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Characteristics of Essential Oils of *Apiaceae* Family: Their Chemical Compositions, *in vitro* Properties and Effects on Broiler Production

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There has been an upsurge of interest in the phytobiotics coincident with the onset of the potential ban on the use of antibiotic growth promoters (AGPs) in the broiler industry and because many kinds of nutraceuticals play an important role in improving growth performance, feed efficiency, and gut health of broilers. In the previous years, significant biological activities of essential oils (EOs) belonging to phytobiotics were observed, including antibacterial, antifungal, antiviral, and antioxidant properties. We found new perspectives on the roles of EOs, particularly extracts from the *Apiaceae* family, which is one of the largest plant families, in potential replacement of AGPs, and on the chemical composition involved in regulating microorganism activity and oxidative damage. Furthermore, the positive effects of EOs on broiler production and the possible mechanisms inducing the involvement of gut health and growth performance have been studied.

Key words: Antibiotic growth promoters, *Apiaceae*, broilers, essential oils, growth performance, gut health

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Introduction

Antibiotic growth promoters (AGPs) have been used in the broiler industry for decades to improve production performance and to minimize morbidity and mortality (Zeng *et al*., 2015; Broom, 2018). However, the use of antibiotics in broiler production has raised problems in the human population due to bacterial resistance to the agents and

transmission via the food chain (Graham *et al*., 2009; Chowdhury *et al*., 2018a). Therefore, the use of AGPs in broilers has been prohibited in several countries. In 2006, the European Union imposed a complete ban on all AGPs. The USA is limiting AGP use and moving towards a significant reduction in general antibiotic usage (Salim *et al*., 2018). Thereafter, many countries have announced AGP restrictions (Goutard *et al*., 2017).

In broiler production, AGP supplementation improves body weight gain (BWG) and feed conversion ratio (FCR), indicating that the withdrawal of AGP may increase production costs (Cardinal *et al*., 2019). This expectation has compelled nutritionists and feed manufacturers to seek the most suitable alternatives to AGPs. Since the early 2000s, researchers have explored the potential of nutraceuticals, such as probiotics, prebiotics, synbiotics, organic acids, and phytobiotics as alternatives to AGPs (Sugiharto, 2016), and the volatile extracts from plant sources have been identified as a new class of phytogenic feed additives (Zeng *et al*., 2015).

The volatile extracts obtained from different plant parts, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots by hydro/steam distillation, are referred to as EOs. EOs have been reported to have antibacterial, antifungal, antiviral, and antioxidant properties as biological actions that depend on their chemical constituents (Al Bayati, 2008). Attention to EOs as a replacement for AGPs in poultry has increased because of their positive effects on production performance (Sugiharto, 2016). However, the mode of action of EOs is yet to be fully elucidated (Zeng *et al*., 2015; Kikusato, 2021).

Apiaceae is one of the largest plant families (Pimenov and Leonov, 1993). Its plants have a characteristic pungent smell, whose extracts are EOs. Several constituents of EOs are believed to be the precursors of biological compounds that exert beneficial effects on gut morphology, nutrient absorption, microbiota, and oxidative status. Therefore, the EOs extracted from the *Apiaceae* family have been considered as a possible replacement for AGPs in broiler production (Acimovic *et al*., 2016).

This review focuses on the characteristics of EOs, particularly the *in vitro* properties of EOs extracted from selected plants of the *Apiaceae* family, such as coriander (*Coriandrum sativum*), ajwain (*Trachyspermum ammi*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), and anise (*Pimpinella anisum*), and their effects on broiler production and possible machineries. Such an endeavor can never be truly comprehensive; however, this review aims to provide an awareness of the current state of the field for readers both inside and outside the phytobiotics community.

1. Chemical compositions and *in vitro* properties of selected essential oils

EOs are synthesized to protect the plant bodies against bacterial and fungal invasions and viruses and protect DNA and photosynthetic apparatus from the oxidative damage caused by ultraviolet radiation (Kikusato, 2021). Therefore, the EOs extracted from the plants of the *Apiaceae* family can perform various biological activities based on their chemical constituents. The relative concentration and overall yield of the constituents differ among plant types, parts, harvesting season, environmental conditions, soil type, storage conditions, and types of processing (Applegate *et al*., 2010; Grashorn, 2010; Kiczorowska *et al*., 2015; Al Yasiry and Kiczorowska, 2016). Most of the published literature describing *in vitro* antibacterial and antifungal properties has focused on the microbial species relevant to food pathogenesis; however, data regarding bacterial species that may influence the intestinal circumstances of broilers are lacking.

In this section, many measurement units are described as used in the literature: minimum inhibitory concentration (MIC) and/or zone of inhibition (ZOI) for antibacterial activity of the EOs. In addition, inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) assay, and Trolox equivalent antioxidant capacity (TEAC) using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), peroxide value (PV), thiobarbituric acid value (TBA), and antioxidant activity in the linoleic acid system are used for antioxidant activity.

1.1. Coriander essential oil (CEO: Table 1)

A. Chemical compositions

Coriander (*Coriandrum sativum*) is a glabrous, aromatic, and herbaceous annual plant with culinary applications and serves as a source of aroma compounds and EO. Coriander seeds contain 0.03 to 2.6% EO, with linalool being the main chemical constituent (Acimovic *et al*., 2016; Jeya *et al*., 2019). Table 1 shows the chemical composition, area of cultivation, extraction method, and yield of CEO from the selected studies. Linalool (Figure 1-1) was the major component of the CEO with a share of 66.3−75.3% of the total composition, whereas α-pinine, γ-terpinene, camphor, geranyl acetate, and cymene were the other major components (Baratta *et al*., 1998; Delaquis *et al*., 2002; Singh *et al*., 2006; Kacaniova *et al*., 2020). Singh *et al*. (2006) reported the presence of more than 52 chemical compounds in CEO.

B. In vitro properties

a) Antibacterial activity: Many studies have shown that the chemical constituents present in CEO have antibacterial properties. Baratta *et al*. (1998) analyzed the CEO (10 μL/disk) against 25 different bacteria, and the reported ZOI for *Bacillus subtilis*, *Clostridium perferengens*, *Escherichia coli*, *Salmonella pullorum*, and *Staphylococcus (Staph.) aureus* were 8.5, 4, 6.5, 7.6, and 16.1 mm, respectively. Kacaniova *et al*. (2020) reported that the ZOI of CEO (10 μL/disk) against *B. subtilis* was 10.7 mm. Delaquis *et al*. (2002) demonstrated that CEO had antibacterial activity against *E. coli*, *Listeria monocytogenes*, and *Staph. aureus* with MIC 0.2, 0.5, and 0.4 mL/dL (% vol/vol), respectively, except for *C. perferengens*. In a recent study, Jeya *et al*. (2019) reported 0.64 mg/mL as the MIC of CEO against *E. coli*.

b) Antifungal activity: The CEO can effectively inhibit the growth of *Aspergillus niger* (inhibition index: 94.8%) at 1 μL/mL concentration (Baratta *et al*., 1998). Singh *et al*.

Table 1. **Chemical Compositions and *in vitro* properties of CEO (Coriander Essential Oil)**

Chemical Composition	Baratta *et al*., 1998	Delaquis *et al*., 2002	Singh *et al*., 2006	Kacaniova *et al*., 2020
linalool	66.3	69.8	75.3	66.1
γ-terpinene	7.1	5.3	0.7	2.0
α-pinene	8.5	5.4	4.1	
geranyl acetate	2.7		8.1	6.9
geraniol	2.0		0.8	2.6
camphor	3.8	5.2	0.1	8.3
limonene	1.9		0.6	3.0
camphene	0.9	1	0.1	
myrcene	0.9	1.5	0.3	0.4
β-pinene	0.6		0.7	
cymene	2.2		0.5	6.4
borneo1	0.6		0.3	
terpinolene	0.4		0.2	
α-terpineol	0.4		0.4	0.9
sabinene	0.3		0.2	
terpinen-4-ol	0.3		0.2	
β-phellandrene	0.2			
trans-geraniol				2.6
1,2-oxolinalool				2.4
β-caryophyllene	0.1		0.4	
2-myristynoyl pantetheine				0.4
citronellol				0.4
terpendiol				0.4
1,8-cineol			0.4	
cis-linalool oxide			0.5	
cuminal			0.6	
α-thujene	0.1			
α-terpinene	0.1			
Cultivation/experimentation area	Italy	Canada	India	Slovakia
Extraction method/ source	Commercial	Hydro distillation	Hydro distillation	Commercial
EO yield (%)	—	0.5	2.2	—

(2006) evaluated the CEO (10 μL) against different fungi and reported good ZOI (more than 70%) against *Curvularia palliscens*, *Fusarium moniliforme*, and *A. terreus*. In addition, Jeya *et al*. (2019) reported that the CEO showed fungicidal effects against *Candida (Can.) albicans* with a MIC of 0.02 mg/mL.

