Implication of chemical compositions and in vitro properties of coriander, ajwain and dill seed essential oils as potential replacement of antibiotic growth promoters in broilers

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SUMMARY

The aim of this study was to evaluate chemical constituents and antimicrobial as well as antioxidant properties of three essential oils (EOs) of Apiaceae family, coriander (*Coriandrum sativum*), ajwain (*Trachyspermum ammi*) and dill seed (*Anethum graveolens*) to assess their in vitro potential to be used as an alternative to antibiotic growth promoters (AGPs) in broiler production. The EOs of coriander, ajwain and dill seed were extracted by hydro-distillation technique and analyzed for their chemical constituents by gas chromatography-mass spectrophotometry (GC-MS) analysis. The GC-MS analysis indicated that the major bioactive compounds in coriander essential oil (CEO), ajwain essential oil (AjEO) and dill seed essential oil (DEO) are linalool (56.8%), thymol (68.2%) and carvone (41.1%), respectively. The antibacterial capacity of these EOs determined against *E. coli* and two *Salmonella* species of poultry origin. In agar well diffusion assay for *E. coli*, AjEO was 2 and 3 folds more potent as compared to CEO and DEO, respectively. For *S. enteritidis,* DEO showed 2 folds more activity than AjEO, whereas for *S. gallinarum* AjEO performed 3 times better than CEO. In agreement with the results of the agar well diffusion assay, minimum inhibitory concentrations (MIC) of AjEO were lowest for *E. coli* and *S. gallinarum* as compared to other EOs, while for *S. enteritidis,* MIC of DEO was found lowest. The antioxidant activities, analyzed by per oxide value (PV), thiobarbituric acid (TBA) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) method showed the AjEO had highest antioxidant potential in comparison to other EOs. During storage period of 28 days, AjEO reduced the PV and TBA values of rapeseed oil by 44.3 and 49.1%, respectively. Overall, the findings of in vitro analysis demonstrated that AjEO in comparison to CEO and DEO, has considerable antibacterial and antioxidant activities and could be a potential replacement of AGPs in broiler production with less amount of supplementation.

KEY WORDS

Essential oil, in vitro, antibacterial, antioxidant, broiler.

1. INTRODUCTION

World population is growing exponentially and predicted to cross nine billion by 2050. In response, agri-food markets have also been expanding rapidly in the last two decades. To meet the worldwide population demand in the next thirty years, a 102% increase in the food supply will be required[1]. Poultry is one of the major contributor to fulfil the protein requirements of the world population. The nutritional and genetic improvements in chickens are the main strategies to increase productivity to accomplish the white meat demands worldwide. However, high growth rates of birds and stocking density negatively affect the health status, which is partially covered by the sub-therapeutic usage of antibiotics, known as antibiotic growth promoters (AGPs)[2].

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The AGPs are potentially used in broiler industry to improve the gut health, production performance and to reduce the morbidity and mortality[3]. These AGPs support the intestinal health by reducing the antimicrobial load and ultimately maximum utilization of the nutrients which results in better growth performance. However, an extensive use of AGPs is leading to development of antibiotic resistance crisis and foodborne-disease outbreaks in broilers as well as human populations. Many of AGPs used in broiler production belong to the same class of antibiotics being practiced treating the human diseases. These facts have high-lighten the serious public health concerns worldwide and question the use of AGPs in broiler production. Consequently, the AGPs use in broiler production has been prohibited in several countries including European Union[4]. Keeping in mind the importance, there is a dire need to find the best suitable alternatives of AGPs for broiler production.

Alternatives to reduce and/or eliminate the AGPs in broiler production include not only probiotics, prebiotics, organic acids, bacteriophages, but also the phytobiotics including essential oils (EOs)[5]. The EOs received much attention due to being natu-

ral, easily available, non-toxic, cost effective and residue-free. A variety of EOs have the potential to replace AGPs in broiler production based on their bioactive constituents and growth promoting activities[3]. Antimicrobial activity is one of the most eminent activities of EOs, while they also have antioxidant, anti-inflammatory, anti-stress, and growth promoting effects. The EOs can also alleviate the oxidative stress by several mechanisms, such as direct antioxidant action and expression of antioxidant enzymes[6]. Therefore, EOs with the antimicrobial and antioxidant properties could support gut health and growth performance of the broilers as a possible replacement of AGPs.

Apiaceae is one of the largest plant families. Several constituents of the EOs extracted from plants of this family are reported to have potential to exert beneficial effects on gut morphology, nutrient absorption, microbiota, and oxidative status[3]. Therefore, the EOs extracted from the Apiaceae family have been considered as a possible replacement for AGPs in broiler production. In this study coriander (*Coriandrum sativum*), ajwain (*Trachyspermum ammi*) and dill seed (*Anethum graveolens*) were employed as representatives from the Apiaceae family because of their well-known antimicrobial and antioxidant potential and common availability mostly in South Asian countries. Most of the published literature covers the in vitro antimicrobial properties of these EOs against the bacterial species relevant to food pathogenesis[1]. There is scarcity of published data for bacteria of poultry origin such as *Escherichia coli, Salmonella enteritidis*, *Salmonella gallinarum* and *Clostridium perfrengens*. Furthermore, as different in vitro test methods as well as different pathogens and culture media conditions exist, a side-by-side comparison of their chemical compositions and in vitro antibacterial and antioxidant properties could not be made between those EOs. Therefore, studies investigating for ranking the capacity of these three EOs i.e., coriander essential oil (CEO), ajwain essential oil (AjEO) and dill seed essential oil (DEO) are of particular importance. The aim of this study was to analyze the chemical constituents and compared antimicrobial as well as antioxidant properties between three EOs, to assess their in vitro potential to be used as an alternative to AGPs in broiler production.

