rot. *Afr. J. Biotechnol.* **2017**, *16*, 2349-2354.
23. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Bampidis, V.; Azimonti, G.; Bastos, M.L.; Christensen, H.; Kos Durjava, M.; Kouba, M.; López-Alonso, M.; López Puente, S.; Marcon, F.; Mayo, B.; Pechová, A.; Petkova, M.; Ramos, F.; Sanz, Y.; Villa, R.E.; Woutersen, R.; Brantom, P.; Chesson, A.; Westendorf, J.; Gregoretti, L.; Manini, P.; Dusemund, B. Safety and efficacy of essential oil, oleoresin and tincture from Zingiber officinale Roscoe when used as sensory additives in feed for all animal species. *EFSA J*. **2020**, *18*, e06147.
24. Daning, D.; Widyobroto, B.; Hanim, C.; Yusiati, L.M. Effect of Galangal (*Alpinia galanga*) essential oil supplementation on milk production, composition, and characteristics of fatty acids in dairy cows. *Adv. Anim. Vet. Sci.* **2021**, *10*, 192-202.
25. Ruiz-Cano, D.; Sánchez-Carrasco, G.; El-Mihyaoui, A.; B. Arnao, M. Essential Oils and Melatonin as Functional Ingredients in Dogs. *Animals* **2022**, *12*, 2089.
26. Ibrahium, S.M.; Wahba, A.A.; Farghali, A.A.; Abdel-Baki, A.-A.S.; Mohamed, S.A.A.; Al-Quraishy, S.; Hassan, A.O.; Aboelhadid, S.M. Acaricidal Activity of Tea Tree and Lemon Oil Nanoemulsions against *Rhipicephalus annulatus*. *Pathogens* **2022**, *11*, 1506.
27. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Bampidis, V.; Azimonti, G.; Bastos, M.L.; Christensen, H.; Fašmon Durjava, M.; Kouba, M.; López-Alonso, M.; López

Puente, S.; Marcon, F.; Mayo, B.; Pechová, A.; Petkova, M.; Ramos, F.; Sanz, Y.; Villa, R.E.; Woutersen, R.; Brantom, P.; Chesson, A.; Schlatter, J.; Schrenk, D.; Westendorf, J.; Manini, P.; Pizzo, F.; Dusemund, B. Safety and efficacy of feed additives consisting of essential oils from the bark and the leaves of *Cinnamomum verum* J. Presl (cinnamon bark oil and cinnamon leaf oil) for use in all animal species (FEFANA asbl). *EFSA J*. **2022**, *20*, e07601.

28. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Bampidis, V.; Azimonti, G.; Bastos, M.L.; Christensen, H.; Durjava, M.; Kouba, M.; López-Alonso, M.; López Puente, S.; Marcon, F.; Mayo, B.; Pechová, A.; Petkova, M.; Ramos, F.; Sanz, Y.; Villa, R.E.; Woutersen, R.; Brantom, P.; Chesson, A.; Schlatter, J.; Westendorf, J.; Manini, P.; Dusemund, B. Safety and efficacy of a feed additive consisting of an essential oil from the seeds of Myristica fragrans Houtt. (nutmeg oil) for all animal species (FEFANA asbl). *EFSA J*. **2023,** *21*, e08066.
29. Čmiková, N.; Galovičová, L.; Schwarzová, M.; Vukic, M.D.; Vukovic, N.L.; Kowalczewski, P.Ł.; Bakay, L.; Kluz, M.I.; Puchalski, C.; Kačániová, M. Chemical Composition and Biological Activities of *Eucalyptus globulus* Essential Oil. *Plants (Basel)* **2023**, *12*, 1076.
30. Kozuharova, E.; Simeonov, V.; Batovska, D.; Stoycheva, C.; Valchev, H.; Benbassat, N. Chemical composition and comparative analysis of lavender essential oil samples from Bulgaria in relation to the pharmacological effects. *Pharmacia* **2023**, *70*, 395-403.
31. Keivanfar, L.; Nateghi, L.; Rashidi Nodeh, H. Comparing two different extraction techniques on chemical composition and antioxidant property of three essential oils of *Ferulago contracta, Rosmarinus officinalis* and *Lavendula sublepoidota*. *J. Food Meas. Charact*. **2023**, *17*, 3579–3591.
