






# Essential oils and essential oil compounds in animal production as antimicrobials and anthelmintics: an updated review

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## Review Article

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### Abstract

Several countries have shown an increased prevalence of drug resistance in animal production due to the indiscriminate use of antibiotics and antiparasitics in human and veterinary medicine. This article aims to review existing methods using naturally occurring essential oils (EOs) and their isolated compounds (EOCs) as alternatives to antimicrobials and antiparasitic compounds in animal production and, consequently, to avoid resistance. The most-reported mechanism of action of EOs and EOCs was cell membrane damage, which leads to the leakage of cytoplasmic content, increased membrane permeability, inhibition of metabolic and genetic pathways, morphologic changes, antibiofilm effects, and damage to the genetic material of infections. In parasites, anticoccidial effects, reduced motility, growth inhibition, and morphologic changes have been reported. Although these compounds regularly show a similar effect to those promoted by traditional drugs, the elucidation of their mechanisms of action is still scarce. The use of EOs and EOCs can also positively influence crucial parameters in animal production, such as body weight gain, feed conversion rate, and cholesterol reduction, which also positively impact meat quality. The application of EOs and EOCs is enhanced by their association with other natural compounds or even by the association with synthetic chemicals, which has been found to cause synergism in their antimicrobial effect. By reducing the effective therapeutical/prophylactic dose, the chances of off-flavors – the most common issue in EO and EOC application – is greatly mitigated. However, there is very little work on the combination of EOs and EOCs in large *in vivo* studies. In addition, research must apply the correct methodology to properly understand the observed effects; for example, the use of only high concentrations may mask potential results obtained at lower dosages. Such corrections will also allow the elucidation of finer mechanisms and promote better biotechnologic use of EOs and EOCs. This manuscript presents several information gaps to be filled before the use of EOs and EOCs are fully applicable in animal production.

## Introduction

Bacterial resistance to antibiotics is increasingly widespread, with intensive antibiotic use in human and animal health as a primary factor of diffusion, representing a serious problem for the economy and public health (Corrêa *et al.*, 2019; Evangelista *et al.*, 2021). Such products are widely used in livestock, and it has been shown that soil in the vicinity of animal production contains microorganisms with a high prevalence of antibiotic-resistance genes (ARGs) (Duan *et al.*, 2019). This and other factors lead to the conclusion that the indiscriminate use of antibiotics in intensive animal production may be one of the leading causes of resistance among zoonotic microbial pathogens.

ARGs have been constantly found in Enteric commensal and pathogenic bacteria from production animals. *Escherichia coli* has been largely studied (Lanz *et al.*, 2003; Zhang *et al.*, 2017a, 2017b, 2020; Poirel *et al.*, 2018; Li *et al.*, 2019) and strains that cause diarrhea in pigs have been identified as resistant to several classes of antibiotics, including colistin, an antibiotic used to control multi-resistant microorganisms (Le Devendec *et al.*, 2018). Strains of *Salmonella* Typhimurium isolated from humans and animals in China have presented genes for resistance to a wide range of quinolones, aminoglycosides, and colistins (He *et al.*, 2020). Polymyxins, which belong to the colistin antimicrobial group, are still extensively used in animal production and have been found ineffective against many enterobacteria isolated from livestock (Giamarellou, 2016). Having ushered in a new era in medicine, the emerging ineffectiveness of antibiotics puts health at risk across the world. It is estimated that the United States spends \$20 billion annually on treating antibiotic-resistant infectious diseases (Dadgostar, 2019).

In addition to antimicrobial resistance, antiparasitic resistance in animal production has also been an emerging problem due to the indiscriminate use of these compounds. Currently, the largest classes of drugs used to control intestinal parasites – imidothiazoles, tetrahydropyrimidines, benzimidazoles, and avermectins – have gradually lost effectiveness (Dey *et al.*, 2020). Resistance to anthelmintics is increasing; among the farms tested, no antiparasitic activity of albendazole or fenbendazole was found against eggs of *Haemonchus contortus*, *Teladorsagia circumcincta*, and *Trichostrongylus* spp. (Claerebout *et al.*, 2020). This also represents a serious problem, and it is estimated that the economic impact in Europe is about 38 million euros annually due to resistant parasites (Charlier *et al.*, 2020). Another reason for the decreased effectiveness of these products is due to soil contamination by antiparasitic agents, as shown by de Oliveira Ferreira *et al.* (2019), who identified avermectins and moxidectin residues in Brazilian soils in concentrations close to 0.1 mg kg<sup>-1</sup>.

