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EFFECT OF DIFFERENT LEVELS OF CLOVE ESSENTIAL OIL ON THE GROWTH PERFORMANCE, LIPID METABOLISM, IMMUNITY, AND INTESTINAL MICROBIAL STRUCTURE OF BROILER CHICKENS

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SUMMARY

This study assessed the effects of varying concentrations of clove essential oil (CEO) on broiler chickens' growth metrics, cecal microbiota composition, and blood serum metabolites, positioning CEO as a potential substitute for antibiotic growth enhancers. A total of 250 one-day-old Ross 308 chicks were evenly distributed into five groups, with each group comprising 50 chicks. The control group, G1, received a basal diet devoid of any supplements; G2 was fed a basal diet plus 20 mg/kg of avilamycin; groups G3, G4, and G5 were administered 100, 200, and 300 mg/kg of CEO, respectively. The study documented a direct correlation between CEO dosage and the birds' growth efficiency. Notably, groups G3 and G4 exhibited significant ($P < 0.05$) improvements in both weight gain and feed conversion ratio compared to the other groups, without a change in feed consumption. While carcass quality remained consistent across the groups, significant increases ($P < 0.05$) in the weights of the intestine and bursa were observed in G4 and G3, respectively. Addition of CEO markedly elevated ($P < 0.05$) serum levels of immunoglobulin (IgA) and high-density lipoprotein (HDL), while concurrently reducing levels of low-density lipoprotein (LDL) and total cholesterol. Additionally, CEO use notably enhanced the cecal microbiota by augmenting *Lactobacillus* spp. populations and reducing ($P < 0.05$) *E. coli* numbers in the ileum of groups G4, G3, and G2. The outcomes suggest that clove essential oil supplementation could bolster growth, immunity, and intestinal health in broiler chickens, offering a viable alternative to antibiotic use.

Key words: *clove essential oil, broilers, performance, lipid profile, digestibility, immunity.*

INTRODUCTION

Chicken meat continues to dominate the global meat market, largely due to its favorable protein-to-fat ratio and affordability, making broiler meat a preferred choice among consumers. Consequently, enhancing poultry production performance is a critical focus for researchers in animal husbandry. The poultry sector has historically seen significant advantages from incorporating antibiotics into feed to promote growth and disease management. These antibiotics have played a crucial role in ensuring poultry health, addressing subclinical infections, and controlling pathogenic microbes (David *et al*., 2012). However, due to escalating concerns over antibiotic resistance and its subsequent effects on poultry products, human health, and antimicrobial resistance, the European Union enacted a ban on the use of antibiotics as growth promoters in 2006 (Franz *et al*., 2010). This led to nutrition specialists in the animal sector exploring viable non-antibiotic alternatives such as probiotics, organic acids, and herbal remedies (Elbaz *et al*., 2021; Abdel-Moneim Eid *et al*., 2020).

Essential oils (EOs), derived from plants and herbs via steam distillation, contain a variety of bioactive terpenoids (e.g., carvacrol, thymol, menthol, linalool, borneol, α-terpineol) with low molecular weight. These oils have been shown to positively influence several metabolic functions, including lipid metabolism, enhancing the activity of digestive enzymes (Ghanima *et al*., 2020), and offering antioxidant, antimicrobial (Arif *et al*., 2022), anti-inflammatory, immunomodulatory benefits, thereby improving gut health and overall broiler growth performance. As a result, EOs are being considered as potential replacements for antibiotics in broiler nutrition, with the effectiveness of each EO varying based on its specific bioactive components. This study focuses on evaluating the impact of clove essential oil (*Syzygium aromaticum*), which is rich in caryophyllene, eugenol, humulene, and humulene epoxide, on broiler growth and health. These components are believed to be critical in promoting animal health and performance. Clove essential oil has been recognized for its antibacterial (Mitsch *et al*., 2004), antifungal, and antioxidant capabilities (Bostoglou *et al*., 2004), positioning it as a promising antibiotic alternative in broiler diets for improving growth, immune function, and gut health.

