

PHYTOCHEMICAL COMPOSITION OF *CORIANDRUM SATIVUM* L. (CORIANDER) SEEDS AND ANTIBACTERIAL EFFECTS ON LAYING HENS

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ABSTRACT

This study was performed to evaluate the antimicrobial activity of *Coriandrum sativum* L. (coriander) seed (CS) extracts and essential oil (EO) *in vitro* and *in vivo* assays. The major fatty acid was petroselinic acid (64%) and the main component of EO was linalool (82.2%). The petroleum ether extract (PE) had a higher antibacterial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 700603 and *Salmonella typhi* NCTC 8394 compared to the methanol extract (ME). However, the ME extract was more effective against *Enterococcus faecium* NJ-1 ATCC than the PE extract. The PE extract increased the susceptibility of *E. coli* compared to other standard strains. The CS extracts and EO exhibited antimicrobial activity against standard strains of *S. aureus*, *E. faecium*, *E. coli*, *K. pneumoniae* and *S. typhi*. *In vivo* assays evaluated the effects of CS supplementation to diets on the antimicrobial activity in the intestines of laying hens. A total of 90 laying hens (Hyline-5 White, 58 weeks old) were used and fed with diets supplemented with 0, 1, 2.5, 5 and 10% of seeds for 10 wk. The diet with Coriander seed decreased the ileal count of *E. coli*, *S. aureus* and *E. faecium* in hens significantly ($p < 0.01$). In conclusion, CS was effective on standard bacteria (*in vitro*) and gastrointestinal microbiota (*in vivo*).

Key words: *Coriandrum sativum*, Laying hens, Fatty acid, Essential oil, Antibacterial activity, Apiaceae.

INTRODUCTION

Coriandrum sativum L. (coriander) belongs to the well-known Apiaceae family, which is common in the eastern Mediterranean and southern Europe. *C. sativum* has been used traditionally as anti-inflammatory (Varier, 1994), antispasmodic (Bruneton, 1995), analgesic (Chaudry and Tariq, 2006), antiseptic (Duke *et al.*, 2002), carminative (Wichtl and Bisset, 1994), and anti-diabetic (Al-Rowais, 2002). The coriander seed (CS) in the form of whole, ground or extract is consumed commonly as a flavouring agent in the food industry (Baytop, 1999). Burdock and Carabin (2009) reported that coriander oil had no adverse effects or toxicity and that it may be used as food and is considered safe for human consumption. Coriander oil was confirmed as a novel food ingredient by the European Council under Regulation (EC) No 258/97 of the European Parliament and of the Council. In the literature, linalool is the major component in EO (Silva *et al.*, 2011a; Beyzi and Gurbuz, 2014) and petroselinic acid is the most abundant fatty acid in CS (Msaada *et al.*, 2009; Kiralan *et al.*, 2009). Typical seed oil is composed of γ -terpinene, camphor, limonene, geraniol and myrcene (Matasyoh *et al.*, 2009). These components were evaluated for different uses (antibacterial, antifungal and antioxidant activities) and most of the studies showed positive effects (Baratta *et al.*, 1998; Alves-Silva *et al.*, 2013).

Recently, plant essential oils (EOs) form a significant potential source of bioactive constituents in the field of health (Burt, 2004; Sahib *et al.*, 2013). Some herbal extracts can prevent pathogenic microbial growth in animal intestines (Bozkurt *et al.*, 2013; Bozkurt *et al.*, 2014) and reduce antibiotic use in poultry production. Antibiotic usage as growth promotor was banned in the poultry industry in EU countries in 2002 (Konca *et al.*, 2009). In broiler production, some medicines are in use regularly to protect against coccidiosis. The studies showed that animals could be protected using some plant EO without drug supplementation (Bozkurt *et al.*, 2014). Biological facts may provide valuable information compared to *in vitro* studies, because there are many interactions in the animal and human body with regard to biological activity. Therefore, this study was performed to evaluate the effectiveness of CS extracts and EO on antimicrobial and antifungal activity under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

Plant material: Coriander seeds (Arslan cultivar) were supplied from plants grown in the Faculty of Agriculture at Erciyes University in 2014. The seeds were kept at normal room temperature for two months until the initiation of the experiments. The dry matter (DM), crude protein (CP), ether extract (EE) and crude ash (CA)

content of seeds were determined according to procedures of AOAC (1980).