As an alternative to the treatment of both bacteria and resistant parasites, the use of essential oils (EOs), which can also be later processed into purified compounds, has been evaluated (Shen *et al.*, 2021; Zhang *et al.*, 2021). For instance, *in vitro* tests using 1 mg ml<sup>-1</sup> of *Ruta chalepensis* (fringed rue) flower EO showed positive effects (87.5% efficacy) leading to the death of *H. contortus* larvae, whilst 1 mg ml<sup>-1</sup> albendazole had relatively lower beneficial effects (75% of efficacy) (Akkari *et al.*, 2015). *Cuminum cyminum* oil (cumin) was responsible for 99% ovicidal activity in *Fasciola hepatica*, using a concentration of 0.03 mg ml<sup>-1</sup> (Silva *et al.*, 2020), which is a parasite that has been found resistant to albendazole (Ceballos *et al.*, 2019). For bacteria, tested EOs also show good results against human and animal pathogens, such as *Melaleuca alternifolia* (tea tree) (Silva *et al.*, 2019), *Syzygium aromaticum* (clove), *Cinnamomum cassia* (Chinese cinnamon) (Khaleque *et al.*, 2016), *Cinnamomum zeylanicum* (true cinnamon tree), *Origanum vulgare* (oregano), *Thymus vulgaris* (thyme) (Mazzarrino *et al.*, 2015), and *Brassica nigra* (black mustard) EO – composed mainly of allyl isothiocyanate, that has one of the greatest antimicrobial potentials among the essential oil compounds (EOCs) studied (Clemente *et al.*, 2016; Reyes-Jurado *et al.*, 2019).

To be able to consider their use in animals, there must be little or no interference in the beneficial animal microbiota (Ambrosio *et al.* (2017). Understanding of EO mechanisms of action is still lacking, but in general, some of the reported effects are the impairment of cell membrane integrity (e.g., cinnamaldehyde, present in cinnamon EO); the inhibition of protein synthesis (e.g., thyme EO); and the inhibition of genetic material repair (e.g., the effect observed in *E. coli* through phenolic compounds and terpenes, which can also affect the transfer of electrons in cellular respiration) (Ju *et al.*, 2019).

This review aims to compile information about EO and EOC, covering their use as alternatives to antimicrobials and antiparasitics, evaluating their mechanisms of action, critically analyzing the information available about this topic, as well as to identify knowledge gaps in the available literature.

## EO and EOC mechanisms of action as antiparasitic and antimicrobial agents

Mechanisms of action differ among EOs and EOCs (Table 1). In general, scientific studies found in the literature show that the main bactericidal activity occurs through plasmic membrane damage with extravasation of intracellular content, or an increase

in its permeability (Lin *et al.*, 2000; Bischoff *et al.*, 2009; Hemaiswarya and Doble, 2009; Bassolé *et al.*, 2010; Tyagi and Malik, 2012; Shen *et al.*, 2015; Xu *et al.*, 2016; Wang *et al.*, 2017; Zhang *et al.*, 2017a, 2017b; Bouyahya *et al.*, 2019; Cui *et al.*, 2019; Hu *et al.*, 2019; Churklam *et al.*, 2020; Liu *et al.*, 2020, 2021). Other effects observed were the inhibition of metabolic and genetic pathways and damage to genetic material (Cui *et al.*, 2019; Hu *et al.*, 2019; Wang *et al.*, 2020; Liu *et al.*, 2021), morphological changes (Clemente *et al.*, 2016), antibiofilm effects (Bouyahya *et al.*, 2019; Liu *et al.*, 2021), and anti-quorum sensing activity (Clemente *et al.*, 2016). The accumulation of monoterpenes and phenylpropanoids (compounds present in many EOs) in the lipid part of the plasma membrane was responsible for destabilizing the structure of the phospholipid bilayer, depolarizing it, and increasing its permeability, compromising its proper functioning, and eventually causing cell death (Hammer and Heel, 2012).

Carvacrol, the major compound from oregano EO, caused the disruption of the cell membrane and increased transmembrane permeability (Cui *et al.*, 2019). This is supported by the presence of free genetic material in the culture medium of treated groups and the increased concentration of carvacrol in the cytoplasm. Anti-quorum sensing activity and inhibition of peptidoglycan synthesis, which avoids repair and maintenance of the cell wall structure, were also reported (Bouyahya *et al.*, 2019; Ni *et al.*, 2021). Similar to carvacrol, *Zingiber officinale* (ginger) EO presented a membrane-related effect against *E. coli* and *Staphylococcus aureus*. The EO also caused metabolism disturbance, compromising the citric acid cycle, and inhibiting DNA repair and replication mechanisms (Wang *et al.*, 2020).

The diversity of compounds present in EOs is extremely high. As an example, *Pinus* spp. (pine) EO was identified with 116 constituents, mostly belonging to the terpene class (Mitić *et al.*, 2018). In the EO of some plants, the prevailing terpene is limonene, a compound that has anthelmintic activity on *H. contortus* and is bactericidal for *Salmonella* Paratyphi A and *Pseudomonas luteola*, with a moderate effect on *Enterococcus faecalis*. The mechanism of limonene is still not fully understood, but studies show that it can destroy cell integrity and cell wall structure of bacteria through an increase in conductivity and the leakage of intracellular biomacromolecules (nucleic acids and proteins) (Squires *et al.*, 2010; Han *et al.*, 2019; Yazgan *et al.*, 2019).