2. MATERIALS AND METHODS

2.1. Plant material and essential oil extraction

The samples (coriander, ajwain and dill seeds) collected from local market of Lahore, Pakistan in summer, 2021, botanically identified and stored after coarse grinding until hydro-distillation process. The EOs from ground material were extracted by hydro-distillation technique using clevenger apparatus. Briefly, 100 g material was subjected to hydro-distillation using 500 mL of distilled water in 1 L flask for 4 hrs until complete recovery of the EO. The yielded EOs were stored in a sealed amber colored vial at 4°C for the further laboratory analysis[7].

2.2. Gas chromatography-mass spectrometry analysis

Chemical constituents present in the extracted EOs were analyzed by GC-MS (QP-2020, Shimadzu Co., Kyoto, Japan). The individual compounds were separated by Shimadzu SH-Rxi-5sil MS (30 m length, 0.25 mm i.d., 0.25 µm df) column. Helium was used as a carrier gas for all the three EOs with flow rate 1.5, 1.8, and 1.5 mL/min for CEO, AjEO, and DEO, respectively. For CEO, the oven temperature was programmed to increase from 35 to 200°C at a rate of 3°C/min, followed by a final hold time of 10 min[8] and the total scan time was 66 min. For AjEO, the temperature program was set at 60°C for 5 min, subsequently increased to 220°C with 4°C/min increase, then 11°C/min up to 280°C with 15min hold time and the total scan time was 65 min[7]. For DEO, the oven temperature program was initially set at 60°C for 2 min, thereafter, increased up to 280°C at 10°C/min, with 5 min of hold time and the total scan time was 28 min[9].

The identification of the EO constituents was based on the comparison of their retention indices (RI) relative to C_8-C_{30} *n*-alkanes and by matching their mass spectral fragmentation patterns with corresponding library (NIST® 2017; National Institute of Standards and Technology, Gaithersburg, MD, USA). The quantity of the identified compounds was calculated by dividing the individual peak area by the total area of all peaks[10].

2.3. Antibacterial activity

a. Bacterial test strains

The polymerase chain reaction (PCR) confirmed strains of *E. coli*, *S. enteritidis* and *S. gallinarum* of poultry origin were obtained from the in-house collection of Department of Microbiology, University of Veterinary and Animal Sciences, Lahore. The bacteria were collected from the liver and cecal samples of chickens arranged from different commercial farms. Briefly, the *E. coli* and *S. enteritidis* were isolated from the cecal and the *S. gallinarum* from the liver tissues of the suspected birds. The isolated and PCR confirmed strains of these three bacteria were stored at -80°C in 50% sterile glycerol for further use.

b. Agar well diffusion assay

The activity of EOs against *E. coli*, *S. enteritidis* and *S. gallinarum* of poultry origin were determined by well diffusion assay on nutrient agar plates. All the analyses of agar well diffusion assay were replicated three times. Briefly, exponentially growing bacteria were re-suspended in phosphate buffer saline (~0.5 McFarland) and swabbed on agar plates to obtain a uniform lawn of bacterial growth. The wells of 6mm size were made on the inoculated plates aseptically, sealed and 100 µL of each EOs (100 µL/mL dimethyl sulfoxide (DMSO)) were added in each well[11]. The gentamycin and ciprofloxacin (15 µL/mL DMSO) were evaluated as standards for *E. coli* and *Salmonella* strains, respectively, and DMSO as a negative control. Plates were incubated at 37°C in an incubator for 24 hrs and the antibacterial activity of EOs were read as zone of inhibition (ZOI) of growth surrounding the wells.

c. Minimum inhibitory concentrations

Minimum inhibitory concentrations of EOs were determined by broth microdilution method using 96 well microtiter plates in triplicates. A twofold serial dilution of EOs in 10% DMSO (10 µL/mL to 0.02 µL/mL) were prepared. A 100 µL of each dilution was added in each well and 100 µL of microbial suspension (1×10^6 CFU/mL) was also mixed. The gentamycin and ciprofloxacin at similar concentrations were evaluated as standards for *E. coli* and *Salmonella* strains, respectively, and DMSO as negative control. The plates were incubated at 37°C in an incubator for 24 hrs. MICs were defined as the lowest concentration of compound able to in-

hibit the growth of the microorganisms.