MATERIALS AND METHODS

Experimental design, chicks, and diets:

Two hundred and fifty commercial Ross 308 broiler chicks, aged one day, were evenly allocated into five distinct groups, with each group comprising five subgroups (10 chicks per subgroup). The treatment regimens were as follows: G1) chicks given a basal diet without any supplements, G2) chicks receiving a basal diet supplemented with 20 mg/kg of avilamycin, G3) chicks fed basal diet supplemented with 100 mg/kg of clove essential oil (CEO), G4) chicks fed a basal diet supplemented with 200 mg/kg CEO, and G5) chicks provided with a basal diet that included 300 mg/kg CEO. The formulated diets adhered to the broiler nutritional guidelines established by the NRC in 1994, detailed in Table 1. The addition of clove essential oil was facilitated by its incorporation into vegetable oil, which was then thoroughly blended with the experimental feeds. The chicks were housed in cages for duration of 35 days, with unlimited access to both feed and water. Initially, the lighting was set to remain on for 24 hours during the first week, after which it was adjusted to 20 hours for the remainder of the study period. The temperature within the experimental facility was systematically decreased from 32°C to 21°C over the first 30 days and then maintained at a constant level.

Table (1): Diet composition for starter and grower of basal diets.

Ingredient	Starter (1–21 days)	Grower (22–35 days)
Yellow Corn	55.10	59.20
Soybean meal	38.6	33.25
Soybean oil	2.38	3.90
Limestone	1.17	1.05
Dicalcium phosphate	2.05	1.83
Premix*	0.25	0.25
Salt	0.25	0.25
DL-methionine	0.16	0.17
L-lysine	0.04	0.10
Total	100	100
Calculated composition		
ME (kcal/kg)	3000	3100
Crude protein	22	20
Calcium (%)	1.04	0.94
Av. Phosphorus (%)	0.52	0.48

**Premix provided per kg of diet. 300 IU vitamin E; 50 mg vitamin K; 4 mg vitamin B6; 3 mg vitamin B12; 1.5 mg vitamin A, 14,000 IU vitamin D3; 6 mg niacin; 60 mg pantothenic acid; 20 mg folic acid; 0.20 mg choline; 150 mg Ca; 48 mg P; 3.18 mg Mn; 100 mg Fe; 50 mg Zn; 80 mg Cu;10 mg Co; 0.25 mg iodine*

Growth performance evaluation:

The chicks were weighed at weekly intervals on the 21st and 35th day of age, and average live body weight was recorded. Body weight gain, feed intake, feed conversion ratio (g feed /g gain) were recorded.

Sample collection:

At 35 days, five chickens from each group were sacrificed by slaughter. Before slaughter, blood samples were withdrawn from the wing of the bird to separate serum by centrifuging for 15 min at 3000 rpm, and stored at -20 °C until use. After slaughter, carcass traits such as carcass, liver, gizzard, and abdominal fat were separated and weighed, and their rates were calculated according to the live body weight. Approximately 3 cm of cecal samples were collected for the determination of microbial count, and samples were kept at -80 °C until analysis.

Analysis of serum:

Serum glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) concentration were determined calorimetrically using an auto-analyzer system by using commercially available kits (Bio Systems Co. Spain). Levels of immunoglobulin G (IgG), Immunoglobulin A (IgA), and Immunoglobulin M (IgM) in serum were determined using immunoglobulin ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX, USA).

Analysis of cecum microbial count:

After the cecal contents (3 gm) were aseptically collected. The cecal content was diluted following the 10-fold serial dilution method using sterile PBS, and 10-5 dilutions were used for drop plating in the agar media. MRS agar, Bifidobacterium agar, De Man-Rogosa-Sharpe (MRS) agar, and Mac Conkey (MC) agar (purchased from HiMedia, Mumbai, India) media were used for the Total lactic acid bacteria, Bifidobacterium, Lactobacillus spp., and E. coli count, respectively. Total lactic acid bacteria and E. coli count were incubated at 37 °C (48 - 72 h) and Bifidobacterium, Lactobacillus spp. at 37 °C (48 - 72 h). The colonies for each bacterial population were counted manually. The bacterial counts were presented as Log10 colony forming units (CFU) per gm of the cecal content.

Statistical analysis:

Data were analyzed with the GLM procedure of SAS (2003) using one-way factorial analysis ($P < 0.05$). The significant differences among means were tested using the Duncan Multiple Range Test (Duncan, 1955).All traits % were analyzed by Chi square analysis.