The action of EO depends on different mechanisms inherent to each compound present or the association of mechanisms from the distinctive compounds, making it difficult to elucidate the exact pathways in which these complex solutions work. The resolution of this knowledge gap is of utmost importance, due to the growing need for alternatives to antimicrobial compounds. Another aggravating factor is the lack of adequate methodologies for identifying mechanisms of antimicrobial activity. Many studies use minimal inhibitory concentrations (MICs) and neglect effects that may not even have been documented because they appeared before the MIC was reached and the occurrence of morphologic alterations occurred in a way in which metabolic dysfunctions and genomic alterations become less noticeable.

EOs and EOCs are also used to control parasites (Table 2). Cinnamaldehyde, for example, had its mechanism of action against *Caenorhabditis elegans* – a free-living nematode used as a model organism – based on an interference of several genes that regulate the expression of glutathione, inhibiting the metabolism of xenobiotics, and leading to death of the organism (Lu *et al.*, 2020).

**Table 1.** Effects of essential oils and isolated compounds on bacteria of relevance to animal production

	Effects	Bacteria	Inhibitory concentrations	Bactericidal concentrations	References
Compounds isolated from essential oils					
Carvacrol	Damage to the cell membrane, inhibition of cellular respiration, and synergism with nisin	<i>Listeria monocytogenes</i>	250 µg ml <sup>-1</sup>	500 µg ml <sup>-1</sup>	Churklam <i>et al.</i> (2020)
Cinnamaldehyde	Disintegration and separation of cell wall and membrane	<i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	0.31 mg ml <sup>-1</sup>	ND	Shen <i>et al.</i> (2015)
Eugenol	Damage to the cell membrane and synergism with antibiotics	<i>E. coli</i> , <i>Pseudomonas aeruginosa</i> and <i>Salmonella Typhimurium</i>	10 mM	ND	Hemaiswarya and Doble (2009)
Allyl isothiocyanate	Damage to the cell membrane (a similar mechanism to polymyxin B)	<i>E. coli</i> , <i>S. Typhimurium</i> and <i>L. monocytogenes</i>	1 µg ml <sup>-1</sup>	ND	Lin <i>et al.</i> (2000)
	Morphological changes and cell cycle arrest (resulting in filamentation)	<i>E. coli</i> , <i>Salmonella enterica</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	100 µg ml <sup>-1</sup> (EC) 100 µg ml <sup>-1</sup> (SE) 12.5 µg ml <sup>-1</sup> (PA) 100 µg ml <sup>-1</sup> (SA)	200 µg ml <sup>-1</sup> (EC) 200 µg ml <sup>-1</sup> (SE) 25 µg ml <sup>-1</sup> (PA) 800 µg ml <sup>-1</sup> (SA)	Clemente <i>et al.</i> (2016)
Linalool	Damage to the cell membrane and inhibition of cellular respiration	<i>P. aeruginosa</i>	431 µg ml <sup>-1</sup>	862 µg ml <sup>-1</sup>	Liu <i>et al.</i> (2020)
Thymol	Damage to the cell membrane, DNA damage by intercalation, reduced motility, and antibiofilm effect	<i>P. aeruginosa</i>	0.125 mg ml <sup>-1</sup>	0.25 mg ml <sup>-1</sup>	Liu <i>et al.</i> (2021)
Essential oils					
<i>Cinnamon zeylanicum</i>	Morphological changes and self-aggregation	<i>E. coli</i> , <i>S. enterica</i> , <i>P. aeruginosa</i> and <i>S. aureus</i>	200 µg ml <sup>-1</sup> (EC) 200 µg ml <sup>-1</sup> (SE) 400 µg ml <sup>-1</sup> (PA) 200 µg ml <sup>-1</sup> (SA)	400 µg ml <sup>-1</sup> (EC) 400 µg ml <sup>-1</sup> (SE) 400 µg ml <sup>-1</sup> (PA) 800 µg ml <sup>-1</sup> (SA)	Clemente <i>et al.</i> (2016)
<i>Cymbopogon citratus</i>	Damage to the cell membrane	<i>E. coli</i>	0.288 mg ml <sup>-1</sup>	ND	Tyagi and Malik (2012)
<i>Dodartia orientalis</i>	Damage to the cell membrane and reduced adhesion	<i>S. aureus</i> , <i>E. coli</i> and <i>Salmonella Enteritidis</i>	0.5 µl ml <sup>-1</sup> (SA) 1 µl ml <sup>-1</sup> (EC) 2 µl ml <sup>-1</sup> (SE)	1 µl ml <sup>-1</sup> (SA) 2 µl ml <sup>-1</sup> (EC) 4 µl ml <sup>-1</sup> (SE)	Wang <i>et al.</i> (2017)
<i>Lippia multiflora</i>	Damage to the cell membrane	<i>S. aureus</i> and <i>S. enterica</i>	1.2 mg ml <sup>-1</sup> (SA) 4.2 mg ml <sup>-1</sup> (SE)	ND	Bassolé <i>et al.</i> (2010)
<i>Litsea cubeba</i>	Damage to the cell membrane, reduced ATPase activity, inhibition of β-galactosidase activity, inhibition of the hexose monophosphate pathway by decreasing the glucose-6-phosphate dehydrogenase enzyme, and structural change in DNA	Methicillin-resistant <i>S. aureus</i> (MRSA)	0.5 mg ml <sup>-1</sup>	1 mg ml <sup>-1</sup>	Hu <i>et al.</i> (2019)
<i>Origanum compactum</i>	Damage to and increase in cell membrane permeability and anti-biofilm effect	<i>E. coli</i>	0.62 mg ml <sup>-1</sup>	0.62 mg ml <sup>-1</sup>	Bouyahya <i>et al.</i> (2019)
<i>Origanum vulgare</i>	Damage to the cell membrane, metabolism inhibition by tricarboxylic acid cycle enzymes, structural alteration of DNA, and inhibition of PVL toxin gene expression	MRSA	0.4 mg ml <sup>-1</sup>	0.4 mg ml <sup>-1</sup>	Cui <i>et al.</i> (2019)