Test microorganisms: Standard strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* NJ-1 ATCC, *Streptococcus pyogenes* ATCC 19615, *Salmonella typhi* NCTC 8394, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, and *Klebsiella pneumoniae* ATCC 700603 were used to evaluate antibacterial effects of CS extracts and EO under *in vitro* conditions using MICs and disc diffusion method. *Candida albicans* ATCC 10231 and *C. glabrata* ATCC 90030 were used for antifungal activity assays. Reference strains obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) were used.

Animals: In animal experiments, a total of 90 laying hens (Hyline-5 White, 58 weeks old) were used. Animals were randomly distributed into 5 treatment groups with 6 replicates of 3 laying hens in each cage (18 laying hens per group) and fed with diets supplemented with 0, 1, 2.5, 5 or 10% coriander seeds for 10 weeks. The animal care protocol was confirmed by Erciyes University number 12/115.

Extraction of seeds: Seeds were extracted by using methanol (ME) and petroleum ether (PE). The coriander seeds were ground, 10 g seed was weighed and extracted with 100 ml methanol for 8 h at room temperature (24 °C). This process was repeated three times. For PE extract, a 10 g ground sample was weighed. Then 100 ml of PE was added and placed into an ultrasonic bath filled with ice water and treated for 15 min. This process was repeated three times. Afterwards, the extracts were filtered, and evaporated for drying. The samples were stored at -20 °C for *in vitro* assays.

In addition, seeds were distilled in water using a Clevenger-type apparatus to produce an EO.

Determination of fatty acid (FA) composition: The standard method of the Association of Official Analytical Chemists (AOAC, 1980) was used to extract lipids from the seeds. Fatty acid composition of the CS samples was determined using a standard analysis procedure as described Konca *et al.* (2014).

Determination of Essential oil (EO) Components: The essential oil analyses were completed as described by Beyzi and Gurbuz (2014).

Antibacterial Activity Assays: EO and extracts of *C. sativum* were dissolved in dimethylsulfoxide (DMSO) and 312.5, 625, 1250, 2500, and 5000 µg/ml concentrations were prepared. Each sample was absorbed on sterile blank discs (Bioanalyse, Turkey) at 20 µg/ml and carefully dried. Sterile discs that contained DMSO were dried and used as negative controls. The appropriate

antibiotic discs were used as positive control for each bacterium. Disc diffusion method and MICs were performed according to Clinical Laboratory and Standards Institute (CLSI, 2012) guidelines.

Antifungal Activity Assays: Antifungal activities were evaluated by using broth microdilution and disc diffusion methods according to CLSI (2008). *L*-glutamine, sodium bicarbonate-free RPMI 1640 broth were added to 0.2% glucose at pH 0.165 molar morpholinepropanesulfonic acid (MOPS) and buffered to 7.0. Inoculum is prepared in 0.85% NaCl as 0.5 McFarland. The same assay was used for the disc diffusion method by adding 1.5% agar (Yucesoy *et al.*, 2001). Amphotericin B was used as a positive and DMSO was used as a negative control. Experiments were completed in duplicate and repeated three times. Results were evaluated visually.

Animal Experiments: A total of 90 laying hens were allocated into 5 treatment groups with 6 replicates. The laying hens were fed with diets supplemented with 0, 1, 2.5, 5 or 10% coriander seeds for 10 weeks. The coriander seeds were ground and homogenously mixed into the diets of laying hens. The feed and water were provided *ad-libitum*.

At the end of 10 weeks, six hens in each group were slaughtered. Immediately a section of ileum was removed and placed in thioglycollate broth (BD, USA) medium. The microbial counts were determined as colony-forming units per gram of sample as described by Konca *et al.* (2009).

Statistical analyses: The data were analysed using one-way ANOVA under the General Linear Models in SPSS computer software (SPSS, 1999). The means were separated using Duncan's multiple range tests. Statistical significance was accepted as $p < 0.05$.

RESULTS

The yields of ME and PE extracts were 3.0% and 3.3%, respectively. The cultivar of coriander was Arslan which was adapted as a Turkish breed. The fatty acid composition of CS used in the experiment is shown in Table 1.

Table 1. Fatty acid composition of coriander seeds (%).

Issue	%
C14:0 (Myristic acid)	0.11
C18:1n-12 (Petroselinic acid)	64.10
C18:2 (Linoleic acid)	19.01
C18:3 (Linolenic acid)	2.12
C18:1n-9 (Oleic acid)	9.22
C18:0 (Stearic acid)	1.20
C16:0 (Palmitic acid)	4.24

In current experiments, the EO composition of CS is given in Table 2. The EO yield (ml/100 g) of CS was 0.31%. Linalool had the highest proportion (82.2%) in EO. Second and third essential oil components were camphor and γ -terpinene, respectively.