RESULTS AND DISCUSSION

Production performance:

Table 2 illustrates the impact of varying concentrations of clove essential oil (EO) on broiler growth performance. The inclusion of different levels of clove EO did not significantly alter feed consumption throughout the study period, yet it had a pronounced effect ($P < 0.05$) on various growth indicators of the broilers. The initial growth rates across the experimental groups were comparable ($P > 0.05$). Notably, there was a marked enhancement in both body weight gain and feed conversion efficiency during the finisher phase as the dosage of clove EO increased. Particularly, the G5 and G4 groups exhibited significantly ($P < 0.05$) greater weight gains compared to other groups, along with a notably better ($P < 0.05$) feed conversion ratio. Conversely, the G2 and G3 groups showed no significant differences ($P > 0.05$) in weight gain or feed conversion efficiency relative to the control group. This finding is supported by various studies that utilized different essential oils, such as Hashemipour *et al*. (2013) with thymol and carvacrol, Nameghi *et al*. (2019) with a mixture of thyme, peppermint, and eucalyptus oils, and Abdel-Wareth *et al*. (2019) with peppermint and menthol oils. The observed improvements in final body weight and feed conversion ratios are attributed to the essential oils' role in enhancing nutrient absorption. Several studies have verified that essential oils improve nutrient digestibility in chickens (Altop *et al*., 2019). Essential oils possess antimicrobial and immunostimulatory properties that significantly contribute to the birds' optimal performance (Arif *et al*., 2022). Additionally, it's suggested that essential oils may enhance feed palatability (Altop *et al*., 2019), further supporting their utility in poultry diets as growth promoters and viable alternatives to antibiotics.

Carcass characteristics:

Regarding carcass traits, the use of clove EO led to an increase in intestinal weight and a reduction in abdominal fat ($p<0.05$), while it did not significantly influence other carcass parameters (such as eviscerated carcass, liver, and gizzard) as indicated in Table 3. Similar findings have been documented, where EO inclusion resulted in decreased abdominal fat content (Amad *et al*., 2011), and some studies have shown that EO supplementation increased eviscerated carcass weight and reduced abdominal fat (Irawan *et al*., 2021). These effects are likely due to improved nutrient absorption, enhanced gastrointestinal tract morphology, and microbial equilibrium. Variations in carcass trait outcomes may be due to the use of different essential oils with distinct bioactive components, differences in animal age, diet composition, and environmental conditions (Hashemipour *et al*., 2013). Nonetheless, our findings highlighted an increase in intestinal weight, potentially reflecting histological enhancements in the gastrointestinal tract as supported by numerous studies (Al-Mufarrej *et al.*, 2019; Amad *et al.*, 2011), which contribute to improved growth performance.

Table (2): Effect of clove essential oil administration on growth performance of broiler chickens at 35 day.

Item	G1	G2	G3	G4	G5	SEM	p-value
			Initial weight				
	40.8	40.6	40.5	40.6	40.7	0.034	0.335
			1-21 days				
LBW	742.5^{b}	755.4^{b}	749.7^{b}	781.4^{ab}	816.9^{a}	2.878	0.031
AWG	33.41^{c}	34.04^{c}	33.77^{c}	35.28^{b}	36.96^{a}	0.780	0.047
AFI	44.85	45.07	44.81	45.16	45.48	0.119	0.106
FCR	1.342^{a}	1.324^{a}	1.327^{a}	1.280^{b}	1.231^{c}	0.006	0.005
			22-35 days				
LBW	1806^{c}	1829^{ab}	1811^{c}	1863^{b}	1904^{a}	4.621	< 0.001
AWG	73.05^{b}	73.79^{b}	72.91^{b}	74.36^{a}	74.74^{a}	0.344	0.025
AFI	152.1	152.7	152.6	153.8	153.4	0.718	0.211
FCR	2.084^{a}	2.070^{ab}	2.093^{a}	2.068^{ab}	2.052^{b}	0.035	0.004
			0-35 days				
AWG	50.43^{c}	51.09^{bc}	50.59^{c}	52.07^{b}	53.24^{a}	0.213	< 0.001
AFI	87.75	88.16	87.94	88.64	88.78	0.357	0.503
FCR	1.740^{a}	1.725^{b}	1.738^{a}	1.702^{c}	1.668^{d}	0.019	< 0.001