(Continued)

Table 1. (Continued.)

	Effects	Bacteria	Inhibitory concentrations	Bactericidal concentrations	References
<i>Piper nigrum</i>	Damage to the cell membrane	<i>E. coli</i>	1.0 $\mu\text{l ml}^{-1}$	2.0 $\mu\text{l ml}^{-1}$	Zhang <i>et al.</i> (2017a)
<i>Syzygium aromaticum</i>	Damage to the cell membrane and destruction of proteins or inhibition of their synthesis	<i>S. aureus</i>	0.625 $\text{mg ml}^{-1}$	1.25 $\text{mg ml}^{-1}$	Xu <i>et al.</i> (2016)
<i>Thymus vulgaris</i>	Damage to the cell membrane, DNA damage by intercalation, reduced motility, and antibiofilm effect	<i>P. aeruginosa</i>	0.25 $\text{mg ml}^{-1}$	0.5 $\text{mg ml}^{-1}$	Liu <i>et al.</i> (2021)
<i>Zingiber officinale</i>	Damage to the cell membrane, metabolism inhibition by tricarboxylic acid cycle enzymes, inhibition of DNA repair and replication mechanisms	<i>E. coli</i> and <i>S. aureus</i>	2.0 $\text{mg ml}^{-1}$ (EC) 1.0 $\text{mg ml}^{-1}$ (SA)	4.0 $\text{mg ml}^{-1}$ (EC) 2.0 $\text{mg ml}^{-1}$ (SA)	Wang <i>et al.</i> (2020)

ND, Not determined.

Many compounds were studied, but only a few had proven antiparasitic effects (Tavares-Dias, 2018), and little is known about their mechanisms of action against these pathogens. Although many studies have shown effectiveness (Camurça-Vasconcelos *et al.*, 2007; Ji *et al.*, 2012; De Aquino Mesquita *et al.*, 2013; De Moraes *et al.*, 2013; Godinho *et al.*, 2014; Qi *et al.*, 2015; Fabbri *et al.*, 2020), some compounds may exhibit an *in vitro* effect but have little or no activity *in vivo*. For example, artemisinin, derived from *Artemisia annua* (sweet wormwood) EO, was tested in rodents to evaluate its action against *H. contortus*, where it showed no beneficial effects (Squires *et al.*, 2011). Even though it does not affect this parasite, the possibility of presenting effects against other species was not excluded, requiring further *in vivo* research.

Encapsulated EO and EOC forms usually stand out over their free forms, due to greater resistance to stomach acids and increased bioaccessibility, which improves their absorption. In addition, capsules can mask adverse sensory effects, improving palatability characteristics (Lu *et al.*, 2019; Amiri *et al.*, 2020).