c) Antioxidant activity: The CEO contains natural antioxidants that can prevent or delay the effects of oxidation processes. Baratta *et al*. (1998) analyzed the antioxidant effectiveness of CEO through the modified thiobarbituric acid reactive species (TBARS) assay using two materials rich in lipids as oxidable substrates (egg yolk and rat liver). The results demonstrated that the CEO at 1000ppm in rat liver exhibited a higher antioxidant index than synthetic antioxidants, α-tocopherol, and butylated hydroxytoluene (BHT) at the same supplementation levels. Singh *et al*. (2006) evaluated the antioxidant capacity of CEO by PV, TBA, and antioxidant activity in the linoleic acid system, revealing that 200 ppm CEO supplementation resulted in a 21% reduction in PV during storage at 80℃ for 28 days. Kacaniova *et al*. (2020) analyzed the radical scavenging activity of the CEO using the DPPH test and Trolox (vitamin E analog) as the standard, showing that 25 μL/mL CEO has 51.1% inhibition efficiency for scavenging free radicals. Moreover, Shahwar *et al*. (2012) and Singh *et al*. (2015) performed the radical scavenging activity of CEO at 500 μg/mL and 50 μL/mL

Table 1. **Chemical Compositions and *in vitro* properties of CEO (Coriander Essential Oil) (continued)**

Chemical Composition	Baratta *et al.*, 1998		Delaquis *et al.*, 2002		Singh *et al.*, 2006		Kacaniova *et al.*, 2020	
Antibacterial activity	**Species**	**ZOI (mm) 10 μL/disk**	**Species**	**MIC (mL/dL)**	**NA**		**Species**	**ZOI (mm) 10 μL/disk**
	B. subtilis	8.5					*B. subtilis*	10.7
	C. perfringens	4.0	*L. monocytogenes*	0.5			*S. maltophilia*	9.2
	E. coli	6.5	*E. coli*	0.2				
	S. pullorurn	7.6	*S. typhi*	No inhibition observed				
	Staph. aureus	16.1	*Staph. aureus*	0.4				
Antifungal activity	**Species**	**% Inhibition index 1 μL/mL**	**NA**		**Species**	**% ZOI⁺ 10 μL**	**NA**	
	A. niger	94.8			*A. flavus*	31.3 (75% by FPT[1])		
					A. terreus	75 (100% by FPT)		
					A. niger	37.5 (100% by FPT)		
Antioxidant activity	**Method**	**Effects**	**NA**		**Method**	**Effects**	**Method**	**Effects**
	Antioxidant index (AI%) using TBARS assay	higher than BHT at 1000 ppm			Peroxide value (PV) method	PV 248 meq/Kg of sunflower oil was reduced to 196 meq/Kg during storage at 80℃ for 28days at 200 ppm dose of CEO	DPPH	CEO radical scavenging activity was 39.4 mg TEAC/L (Trolox equivalent antioxidant activity) equivalent to 51.1% of inhibition
					TBA value	TBA value 4 meq/kg of sunflower was reduced to 2.5 meq/Kg during storage at 80℃ for 21days by 200 ppm dose of CEO		

ZOI=zone of inhibition, MIC=minimum inhibitory concentration, FPT=food poison technique, *S. maltophilia*=*stenotrophomonas maltophilia*
[1] Mycelial inhibition zone (%) at dose 10 *μl* by inverted petri plate method

using the DPPH test and reported 66.5% and 54.6% inhibition in DPPH-derived free radicals, respectively.

1.2. Ajwain essential oil (AjEO: Table 2)

A. Chemical compositions

Ajwain (*Trachyspermum ammi*) is an important plant with spice, aromatic, and medicinal properties. It originated in

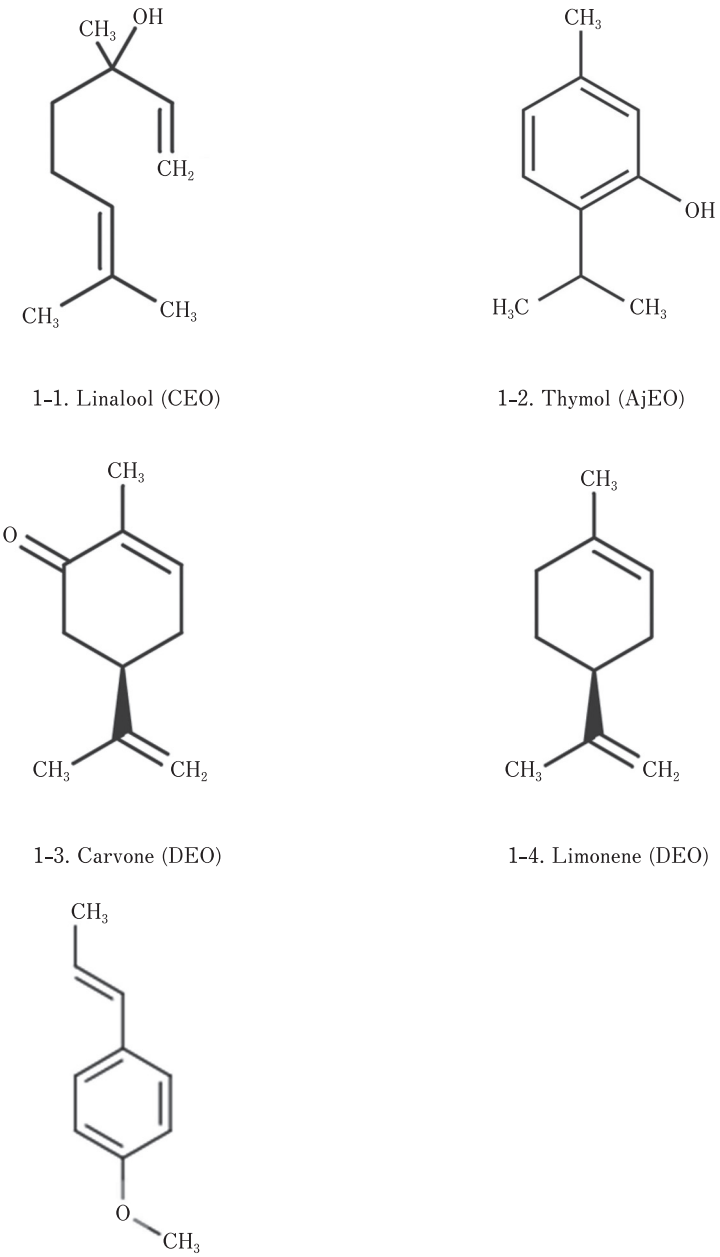

Fig. 1. **Chemical structure of main compounds found in the selected essential oils.**

Egypt and is found worldwide. Ajwain seeds contain 2%–5% EO, with thymol (Fig. 1–2) as a major bioactive compound with a share of 39.1–67.4% of the total composition, followed by *p*-cymene, γ-terpinene, β-pinene, carvacrol, α-phellandrene, β-phellandrene, α-terpinene, α-pinene, and sabinene (Singh *et al*., 2004; Vitali *et al*., 2016; Gradinaru *et al*., 2018). However, Patil *et al*. (2016) reported that *p*-cymene (15.6%) was the major component in AjEO, followed by thymol (15.5%), by analyzing the peak area percentage of GC/MS results.

B. In vitro properties

a) Antibacterial activity: The MIC of AjEO against *Staph. aureus* and *E. coli* were 500 μg/mL (Vitali *et al*., 2016). However, Paul *et al*. (2011) showed stronger antibacterial activity against gram-positive bacteria than against gram-negative bacteria. The MIC of AjEO against *Streptococcus*

(Strep.) mutans, *E. coli*, *S. typhi*, *S. parathyphi*, *P. vulgaris*, and *P. aeruginosa* was 12.5 μL/mL (Patil *et al*., 2016). Considering the composition of AjEO, thymol may be the main component to induce antibacterial activity. In a recent study, Gradinaru *et al*. (2018) revealed that AjEO has the potential to limit the growth of respiratory pathogens (*Staph. aureus, Strep. pneumoniae, P. aeruginosa*) and discovered the combined effects of AjEO/thymol and conventional antibiotics against multidrug-resistant respiratory pathogens.

b) Antifungal activity: Singh *et al*. (2004) showed that the AjEO at 6 μL dose rate is 100% fungicidal for all the tested pathogenic fungal species. In contrast, Vitali *et al*. (2016) reported limited activity of AjEO against *Can. albicans* with a MIC of 500 μg/mL, which is 125 times higher than nystatin (reference anti-fungal drug).

c) Antioxidant activity: According to Singh *et al*. (2004), AjEO has good antioxidant properties, as analyzed by the PV, TBA, and linoleic acid system. Patil *et al*. (2016) demonstrated that AjEO is a strong antioxidant with 71.7% efficacy using the DPPH method, whereas the antioxidant activity of ascorbic acid (standard) was 20.2%. Vitali *et al*. (2016) evaluated the antioxidant properties of AjEO using DPPH, ABTS, and FRAP assays. The ability of AjEO to scavenge the different radicals in all assays was compared with Trolox (vitamin E analog) and expressed as TEAC. The results revealed that the AjEO showed good antioxidant activity as the TEAC of ABTS, FRAP, and DPPH assays were 266.7 μmol TE/g, 90.6 μmol TE/g, and 72.6 μmol TE/g, respectively. The free radical scavenging activities of AjEO in all the studies mentioned above proved its potential as a natural antioxidant substance, which can be used as an efficient antioxidant agent.