2.4. Antioxidant activity

a. Peroxide value

To measure primary lipid oxidation that indicates the number of peroxides formed in the fats and oils during oxidation, PV was analyzed by using a modified oven test[12]. Briefly, 200 ppm of EOs or the butylated hydroxytoluene (BHT, commercial antioxidant) were added to 30 g of the rapeseed oil in a 100 mL glass beaker. BHT was used as a positive control to confirm the effectiveness of EOs[13]. The mixtures were thoroughly homogenized and placed into the thermostatic oven at 80°C for four weeks[12]. A control sample (without additive) was also prepared and evaluated under similar conditions. The PV of the samples was measured on weekly basis at 0, 7th, 14th, 21st and 28th day and replicated for 3 times. For this purpose, 1 g sample was dissolved in a solution of CH_3COOH:$CHCl_3$ (3:2 v/v) and then 1 ml saturated solution of potassium iodide was added and titrated against 0.1 N sodium thiosulphate, using starch as an indicator. A blank titration was also run parallel to the treated samples and the PV (meq/kg) was calculated as described by Singh et al.[13].

b. Thiobarbituric acid value

The sample for TBA analysis was also prepared by using the same technique as for PV test and replicated for 3 times. For TBA value estimation, a 10 g sample was mixed with 20 mL 0.67% aqueous thiobarbituric acid and 25 mL benzene solution, and this mixture was shaken continuously by mechanical shaker for 2 hrs. The supernatant was separated after shaking and placed in water bath (95°C) for 60 min. After cooling, absorbance of the supernatant was measured at 540 nm with SPECORD® 200 Plus spectrophotometer as described by Singh et al.[12]. The TBA value of the samples were measured on weekly basis at 0, 7th, 14th, 21st and 28th day.

c. DPPH free radical scavenging activity

The reducing efficacy of DPPH in the presence of antioxidant is one of the several methods proposed for antioxidant assay whose characteristic color transformation from purple to yellow is measured spectrophotometrically. 1 mL methanolic solution of the EOs or BHT at five different concentrations (5-25 µL/mL) was thoroughly mixed with 4 mL of 0.004% methanolic solution of DPPH and replicated 3 times. The mixtures were than kept in dark for 30 min and absorbance was measured using SPECORD® 200 Plus spectrophotometer at 517 nm wavelength[14]. The ability of additives to scavenge the DPPH radicals was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = A_c - A_t / A_c \times 100$$

Where, A_c = absorbance of control sample

A_t = absorbance of test sample

d. Oxidative stability index (OSI)

The OSI was determined by using the Professional Rancimat 892 (Metrohm, Herisan, Switzerland) instrument. Five samples of rapeseed oil were prepared out of which three were supplemented with 200 ppm of each EO, fourth had BHT and the fifth was the negative control[15]. Three gram of each sample was subjected to determine the oxidative stability at 120°C under a constant oxygen flow (20 L/hr) and replicated 3 times. The results are indicated as the induction period (IP), which is known as the time required for reaching an endpoint of oxidation corresponding to either a level of detectable rancidity or a sudden change in the oxidation rate as described by Cordeiro et al.[15].

2.5. Statistical analysis

The data were analyzed for normality and for homogeneity.

Table 1 - Major phyto-constituents of coriander, ajwain and dill seed essential oils analyzed by gas chromatography-mass spectrometry.

RI*	Compound Name	Concentrations (%)		
		CEO	AjEO	DEO
948	α-pinene	3.34	-	0.07
998	δ-terpinene	1.65	12.7	0.67
1018	D-limonene	1.01	-	19.9
1042	Cymene	0.72	13	0.16
1082	Linalool	56.8	-	3.7
1104	Nonanal	2.63	-	-
1121	Camphor	2.33	-	0.02
1138	Borneol	3.11	-	-
1179	Dihydro-carvone	-	-	11.2
1190	Carvone	1.09	-	41.1
1228	Geraniol	2.94	-	-
1262	Thymol	-	68.2	-
1352	Geranyl acetate	17.6	-	0.06
1705	Dill apiol	-	-	6.57
2704	Phthalic acid	-	-	9.05
Total (%)		**93.2**	**93.9**	**92.5**

CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; *RI: retention index observed; (-): not detected

Analysis of variance was performed by ANOVA procedure using SPSS 16.0 (SPSS Inc., Chicago, IL) statistical package. Probability values of $p \leq 0.05$ were declared as significant. When the effect was significant, the difference among the treatment means were detected using Duncan's multiple range test.

3. RESULTS AND DISCUSSION

3.1. Essential oil yields and the chemical compositions

The hydro-distillation of coriander, ajwain, and dill seed yielded 0.35% (yellowish), 1.96% (brownish), and 2.24% (light yellowish) EOs, respectively. A total of 28, 25, and 31 volatile bioactive compounds covering >99% of the GC-MS peaks were identified in CEO, AjEO and DEO, respectively. The concentrations (%) of major chemical constituents (>92.5% in total) of EOs along with their retention indices (RI) are presented in Table 1. The main volatile compounds in CEO were linalool (56.8%), geranyl acetate (17.6%), -pinene (3.34%), borneol (3.11%), and geraniol (2.94%), which belong to monoterpenes (linalool, geranyl acetate, -pinene, borneol) and monoterpenoid alcohols (geraniol) groups. In AjEO, the major identified compounds were thymol (68.2%), cymene (13%) and δ-terpinene (12.7%). Thymol in AjEO is phenolic in nature while the other two are monoterpenes. In DEO, carvone (41.1%), D-limonene (19.9%), dihydro-carvone (11.2%), phthalic acid (9.05%), dillapiol (6.57%), and linalool (3.7%) were identified as main bioactive compounds-belonging to monoterpenes group.