Most of the studies found in this literature review only present the inhibitory activity of EOs against the parasites tested. However, it is essential to improve the current knowledge of how inhibition works to enhance parasite-control techniques that use these compounds. Some of the works showed morphologic changes, such as damage to the integument (Machado *et al.*, 2011; Ullah *et al.*, 2017; Woolsey *et al.*, 2019; Dominguez-Uscanga *et al.*, 2021), reduction in the numbers of hatching of eggs (Macedo *et al.*, 2010; Katiki *et al.*, 2011; Carvalho *et al.*, 2012; Ribeiro *et al.*, 2013; Zhu *et al.*, 2013a, 2013b; Oliveira *et al.*, 2014; Gaínza *et al.*, 2015; Qi *et al.*, 2015), inhibition or reduction of motility (Singh *et al.*, 2009; Zhu *et al.*, 2013a, 2013b; Ullah *et al.*, 2017), and changes in the genetic material and inhibition of metabolic pathways (Machado *et al.*, 2011; Ullah *et al.*, 2017; Dominguez-Uscanga *et al.*, 2021; Khamesipour *et al.*, 2021), but there is a lack of research that addresses *in vivo* tests and different application techniques for the use of these compounds, which are needed before they become feasible techniques for animal management.

The development of EO resistance is improbable, due to the multifactorial nature of their mechanisms caused by this diversity in substances. This is an advantage in opposition to EOCs which are more susceptible to the development of resistance and have a higher cost of production and purification. On the other hand, EOCs usually require lower concentrations for bacterial inhibition in comparison to EOs, which reduces sensory alterations in animal feed or drinking water and improves palatability (Janz *et al.*, 2007; Franz *et al.*, 2010). When animals are treated with either EOs or EOCs, they must be closely monitored during the initial administration to assess the efficacy of these treatments and to promote a healthy transition from conventional drugs.

### Zotechnical benefits of EOs and EOCs

The beneficial effects of EOs and EOCs, in addition to promoting the biosafety of breeding stock, are also reflected in the zotechnical indexes and may act to replace performance-enhancing additives. Moreover, Hernández-Coronado *et al.* (2019) showed improved sensory evaluation of chicken meat when EO extracted from *Poliomintha longiflora* (rosemary-mint) (400  $\text{mg l}^{-1}$ ) was used as an additive to chicken feed.

The addition of nanoencapsulated cumin EO with chitosan at a concentration of 200  $\text{mg kg}^{-1}$  in broiler feed resulted in better

**Table 2.** Effects of essential oils and isolated compounds on parasites of relevance in animal production

Effects		Parasite	Optimal inhibition concentration	Type of test	References
Compounds isolated from essential oils					
Carvacrol	Inhibition of muscle contractions and synergism with gamma-aminobutyric acid	<i>Ascaris suum</i>	300 $\mu$ M	<i>In vitro</i>	Trailović <i>et al.</i> (2015)
	Anticoccidial	<i>Cryptosporidium</i> spp.	1 mg ml <sup>-1</sup>	<i>In vitro</i>	Tanghort <i>et al.</i> (2019)
Cinnamaldehyde	Increased production of specific antibodies	<i>A. suum</i>	1000 mg kg <sup>-1</sup>	<i>In vivo</i>	Williams <i>et al.</i> (2017)
	Activity against larvae, subcuticular muscle injury, and intestinal injury	<i>A. suum</i>	236 $\mu$ M	<i>In vitro</i>	(Williams <i>et al.</i> , 2015)
Curcumin	Morphological changes in the tegument, reduced motility, inhibition of cathepsin L gene, reduced glutathione levels, inhibition of superoxide dismutase activity, oxidative damage by protein carbonylation, and inhibition of glutathione S-transferase	<i>Fasciola gigantica</i>	60 $\mu$ M	<i>In vitro</i>	Ullah <i>et al.</i> (2017)
Eugenol	Anticoccidial	<i>Cryptosporidium</i> spp.	0.5 mg ml <sup>-1</sup>	<i>In vitro</i>	Tanghort <i>et al.</i> (2019)
Thymol	Membrane damage, inhibition of lipid metabolism, interference with enzymes responsible for energy production, and free radical production	<i>Cryptosporidium parvum</i>	500 $\mu$ g ml <sup>-1</sup>	<i>In vitro</i>	Dominguez-Uscanga <i>et al.</i> (2021)
Thymoquinone	Morphological changes in the tegument, reduced motility, inhibition of cathepsin L gene, reduced glutathione levels, inhibition of superoxide dismutase activity, and oxidative damage by protein carbonylation	<i>F. gigantica</i>	60 $\mu$ M	<i>In vitro</i>	Ullah <i>et al.</i> (2017)
Essential oils					
<i>Allium sativum</i>	Irreversible paralysis	<i>F. gigantica</i>	3 mg ml <sup>-1</sup>	<i>In vitro</i>	Singh <i>et al.</i> (2009)
	Total trophozoite growth inhibition within 24 h	<i>Entamoeba histolytica</i>	0.4 mg ml <sup>-1</sup>	<i>In vitro</i>	Behnia <i>et al.</i> (2008a, 2008b)
	Antiparasitic activity	<i>E. histolytica</i> e <i>Giardia lamblia</i>	0.2 $\mu$ g ml <sup>-1</sup>	<i>In vitro</i>	Azadbakht <i>et al.</i> (2019)
<i>Arisaema</i> spp.	Egg hatching reduction and inhibition of larval development and migration	<i>Haemonchus contortus</i>	10 mg ml <sup>-1</sup>	<i>In vitro</i>	Zhu <i>et al.</i> (2013a, 2013b)
<i>Artemisia absinthium</i>	Reduction in the number of oocysts by 70%	<i>Eimeria</i> sp.	4 mg ml <sup>-1</sup>	<i>In vitro</i>	Remmal <i>et al.</i> (2011)
<i>Artemisia lancea</i>	Egg hatching reduction and inhibition of larval motility and development	<i>H. contortus</i>	10 mg ml <sup>-1</sup>	<i>In vitro</i>	Zhu <i>et al.</i> (2013a, 2013b)
<i>Cichorium intybus</i>	Growth inhibition, no cytotoxic effect	<i>Cryptosporidium parvum</i>	300 $\mu$ g ml <sup>-1</sup>	<i>In vitro</i>	Woolsey <i>et al.</i> (2019)
<i>Citrus sinensis</i>	Egg hatching reduction and possible inhibition of embryogenesis	<i>H. contortus</i>	1.56 mg ml <sup>-1</sup>	<i>In vitro</i>	Gainza <i>et al.</i> (2015)
<i>Citrus</i> spp.	Reduction in fecal egg count by 71% and prevalence reduction by 68%	<i>Ascaridia galli</i>	1200 mg kg <sup>-1</sup>	<i>In vivo</i>	Abdelqader <i>et al.</i> (2012)
<i>Clausena anisata</i>	Inhibition of larval migration	<i>A. suum</i>	1 mg ml <sup>-1</sup>	<i>In vitro</i>	Williams <i>et al.</i> (2016)
<i>Cuminum cyminum</i>	Ovicidal activity	<i>Fasciola hepatica</i>	0.016 mg ml <sup>-1</sup>	<i>In vitro</i>	Silva <i>et al.</i> (2020)