1.3. Dill essential oil (DEO: Table 3)

A. Chemical compositions

Dill (*Anethum graveolens*) is one of the most useful spices with medicinal properties. It is cultivated worldwide, and its EO has flavoring and medicinal effects. Dill seeds yield 2%−4.2% EO with carvone (Fig. 1−3) as a major chemical component with a share of 47.7−73.6% in total composition, followed by limonene (Fig. 1−4), dill apiol, and α-phellendrene (Singh *et al*., 2005; Yili *et al*., 2009; Chahal *et al*., 2017; Singh *et al*., 2017). In contrast to previous studies, Kazemi (2015) reported thymol (20.1%) as the major component of DEO, followed by limonene, α-pinene, and carvacrol. He justified that his results are in contrast with those of other studies because of the genetic, environmental, chemotypes, and nutritional status of the plants. Since the chemical composition of DEO varies considerably between different studies, more comprehensive studies on chemical constituents are required.

B. In vitro properties

a) Antibacterial activity: Singh *et al*. (2005) analyzed the antimicrobial activity of DEO against six pathogenic bacteria. They reported it as an effective antibacterial agent against *P. aeruginosa* and *E. coli* with ZOI 25.3 mm and 18.5 mm, respectively, although ineffective against *S. typhi.* DEO also showed effective antibacterial activity against *Staph. aureus* with MIC 0.27 mg/mL (Yili *et al*., 2009). According to Kazemi (2015), DEO performed best against *E. coli* at a MIC of 5 μg/mL. In contrast, the MIC for other tested bacteria (*B. cereus, Enterococcus (En.) facealis, S. aureus, P. aerogenosa, and S. typhi*) ranged between 10−40 μg/mL. In a recent study, DEO showed better inhibitory effects against gram-positive bacteria than gram-negative bacteria at 10 μL dose/disk (Singh *et al*., 2017). ZOI for *B. subtilis, Staph. aureus, E. coli*, and *P. aerogenosa* were 15.6, 20.3, 7.5, and 8.9 mm, respectively.

b) Antifungal activity: DEO has the potential to produce antifungal effects. It has shown 100% fungicidal activity for *Penicillium (Pen.) citrinum* and *A. niger* at 6 μL concentration out of eight tested pathogenic fungi. The activity against other fungi was also considerable (Singh *et al*., 2005). The *Can. albican* was also found to be very sensitive to DEO with a MIC value of 2.7 μg/mL (Yili *et al*., 2009). Kazemi (2015) reported the significant fungicidal effects of DEO against *Can. albicans* and *A. fumigatus* at MIC 10 and 20 μg/mL, respectively. Singh *et al*. (2017) reported the significant antifungal activity of DEO against five tested pathogenic fungi. Among the tested fungi, *A. flavus* was the most sensitive (more than 80% ZOI) to DEO at 10 μL, followed by the other tested fungi. More recently, ten *Candida* species were examined against DEO and found very significant fungicidal effects with a MIC of 8.75 mg/mL for all tested fungi (Vieira *et al*., 2019).

c) Antioxidant activity: Singh *et al*. (2005) evaluated the antioxidant properties of DEO by PV, TBA, and DPPH methods, revealing that 200 ppm DEO supplementation resulted in a 10.6% reduction in PV during storage at 80℃ for 28 days. The TBA value of rapeseed oil was also reduced by approximately 50% during this storage period. Moreover, the radical scavenging activity of DEO by the DPPH method was 81.6% compared to butylated hydroxyanisole (BHA) (88.5%) and BHT (90.3%). Kazemi (2015) reported that the DPPH value of DEO (IC_{50}＝34.4 mg/mL) is comparable to that of Trolox (IC_{50}＝28.3 mg/mL), suggesting the antioxidant properties of this EO.

In a recent study, Singh *et al*. (2017) evaluated the antioxidant activity of DEO by PV, TBA, and DPPH methods. They proved that it is a good natural antioxidant, similar to commercial antioxidant products. Briefly, 200 ppm DEO supplementation in mustard oil resulted in a 45% reduction in PV during storage at 60℃ for 28 days, and the TBA value was reduced by approximately 50% and 25% on the 21st and 28th day of storage, respectively, compared to the control group. Moreover, DEO showed 75% radical scavenging activity, which was higher than that of the other tested commercial antioxidants. The conclusion of the studies mentioned above indicated the presence of carvone, limonene, and dill apiole in DEO, which may be the main reason for the antioxidant properties.

1.4. Fennel essential oil (FEO: Table 4)

A. Chemical compositions

Fennel (*Foeniculum vulgare*) is one of the oldest spice plants with considerable medicinal properties. The fennel

Table 2. **Chemical Compositions and *in vitro* properties of AjEO (Ajwain Essential Oil)**

Chemical Composition	Singh *et al*., 2004	Patil *et al*., 2016	Vitali *et al*., 2016	Gradinaru *et al*., 2018
thymol	39.1	15.5	67.4	50.8
γ-terpinene	23.2	9.3	11.3	26.0
ρ-cymene	30.8	15.6	17.9	18.3
α-phellandrene		8.7		
α-pinene	0.2	4.7	0.1	0.2
carvacol	0.3	10.7	0.9	
sabinene		4.2		
β-phellandrene	0.6	7.6	0.3	
β-pinene	1.7	10.6	0.7	2.3
α-terpinene	0.2	6.7	0.3	0.3
α-thujene	0.2		0.2	0.4
α-pinene	0.2			
myrcene	0.4		0.2	0.5
terpinolene	0.2			
trans-sabinene hydrate	0.1		0.1	
linalool	0.1			
terpenen-4-ol	0.8		0.2	0.1
α-terpineol	0.1		0.1	
β-selinene	0.1			
Cultivation/experimentation area	India	India	Iran	India (Romania)
Extraction method/ source	Hydrodistillation	Hydrodistillation	Hydrodistillation	Hydrodistillation
EO yield (%)	2.2	5.1	2.7	7.4

contains 4%−6% EOs with more than 30 types of chemical constituents (Kooti *et al*., 2015). Trans-anethole (Fig. 1−5) was identified as a major component with a share of 56.4−69.9% in total composition, whereas fenchone, estragole, and limonene were the other main components (Anwar *et al*., 2009; Roby *et al*., 2013; Diao *et al*., 2014; Ilic *et al*., 2019).

B. In vitro properties

a) Antibacterial activity: According to Anwar *et al*. (2009), FEO showed considerable antibacterial activity against *B. subtilis* and *E. coli* with ZOI of 29 mm and 14 mm, respectively. Roby *et al*. (2013) demonstrated the antibacterial effects of FEO against gram-positive bacteria (*B. cereus*, *Staph. aureus*) and gram-negative (*E. coli*, *S. typhi*) bacteria with MICs ranging from 10 to 15 μg/mL. In another study, FEO showed antibacterial activity against *E. coli*, *B. subtilis*, and *S. typhi* at a MIC of 0.25 mg/mL; however, *Staph. aureus* and *P. aurogenosa* did not respond to it even at the highest tested concentration (10 mg/mL) (Diao *et al*., 2014). More recently, Ilic *et al*. (2019) reported that *B. subtilis* (MIC; 25 μg/mL) was the most sensitive bacteria to FEO, followed by *Staph. aureus* (50 μg/mL), *E. coli* (75 μg/mL), and *Klebsiella pneumoniae* (75 μg/mL). The authors concluded that the antibacterial activity of FEO depends on its chemical composition and the synergistic effects of the major chemical constituents.

b) Antifungal activity: Several studies have reported the significant antifungal properties of FEO, as shown by its activity against various fungal species such as *Can. albicans*, *Aspergillus* species, and *dermatophytes* (Kooti *et al*., 2015). Anwar *et al*. (2009) reported FEO as an efficient antifungal against the three tested fungi, particularly *A. niger,* showing the highest sensitivity with 28 mm ZOI and 80.6 mg/mL a MIC value. In another study, *Can. albicans* and *A. flavus* were sensitive to FEO at a MIC of 10 μg/mL (Roby *et al*., 2013). More recently, Ilic *et al*. (2019) reported that the *Can. albicans* was the most sensitive of the seven tested microorganisms in their study, with clear ZOI and 25 μg/mL MIC.

c) Antioxidant activity: Limited data are available regarding the antioxidant properties of FEO; however, in some studies, it has been proven to be a strong antioxidant agent. Anwar *et al*. (2009) evaluated the antioxidant properties of FEO using DPPH assay. They concluded that it has good radical scavenging activity with IC_{50}=32.32 μg/mL. Moreover, FEO can replace commercial synthetic antioxidants such as BHA and BHT, which are discouraged because of their perceived carcinogenic potential and safety concerns (Anwar *et al*., 2009).