Results of MICs and disc diffusion methods are given in Table 3-4. *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *E. faecium* NJ-1 ATCC, *S. typhi* NCTC 8394, and *K. pneumoniae* ATCC 700603 were significantly affected by CS and CS EO at varying concentrations. Furthermore, there were no inhibition zones against *S. pyogenes* ATCC 19615, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *C. glabrata* ATCC 90030 at specified concentration.

Table 2. Coriander seed EO proportions and components.

Components	Retention time (RT)	%
α -pinene	9.64	1.99
β -myrcene	12.15	0.33
p-cymene	13.62	0.72
limonene	13.81	0.63
γ -terpinene	15.23	2.6
linalool	17.42	82.2
camphor	19.23	3.21
α -terpineol	21.42	0.32
geraniol	24.38	2.20
geranyl acetate	30.05	2.18
Total		96.38
Rate of essential oil (% ml/100 g)		0.31

Table 3. Inhibition zones for different concentrations of coriander seed extracts and essential oil *in vitro* (mm).

Extract	Concentrations (μ g/ml)					SEM	P
	312.5	625	1250	2500	5000		
<i>E. coli</i> ATCC 25922							
ME	10.00	12.00	12.67 ^c	15.33 ^b	17.67 ^a	0.37	*
PE	12.33	16.67	17.67	20.33	20.67	0.42	*
EO	NI	10.33	14.67	18.67	22.33	0.63	*
<i>S. aureus</i> ATCC 25923							
ME	NI	10.33	12.00	13.00	14.67	0.24	**
PE	NI	13.00	14.00	15.33	18.00	0.17	**
EO	NI	NI	9.33	14.33	17.66	0.43	*
<i>E. faecium</i> NJ-1 ATCC							
ME	NI	11.67	13.00	14.33	16.33	0.29	*
PE	NI	9.667	11.00	12.00	14.33	0.24	*
EO	NI	NI	11.33	14.67	19.33	0.33	*
<i>K. pneumoniae</i> ATCC 700603							
ME	NI	NI	NI	13.67	16.67	2.01	**
PE	NI	NI	15.33	20.33	23.33	2.67	**
EO	11.33	15.00	19.33	23.33	27.67	0.42	*
<i>S. typhi</i> NCTC 8394							
ME	NI	NI	NI	13.00	16.33	1.95	**
PE	NI	NI	12.33	15.00	19.33	2.13	**
EO	NI	NI	11.33	17.33	22.67	0.42	*

ME: Methanol extract, PE: Petroleum ether extract, SEM: Pooled standard error of means, p: Probability, NI: no inhibition, No inhibition zones against *S. pyogenes* ATCC 19615, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *C. glabrata* ATCC 90030 at specified concentration.

^{a, b, c}: Values with different superscript in a row differ significantly; SEM: Pooled standard error of means, P: probability, *:0.05, **: p<0.01.

Table 4. MIC values of coriander extract and essential oil against standard strains.

Standard strains	Methanol extract (μ g/ml)	Petroleum ether extract (μ g/ml)	Essential oil (μ g/ml)
<i>E. coli</i> ATCC 25922	2500	1250	1250
<i>S. aureus</i> ATCC 25923	>5000	2500	2500
<i>E. faecium</i> NJ-1	2500	5000	2500
<i>K. pneumoniae</i> ATCC 700603	2500	1250	625
<i>S. typhi</i> NCTC 8394	2500	2500	1250
<i>P. aeruginosa</i> ATCC 27853	>5000	>5000	>5000

<i>S. pyogenes</i> ATCC 19615	>5000	>5000	>5000
<i>B. cereus</i> ATCC 11778	>5000	>5000	>5000
<i>B. subtilis</i> ATCC 6633	>5000	>5000	>5000
<i>C. albicans</i> ATCC 10231	>5000	>5000	>5000
<i>C. glabrata</i> ATCC 90030	>5000	>5000	>5000

The *E. coli*, *S. aureus* and *E. faecium* counts were significantly decreased in the ileum by CS supplementation to diets. The composition of the diets is illustrated in Table 5. In the *in vivo* experiment using laying hens, the effects of CS in diets on the *E. coli*, *S. aureus* and *E. faecium* in ileum of laying hens are shown in Table 6.

Table 5. Composition of experimental diets.