Linalool, thymol and carvone are considered as the major bioactive constituents of the CEO, AjEO and DEO, respectively, with variable relative concentrations. In the current study, linalool concentration (56.8%) in CEO was relatively lower than the reported concentrations (66.1 - 75.3%) in some studies[8,10,13], whereas the geranyl acetate (17.6%) concentration in CEO was higher than the reported concentrations (0 - 8.1%).

The thymol concentration (68.2%) in AjEO agrees with the findings (67.4%) of Vitali et al.[7], but not in line with the reported concentrations (15.5-50.8%) in other studies[16,17,18]. This variation might be related with the differences in rapness of the seeds, area of cultivation and conditions and duration of hydrodistillation process.

The carvone concentration (41.1%) in DEO was similar to the result of Singh et al.[19], who showed 47.7% carvone, but they reported dillapiole as the second component with 32.7%, which is much higher than our result with 6.57%. This may be due to differences in growth stage of seeds, considering that the conversion of dillapiole to oxygenated terpenes might increase during the developmental growth of seeds. The presence of limonene as second major component in DEO is in accordance with the findings of most of studies[12,20,21] except for results of Singh et al.[19], who showed dillapiole as the second one as mentioned above.

3.2. Antibacterial activity

a. Agar well diffusion assay

Table 2 shows the results of antibacterial activity of the EOs determined by well diffusion assay against *E. coli, S. enteritidis* and *S. gallinarum* of poultry origin. Each of the three EOs exhibited different antibacterial activities in terms of ZOI based on their bioactive constituents. The AjEO performed better against *E. coli* (10.83 mm) and *S. gallinarum* (13.93 mm) than the other EOs. Our results are line with the data presented in previous studies[7,16]. These findings supported by the fact that thymol, the major bio active compound of AjEO, has the bacteriostatic or bactericidal properties due to its phenolic nature[22]. The cymene and δ-terpinene are the major components of AjEO after thymol and their antibacterial activities have also been reported previously[23] which support our findings. DEO oil showed higher antibacterial activity ($p < 0.001$) against *S. enteritidis* than CEO and AjEO, while Singh et al.[12] reported DEO as ineffective against *Salmonella typhimurium.* The CEO showed the narrower ZOI against all the bacteria tested in this study which can be justified with the fact that it is a potent antibacterial against gram positive than gram negative bacteria due to structural differences in bacterial membranes as the gram-negative bacteria are naturally more resistant due to the presence of an outer membrane made up of lipoproteins and lipopolysaccharides which reduce the entry of certain lipophilic molecules inside the bacterial cell[24]. Additionally, this finding can be supported by the fact that the linalool has weaker antibacterial but stronger antifungal activity[25].

Table 2 - The zone of inhibition (mm) of coriander, ajwain and dill seed essential oil, and standard antibiotics against three different bacteria.

Test material	*E. coli*	*S. enteritidis*	*S. gallinarum*
CEO	5.33[b]	0[d]	4.67[c]
AjEO	10.83[a]	5.67[c]	13.93[a]
DEO	3.33[b]	11.1[b]	0[d]
Gentamycin	9.26[a]	NA	NA
Ciprofloxacin	NA	15.34[a]	9.37[b]
SEM	0.54	0.46	0.53
p-value	<0.001	<0.001	<0.001

CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; CEO, AjEO, DEO (100 µL/mL DMSO); gentamycin and ciprofloxacin (15 µL/mL DMSO); ZOI exclude the diameter (6mm) of well; NA: not evaluated; Means with different superscript letters are significantly different ($p \leq 0.05$) from each other; SEM=standard error of the mean

b. Minimum inhibitory concentrations

The MICs of CEO, AjEO and DEO required to inhibit the bac-

Table 3 - Minimum inhibitory concentration (µL/mL) of coriander, ajwain and dill seed essential oils and standard antibiotics against three different bacteria.