Table 2. **Chemical Compositions and *in vitro* properties of AjEO (Ajwain Essential Oil) (continued)**

Chemical Composition	Singh *et al*., 2004		Patil *et al*., 2016			Vitali *et al*., 2016			Gradinaru *et al*., 2018	
Antibacterial activity	**NA**		**Species**	**ZOI (mm) 20 μL/disk**	**MIC (μL/mL)**	**Species**	**ZOI (mm) 10 μL/disk**	**MIC (μg/mL)**	**Species**	**MIC (mg/mL)**
			S. paratyphi A	52	12.5	*Staph. aureus*	34.7	500	*Staph. aureus*	4
			S. typhi	54	12.5	*E.coli*	29.3	500		
			E. coli	66	12.5					
Antifungal activity	**Species**	**ZOI (%) 6 μL/disk**	**NA**			**Species**	**ZOI (mm) 10 μL/disk**	**MIC (μg/mL)**	**NA**	
	A. flavus	100				*Can. albicans*	54.3	500		
	A. niger	100								
Antioxidant activity	**Method**	**Effects**	**Method**	**Effects**		**Method**	**Effects**		**NA**	
	PV method	PV 248 meq/Kg of linseed oil was reduced to 150 meq/Kg at 80℃ during storage of 28days by 200 ppm addition of EO	DPPH	Strongest antioxidant activity (71.68%) noted at 1000 mg/L concentration and was three times greater than the effect produced by standard; ascorbic acid (20.24%)		ABTS	AjEO showed strong antioxidant activity with [1] C_{50}=22.4 μg/mL and TEAC= 266.7 μmol TE/g			
						FRAP	AjEO showed antioxidant activity with TEAC=90.6 μmol TE/g			
	TBA method	TBA value 3.8 and 5 meq/kg of linseed oil was reduced to 3.0 and 3.8 meq/Kg at 80℃ during storage for 21 and 28 days, respectively, by 200 ppm addition of EO				DPPH	AjEO showed weak antioxidant activity with [a] IC_{50}=239.3 μg/mL and [b] TEAC= 72.6 μmol TE/g			

ZOI=zone of inhibition, MIC=minimum inhibitory concentration, TEAC=Trolox equivalent antioxidant concentration
[1] Concentration of compound that affords a 50% reduction in the assay

1.5. Anise essential oil (AnEO: Table 5)

A. Chemical compositions

Anise (*Pimpinella anisum*) is an annual aromatic spice known for its medicinal and aromatic properties and is found worldwide. The fruit or seed of anise yields 2.1%−3.3% EO, and the important chemical components are trans-anethole (Fig. 1−5), methyl chavicol, and anisaldehyde (Arslan *et al*., 2004; Sharifi *et al*., 2008). Trans-anethole was identified as a major component with a share of 79−92.9% of the total composition, whereas estragole, 3, 4-dimethoxystyrene, α-gurjunene, and α-bisabolene were the other main components (Sharifi *et al*., 2008; Topal *et al*., 2008; Foroughi *et al*., 2016; Asadollahpoor *et al*., 2017). In contrast to the studies mentioned above, De Martino *et al*. (2009) reported a slightly different chemical composition of AnEO and stated that the major chemical constituent of this EO is cis-anethole (97.1%).

B. In vitro properties

a) Antibacterial activity: Al Bayati (2008) reported the moderate antibacterial activity of AnEO against nine pathogenic bacteria with MIC ranging from 62.5−500 μg/mL, where gram-positive bacteria were more sensitive than gram-negative bacteria. In another study, a wide range of gram-positive and gram-negative bacterial species were found to be sensitive to AnEO, with MICs ranging from 25−100 mg/mL (Al Maofari, 2013). More recently, Foroughi *et al*. (2016) confirmed the antibacterial effectiveness of AnEO against *E. coli* and *Staph. aureus*. The AnEO (31 mg/mL) performed better than the positive controls (kanamycin and cephalexin) in ZOI for *E. coli* and *Staph. aureus*. Moreover, the MICs for *E. coli* and *Staph. aureus* were 3 mg/mL and 7 mg/mL, respectively.

Table 3. **Chemical Compositions and *in vitro* properties of DEO (Dill Essential Oil)**

Chemical Composition	Singh *et al*., 2005	Yili *et al*., 2009	Kazemi, 2015	Singh *et al*., 2017
carvone	55.2	73.6		47.7
limonene	16.6	14.7	16.3	12.4
thymol			20.1	
carvacrol			8.3	
dill ether	0.2		3.1	
dill apiole	14.4			32.7
α-pinene	0.1		8.7	
linalool	3.7			
trans-dihydrocarvone	2.8			2.7
cis-dihydrocarvone	2.6	5.9		2.1
α-phellandrene		0.03	2.4	1.3
sabinene	0.1		1.0	
β-pinene	0.1			
myrcene	0.1		0.7	
γ-terpenene	0.3			
terpinen-4-ol	0.1			
iso-dihydrocarveol	0.1			
cis-dihydrocraveol		0.2		
trans-dihydrocarveol	0.1			
geranyl acetate	0.3			
β-caryophylene	0.6			
β-bisabolene	0.3			
δ-cadinene	0.1			
trans-isocroweacin	0.8			
1,2-diethoxyethane		1.4		
dihydrocarvone		1.4		
diplaniol		2.2		
α-thujene			0.1	
neophtadiene			1.4	
n-nonadecane			1.0	
n-eicosane			0.9	
n-heneicosane			0.7	
n-docosane			1.0	
n-tricosane			1.0	
n-tetracosane			1.5	
ρ-cymenene				0.2
menthol				0.7
myristicin				0.9
Cultivation/experimentation area	India	Uzbekistan	Iran	India
Extraction method/source	Hydrodistillation	Hydrodistillation	Hydrodistillation	Hydrodistillation
EO yield (%)	**2.6**	**4.2**	**3.2**	**2.4**

Table 3. **Chemical Compositions and *in vitro* properties of DEO (Dill Essential Oil) (continued)**

Chemical Composition	Singh *et al.*, 2005		Yili *et al.*, 2009		Kazemi, 2015		Singh *et al.*, 2017	
Antibacterial activity	**Species**	**ZOI (mm) 6 μL/disk**	**Species**	**MIC (mg/mL)**	**Species**	**MIC (μg/mL)**	**Species**	**ZOI (mm) 10 μL/disk**
	B. subtilis	16.2					*B. subtilis*	15.6
	Staph. aureus	13.2	*Staph. aureus*	0.27	*Staph. aureus*	20	*Staph. aureus*	20.3
	S. typhi	No ZOI			*S. typhi*	40		
	E. coli	18.5			*E. coli*	5	*E. coli*	7.5
	P. aeruginosa	25.3					*P. aeruginosa*	8.9
Antifungal activity	**Species**	**% ZOI 6 μL/disk**	**Species**	**MIC (μg/mL)**	**Species**	**MIC (μg/mL)**	**Species**	**% ZOI (FPT[1]) 10 μL**
	A. niger	100	*Can. albican*	2.7	*A. fumigates*	20	*A.niger*	63.9
	A. flavus	82.5			*Can. albicans*	10	*A. flavus*	89.7
	Pen. citrinum	100					*Pen. viridicatum*	17.6
Antioxidant activity	**Method**	**Effects**	**Method**	**Effects**	**Method**	**Effects**	**Method**	**Effects**
	PV	PV 239.2 meq/Kg of rapeseed oil was reduced to 213.9 meq/Kg during storage at 80℃ for 28days by 200 ppm addition of EO	**NA**		FRAP	DEO= Antioxidant activity301 μmol Fe^{2+}/g EO, Trolox (standard)= 321 μmol Fe^{2+}/g EO	PV	PV 181.8 meq/Kg of mustard oil was reduced to 100 meq/Kg during storage at 60℃ for 28days by 200 ppm addition of EO
	TBA value	TBA value 6.9 meq/kg of rapeseed oil was reduced to 3.4 meq/Kg during storage at 80℃ for 28 days by 200 ppm addition of EO			DPPH	DEO scavenging activity [1] C_{50}= 34.41 mg/mL, Trolox (standard) IC_{50}=28.32 mg/mL	TBA value	TBA value 0.18 and 0.21 meq/kg of mustard oil was reduced to 0.092 and 0.16 meq/Kg during storage at 60℃ for 21 and 28 days, respectively, by 200 ppm addition of EO
	DPPH	DEO showed 81.6% radical scavenging activity in comparison to BHA (88.5%) and BHT (90.3%)					DPPH	DEO showed 75% radical scavenging activity