$^{a-c}$ Within a row, values with different alphabetic superscripts differ significantly (p < 0.05); SEM: standard error of means; G1: a basal diet without addition; G2: a basal diet with 20 mg/kg avilamycin; G3: a basal diet with 100 mg/ kg CEO; G4: a basal diet with 200 mg/ kg CEO; G5: a basal diet with 300 mg/ kg CEO; LBW: live body weight; AWG: average weight gain; AFI: average feed intake; FCR: feed conversion ratio.

Table (3): Effect of clove essential oil administration on carcass traits (% BW) of broiler chickens at 35 d.

Item	G1	G2	G3	G4	G5	SEM	p-value
Carcass %	68.71	68.58	68.60	68.74	68.59	1.261	0.472
Gizzard %	3.03	2.91	3.11	3.06	2.93	0.703	0.329
Liver %	2.26	2.29	2.25	2.24	2.25	0.084	0.173
Abdominal fat %	2.64^{a}	2.58^{a}	2.45^{a}	2.21^{ab}	1.98^{b}	0.152	0.041
Intestine %	6.35^{c}	6.27^{c}	6.38^{c}	6.85^{b}	7.16^{a}	0.935	0.006

$^{a-c}$ Within a row, values with different alphabetic superscripts differ significantly (p < 0.05); SEM: standard error of means; G1: a basal diet without addition; G2: a basal diet with 20 mg/kg avilamycin; G3: a basal diet with 100 mg/ kg CEO; G4: a basal diet with 200 mg/ kg CEO; G5: a basal diet with 300 mg/ kg CEO

Lipid metabolism:

Our current study results showed an increase in the concentration of HDL, while the concentration of LDL and cholesterol linearly decreased in response to increasing the level of clove EO ($p<0.05$), as shown in Table 4. However, adding clove essential oil did not affect glucose and triglyceride concentration. It is obvious that bioactive components of clove EOs such as caryophyllene, eugenol, humulene, and humulene epoxide are involved in lipids metabolism, particularly in serum cholesterol (Arif *et al.*, 2022). This is consistent with numerous studies using different clove EO sources (Amad *et al.*, 2011; Abdel-Wareth *et al.*, 2019). Studies by Amad *et al.* (2011); Nameghi *et al.* (2019) showed a reduction in total serum cholesterol and LDL levels when the chickens were fed a diet including EO. It was found that some biologically active compounds of EO reduced rate-limiting enzymes involved in cholesterol synthesis, 3 hydroxy-3-methylglutaryl coenzymes A (HMG-CoA) reductase because it is a major enzyme for cholesterol production (Irawan *et al.*, 2021). The change in lipid metabolism levels may be from the effect of essential oils antioxidants and anti-peroxide on liver activity, which affects the biosynthesis of cholesterol (Elbaz *et al.*, 2022). These results show that the addition of clove essential oils has an effective lipid metabolism-enhancing effect by increasing lipid stability and reducing cholesterol, thus reducing lipid oxidation problems during hot climates.

Table (4): Effect of clove essential oil administration on lipid metabolism (mg/dl) of broiler chickens at 35 d.

Item	G1	G2	G3	G4	G5	SEM	p-value
Glucose	218	207	216	215	212	2.091	0.250
Triglycerides	201	206	203	198	191	1.550	0.093
Cholesterol	253^{a}	249^{a}	236^{a}	197^{b}	169^{c}	3.754	0.021
HDL	42.9^{c}	43.1^{c}	45.8^{b}	46.2b	48.5^{a}	0.518	0.044
LDL	128^{a}	121^{ab}	117^{ab}	109^{b}	88.7^{c}	0.636	0.037

[a-c] Within a row, values with different alphabetic superscripts differ significantly (p < 0.05); SEM: standard error of means; G1: a basal diet without addition; G2: a basal diet with 20 mg/kg avilamycin; G3: a basal diet with 100 mg/ kg CEO; G4: a basal diet with 200 mg/ kg CEO; G5: a basal diet with 300 mg/ kg CEO; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Immunity response:

The current study focused on the changes in the function of the immune system, especially since many studies have proven the beneficial effect of EOs on the immune system in animals. This explains the reason for the increase in the relative weight of the bursa of Fabricius in chicks fed a diet containing clove EO, while the relative weight of the spleen and thymus was not affected with the experimental treatments (Table 5). The results were consistent with previous investigations, by Hanieh *et al.* (2010) who illustrated that essential oil supplementation in broilers increased the relative weights of the immune organs.