Test material	*E. coli*	*S. enteritidis*	*S. gallinarum*
CEO	0.63[b]	2.5[a]	0.31[b]
AjEO	0.31[c]	0.63[c]	0.08[c]
DEO	2.5[a]	0.31[d]	0.63[a]
Gentamycin	0.63[b]	NA	NA
Ciprofloxacin	NA	1.25[b]	0.02[d]
SEM	0.26	0.25	0.07
p-value	<0.001	<0.001	<0.001

CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; NA: not evaluated; Means with different superscript letters are significantly different ($p \leq 0.05$) from each other; SEM=standard error of the mean

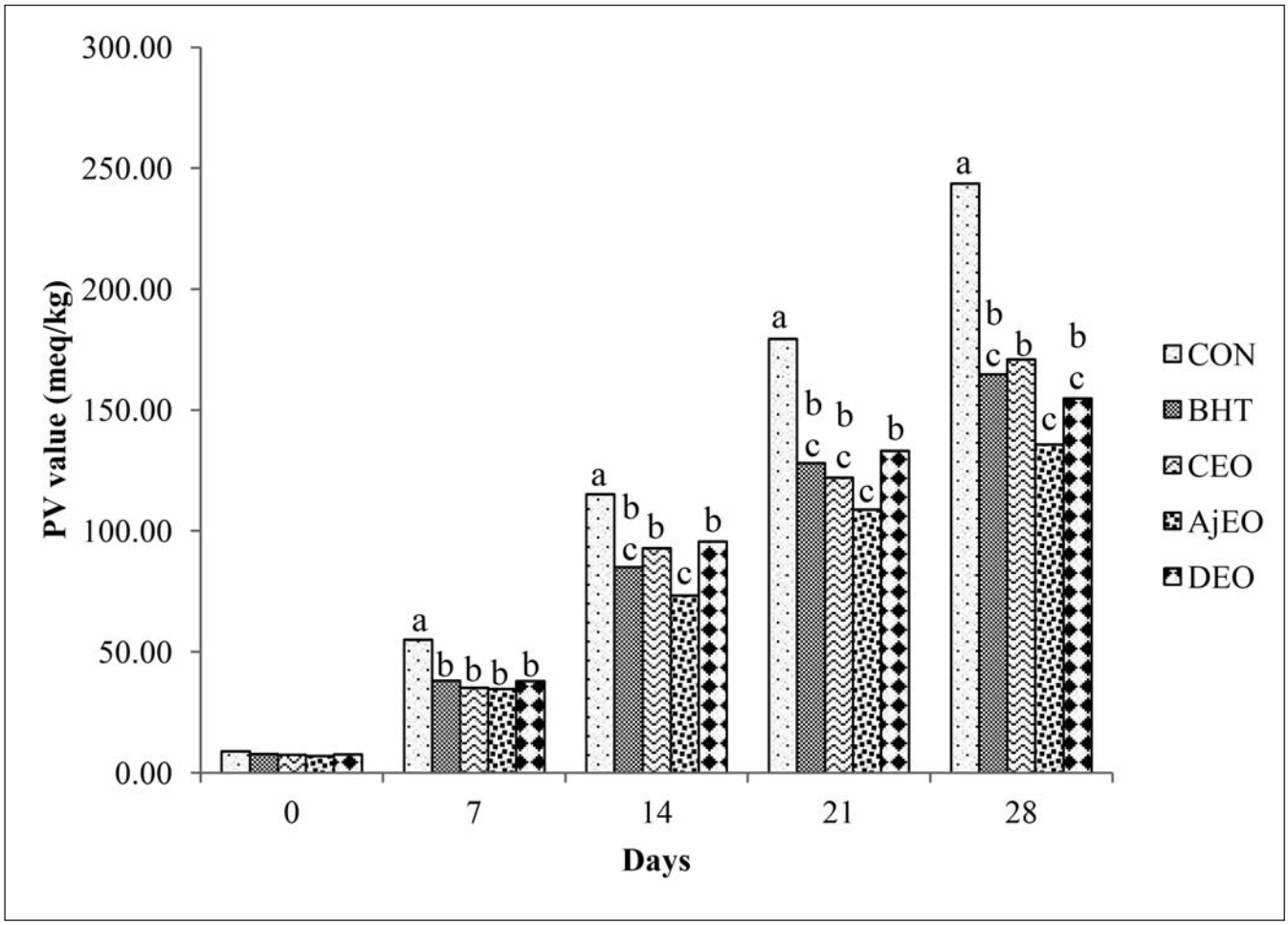

Figure 1 - Inhibitory effects on peroxide accumulation of coriander, ajwain and dill seed essential oils, and BHT in term of rapeseed oil at 80 C iCON=control; BHT=butylated hydroxytoluene; CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; *p*-value (0.52 for day 0; <0.001 for day 7; <0.001 for day 14; <0.001 for day 21; <0.001 for day 28); Significant differences ($p \leq 0.05$) between means are indicated by different letters

terial growth are shown in Table 3. The AjEO showed lower MIC against *E. coli* (0.31 µL/mL) and *S. gallinarum* (0.08 µL/mL) in comparison to other EOs tested in this study. Our result confirmed the findings of Upadhyay et al.[26], who reported the low MIC (0.13 µL/mL) of AjEO against *E. coli*, emphasizing AjEO can be a potent antimicrobial agent.

DEO showed better action against *S. enteritidis* than the others. The CEO showed 0.63 µL/mL MIC against *E. coli.* Our findings did not agree with some of the previous studies investigating antibacterial activity of CEO[8,27]: Delaquis et al.[8] and Ozkinali et al.[27] reported higher MIC of CEO (2.3 µL/mL and 50 µg/mL, respectively) against *E. coli* in comparison to our results. This can be related with the variation in chemical composition of CEO as Delaquis et al.[8] and Ozkinali et al.[27] reported 70% linalool and no geranyl acetate in their findings which in much different from current data.