ZOI=zone of inhibition, MIC=minimum inhibitory concentration, FPT=food poison technique
[1] Concentration of compound that affords a 50% reduction in the assay

Table 4. **Chemical Compositions and *in vitro* properties of FEO (Fennel Essential Oil)**

Chemical Composition	Anwar *et al*., 2009	Roby *et al*., 2013	Diao *et al*., 2014	Ilic *et al*., 2019
trans-anethole	69.9	56.4	68.5	64.9
estragole	5.5	5.2	10.4	2.6
limonene	5.1	4.2	6.2	2.3
fenchone	10.2	8.3	5.5	23.1
δ-3-carene			1.2	
o-cymene			0.6	
α-pinene	0.6	1.6	0.4	2.0
methyl chavicol		5.2		
β-farnesene		3.0		
γ-terpinene	0.2	1.4		0.7
camphene	0.1			0.2
sabinene	0.2			0.1
β-pinene	0.1		0.4	
β-myrcene	0.9	0.6	0.2	1.0
α-phellandrene	0.2		0.1	0.4
β-ocimene	0.6			
1,8-cineol	0.2	0.9		
fenchyl alcohol	0.4			
fenchyl acetate	0.5		0.1	
cis-anethol	0.3		0.5	0.1
ρ-anisaldehyde	0.2		0.3	0.1
β-caryophyllene	0.3			
germacrene	0.1		0.2	
α-terpinin		0.6		
terpin-4-ol		2.8		
myrcenol		1.0		
bergamoil		0.6		
2,5-diethyl phenol		0.8		
β-farnesene		3.0		
α-farnesene		1.3		
camphor			0.2	0.5
Cultivation/experimentation area	Pakistan	Egypt	China	Serbia
Extraction method/source	Hydrodistillation	Hydrodistillation	Hydrodistillation	Hydrodistillation
EO yield (%)	2.8	2.0	1.7	4.0

b) Antifungal activity: The antifungal activity of AnEO has been proven by many researchers. Elgayyar *et al*. (2001) reported the antifungal potential of AnEO against *A. niger* with a significant zone of growth inhibition. Ozcan and Chalchat (2006) proved that AnEO is an effective antifungal agent against *A. parasiticus*, *A. niger*, and *Alternaria alternate* at 10−100 ppm doses. In another study, AnEO was reported as an antifungal agent against *A. niger* with a MIC of 2000 μL/L and EC_{50} of 400 μL/L (half-maximal effective concentration) (Sharifi *et al*., 2008). In a study by Nanasombat and Wimuttigosol (2011), AnEO produced strong antifungal effects with clear ZOI against the six yeast and four mold species, and the MIC ranged from 1 mg/mL to 6 mg/mL for all the tested microbes.

c) Antioxidant activity: Many studies have proved the antioxidant activity of AnEO and its ability to be used as a replacement for commercial antioxidants. According to Singh *et al*. (2008), 200 ppm AnEO supplementation in mustard oil resulted in a 28% reduction in PV during storage for 28 days at 70℃, which obtained a better result than commercial antioxidants. In addition, the DPPH assay proved the stronger antioxidant activity of AnEO than BHA

Table 4. **Chemical Compositions and *in vitro* properties of FEO (Fennel Essential Oil) (continued)**

Chemical Composition	Anwar *et al*., 2009			Roby *et al*., 2013			Diao *et al*., 2014			Ilic *et al*., 2019		
Antibacterial activity	**Species**	**ZOI (mm) 15 μL/ disk**	**MIC (mg/ mL)**	**Species**	**ZOI (mm) 20 μg/ disk**	**MIC (μg/ mL)**	**Species**	**ZOI (mm) 100 μL in DMSO**	**MIC (mg/ mL)**	**Species**	**ZOI (mm) 60 μL/ disk**	**MIC (μg/ mL)**
	B. subtilis	29	62.6				*B. subtilis*	15.8	0.25	*B. subtilis*	32	25
	E. coli	14	259.3	*E. coli*	17	12.5	*E. coli*	19.1	0.25	*Staph. aureus*	23	50
				S. tyhpi	18	15	*S. typhi*	20.2	0.25	*E. coli*	20	75
				Staph. aureus	19	10	*Staph. aureus*	11.5	>10	*k. pneumoniae*	21	75
							P. aeruginosa	12.3	>10			
Antifungal activity	**Species**	**ZOI (mm) 15 μL/ disk**	**MIC (mg/ mL)**	**Species**	**ZOI (mm) 20 μg/ disk**	**MIC (μg/ mL)**	**NA**			**Species**	**ZOI (mm) dose rate: 60 μL/ disk**	**MIC (μg/ mL)**
	A. niger	28	80.6	*Can. albicans*	22	10				*Can. albicans*	100%	25
				A. flavus	20	10						
Antioxidant activity	**Method**	**Effects**		**NA**			**NA**			**NA**		
	DPPH	FEO scavenging activity [1] IC_{50}= 32.32 μg/mL, BHT (standard) IC_{50}=19.00 μg/mL										

ZOI=zone of inhibition, MIC=minimum inhibitory concentration
[1] Concentration of compound that affords a 50% reduction in the assay

and BHT. In a study by Topal *et al*. (2008), AnEO showed 77.5% free radical scavenging activity using DPPH assay, which was slightly lower than that of BHT (91%). In contrast, De Martino *et al*. (2009) noted the least free radical scavenging activity of this EO (DPPH inhibition=19%) and speculated the reason is the low percentage of monoterpenes (1.2%) in its chemical constituents. They discussed that antioxidant activity is directly related to the monoterpene content of EOs. Nanasombat and Wimuttigosol (2011) also reported the antioxidant activity of AnEO measured by DPPH assay with IC_{50}=86.88 mg/mL.

Thus, the chemical composition and *in vitro* properties of EOs are very unstable and depend on the genetic factors, environmental conditions, geographical location, harvest time, plant part used, and method of extraction. The EOs may consist of 20−60 chemical compounds with two or three major components present at high concentrations (70%−80%). The potential antibacterial, antifungal, and antioxidant activities of EOs rely entirely on their major bioactive chemical compounds, functional groups, and synergistic interactions between components (Chouhan *et al*., 2017). Due to the variable nature of the chemical composition of EOs, it is difficult to determine the exact mechanism of action and dose rates for a specific activity (Kikusato, 2021).

2. Effects of selected essential oils on broiler performance, carcass characteristics and serum traits

Although several *in vitro* studies have shown the antimicrobial and antioxidant activities of EOs, the *in vivo* knowledge on the whole body of broiler health and growth performance is relatively less based on their chemical compositions and *in vitro* properties; however, possible mechanisms underlying the positive effects of EOs on biological actions can be generally hypothesized, including membrane disruption of pathogens, immunity-boosting activities, improvement of beneficial gut microbiota, appetite stimulation, and enhancement in the secretion of endogenous

Table 5. **Chemical Compositions and *in vitro* properties of AnEO (Anise Essential Oil)**

Chemical Composition	Sharifi *et al*., 2008	Topal *et al*., 2008	De Martino *et al*., 2009	Foroughi *et al*., 2016
trans-anethole	92.9	79.0		89.7
cis-anethole	0.1		97.1	0.4
estragole	2.2	3.6		
α-pinene			0.3	0.1
anisaldehyde	0.1	0.7		0.4
α-himachalene		0.5		
carvone	0.8			
α-bisabolene	1.8			
zingiberene	0.4			
methyl-chavicol				2.2
3,4-dimethoxystyrene		5.2		
α-gurjunene		4.0		
limonene	0.8			0.8
fenchone			0.2	4.6
linalool		0.3	0.4	
ρ-allyanisole	2.2			
cis-dihydrocarvone	0.1			
δ-element	0.1			
aromadendrene	0.1			
ar-curcumene	0.2			
β-bisabolene	0.2			
β-sesaquiphellandrene	0.1			
α-terpinene		0.2		
ylangene		0.2		
elemene		0.2		
β-caryophyllene		0.2		
α-cis-himachalene		0.5		
α-ethyl-*ρ*-anisyl alcohol		0.3		
1-methylguanine		0.1		
spathulenol		0.2		
3-hydroxycarbofuran		0.8		
ethyl oleate		0.9		
methyl 1-phenylallyl ether		1.7		
α-phellandrene			0.1	0.01
Δ3-carene			0.1	
o-cymene			0.1	
ρ-cymene				0.1
1,8-cineole				0.1
camphor				0.2
β-monopalmitate		0.2		
di-*α*-furylmethane		0.2		
Cultivation/experimentation area	Iran	Turkey	Italy	Iran
Extraction method/source	Hydrodistillation	Hydrodistillation	Commercial	Hydrodistillation
EO yield (%)	**3.3**	**Not given**	—	**Not given**