32. Afkar, S. Assessment of chemical compositions and antibacterial activity of the essential oil of Mentha piperita in response to salicylic acid. *Nat. Prod. Res.* **2023**, *13*, 1-12.
33. Dangol, S.; Poudel, D.K.; Ojha, P.K.; Maharjan, S.; Poudel, A.; Satyal, R.; Rokaya, A.; Timsina, S.; Dosoky, N.S.; Satyal, P.; Setzer, W.N. Essential Oil Composition Analysis of *Cymbopogon* Species from Eastern Nepal by GC-MS and Chiral GC-MS, and Antimicrobial Activity of Some Major Compounds. *Molecules* **2023**, *28*, 543.
34. Brah, A.S.; Armah, F.A.; Obuah, C.; Akwetey, S.A.; Adokoh, C.K. Toxicity and therapeutic applications of citrus essential oils (CEOs): A review. *Int. J. Food Prop*. **2023,** *26*, 301-26.
35. Paiano, R.B.; de Sousa, R.L.M.; Bonilla, J.; Moreno, L.Z.; de Souza, E.D.F.; Baruselli, P.S.; Moreno, A.M. In vitro effects of cinnamon, oregano, and thyme essential oils against *Escherichia coli* and *Trueperella pyogenes* isolated from dairy cows with clinical endometritis. *Theriogenology* **2023**, *196*, 106-111.
36. Ebani, V.V.; Najar, B.; Bertelloni, F.; Pistelli, L.; Mancianti, F.; Nardoni, S. Chemical Composition and In Vitro Antimicrobial Efficacy of Sixteen Essential Oils against Escherichia coli and Aspergillus fumigatus Isolated from Poultry. *Vet. Sci.* **2018**, *5*, 62.
37. Ebani, V.V.; Nardoni, S.; Bertelloni, F.; Tosi, G.; Massi, P.; Pistelli, L.; Mancianti, F. In Vitro Antimicrobial Activity of Essential Oils against *Salmonella enterica* Serotypes Enteritidis and Typhimurium Strains Isolated from Poultry. *Molecules* **2019**, *24*, 900.
38. Ebani, V.V.; Nardoni, S.; Bertelloni, F.; Najar, B.; Pistelli, L.; Mancianti, F. Antibacterial and Antifungal Activity of Essential Oils against Pathogens Responsible for Otitis Externa in Dogs and Cats. *Medicines* **2017**, *4*, 21.
39. Ebani, V.V.; Nardoni, S.; Bertelloni, F.; Pistelli, L.; Mancianti, F. Antimicrobial Activity of Five Essential Oils against Bacteria and Fungi Responsible for Urinary Tract Infections. *Molecules* **2018**, *23*, 1668.
40. Sharma, G.; Sharma, R.; Rajni, E.; Saxena, R. Synergistic, antidermatophytic activity and chemical composition of essential oils against zoonotic dermatophytosis. *Russ. J. Bioorganic Chem.* **2022**, *48*, 1338-47.
41. Stringaro, A.; Colone, M.; Cecchetti, S.; Zeppetella, E.; Spadaro, F.; Angiolella, L. "*In vivo*" and "*in vitro*" antimicrobial activity of *Origanum vulgare* essential oil and its two phenolic compounds on clinical isolates of *Candida* spp. *Arch. Microbiol.* **2022**, *205*, 15.
42. Zhao, Y.; Yang, Y.H.; Ye, M.; Wang, K.B.; Fan, L.M.; Su, F.W. Chemical composition and antifungal activity of essential oil from Origanum vulgare against Botrytis cinerea. *Food Chem.* **2021**, *365*, 130506.
43. Bismarck, D.; Dusold, A.; Heusinger, A.; Müller, E. Antifungal in vitro Activity of Essential Oils against Clinical Isolates of *Malassezia pachydermatis* from Canine Ears: A Report from a Practice Laboratory. *Complement. Med. Res*. **2020**, *27*, 143-154.