The available data on antibacterial properties of CEO, AjEO and DEO is not always certain due to not only variable chemical compositions and analytical procedures, but also the variability in bacterial strains and concentration in cultures, affecting the MIC results. As per authors' knowledge, there is a scarcity of data regarding antibacterial properties of these EOs against bacterial strains of poultry origin. Based on the ZOI and MIC results of this study, it would be conceivable that the AjEO might be an appropriate replacement for antibiotics in the chicken diets with less amount of supplementation than CEO and DEO.

3.3. Antioxidant activity

a. Peroxide value

Figure 1 demonstrates PV changes in rapeseed oil of all investigated samples at 80°C. Peroxide value is a widely known measure of the primary lipid oxidation, showing the number of peroxides formed in the fats and oils during oxidation. Rapeseed oil oxidation was measured at time intervals of 7 days during 28 days of storage. The oxidation status of all the samples was low in start and linearly increased with the storage duration, however, the extent of increase is variable due to additives. For 28 days storage period the PV of control rapeseed oil increased to 243.8 meq/Kg, whereas the oil supplemented with BHT, CEO, AjEO, and DEO showed 164.8, 170.9, 135.7, and 154.8 meq/Kg PV at concentration of 200 ppm, respectively. Our findings of reduction in PV with EOs supplementing supported the results from previous studies using different dietary oils[12,13,17]: briefly, the CEO reduced the PV of sunflower oil by 21%[13], AjEO reduced the PV of linseed oil by 39.5%[17], and DEO reduced the PV of rapeseed oil by 10.6%[12]. The present results showed that the BHT, CEO, AjEO and DEO reduced the PV of rapeseed oil by 32.4, 29.8, 44.3, and 36.5%, respectively in comparison to control on day 28. In terms of retarding the formation of primary oxidation products, the effectiveness of the addi-