Table 5. **Chemical Compositions and *in vitro* properties of AnEO (Anise Essential Oil) (continued)**

Chemical Composition	Sharifi *et al*., 2008		Topal *et al*., 2008		De Martino *et al*., 2009		Foroughi *et al*., 2016		
Antibacterial activity	**NA**		**NA**		**Species**	**ZOI (mm) 490 μg/disk**	**Species**	**ZOI (mm) 31 mg/mL**	**MIC (mg/mL)**
					B. cereus	6			
					E. coli	0	*E. coli*	22	3
					Staph. aureus	0	*Staph. aureus*	22	7
Antifungal activity	**Species**	**MIC (μL/L)**	**NA**		**NA**		**NA**		
	A. niger	2000 ([1] EC_{50} =400 μL/L)							
Antioxidant activity	**NA**		**Method**	**Effects**	**Method**	**Effects**	**NA**		
			DPPH	AnEO showed 77.5% free radical scavenging activity, BHT (Standard) showed 91.4%	DPPH	AnEO showed 19% free radical scavenging activity			

ZOI=zone of inhibition; MIC=minimum inhibitory concentration
[1] Half maximal effective concentration

digestive enzymes (Williams, 2001; Cross *et al*., 2007; Hong *et al*., 2012; Sugiharto, 2016). Thus, some of this information is valuable to the application of EOs in the development of feed additives. It should also be noted that excess supplementation could decrease growth performance, possibly due to the potent nature of EOs, which negatively affects the digestive system by reducing FI and disturbing gut microflora at higher dose rates (Falaki *et al*., 2016).

2.1. Broiler performance (Table 6)

For CEO supplementation, Ghazanfari *et al*. (2015) reported a significant decrease in feed intake (FI), increase in body weight gain (BWG), and better feed conversion ratio (FCR) at 0.01, 0.02, and 0.03% of CEO in broiler diets compared to the negative control (NC: no supplementation of any EO or AGP). The highest output was noted with 0.03% supplementation, where a 9% increase in BWG and an 8% decrease in FCR were observed at the end of the experiment.

Falaki *et al*. (2016) showed that the BWG of broilers increased by supplementing the AjEO up to 0.025% in the diet and started to decrease at 0.035% supplementation, although the FI was unchanged. One possible reason why growth performance was reduced by the overdose may be involved in thujone in AjEO, considering that this chemical component in sage oil is responsible for renal and liver dysfunction (Traesel *et al*., 2011). In contrast, Chowdhury *et al*. (2018a) reported neither positive nor negative effects of 0.04% AjEO supplementation on growth performance compared to NC, although this supplementation level (0.04 %) was even higher than the level at which Falaki *et al*. (2016) have negative effects on performance. The AjEO used in their study was not extracted by themselves; however, it was procured from a commercial company and was not chemically analyzed to check the purity and chemical composition. This suggests that the purity and chemical composition of EOs should be clarified to determine their effects on growth performance and other parameters in broilers.

Supplementation with FEO improved the BWG by up to 9% and reduced the FCR by up to 6% with 0.025% supplementation in broiler diets (Gharehsheikhlou *et al*., 2018). In contrast, Stef *et al*. (2018) reported the non-significant positive effects of FEO on the growth performance supplemented with 0.0125% and 0.025% in broiler diets. In both studies, the authors did not mention the purity and chemical composition of the FEO examined. The discrepancies in the results might be due to differences in the chemical composition and purity of the EOs. More detailed studies are required to clarify the reasons for these differences.

Several studies on AnEO supplementation have been conducted. In the 2000s, 0.04% AnEO-supplemented feed exhibited significantly improved body weight gain (Ciftci *et al*., 2005; Simsek *et al*., 2007). These observations were confirmed by Bhandari and Yadav (2013) and Eltazi (2014) using 0.04% AnEO. However, the effects of AnEO on feed intake are controversial: Bhadrai and Yadav (2013), Eltazi (2014), and Stef *et al*. (2018) showed no changes, increases, or decreases in feed intake containing AnEO at 0.02−0.025% of diet, respectively.

Thus, many researchers have reported the positive effects of selected EOs obtained from the *Apiaceae* family on the

Table 6. **Effects of selected essential oils on broiler performance**

EO	Actual data				Percent increase (+) or decrease (-) VS NC			Age	References
	Dose rate (%)*	FI (g)	BWG (g)	FCR	FI	BWG	FCR		
CEO	0 (control)	4082^{ab}	2122^{b}	1.92^{a}				0–42	Ghazanfari *et al*., 2015
	PC[1]	4125^{a}	2311^{a}	1.78^{b}	1.05	8.91	−7.29		
	0.01	3973^{c}	2169^{b}	1.83^{b}	−2.67	2.21	−4.69		
	0.02	4013^{bc}	2219^{ab}	1.8^{b}	−1.69	4.57	−6.25		
	0.03	4082^{ab}	2309^{a}	1.76^{b}	0	8.81	−8.33		
AjEO	0 (control)	4317	2277^{b}	1.89				0–42	Falaki *et al*., 2016
	PC[2]	4243	2305^{ab}	1.84	−1.71	1.23	−2.65		
	0.015	4170	2329^{a}	1.79	−3.41	2.28	−5.29		
	0.025	4312	2288^{ab}	1.88	−0.12	0.48	−0.53		
	0.035	4323	2268^{b}	1.9	0.14	−0.4	0.53		
AjEO	0 (control)	3678	2188^{b}	1.72^{a}				0–39	Chowdhury *et al*., 2018a
	PC[3]	3721	2304^{a}	1.65^{b}	1.17	5.3	−4.07		
	0.04	3650	2164^{b}	1.73^{a}	−0.76	−1.10	0.58		
FEO	0 (control)	4773	2418	1.61				0–42	Gharehsheikhlou *et al*., 2018
	0.015	4809	2484	1.58	0.75	2.73	−1.86		
	0.025	4896	2633	1.51	2.58	8.89	−6.21		
FEO	0 (control)	4633	2606	1.78				0–42	Stef *et al*., 2018
	0.0125	4437	2537	1.75	−4.23	−2.65	−1.69		
	0.025	4517	2578	1.75	−2.50	−1.07	−1.69		
AnEO	0 (control)	3450	2146^{c}	1.61^{a}				0–35	Ciftci *et al*., 2005
	PC[4]	3457	2304^{b}	1.50^{b}	0.20	7.36	−6.83		
	0.01	3433	2190^{c}	1.57^{a}	−0.49	2.07	−2.48		
	0.02	3449	2186^{c}	1.58^{a}	−0.03	1.91	−1.86		
	0.04	3470	2462^{a}	1.41^{c}	0.58	14.76	−12.42		
AnEO	0 (control)	NA	2256^{c}	NA				0–40	Simsek *et al*., 2007
	PC[4]		2414^{b}			7.00			
	0.01		2300^{c}			1.95	NA		
	0.02		2296^{c}			1.77			
	0.04		2572^{a}			14.04			
AnEO	0 (control)	4633^{a}	2606	1.78^{a}				0–42	Stef *et al*., 2018
	0.0125	4326^{b}	2690	1.61^{b}	−6.63	3.22	−9.55		
	0.025	4302^{b}	2672	1.61^{b}	−7.14	2.53	−9.55		
AnEO	0 (control)	3479^{d}	1697^{d}	2.05^{a}				0–42	Eltazi, 2014
	PC[5]	3719^{b}	1937^{b}	1.92^{b}	6.90	14.14	−6.34		
	0.015	3491^{c}	1828^{c}	1.91^{b}	0.34	7.72	−6.83		
	0.025	3545^{c}	1846^{c}	1.92^{b}	1.90	8.78	−6.34		
	0.04	3852^{a}	2105^{a}	1.83^{c}	10.72	23.98	−10.73		
AnEO	0 (control)	3394	1796^{d}	1.97				0–35	Bhandari and Yadav, 2013
	PC[6]	3437	1835^{bc}	1.95	1.27	2.17	−1.02		
	0.01	3408	1809^{cd}	1.96	0.41	0.72	−0.51		
	0.02	3412	1825^{bcd}	1.95	0.53	1.61	−1.02		
	0.04	3471	1883^{a}	1.92	2.27	4.84	−2.54		
	0.06	3450	1847^{b}	1.94	1.65	2.84	−1.52		

PC＝positive control

* All values are in percentage except PC.

[1] Flavophospholipol, 600 mg/kg; [2] Virginiamycin, 200 mg/kg; [3] Bacitracin methylene disalicylate, 500 mg/kg; [4] Avilamycin, 1000 mg/kg; [5] Neomycin sulfate, 1000 mg/kg; [6] Cholotetracycline, 5 mg/kg

[a–d] Mean values sharing a common superscript letter are not statistically different at $P < 0.05$.

growth performance of broilers. Therefore, detailed data from animal experiments should be carefully understood considering the dose rate and purity of each EO examined and their active compounds, including phenolics, terpenoids, glycosides, and alkaloids present as secondary plant metabolites.