Table (5): Effect of clove essential oil administration on immune response, Lymphoid organ (%) and Immunoglobulin (mg/dl), of broiler chickens at 35 d.

Item	G1	G2	G3	G4	G5	SEM	p-value
			Lymphoid organ				
Spleen %	0.25	0.24	0.26	0.24	0.25	0.127	0.519
Thymus %	1.06	1.13	1.11	1.08	1.14	0.090	0.141
Bursa of Fabricius %	0.21^{b}	0.19^{b}	0.22^{b}	0.26^{ab}	0.31^{a}	0.025	0.033
			Immunoglobulin				
IgG	322.7	316.5	324.1	321.9	327.5	0.224	0.205
IgA	126.5^{d}	131.4^{d}	174.2^{c}	225.1^{b}	264.8^{a}	0.054	0.001
IgM	117.3	109.8	115.0	113.6	118.2	0.585	0.067

Within a row, values with different alphabetic superscripts differ significantly (p < 0.05); SEM: standard error of means; G1: a basal diet without addition; G2: a basal diet with 20 mg/kg avilamycin; G3: a basal diet with 100 mg/ kg CEO; G4: a basal diet with 200 mg/ kg CEO; G5: a basal diet with 300 mg/ kg CEO

A boost in the immune response and disease resistance by adding essential oil to the broiler diet has been reported in several studies (Zhang *et al.*, 2016; Elbaz *et al.*, 2022), which in consistent with our study. It is suggested that the antimicrobial effect of essential oils could play a beneficial role in the development of the immune system (Batiha *et al.*, 2020), and this may be due to the effect of essential oils on increasing the available nutrients necessary for the development of the immune system. In our results, chickens fed clove EO diets had a significant improvement in immunoglobulin which is due to the increase in the concentration of IgA, while the concentration of IgM and IgG was not changed by the experimental treatments, as is shown in Table 5. IgA is the main effector of the intestinal immune response system, which prevents bacteria, viruses, and some harmful antigens from adhering to the intestinal epithelial, thereby enhancing immunity. This shows that supplementation of clove EO promoted immune responses which led to an increase in the IgA secretions. In line with our results, Liu *et al.* (2019) found that adding essential oils increased SIgA gene expression in the intestine, which maintains gut integrity in chickens. Likewise, essential oil supplementation in the diet increased the levels of IgA, IgG, and IgM in broiler chickens (Di, Yuting, *et al.*, 2022). The addition of EOs has a significant role in activating the cellular and intestinal immunity of the broiler, as shown by the current study.

Microbial status of the cecum:

Broiler chickens gut is considered the major site for the digestion, absorption of dietary nutrients, and immune support (Svihus, 2014). Microbial structure and morphometric properties of the gut reflect the broilers' health status and are associated with the nutrient assimilation capacity (Nicholson *et al.*, 2012). Herbs and their extract compounds have been proven to have antimicrobial effects in broilers through their bioactive compounds which strongly inhibit harmful microbes (Chowdhury *et al.*, 2018). These properties of selective inhibition of intestinal pathogenic bacteria can be exploited to balance the gut microbial population in poultry. In the current study, we examined the gut microbiota to evaluate the functional changes that happened while feeding different levels of clove essential oil (Table 6). The microbial structure of the intestine was significantly influenced by the treatments in our study. Lactobacillus ssp. population in the intestine increased linearly with the increase in the level of clove essential oil in broiler chicken diets, while the number of E. coli decreased. Irawan *et al.* (2021) also reported a higher population of Lactobacillus and a lower population of E. coli in broilers fed clove with their diet. Likewise, Al-Mufarrej *et al.* (2019) demonstrated that the inclusion of clove powder with feed improved microbial content by modulating gut microbiota. The improved microbial content and the enhanced immunity indicate an improvement in nutrient absorbability and gut health (Mohammadi *et al.*, 2014). According to previous study reports, intestinal digestibility and absorbability increase with the improved microbial content, and thus the productive performance enhances when broilers fed on dietary included clove EO.