(Continued)

**Table 2.** (Continued.)

	Effects	Parasite	Optimal inhibition concentration	Type of test	References
<i>Curcuma longa</i>	Activity against adult worms	<i>A. galli</i>	100 mg ml <sup>-1</sup>	<i>In vitro</i> ; <i>In vivo</i>	Bazh and El-Bahy (2013)
<i>Cymbopogon schoenanthus</i>	Egg hatching reduction, inhibition of larval development, and inhibition of larvae feeding	<i>H. contortus</i>	0.27 mg ml <sup>-1</sup>	<i>In vitro</i>	Katiki et al. (2011)
<i>Dracocephalum kotschyi</i>	Activity against tachyzoites and reduction in ATP levels	<i>Toxoplasma gondii</i>	24.49 µg ml <sup>-1</sup>	<i>In vitro</i>	Khamesipour et al. (2021)
<i>Eucalyptus globulus</i>	Antiparasitic activity	<i>E. histolytica</i> e <i>G. lamblia</i>	0.2 µg ml <sup>-1</sup>	<i>In vitro</i>	Azadbakht et al. (2019)
<i>Eucalyptus staigeriana</i>	Egg hatching reduction and inhibition of larval development	<i>H. contortus</i>	8 mg ml <sup>-1</sup> ( <i>in vitro</i> ) 500 mg kg <sup>-1</sup> ( <i>in vivo</i> )	<i>In vitro</i> <i>In vivo</i>	Ribeiro et al. (2013)
	Egg hatching reduction and inhibition of larval development	<i>H. contortus</i>	5.4 mg ml <sup>-1</sup> ( <i>in vitro</i> ) 500 mg kg <sup>-1</sup> ( <i>in vivo</i> )	<i>In vitro</i> <i>In vivo</i>	Macedo et al. (2010)
<i>Lippia sidoides</i>	Egg hatching reduction and inhibition of larval development	<i>H. contortus</i>	0.13 mg ml <sup>-1</sup>	<i>In vivo</i> <i>in vitro</i>	Carvalho et al. (2012)
<i>Melaleuca alternifolia</i>	Reduction in the number of oocysts by 70%	<i>Eimeria</i> sp.	4 mg ml <sup>-1</sup>	<i>In vitro</i>	Remmal et al. (2011)
<i>Melaleuca quinquenervia</i>	Inhibition of larval development	<i>H. contortus</i>	1.56 mg ml <sup>-1</sup>	<i>In vitro</i>	Gaíza et al. (2015)
<i>Mentha longifolia</i>	Activity against trophozoites	<i>E. histolytica</i> e <i>Giardia duodenalis</i>	200 µg ml <sup>-1</sup>	<i>In vitro</i>	El-Badry and Al Ali (2010)
<i>Mentha piperita</i>	Egg hatching reduction and inhibition of larval development	<i>H. contortus</i>	0.10 mg ml <sup>-1</sup>	<i>In vitro</i>	Carvalho et al. (2012)
	Egg hatching reduction, inhibition of larval development, and inhibition of larvae feeding	<i>H. contortus</i>	1 mg ml <sup>-1</sup>	<i>In vitro</i>	Katiki et al. (2011)
<i>Ocimum basilicum</i>	Activity against trophozoites	<i>E. histolytica</i> e <i>G. duodenalis</i>	200 µg ml <sup>-1</sup>	<i>In vitro</i>	El-Badry and Al Ali (2010)
<i>Origanum Vulgare</i>	Reduces post-infection oxidative stress and anticoccidial effect	<i>Eimeria</i> spp.	12 mg kg <sup>-1</sup>	<i>In vivo</i>	Bozkurt et al. (2016)
	Anticoccidial	<i>Eimeria</i> sp.	300 mg kg <sup>-1</sup>	<i>In vivo</i>	Alp et al. (2012)
	Cocciostatic effect	<i>Eimeria</i> spp.	0.5 mg ml <sup>-1</sup>	<i>In vivo</i>	Mohiti-Asli and Ghanaatparast-Rashti (2015)
<i>Origanum compactum</i>	Anticoccidial	<i>Cryptosporidium</i> spp.	1 mg ml <sup>-1</sup>	<i>In vitro</i>	Tanghort et al. (2019)
<i>Pimpinella anisum</i>	Activity against trophozoites	<i>E. histolytica</i>	5 mg ml <sup>-1</sup>	<i>In vitro</i>	Quintilde ones Gutieacute rez et al. (2013)
<i>Piper aduncum</i>	Egg hatching reduction	<i>H. contortus</i>	12 mg ml <sup>-1</sup>	<i>In vitro</i>	Oliveira et al. (2014)
<i>Piper longum</i>	Irreversible paralysis	<i>F. gigantica</i>	3 mg ml <sup>-1</sup>	<i>In vitro</i>	Singh et al. (2009)
<i>Syzygium aromaticum</i>	Reduction in the number of oocysts by 70%	<i>Eimeria</i> sp.	4 mg ml <sup>-1</sup>	<i>In vitro</i>	Remmal et al. (2011)
	Growth inhibition, morphological changes, flagellum internalization, and the presence of autophagic vacuoles	<i>G. lamblia</i>	300 µg ml <sup>-1</sup>	<i>In vitro</i>	Machado et al. (2011)
	Anticoccidial	<i>Cryptosporidium</i> spp.	1 mg ml <sup>-1</sup>	<i>In vitro</i>	Tanghort et al. (2019)