2.2. Carcass characteristics (Table 7)

Although the CEO having linalool as a major chemical component has been proved a potent antimicrobial and growth promoter in broilers (Çabuk *et al*., 2003; Ghazanfari *et al*., 2015), there is a lack of published data regarding its effects on carcass characteristics of broilers according to the authors' knowledge. No positive effects of AjEO and FEO supplementation have been observed on the carcass characteristics of broilers, as well (Falaki *et al*., 2016; Chowdhury *et al*., 2018a).

Simsek *et al*. (2007) reported improved hot and cold carcass yields by supplementing broiler diets with 0.04% AnEO. This observation was confirmed by the results of Eltazi (2014) using the same level of supplementation. Moreover, the relative percentages of breast, thigh, and drumstick and the weight of the liver and gizzard were also improved by supplementing broiler diets with 0.04% AnEO (Simsek *et al*., 2007; Eltazi, 2014). The highest FI was noted with 0.04% AnEO supplementation in the study by Eltazi (2014), which may be a possible reason for the improved liver and gizzard weight. The positive effects on carcass characteristics may be related to the effects of anethol, a major bioactive compound in AnEO, on the digestive system and liver metabolism of broilers.

2.3. Serum traits (Table 8)

Supplementation of CEO at 0.01% to 0.03% in broiler diets did not lead to significant changes in serum traits in broilers, including total cholesterol, triglycerides, glucose, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) (Ghazanfari *et al*., 2015). Chowdhury *et al*. (2018b) reported a reduced blood total cholesterol level of up to 19% in comparison to NC by the diet supplemented with 0.04% AjEO. The concentrations of triglycerides, glucose, and total proteins, however, remained unaffected in their study. The decrease in total cholesterol levels may be due to thymol, a major component of AjEO, which can act as an inhibitor of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis (Lee *et al*., 2003).

3. Effects of selected essential oils on intestinal microbiota and gut morphology of broilers

The status of intestinal microbiota and gut morphology are important factors for evaluating gut health, including different aspects of the gastrointestinal tract (GIT), such as effective digestion of feed, absence of GIT ailment, normal and stable intestinal microbiota, and effective immune status

Table 7. **Effects of selected essential oils on carcass characteristics**

EO	dietary dose %*	Hot dressing	Breast	Thigh	Wing	Gizzard	Liver	Heart	Abdominal fat	Spleen	Bursa	Age	Reference
		% of slaughter body weight											
AjEO	0 (control)	65.5	22.2	8.73	5.30	2.27	1.71	0.48	1.71	0.11^{ab}	0.11	0–39	Chowdhury *et al*., 2018ab
	PC[1]	66.0	21.0	8.99	5.61	2.24	1.78	0.48	2.11	0.13^{a}	0.08		
	0.04	66.6	22.4	9.65	5.79	2.22	1.77	0.48	2.15	0.10^{b}	0.07		
AnEO	0 (control)	67.5^{c}	24.6^{c}	15.0^{c}								0–42	Eltazi, 2014
	PC[4]	68.8^{b}	25.8^{b}	15.8^{b}									
	0.015	68.7^{b}	25.0^{b}	15.8^{b}				**NA**					
	0.025	68.8^{b}	25.5^{b}	15.9^{b}									
	0.040	69.1^{a}	26.5^{a}	16.8^{a}									
	dietary dose %*	% of live body weight											
AjEO	0 (control)	63.8	19.9	17.4		1.95	2.61	0.65	1.78	0.11	0.21	0–42	Falaki *et al*., 2016
	PC[2]	65.3	21.8	18.1		1.86	2.57	0.59	1.68	0.13	0.24		
	0.015	66.0	21.2	18.2	**NA**	1.86	2.61	0.58	1.61	0.15	0.20		
	0.025	64.6	21.4	17.6		1.88	2.47	0.6	1.45	0.11	0.18		
	0.035	64.0	20.0	17.8		1.89	2.44	0.69	1.52	0.1	0.22		
AnEO	0 (control)	73.7^{ab}	28.5	22.2	10.8^{ab}	2.06^{b}	2.4^{ab}	0.51	2.34	0.13		0–40	Simsek *et al*., 2007
	PC[3]	72.9^{b}	29.0	21.31	10.7^{ab}	2.12^{bc}	2.27^{b}	0.51	2.45	0.14			
	0.01	74.5^{ab}	28.8	21.36	11.3^{a}	2.48^{ac}	2.43^{ab}	0.49	2.44	0.14	**NA**		
	0.02	73.1^{ab}	28.7	21.11	10.6^{ab}	2.36^{abc}	2.42^{ab}	0.47	2.62	0.13			
	0.04	74.6^{a}	29.5	21.46	9.8^{b}	2.53^{a}	2.67^{a}	0.41	2.75	0.12			

PC＝positive control

* All values are in percentage except PC.

[1] Bacitracin methylene disalicylate, 500 mg/kg; [2] Neomycin sulfate, 1000 mg/kg; [3] Virginiamycin, 200 mg/kg; [4] Avilamycin, 1000 mg/kg

[a–c] Mean values sharing a common superscript letter are not statistically different at $P<0.05$.

(Bischoff, 2011). Intestinal microbiota play a crucial role in maintaining the health of broilers by altering several physiological functions, including digestion, metabolism, and immune responses (Carrasco *et al*., 2019). Broilers are vulnerable to potentially harmful bacteria such as *E. coli*, *Salmonella* species, and *C. perfringens*, which compete with the host in GIT for nutrients, ultimately leading to poor growth performance and greater risk of disease incidence (Gunal *et al*., 2006). The EO supplements can probably control intestinal microbiota, as these phytochemicals perform beneficial functions in the intestine, similar to prebiotics, even remaining less absorbed in the small intestine (Martel *et al*., 2020). It should be noted that the absorption of phytobiotics, including EOs, is very low in the small intestine, as only 2%–15% of the compounds can be absorbed. This fact has been supported by recent studies revealing that phytochemicals may not need to be absorbed in the body to perform beneficial functions (Kikusato, 2021). Iqbal *et al*. (2020) claimed that the intestinal microbiota would convert the phytochemicals into simpler metabolites to some extent to make them absorbable compounds, which may increase their bioavailability and improve the health-promoting effects in the intestine and inside the body. Furthermore, along with microbial community structure, EO supplementation could also be related to the microbial metabolites that improve the nutritional status of birds as well as GIT function and health (Ghazanfari *et al*., 2015). Thus, the way of phytochemicals where to work on the host or microbiota, and what substrates are decomposed from it to absorb in the intestinal tract might be an important factor when the mechanism underlying their effect on either the host or microbiota is discussed.

3.1. Intestinal microbiota (Table 9)

Decreased numbers of pathogenic bacteria and an increased number of beneficial bacteria in the gut may improve the ability of epithelial cells to regenerate villi and thus enhance intestinal absorptive capacity (Mourao *et al*., 2006). Considering the properties of phytobiotics, it is reasonable to expect such an effect by EOs due to their well-documented inhibitory effects against pathogenic microbiota. However, to the best of our knowledge, studies regarding the effects of selected EOs on the gut microbiota are limited.

CEO supplementation with 0.03% in broiler diets reduced the concentration of *E. coli* (log cfu/g) in caecum content by

Table 8. **Effects of selected essential oils on serum traits**

EO	Dietary dose %*	Cholesterol (mg/d*l*)	Triglyceride (mg/d*l*)	glucose (mg/d*l*)	HDL (mg/d*l*)	LDL (mg/d*l*)	VLDL (mg/d*l*)	Total Protein (mg/d*l*)	Age	Reference
CEO	0 (control)	129	138	280	55	47	27	**NA**	0–42	Ghazanfari *et al*., 2015
	PC[2]	114	82	240	52	46	16			
	0.01	111	112	237	49	41	22			
	0.02	130	119	229	53	54	23			
	0.03	121	114	235	54	44	23			
AjEO	0 (control)	184[a]	91	216	**NA**			2780	0–39	Chowdhury *et al*., 2018b
	PC[1]	194[a]	90	220				2660		
	0.04	148[b]	100	238				2810		

PC=positive control
* All values are in percentage except PC.
[1] Flavophospholipol, 600 mg/kg; [2] Bacitracin methylene disalicylate, 500 mg/kg

Table 9. **Effects of selected essential oils on intestinal microbiota**

EO	Intestine part	Dietary dose %*	Lactobacillus (log cfu/g)	E. coli (log cfu/g)	Clostridium (log cfu/g)	Age	Reference
CEO	Caecum content	0 (control)	4.46	4.44[a]	**NA**	0–42	Ghazanfari *et al*., 2015
		PC[2]	4.47	4.23[b]			
		0.01	4.47	4.36[ab]			
		0.02	4.46	4.29[b]			
		0.03	4.51	4.25[b]			
AjEO	Pre-caecal digesta	0 (control)	7.77	7.91[a]	7.27[a]	0–39	Chowdhury *et al*., 2018b
		PC[1]	4.40	7.29[b]	6.63[b]		
		0.04	7.74	7.97[a]	7.26[a]		

PC=positive control
* All values are in percentage except PC.
[1] Flavophospholipol, 600 mg/kg; [2] Bacitracin methylene disalicylate, 500 mg/kg
[a–b] Mean values sharing a common superscript letter are not statistically different at $P<0.05$.