Item	kg/ ton
Corn	258.0
Wheat	233.15
Sunflower meal	150.0
Soybean meal	63.0
Corn meal	60.0
Corn bran	55.0
Meat-bone meal	35.0
Animal fat	35.0
Corn gluten	25.0
Limestone	77.0
Sodium chloride	2.50
Vitamin-mineral premix	2.0
Lysine	1.75
Enzyme mixture	0.50
Methionine	0.90
Phytase	0.70
Sodium bicarbonate	0.50
	1000
Analysed composition	
Dry matter, %	89.41
Crude protein, %	17.50
Crude ash, %	13.10
Crude cellulose, %	6.00
Calculated composition	
Available phosphorus, %	0.50
Methionine, %	0.40
Lysine, %	0.80
Calcium, %	3.55
Metabolizable energy, kcal/kg	2820.0

¹Vitamin-mineral premix assured in one of the diet, Vitamin A, 12000 IU; Vitamin D3, 2000 IU; Vitamin E, 20.0 mg; Vitamin K, 3.0 mg; Riboflavin, 3.0 mg; Niacin, 20.0 mg; Vitamin B6, 5.0 mg; Thiamin, 3.0 mg; Vitamin C, 50 mg; Vitamin B12, 0.15 mg; Biotin, 0.05 mg; Folic acid, 0.75 mg; Choline Chloride, 150 mg; Calcium D-pantothenate, 6 mg; Selenium, 0.15 mg; Copper, 5.0 mg; Zinc, 60.0 mg; Iron, 60.0 mg; Manganese, 80.0 mg; Iodine, 1.0 mg; Cobalt, 0.2 mg. ²Nutrient composition (dry matter, crude protein, fat and ash determined according to AOAC (1980) and calculated composition based on NRC (1994) data.

Table 6. The effects of coriander seed on *E. coli*, *S. aureus* and *E. faecium* in ileum of laying hens.

Coriander amount, %	<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecium</i>
0	26666.7 ^a	2450.0 ^a	37766.7 ^a
1	22550.0 ^b	2133.3 ^b	26083.3 ^b
2.5	18616.7 ^c	1733.3 ^c	17433.3 ^c
5	14750.0 ^d	1300.0 ^d	11966.7 ^d
10	4633.3 ^e	650.0 ^e	7550.0 ^e
SEM	402.5	55.18	1306.0
P	**	**	**

^{a, b, c, d, e}: Values with different superscript in a column differ significantly; SEM: Pooled standard error of means, p: Probability, **: p<0.01.

DISCUSSION

In the present study, petroselinic fatty acid (C18:1n-12) was the most abundant fatty acid (64.10%) in the seed oil. Msaada *et al.* (2009), Kiralan *et al.* (2009), Alves-Silva *et al.* (2013) and Sriti *et al.* (2010) reported that petroselinic acid was abundant in CS oil with content of 50 to 70%.

In EO studies of coriander, Beyzi and Gurbuz (2014) reported that linalool was the major component (78.94 to 85.66%) and essential oil components were γ -terpinene and camphor in CS, respectively. Grosso *et al.* (2008) found that major components of EO were linalool 65-79%, γ -terpinene 4-7% and camphor 3%. It was reported that coriander oil contains about 50 to 70% linalool and this content may change depending on the cultivar and harvest year (Grosso *et al.*, 2008; Misharina, 2001). These results comply with this study.

The inhibition zones for *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *E. faecium* NJ-1 ATCC were significantly increased depending on concentrations of ME, PE extracts (p<0.01) and EO (p<0.05). However, no inhibition zones were detected for *E. coli* with EO of 312.5 μ g/ml; *S. aureus* and *E. faecium* with PE and ME extract of 312.5 μ g/ml and with EO at 625 and 312.5 μ g/ml concentrations. It was determined that *K. pneumoniae* and *S. typhi* were affected by the ME extract of CS at 5000 and 2500 μ g/ml, and PE extract at 5000, 2500 and 1250 μ g/ml concentrations. However, at lower concentrations (625 and 312.5 μ g/ml) of ME and PE extracts, inhibition zones were not detected. The inhibition zones of EO were increased at each concentration for *K. pneumoniae* strain (p<0.05).