44. Loizzo, M.R.; Saab, A.M.; Tundis, R.; Statti, G.A.; Menichini, F.; Lampronti, I.; Gambari, R.; Cinatl, J.; Doerr, H.W. Phytochemical analysis and *in vitro* antiviral activities of the essential oils of seven Lebanon species. *Chem. Biodivers*. **2008**, *5*, 461-470.
45. Senthil Kumar, K.J.; Gokila Vani, M.; Wang, C.-S.; Chen, C.-C.; Chen, Y.-C.; Lu, L.-P.; Huang, C.-H.; Lai, C.-S.; Wang, S.-Y. Geranium and Lemon Essential Oils and Their Active Compounds Downregulate Angiotensin-Converting Enzyme 2 (ACE2), a SARS-CoV-2 Spike Receptor-Binding Domain, in Epithelial Cells. *Plants* **2020**, *9*, 770.
46. Battistini, R.; Rossini, I.; Ercolini, C.; Goria, M.; Callipo, M.R.; Maurella, C.; Pavoni, E.; Serracca, L. Antiviral Activity of Essential Oils Against Hepatitis A Virus in Soft Fruits. *Food Environ. Virol.* **2019**, *11*, 90-95.
47. Ferreira, L.E.; Benincasa, B.I.; Fachin, A.L.; Contini, S.H.T.; França, S.C.; Chagas, A.C.S.; Beleboni, R.O. Essential oils of *Citrus aurantifolia*, *Anthemis nobile* and *Lavandula officinalis*: in vitro anthelmintic activities against *Haemonchus contortus*. *Parasit. Vectors* **2018**, *11*, 269.
48. Schlieck, T.M.M.; Petrolli, T.G.; Bissacotti, B.F.; Copetti, P.M.; Bottari, N.B.; Morsch, V.M.; da Silva, A.S. Addition of a blend of essential oils (cloves, rosemary and oregano) and vitamin E to replace conventional chemical antioxidants in dog feed: effects on food quality and health of beagles. *Arch. Anim. Nutr*. **2021,** *75*, 389-403.
49. Silva, A.M. de O e.; Andrade-Wartha, E.R.S.; Carvalho, E.B.T.; Lima, A.; Novoa, A.V.; Mancini-Filho, J. Effect of the aqueous extract of rosemary (*Rosmarinus officinalis* L.) on oxidative stress in diabetic rats. *Rev. Nutr., Campinas* **2011**, *24*, 121–130.
50. Xie, Y.J.; Yang, Z.; Cao, D.; Rong, F.; Ding, H.; Zhang, D. Antitermitic and antifungal activities of eugenol and its congeners from the flower buds of *Syzgium aromaticum* (clove). *Ind. Crops Prod.* **2015**, *77*, 780–786.
51. Burt, S. Essential oils: their antibacterial properties and potential applications in foods--a review. *Int. J. Food Microbiol*. **2004**, *94*, 223-253.
52. Zhang, L.Y.; Peng, Q.Y.; Liu, Y.R.; Ma, Q.G.; Zhang, J.Y.; Guo, Y.P.; Xue, Z.; Zhao, L.H. Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. *Poult. Sci.* **2021**, *100*, 101163.
53. Johnson, A.M.; Anderson, G.; Arguelles-Ramos, M.; Ali, A.A.B. Effect of dietary essential oil of oregano on performance parameters, gastrointestinal traits, blood lipid profile, and antioxidant capacity of laying hens during the pullet phase. *Front. Anim. Sci.* **2022**, *3*, 1072712.
54. Gumus, R.; Gelen, S.U. Effects of dietary thyme and rosemary essential oils on performance parameters with lipid oxidation, water activity, pH, colour and microbial quality of breast and drumstick meats in broiler chickens. *Arch. Anim. Breed* **2023,** *66*, 17-29.
55. Righi, N.; Deghima, A.; Ismail, D.; Fernandes, P.A.; Baali, F.; Boumerfeg, S.; Baghiani, A.; Coimbra, M.A.; Coelho, E. Chemical composition and in vivo/in silico anti-inflammatory activity of an antioxidant, non-toxic essential oil from *Thymus algeriensis* Boiss & Reut. S. *Afr. J. Bot.* **2023,** *157*, 64-74.