4% in comparison to NC; however, the concentration of *Lactobacillus* (log cfu/g) remained unchanged (Ghazanfari *et al*., 2015). Previously, it was observed that linalool, a major bioactive compound of CEO, inhibits the pathogenic microorganisms in the digestive system, which is possibly related to the reduction in the concentration of *E. coli* in the gut (Çabuk *et al*., 2003; Lee *et al*., 2004). Chowdhury *et al*. (2018b) reported no significant reduction in the concentration of *E. coli*, *Clostridium,* and *Lactobacillus* bacteria in precaecal digesta by supplementing broiler diets with 0.04% AjEO. They suggested that the low dose rate (0.04%) might be the reason for the unaffected concentration of *E. coli* and *Clostridium* bacteria; otherwise, thymol, a major bioactive compound of AjEO, is a potent antibacterial agent for these bacteria.

3.2. Gut morphology (Table 10)

The intestinal mucosal status and its microscopic structure may be a good indicator of the response of the GIT to active substances present in feed and the intestinal content (Viveros *et al*., 2011). This mucosa is one of the main barriers in the intestine that prevents the invasion of pathogens and toxins in the GIT; therefore, these barriers can be destroyed by environmental, dietary, and oxidative stress, which results in systemic and intestinal inflammation (Kikusato, 2021). According to Huang and Lee (2018), phytobiotics, including EOs, have the potential to modulate inflammation-inducing factors in the intestine and can alleviate the inflammation cascade (For detail: Kikusato, 2021) and support gut health. Regarding changes in mucosal microscopic structure with EOs, the increased VH was reported to be related to enhanced digestive and absorptive functions of the intestine due to larger absorptive surface area and higher expression of brush border enzymes and nutrient transport systems (Pluske *et al*., 1996).

Supplementation of CEO in broiler diets significantly affected VH, CD, and the VH/CD ratio in the duodenum, jejunum, and ileum parts of the intestine (Ghazanfari *et al*., 2015). VH and CD increased significantly, whereas the VH/CD ratio decreased with CEO supplementation compared to NC. Çabuk *et al*. (2003) demonstrated that linalool, a major component of CEO, can enhance VH in the intestine of broilers, and the activity of digestive enzymes, possibly improving digestibility and absorption of nutrients. Moreover, amylase concentration in the broiler intestine increases

Table 10. **Effects of selected essential oils on gut morphology**

EO	Intestinal site	Dietary dose %*	VH		CD		VH/CD		Age	Reference
			(μm)	% change VS NC	(μm)	% change VS NC	Ratio	% change VS NC		
CEO	Doudenum	0 (control)	1759^{c}		147.8^{c}		11.91^{a}		0−42	Ghazanfari *et al*., 2015
		PC[2]	1912^{a}	8.7	157.6^{ab}	6.6	12.15^{a}	2.0		
		0.01	1798^{bc}	2.2	150.8^{bc}	2.0	11.94^{a}	0.3		
		0.02	1810^{b}	2.9	157.2^{ab}	6.4	11.53^{ab}	−3.2		
		0.03	1805^{b}	2.6	161.6^{a}	9.3	11.18^{b}	−6.1		
	Jejunum	0 (control)	849^{d}		107.6^{d}		7.9^{a}			
		PC[2]	877^{a}	3.3	133.4^{a}	24.0	6.58^{c}	−16.7		
		0.01	858^{cd}	1.1	109.4^{cd}	1.7	7.85^{ab}	−0.6		
		0.02	866^{bc}	2.0	114.2^{bc}	6.1	7.59^{ab}	−3.9		
		0.03	872^{ab}	2.7	116.0^{b}	7.8	7.53^{b}	−4.7		
	Ileum	0 (control)	757^{c}		97^{d}		7.88^{a}			
		PC[2]	829^{a}	9.5	129.8^{a}	33.8	6.4^{c}	−18.8		
		0.01	770^{bc}	1.7	106.4^{c}	9.7	7.26^{ab}	−7.9		
		0.02	799^{ab}	5.5	116.2^{b}	19.8	6.89^{bc}	−12.6		
		0.03	783^{bc}	3.4	118.4^{b}	22.0	6.61^{bc}	−16.1		
AjEO	Doudenum	0 (control)	1307		70.6		19.5^{b}		0−39	Chowdhury *et al*., 2018b
		PC[1]	1426	9.1	64.8	−8.2	22.9^{a}	17.4		
		0.04	1230	−5.9	64.1	−9.2	19.4^{b}	−0.5		
	Jejunum	0 (control)	1070^{b}		63.7		17.0^{b}			
		PC[1]	1261^{a}	17.9	67.5	6.0	18.7^{a}	10		
		0.04	1036^{b}	−3.2	69.0	8.3	15.9^{b}	−6.5		
	Ileum	0 (control)	865^{b}		62.1^{b}		14.3^{b}			
		PC[1]	1012^{a}	17.0	68.4^{a}	10.1	14.5^{b}	1.4		
		0.04	959^{a}	10.9	54.8^{b}	−11.8	17.7^{a}	23.8		

PC＝positive control

* All values are in percentage except PC.

[1] Flavophospholipol, (600 mg/kg); [2] Bacitracin methylene disalicylate, 500 mg/kg

[a−d] Mean values sharing a common superscript letter are not statistically different at $P<0.05$.

after dietary supplementation with CEO, which induces the villi to grow longer. According to Chowdhury *et al*. (2018b), AjEO supplementation at 0.04% of the diet in broilers increased the VH and VH/CD ratio in the ileum by up to 27% and 24%, respectively. However, the morphology of the duodenum and jejunum remained unaffected.

How do EOs, such as CEO or AjEO, work on the mucosal structure? Windisch *et al*. (2008) suggested that EOs increase VH due to their antioxidant properties. EOs can exhibit antioxidant effects through several mechanisms. These compounds contribute to the elimination of the reactive oxygen species (ROS) produced due to oxidative stress, not only by direct antioxidant action, but also by inducing the expression of antioxidant enzymes, such as catalase and superoxide dismutase (Windisch *et al*., 2008; Kikusato, 2021). These antioxidant enzymes neutralize the ROS released during digestive processes, which can cause damage to the intestinal mucosa and ultimately shorten the villi. The EOs may protect the villi from oxidative damage by stimulating the activity of the antioxidant enzymes, and the phenolic group of the EOs may act as hydrogen donors showing antioxidant activity (Windisch *et al*., 2008). The involvement of antioxidants was confirmed by Valenzuela-Grijalva *et al*. (2017), who speculated that the supplemented EOs can enhance the production performance not only by better FI, possibly due to improved flavor and palatability of diet, better intestinal functions, and activation of the endocrine system, but also by anti-oxidative defense mechanisms.

Based on the discussed literature, it is clear that all the EOs are not equally effective in the antimicrobial, antioxidant, and growth-promoting effects inside the body of broilers. The benefits of EOs in terms of growth performance may depend on their biological activities. Moreover, it is difficult to determine the precise and invariant effects of each EO, as they constitute variable percentages of mixtures in EOs for each plant. In addition to effectiveness, EOs are safe to be used as growth promoters for broilers and for the user (feed manufacturers/farm managers) and the consumers of the meat products compared to AGPs. In any case, as long as antimicrobial resistance will never emerge in response to their usage, EOs can be supplemented to the broiler diet throughout the rearing period without following the withdrawal period to guarantee food safety.

Conclusions

The potential ban on the use of AGPs in the broiler industry has highlighted the development of alternatives to supplement in broiler diets to support gut health and growth performance. We have endeavored to demonstrate several key themes.

1. The published data suggest that the chemical composition and yield of EOs from selected members of the *Apiaceae* family are quite variable depending on the geographical origin, environmental conditions, sowing/harvesting time of the plants, and the extraction method.
2. The *in vitro* antibacterial, antifungal, and antioxidant properties vary between the same EO of different origins. The relative percentage of bioactive compounds in EOs determines the extent and type of biological activity.
3. The results of the literature regarding supplementation of selected EOs in broiler diets are arbitrary and suggest ambiguous results regarding growth performance and feed efficiency.
4. The EOs extracted from the plant parts of the *Apiaceae* family have the potential to be utilized as a replacement for AGPs in broiler production.
5. Although these EOs have proven beneficial effects in broilers, the literature is so limited that further investigations regarding dose rate, combination of different EOs, and possible mechanisms of action are required.

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Conflicts of Interest

The authors declare no conflict of interest.

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