Table (6): Effect of clove essential oil administration on Cecal microbes of broiler chickens at 35 d.

Item	G1	G2	G3	G4	G5	SEM	p-value
Total LAB	7.26	7.09	7.12	7.30	7.23	2.066	0.105
Bifidobacterium	5.14	5.22	5.17	5.28	5.09	0.190	0.417
Lactobacillus ssp.	6.38^{c}	5.91^{d}	6.45^{c}	7.59^{b}	8.06^{a}	0.306	0.001
E. coli	6.74^{a}	6.05^{b}	6.61^{a}	6.11^{b}	5.47^{c}	0.088	0.016

$^{a\text{-}c}$ Within a row, values with different alphabetic superscripts differ significantly ($p < 0.05$); SEM: standard error of means; G1: a basal diet without addition; G2: a basal diet with 20 mg/kg avilamycin; G3: a basal diet with 100 mg/ kg CEO; G4: a basal diet with 200 mg/ kg CEO; G5: a basal diet with 300 mg/ kg CEO; Total LAB: Total lactic acid bacteria; E. coli: Escherichia coli.

CONCLUSION

It can be concluded that the addition of clove essential oil (up to 300 mg/kg) to broiler chicken diets has a positive effect on growth performance, lipid metabolism, immune status, and intestinal health through manipulating the microbial content.

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تأثير إضافة مستويات مختلفة من زيت القرنفل على الأداء الإنتاجي، تمثيل الدهون، الحالة المناعية، التركيب الميكروبي المعوي لدجاج التسمين

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تهدفت هذه الدراسة إلى تقييم استخدام مستويات مختلفة من زيت القرنفل (CEO)على الأداء الإنتاجي ، والحالة الميكروبية للأمعاء ، ومكونات بلازما الدم لدجاج التسمين. تم تقسيم مائتين وخمسين كتكوتًا عمر يوم من سلالة روس 308 بشكل عشوائي إلى خمس مجموعات ، كل منها بها 50 كتكوت. كانت المجموعات التجريبية كالتالي:
G1 عليقة قاعدية بدون مواد مضافة ، G2 العليقة القاعدية مضافا إليها 20 ملجم / كجم من مضاد حيوي الأفيلاميسين ؛ والعلائق G3 و G4 و G5 عبارة عن العليقة القاعدية مضاقا إليها زيت القرنفل بمستويات 100 و 200 و 300 مجم / كجم على التوالي.
أظهرت النتائج وجود علاقة خطية بين تركيز زيت القرنفل في العليقة والأداء الإنتاجي للطيور حيث أظهرت النتائج تحسم وزن الجسم ومعامل التحويل الغذائي معنويا (P <0.05) في مجموعتى G3 و G4 مقارنة بالمجموعات الأخرى ، بينما لم يتأثر معدل الإستهلاك الغذائي وكذلك لم تظهر صفات الذبيحة أي اختلافات ملحوظة بين المعاملات، ولكن حدثت زيادة معنوية (P <0.05) في وزن كلا من الأمعاء وغدة البرسا لمجموعتي الـ G4 و G3 . زادت مستوى كلا من (IgA) و (HDL) معنويا بإضافة زيت القرنفل وفي الوقت نفسه أدت إلى انخفاض كبير في (LDL) والكوليسترول الكلي. علاوة على ذلك حسنت إضافة زيت القرنفل بشكل كبير من الحالة الميكروبية للأمعاء في G4 و G3 و G2 عن طريق زيادة أعداد Lactobacillus spp. وانخفاض أعداد بكتيريا الـ *E. coli* (P <0.05)، وبناءاً على تلك النتائج ، يمكن الاستنتاج أن إضافة زيت القرنفل يمكن أن يؤدي إلى تحسن الأداء الإنتاجي للطيور ويعززمن المناعة والحالة الصحية للأمعاء وبالتالي يمكن إستخدامه كبديل محتمل للمضادات الحيوية.