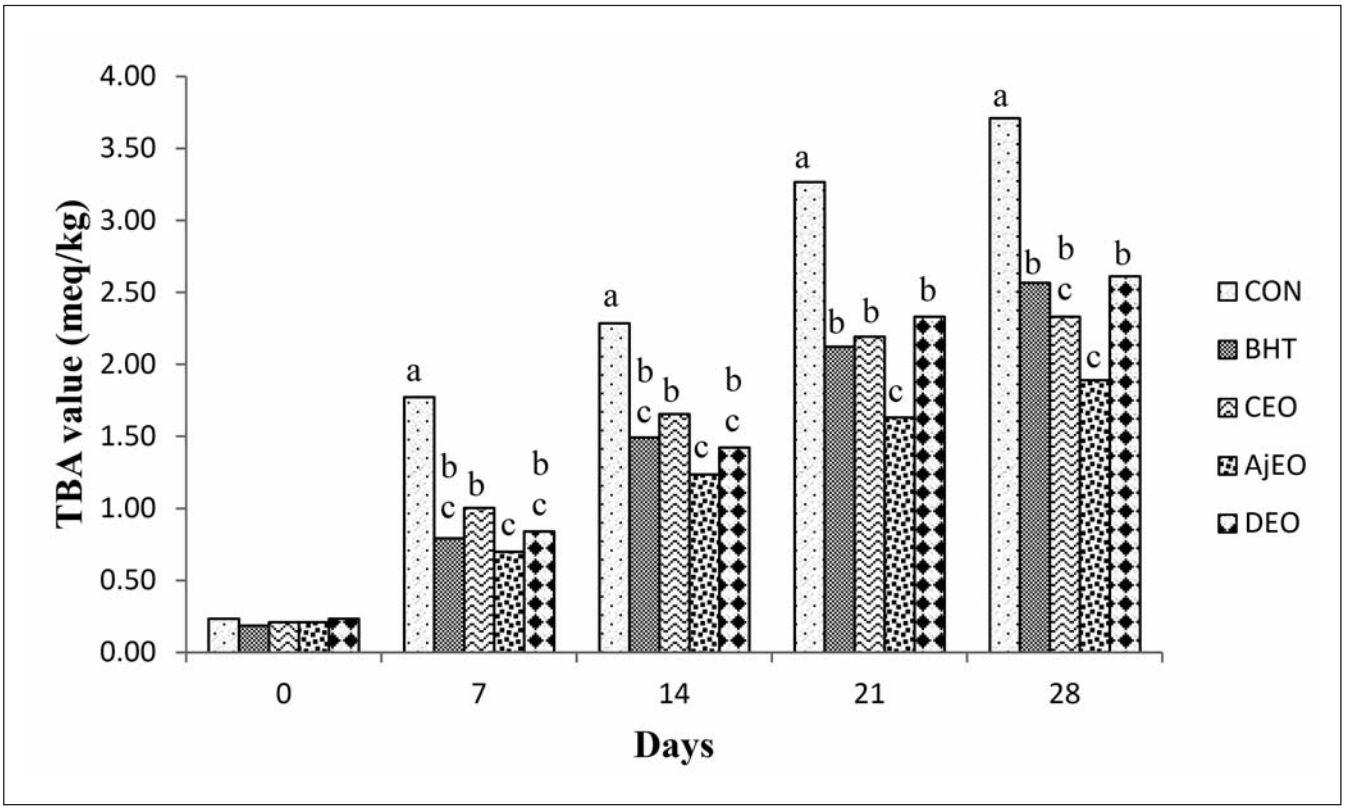

Figure 2 - Inhibitory effects of coriander, ajwain and dill seed essential oils, and BHT for rapeseed oil measured using thiobarbituric acid value method at 80 C
CON=control; BHT= butylated hydroxytoluene; CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; *p*-value (0.75 for day 0; <0.001 for day 7; <0.001 for day 14; <0.001 for day 21; <0.001 for day 28); Significant differences ($p \leq 0.05$) between means are indicated by different letters

tives at concentration of 200 ppm can be put into the following order: AjEO>DEO>BHT>CEO. This performance of AjEO may be involved in the fact that the phenolic group in thymol has the greater radical trapping abilities[28].

b. Thiobarbituric acid value

Figure 2 shows the effects of three EOs and BHT on malonaldehyde formation in rapeseed oil at 80°C measured using thiobarbituric acid value method in terms of incubation time versus TBA value. Note: Primary oxidation products are unstable compounds that produce several secondary products during the oxidation chain process, such as malonaldehyde and 2-alkenals, which are measured by TBA method[19]. TBA value in rapeseed oil for control group linearly increased during period of 7 to 28 days, where more secondary products were formed from the primary oxidation products, peroxides, implying quick formation of malonaldehyde during the oxidation chain process. For 28 days storage period the TBA value of control rapeseed oil increased to 3.71 meq/kg, whereas the oil supplemented with BHT, CEO, AjEO, and DEO showed 2.57, 2.33, 1.89 and 2.61 meq/kg TBA values at concentration of 200 ppm, respectively.

Our findings for EOs effects on TBA value agree with the results of some previous studies[12,13,17]. The CEO reduced the TBA value of sunflower oil by 37.5% during storage of 21 days[13], AjEO reduced the TBA value of linseed oil by 24% for 28 days storage[17], and DEO reduced the TBA value of rapeseed oil by 50% during the storage of 28 days at 80°C[12]. The present results showed that inhibitory effects of BHT, CEO, AjEO and DEO on TBA value were 30.8, 37.1, 49.1, and 29.6%, respectively in comparison to control. Therefore, the effectiveness of the additives at concentration of 200 ppm can be put into the following order: AjEO > CEO > BHT > DEO. Like PV, AjEO performed better than all other additives for TBA which can be related to the phenomenal antioxidant properties of thymol[28] meaning that AjEO might work very well for retarding the formation of both primary and secondary oxidation products in comparison with other EOs.

c. DPPH free radical scavenging activity

Figure 3 shows the free radical scavenging activity of the EOs and BHT in a concentration range 5-25 µL/mL. Percentage DPPH radical scavenging activity of all EOs and BHT gradually increased with increasing the concentrations and was found to be the dose dependent. The AjEO showed highest radical scavenging potential out of all the three EOs and the activity is comparable to the BHT. These findings are in line with some previous studies[16], who reported the higher antioxidant activity of AjEO than ascorbic acid and BHT, respectively. The antioxidant activity is mainly related to the phenolic components of EOs[29]. Thymol, the major component of AjEO, is phenolic in nature and a powerful scavenger of DPPH[29], which support the findings of current study.

For CEO, in line to current findings some studies have reported the considerable radical scavenging activity in comparison to different standards[10,30]. Kacaniova et al.[10] and Shahwar et al.[30] reported the percentage DPPH radical scavenging activity of CEO as 51.1 and 66.5%, respectively. In accordance to our findings, DEO also showed significant DPPH radical scavenging activity in some studies[12,19], though Kazemi[21] reported it as weak-