56. Grando, M.A.; Costa, V.; Genova, J.L.; Rupolo, P.E.; Azevedo, L.B.; Costa, L.B.; Carvalho, S.T.; Ribeiro, T.P.; Monteiro, D.P.; Carvalho, P.L.O. Blend of essential oils can reduce diarrheal disorders and improve liver antioxidant status in weaning piglets. *Anim. Biosci*. **2023**, *36*, 119-131.
57. Arooj, B.; Asghar, S.; Saleem, M.; Khalid, S.H.; Asif, M.; Chohan, T.; Khan, I.U.; Zubair, H.M.; Yaseen, H.S. Anti-inflammatory mechanisms of eucalyptol rich Eucalyptus globulus essential oil alone and in combination with flurbiprofen. *Inflammopharmacology* **2023**, *31*, 1849-1862.
58. Silva, J.; Abebe, W.; Sousa, S.M.; Duarte, V.G.; Machado, M.I.; Matos, F.J. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *J. Ethnopharmacol*. **2003**, *89*, 277-83.
59. Zhang, W.; Shi, R.; Gao, T.; Hu, Y.; Zhou, J.; Li, C.; Wang, P.; Yang, H.; Xing, W.; Dong, L.; Gao, F. Repeated Inhalation of Peppermint Essential Oil Improves Exercise Performance in Endurance-Trained Rats. *Nutrients* **2023**, *15*, 2480.
60. Medeiros, M.T.; Campos, D.R.; Soares, E.F.M.S.; Assis, J.D'.; Oliveira, G.F.; Santos, L.O.; Silva, T.M.E.; Silva, M.P.D.; Cid, Y.P.; Scott, F.B.; Comendouros, K. Larvicidal activity in vitro of essential oils against Cochliomyia hominivorax. *Vet. Parasitol.* **2023**, *322*, 110020.
61. Costa-Júnior, L.M.; Chaves, D.P.; Brito, D.R.B.; Santos, V.A.F.D.; Costa-Júnior, H.N.; Barros, A.T.M. A review on the occurrence of *Cochliomyia hominivorax* (Diptera: Calliphoridae) in Brazil. *Rev. Bras. Parasitol Vet.* **2019**, *28*, 548-562.
62. Escobar, A.; P´erez, M.; Romanelli, G.; Blustein, G. Thymol bioactivity: A review focusing on practical applications. *Arab. J. Chem*. **2020**, *13*, 9243–9269.

63. Dos Santos, J.V.B.; de Almeida Chaves, D.S.; de Souza, M.A.A.; Riger, C.J.; Lambert, M.M.; Campos, D.R.; Moreira, L.O.; Dos Santos Siqueira, R.C.; de Paulo Osorio, R.; Boylan, F.; Correia, T.R.; Coumendouros, K.; Cid, Y.P. In vitro activity of essential oils against adult and immature stages of *Ctenocephalides felis felis*. *Parasitology* **2020**, *147*, 340-347.
64. Basij, M.; Sahebzadeh, N.; Shahriari, M.; Panahandeh, S. Insecticidal potential of Ajwain essential oil and its major components against *Chilo suppressalis* Walker. *J. Plant Dis. Prot.* **2023**, *130*, 735-745.
65. Pedroso, A.L.; Schonwald, M.K.; Dalla Corte, C.L.; Soares, F.A.A.; Sperança, A.; Godoi, B.; de Carvalho, N.R. Effects of *Rosmarinus officinalis* L. (Laminaceae) essential oil on adult and larvae of Drosophila melanogaster. *Toxicol. Res. (Camb)* **2023**, *12*, 913-921.
66. Nardoni, S.; Pistelli, L.; Baronti, I.; Najar, B.; Pisseri, F.; Bandeira Reidel, R.V.; Papini, R.; Perrucci, S.; Mancianti, F. Traditional Mediterranean plants: characterization and use of an essential oils mixture to treat *Malassezia otitis* externa in atopic dogs. *Nat. Prod. Res*. **2017**, *31*, 1891-1894.
67. Soares, N.M.M.; Bastos, T.S.; Kaelle, G.C.B.; de Souza, R.B.M.d.S.; de Oliveira, S.G.; Félix, A.P. Digestibility and palatability of the diet and intestinal functionality of dogs fed a blend of yeast cell wall and oregano essential oil. *Animals* **2023**, *13*, 2527.