However, with EO at 5000, 2500 and 1250 µg/ml concentrations, inhibition was detected for *S. typhi*, but there was none at the 625 and 312.5 µg/ml concentrations. According to plant derivative substances and based on their antibacterial activity, MIC values can be classified as strong and weak activity. Aligiannis *et al.* (2001) described that MIC values of plant extracts above 1.50 mg/mL can be considered poor activity; MIC values between 0.6-1.500 mg/mL as moderate activity and MIC values between 0.05-0.6 mg/mL as strong activity against microorganisms. According to these results, CS extracts showed moderate or weak antibacterial activity in this experiment. Other researchers reported that the CS extracts (Duarte *et al.*, 2011; Silva *et al.*, 2011a; Zardini *et al.*, 2012) and EO had weak activity against *Bacillus subtilis*, *K. pneumoniae*, *P. aeruginosa* and *E. coli*; and moderate activity for *S. aureus* (Baratta *et al.*, 1998). Also, Khan *et al.* (2013) reported that coriander EO were effective against *B. cereus*. Singh *et al.* (2002) noted that EO had moderate activity against *S. aureus* and *E. coli*, for *B. subtilis*, *P. aeruginosa* and weak activity against *K. pneumoniae*. In another experiment, it was ineffective against *Enterococcus spp.* and had moderate activity for *C. albicans* and weak activity for *S. aureus* and *E. coli* (Bogavac *et al.*, 2015). Moreover, Silva *et al.* (2011a) reported that lower concentrations of EO were more effective on gram negative bacteria using the MIC method. Matasyoh *et al.* (2009) and Singh *et al.* (2002) showed that EO affected clinical susceptibility of gram negative and positive microorganisms. Burdock and Carabin (2009) demonstrated that EO had broad-spectrum antimicrobial activity. Sahib *et al.* (2013) and Duarte *et al.* (2016) showed the effectiveness of EO against both gram positive and negative microorganisms.

In the *in vivo* experiment in laying hens, there was a linear decrease in *E. coli*, *S. aureus* and *E. faecium* counts with the increase in concentration of CS in the diet ($p < 0.01$). The proportion of decrease was 15.4, 30.20, 44.69, 82.63% for *E. coli*; 12.93, 29.25, 46.94, 73.47% for *S. aureus*; and 30.94, 53.84, 68.31, 80.01% for *E. faecium* at 1, 2.5, 5 and 10% coriander levels in diet, respectively. Daily feed consumption of layer hens was calculated as 100 g and therefore each concentration of CS with diet was 0, 1, 2.5, 5 and 10 g. Yields of CS was 3 to 3.3% for ME and PE extracts, respectively. Therefore, CS consumption in diets was estimated as 0, 0.03, 0.08, 0.15 and 0.3 g, respectively. Coriander seeds were given directly (in mash form) with feed to the animals, but the calculation was performed on the percentage yield and the results were consistent with each other.

Herbal extracts are promising to prevent growth of pathogen microorganisms in the intestines of poultry (Bozkurt *et al.*, 2013; Bozkurt *et al.*, 2014) and therefore increase performance traits in broilers and layers (Alçiçek *et al.*, 2004). There are a number of results about CS

usage in poultry diets and their effect on intestinal microbiota. The current experimental results are in agreement with the results of Hosseinzadeh *et al.* (2014) who reported that CS extract in water (750, 1000, 1250 ppm) and seed powder (1.5, and 2%) in broiler diets caused a decrease in gastrointestinal *E. coli* counts compared to a control group at 21 and 42 days. There are some reports claiming that EO of plants decreases pathogenic bacteria (such as *E. coli*) and increases beneficial bacteria (such as *Lactobacillus*) (Guo *et al.*, 2004). When the plant seed or leaf EOs were used in diets, they increased lactobacilli counts in the intestine and decreased pH; and so decreased pathogenic bacteria and contributed to a balanced gut microflora (Hosseinzadeh *et al.*, 2014; Vidanarachchi *et al.*, 2005). Ghazanfari *et al.* (2015) reported that 200 and 300 mg/kg coriander EO supplementation to diet caused a decrease in *E. coli* counts in the cecum of broilers. In contrast to these results, Esteghamat (2014) noted that inclusion of 1, 2.3 and 4% coriander into diets did not change the *E. coli* bacteria count in Japanese quail. Also, studies have emphasized antimicrobial activity and gut microbiota modulation based on essential oils of coriander (Skandamis *et al.*, 2001; Carson *et al.*, 2002). The bacterial cell wall is disturbed by the hydrophobicity of these essential oils and partitions lipids in the structures, thus leading to the death of pathogenic bacteria (Brul and Coote, 1999). Therefore, CS used in poultry diets due to the effectiveness of coriander EO components such as linalool, camphor and geraniol can control the growth of pathogenic gram positive and gram negative microorganisms (Sahib *et al.*, 2013; Duarte *et al.*, 2016).

In conclusion, the results revealed that coriander extracts and EO have potential antimicrobial activity *in vitro* and *in vivo* conditions. So, they may be used in the poultry industry as natural source of protection against gram negative and positive bacteria that cause some diseases in the gastrointestinal system.

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