<i>Thymus vulgaris</i>	Reduction in the number of oocysts by 70%	<i>Eimeria</i> sp.	4 mg ml <sup>-1</sup>	<i>In vitro</i>	Remmal <i>et al.</i> (2011)
<i>Zanthoxylum simulans</i>	Total trophozoite growth inhibition within 48 h	<i>E. histolytica</i>	0.7 mg ml <sup>-1</sup>	<i>In vitro</i>	Behnia <i>et al.</i> (2008a, 2008b)
<i>Zingiber officinale</i>	Egg hatching reduction and inhibition of larval development	<i>H. contortus</i>	40 mg ml <sup>-1</sup>	<i>In vitro</i>	Qi <i>et al.</i> (2015)
	Activity against adult worms	<i>A. galli</i>	100 mg ml <sup>-1</sup>	<i>In vitro</i> <i>In vivo</i>	Bazh and El-Bahy (2013)

body weight gain (BWG) and feed conversion ratio when compared to flavomycin at 650 mg kg<sup>-1</sup> (Amiri *et al.*, 2020). The use of *Eucalyptus globulus* (southern blue gum) EO, promoted dose-dependent effects; for instance, it was able to promote the growth of beneficial microbiota and reduce the *E. coli* population. Moreover, it caused an increase in organic matter digestibility, which can lead to an increase in the absorption of nutrients, a decreased serum cholesterol, and increased superoxide dismutase activity. All these factors improved BWG and feed conversion, while cholesterol reduction enhanced the nutritional profile of the meat produced, and antioxidant activity was enhanced by superoxide dismutase inhibiting free radicals (Mohebodini *et al.*, 2021).