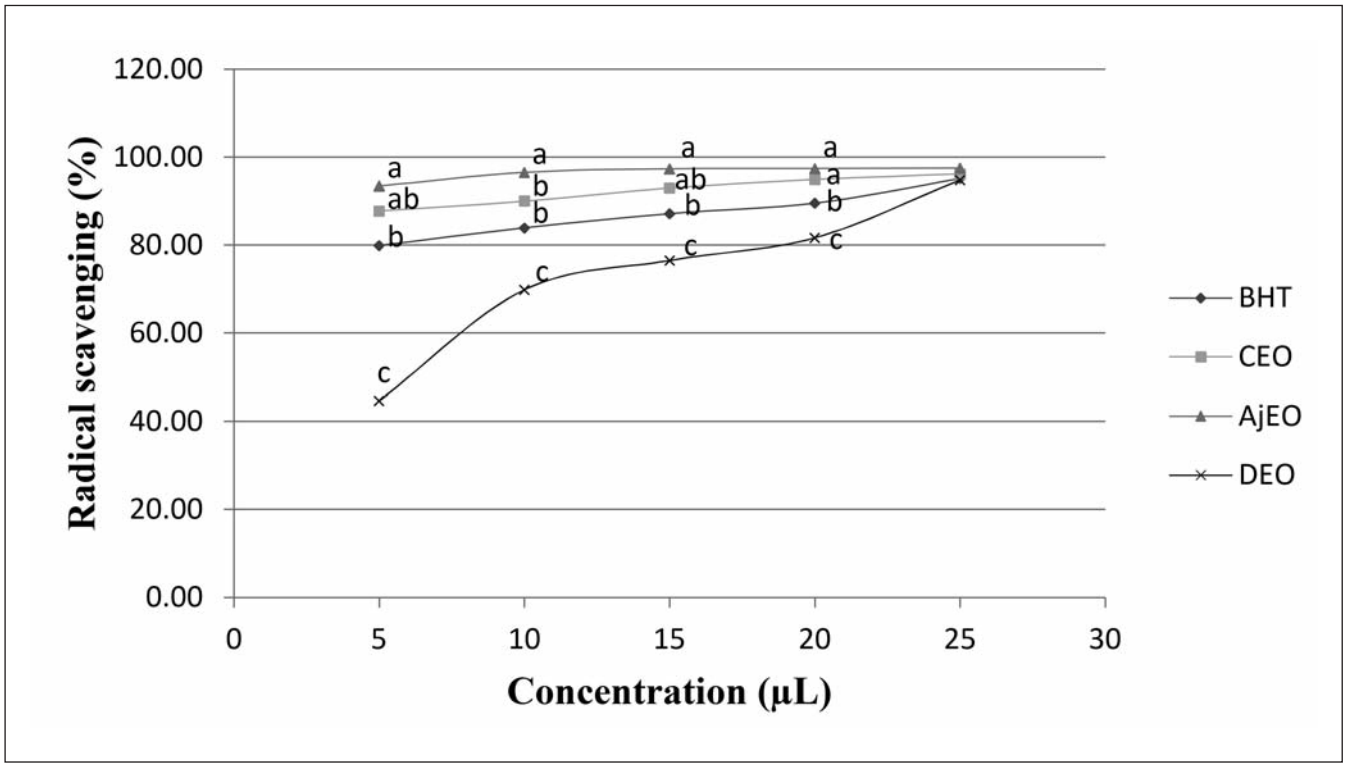

Figure 3 - Radical scavenging effect of coriander, ajwain and dill seed essential oils, and BHT on DPPH radicals
CON=control; BHT= butylated hydroxytoluene; CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; *p*-value (<0.001 for 5 µL; <0.001 for 10 µL; <0.001 for 15 µL; <0.001 for 20 µL; 0.25 for 25µL); Significant differences ($p \leq 0.05$) between means are indicated by different letters

er DPPH scavenger, which can be related to the contrasting chemical composition with no carvone in his study. All studies including ours demonstrating the free radical scavenging activity reported carvone as major bioactive compound with variable concentration between 41 to 74%[12,19].

d. Oxidative stability index

Table 4 shows the effects of EOs and BHT incorporation on oxidative stability of rapeseed oil. This test measures the secondary and volatile products resulting from the oxidation of oils and fats by trapping in and increasing the conductivity of deionized water[31]. The control sample had the lowest IP (3.98 hr) and the CEO showed highest IP (5.1 hr). Ghazanfari et al.[31] evaluated the oxidative stability of soybean oil by supplementing CEO, being effective (IP=5.20 hr) in comparison to control (IP=4.60 hr). As per authors' knowledge, there is no Rancimat published data available regarding use of CEO, AjEO and DEO as oxidative stability agent in rapeseed oil. The present study provides the evidence that the OSI of all the tested additives at concentration of 200 ppm can be in the following order: CEO>BHT>AjEO>DEO.

Table 4 - Stability of rapeseed oil supplemented with coriander, ajwain and dill seed essential oils and BHT estimated by Rancimat at 120 C.

Test material	IP (hr)
CON	3.98^{c}
BHT	4.48^{b}
CEO	5.1^{a}
AjEO	4.29^{bc}
DEO	4.13^{bc}
SEM	0.11
p-value	<0.001

CON=control; BHT= butylated hydroxytoluene; CEO=coriander essential oil; AjEO=ajwain essential oil; DEO=dill seed essential oil; IP (hr): induction period in hours; Means with different superscript letters are significantly different ($p \leq 0.05$) from each other; SEM=standard error of the mean

4. CONCLUSIONS

It is concluded that the tested EOs have diverse phyto-constituents and shown potential antibacterial and antioxidant properties. The AjEO showed better antibacterial and antioxidant potential overall based on its major bioactive compound, thymol. It is a positive indication that these EOs can be included in broilers feed as replacement of AGPs and the antibiotic resistance issue may be addressed. They may support gut health of broilers with their antibacterial and antioxidant properties. Moreover, in vitro studies are not enough to support this cause because during the in vivo conditions, the results are often contrasting. Therefore, in vivo studies are recommended to find out the exact effects of these EOs on broiler production.

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