68. Graham, L.; Wells, D.L.; Hepper, P.G. The influence of olfactory stimulation on the behaviour of dogs housed in a rescue shelter. *Appl. Anim. Behav. Sci.* **2005,** *91*, 143-153.
69. Blaskovic, M.; Rosenkrantz, W.; Neuber, A.; Sauter-Louis, C.; Mueller, R.S. The effect of a spot-on formulation containing polyunsaturated fatty acids and essential oils on dogs with atopic dermatitis. *Vet. J.* **2014**, *199*, 39-43.
70. Low, S.B.; Peak, R.M.; Smithson, C.W.; Perrone, J.; Gaddis, B.; Kontogiorgos, E. Evaluation of a topical gel containing a novel combination of essential oils and antioxidants for reducing oral malodor in dogs. *Am. J. Vet. Res.* **2014,** *75*, 653-657.
71. Goode, P.; Ellse, L.; Wall, R. Preventing tick attachment to dogs using essential oils. *Ticks Tick Borne Dis*. **2018,** *9*, 921-926.
72. Monteiro, C.; Ferreira, L.L.; de Paula, L.G.F.; de Oliveira Filho, J.G.; de Oliveira Silva, F.; Muniz, E.R.; Menezes, K.M.F.; de Camargo, F.R.; de Oliveira Nonato, R.; Martins, D.B.; Marreto, R.N.; Borges, L.M.F. Thymol and eugenol microemulsion for *Rhiphicephalus sanguineus* sensu lato control: Formulation development, field efficacy, and safety on dogs. *Vet. Parasitol.* **2021,** 296, 109501.
73. Schlieck, T.M.M.; Petrolli, T.G.; Bissacotti, B.F.; Copetti, P.M.; Bottari, N.B.; Morsch, V.M.; da Silva, A.S. Addition of a blend of essential oils (cloves, rosemary and oregano) and vitamin E to replace conventional chemical antioxidants in dog feed: effects on food quality and health of beagles. *Arch. Anim. Nutr*. **2021**, 75(5), 389-403.
74. Batista, L.C.; Cid. Y.P.; De Almeida, A.P.; Prudêncio, E.R.; Riger, C.J.; De Souza, M.A.; Coumendouros, K.; Chaves, D.S. *In vitro* efficacy of essential oils and extracts of *Schinus molle* L. against *Ctenocephalides felis felis*. *Parasitology* **2016,** *143*, 627-638.
75. Nardoni, S.; Costanzo, A.G.; Mugnaini, L.; Pisseri, F.; Rocchigiani, G.; Papini, R.; Mancianti, F. Open-field study comparing an essential oil-based shampoo with miconazole/chlorhexidine for haircoat disinfection in cats with spontaneous microsporiasis. *J. Feline Med. Surg*. **2017**, *19*, 697-701.
76. Mugnaini, L.; Nardoni, S.; Pinto, L.; Pistelli, L.; Leonardi, M.; Pisseri, F.; Mancianti, F. *In vitro* and *in vivo* antifungal activity of some essential oils against feline isolates of *Microsporum canis*. *J. Mycol Med*. **2012**, *22*, 179-184.
77. Ellis, S.L.; Wells, D.L. The influence of olfactory stimulation on the behaviour of cats housed in a rescue shelter. *Appl. Anim. Behav. Sci*. **2010**, *123*, 56-62.
78. Štrbac, F.; Krnjajić, S.; Stojanović, D.; Ratajac, R.; Simin, N.; Orčić, D.; Rinaldi, L.; Ciccone, E.; Maurelli, M.P.; Cringoli, G.; Bosco, A. *In vitro* and *in vivo* anthelmintic efficacy of peppermint (Mentha x piperita L.) essential oil against gastrointestinal nematodes of sheep. *Front. Vet. Sci.* **2023**, *10*, 1232570.