When fed to Japanese quail, *S. aromaticum* (clove) EO, at a concentration of 1.5 ml kg<sup>-1</sup>, increased growth performance indices due to observed antioxidant effects and increased levels of insulin, somatotropin (growth hormone), and thyroxine (Hussein *et al.*, 2019). In mammals, the effect of an oil blend (300 mg kg<sup>-1</sup> starter) composed of *Rosmarinus officinalis* (rosemary), *Zataria multiflora* (Shirazi thyme) and *Mentha pulegium* (pennyroyal) (1:1:1) in calves showed an 11.5% body weight increase compared to the control group (Jeshari *et al.*, 2016). A thymol and cinnamaldehyde blend (0.05 g kg<sup>-1</sup> feed) was evaluated in piglets, and the experiment revealed bactericidal and immunomodulatory effects, a decrease in mucosal macrophages, and a reduction in intestinal inflammation by the suppression of interleukin expression (Jiang *et al.*, 2015). Another experiment was carried out in calves using a mix (300 mg day<sup>-1</sup>) of *Thymus kotschyanus* (thyme), *Lavandula angustifolia* (lavender), *Salvia officinalis* (common sage), and *Capparis spinosa* (caper bush) EOs, and showed an optimization of the animals' performance (59.1 kg control group final body weight and 62.3 kg EO group final body weight) due to the antioxidant and bactericidal effects (Asghari *et al.*, 2021).

According to the data presented, the use of EOs in the development of biotechnological alternatives to conventional treatments is largely plausible. In addition, EOs can also act as immunomodulators, detoxifiers, performance enhancers, and are highly versatile compounds (Lopes *et al.*, 2020; Evangelista *et al.*, 2021).

### EO and EOC use with conventional antimicrobial and anthelmintic therapy

EOs present remarkable aromas and flavors, which can reduce their feasibility as commercial products. To circumvent this problem, EO associations with traditional medicines or other bioactive compounds can be used to reduce their recommended doses if they have an additive or synergistic effect. This combination can significantly reduce the necessary dosage of EOs, consequently mitigating changes in sensory properties (Sharma *et al.*, 2020). Although research involving the combination of EOs with conventional drugs still has unknown mechanisms of interactions, the results have shown synergisms and reductions in the appearance of antimicrobial/antiparasitic resistance (Lahmar *et al.*, 2017).

*In vitro* studies conducted by Ait Dra *et al.* (2017) showed a synergistic effect between gentamicin or ciprofloxacin associated with *Periploca laevigata* (*cornicabra*) EO against *S. aureus* and *E. coli*. Combination of *Foeniculum vulgare* (fennel) and antibiotics (cefoxitin, mupirocin, cotrimoxazole, or ciprofloxacin) showed synergism against *S. aureus*, with a significant increase

in the inhibition zones, especially with mupirocin combination, with an increase from 30 mm to approximately 42 mm of diameter (Kwiatkowski et al., 2017). Ciprofloxacin combined with *Lavandula maroccana* (lavender) EO resulted in a 4-fold MIC decrease for *E. coli*, 16-fold for *S. aureus*, and 8-fold for *Pseudomonas aeruginosa* (Soulaïmani et al., 2019).

A multidrug-resistant strain of *Acinetobacter baumannii* was exposed to polymyxin B at  $1 \mu\text{g ml}^{-1}$  and was able to grow during the entire evaluation period (25 h) up to a maximum population of  $\sim 10 \log \text{CFU ml}^{-1}$ . When the strain was exposed to the same concentration of Polymyxin B with  $0.5 \mu\text{g ml}^{-1}$  of *Eucalyptus camaldulensis* (river red gum) EO, the population reached levels below the experimental detection limit ( $2 \log \text{CFU ml}^{-1}$ ) in 6 h. In the same period, the treatment with Polymyxin B alone presented a population of over  $7 \log \text{CFU ml}^{-1}$  (Knezevic et al., 2016).

Although EOs present antiparasitic effects, there is little information about their association with classical drugs. The only article found about this particular subject showed an association between thymol and albendazole *in vivo*; nevertheless, this combination did not obtain satisfactory results, even with *in vitro* data attesting to the potential of the combination (Miró et al., 2020).

Most farmers have not adopted the use of natural alternatives in countries where classical drugs are still allowed as growth promoters. The well-known effect caused by antibiotics and antiparasitic drugs in animal production, their relatively low prices and their ease to use are still stronger arguments to the producers than the problems that they may cause to the environment and public health (Ryan, 2019). Therefore, a gradual transition from the current form of treatment to an approach that uses the association of natural compounds may educate producers and build trust that these molecules can improve zootechnical indices, enhance feed palatability, and reduce the need for conventional drugs.

## Conclusion

EOs and EOCs have great potential to be used in animal production, with several benefits over conventional treatments. They have provided reductions of antimicrobial and anthelmintic resistance, a more effective treatment against resistant organisms, and when used in combination with traditional products, several compounds presented synergisms that substantially reduced the dose required to achieve the desired effect.

The application is not limited to microbiological and parasitic control, EOs and EOCs also show good results as performance-enhancers in animal production. There is still a dearth of scientific literature about animal applications complementary to *in vivo* testing, as well as further elucidation about mechanisms of action, recommended doses, synergistic effects, and supplementation vehicles to maximize their activities and thus reach their best potential.

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