79. Hassan, M.A.; Abo-Elmaaty, A.M.A.; Zaglool, A.W.; Mohamed, S.A.M.; Abou-Zeid, S.M.; Farag, M.R.; Alagawany, M.; Di Cerbo, A.; Azzam, M.M.; Alhotan, R.; El-Hady, E. *Origanum vulgare* Essential Oil Modulates the AFB1-Induced Oxidative Damages, Nephropathy, and Altered Inflammatory Responses in Growing Rabbits. *Toxins* **2023**, *15*, 69.
80. Štrbac, F.; Bosco, A.; Pušić, I.; Stojanović, D.; Simin, N.; Cringoli, G.; Rinaldi, L.; Ratajac, R. The use of essential oils against sheep gastrointestinal nematodes. In *Animal Health Perspectives,* 1st ed.; Abbas R, Z.,

Khan A., Liu P., Saleemi M, K., Eds.; Unique Scientific Publishers: Faisalabad, Pakistan, 2022; Volume 1, pp: 86-94.

81. Vostinaru, O.; Heghes, S.C.; Filip, L. Safety profile of essential oils. In *Essential Oils-Bioactive Compounds, New Perspectives and Applications.* 1st ed.; de Oliveira M.S., da Costa W.A., Silva S.G., Eds.; IntechOpen: London, United Kingdom, **2020;** Volume 1, doi: 10.5772/intechopen.87266
82. Katiki, L.M.; Chagas, A.C.; Takahira, R.K.; Juliani, H.R.; Ferreira, J.F.; Amarante, A.F. Evaluation of *Cymbopogon schoenanthus* essential oil in lambs experimentally infected with *Haemonchus contortus*. *Vet. Parasitol*. **2012**, *186*, 312-318.
83. Katiki, L.M.; Araujo, R.C.; Ziegelmeyer, L.; Gomes, A.C.P.; Gutmanis, G.; Rodrigues, L.; Bueno, M.S.; Veríssimo, C.; Louvandini, H.; Ferreira, J.F.S.; Amarante, A.F.T. Evaluation of encapsulated anethole and carvone in lambs artificially- and naturally-infected with *Haemonchus contortus*. *Exp. Parasitol*. **2019**, *197*, 36-42.
84. Woolf, A. Essential oil poisoning. *J. Toxicol. Clin. Toxicol*. **1999,** *37*, 721-727.
85. Genovese, A.G.; McLean, M.K.; Khan, S.A. Adverse reactions from essential oil-containing natural flea products exempted from Environmental Protection Agency regulations in dogs and cats. *J. Vet. Emerg. Crit. Care* (San Antonio). **2012**, *22*, 470-475.
86. Lans, C.; Turner, N.; Khan, T. Medicinal plant treatments for fleas and ear problems of cats and dogs in British Columbia, Canada. *Parasitol. Res.* **2008**, *103*, 889-898.
87. Veerakumari, L. Botanical anthelmintics. *Asian J. Sci. Technol.* **2015**, *6*, 1881-1894.
88. Prakash, P.; Radha Kumar, M.; Pundir, A.; Puri, S.; Prakash, S.; Kumari, N.; Thakur, M.; Rathour, S.; Jamwal, R.; Janjua, S. Documentation of commonly used ethnoveterinary medicines from wild plants of the high mountains in Shimla District, Himachal Pradesh, India. *Horticulturae* **2021**, *7*, 351.
89. Borges, D.G.L.; Borges, F.D.A. Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants. Nematoda **2016**, *3*, e92016.
90. de Aquino Mesquita, M.; E Silva Júnior, J.B.; Panassol, A.M.; de Oliveira, E.F.; Vasconcelos, A.L.; de Paula, H.C.; Bevilaqua, C.M. Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes. *Parasitol. Res*. **2013,** *112*, 3161-3165.
91. Maes, C.; Bouquillon, S.; Fauconnier, M.-L. Encapsulation of Essential Oils for the Development of Biosourced Pesticides with Controlled Release: A Review. *Molecules* **2019**, *24*, 2539.
92. Junkuszew, A.; Milerski, M.; Bojar, W.; Szczepaniak, K.; Le Scouarnec, J.; Tomczuk, K.; Dudko, P.; Studzińska, M.B.; Demkowska-Kutrzepa, M.; Bracik, K. Effect of various antiparasitic treatments on lamb growth and mortality. *Small Rumin. Res.* **2015,** *123*, 306-313.