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In Vitro Evaluation of Aromatic Galangal Extract as a Feed Additive on Pathogenic Bacteria in the Super Kampong Chicken Digestive Tract

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Abstract | Aromatic galangal as one of the herbal ingredients has a relatively high amount of curcumin compounds that can be used as a feed additive. This curcumin can inhibit and kill pathogenic bacteria in the digestive tract of Super Kampong chickens. The purpose of this study was to evaluate aromatic galangal (*Kaempferia galanga*) extract. Aromatic galangals from Lumajang Regency, East Java Indonesia – a high curcumin content of 39.5 μg g^{-1} used in this study. The concentrations of 0.78 %, 1.56 %, 3.12 %, 6.25 %, 12.50 %, 25.00 %, and 50.00 % used for the inhibition zone variables. The Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (MKC) were 0.125 %, 0.250 %, 1.500 %, 3.125 %, 6.250 %, 12.500 %, 25.000 % and 50.000 %. The treatment was performed in duplicate. The bacteria used in this test were *Clostridium, Campylobacter, Pseudomonas multocida, Salmonella, Staphylococcus aureus, Streptococcus* spp., and *Escherichia coli*. The quantitative descriptive data analysis was presented in both formal and informal forms. The result of study showed that *E. coli* had the highest sensitivity to aromatic galangal extracts compared to the other bacteria. At the same concentration of aromatic galangal extract, which was 0.75 %, the inhibition zone was the highest, which was 7.98 mm ± 1.77 mm, at a concentration of 50 %, the inhibition zone was 19.67 mm ± 1.71 mm. Aromatic galangal extract can inhibit *E. coli* at a concentration of 0.125 % but cannot kill bacteria. As conclusion demonstrated that aromatic galangal extract is only associated with the inhibition of pathogenic bacteria but also have the ability to kill theses pathogenic bacteria isolated from the digestive tract of Super Kampong chickens.

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Introduction

Aromatic galangal (*Kaempferia galanga* L.) is a tuber plant in Indonesia, Southern China, Taiwan, Cambodia, and India, but is also widely cultivated throughout Southeast Asia. It has also been used as a medicinal plant. All parts of the aromatic galangal plant can be used fresh or dried because this

plant is useful for increasing appetite and improving blood flow and digestion. This is because aromatic galangal contains several active compounds such as curcumin, saponins, flavonoids, polyphenols, and essential oils, which have particular roles (Ganapathy and Nair, 2017; Kapitan *et al.*, 2018; Rajput *et al.*, 2013; Riasari *et al.*, 2019). The statement of the benefits of galangal is supported by several experts, *i.e.*, Al-Salhi *et al.* (2020), Asmare *et al.* (2017), Channabasayaiah *et al.* (2016), Edi (2020), Hertiani *et al.* (2010), Kapitan *et al.* (2018), Khiyaaroh and Triratnawati (2021), Silalahi (2019), and Sun *et al.* (2018). They stated, curcumin active compounds to inhibit the growth of viruses, fungi, and Gram-positive and Gram-negative bacteria, such as *Escherichia coli, Shigella dysenteriae,* and *Staphylococcus aureus*, which cause bacterial diseases and colibacillosis in poultry.

Previous studies have shown that aromatic galangal production in East Java, Indonesia varies significantly between districts. The main producer of aromatic galangal in East Java in 2018 was the Pacitan Regency. The study results from five regencies, Malang, Lumajang, Jember, Probolinggo, and Pacitan, showed that aromatic galangal from the Lumajang Regency (112˚53' to 113˚23' East Longitude and 7˚54' to 8˚23' South Latitude) displayed the best physical characteristics. The physical characteristics of the aromatic galangal from Lumajang were superior in all parameters, including yellow color, distinctive pungent aroma, clean appearance, spicy aromatic galangal taste, large size, and freshness. The average curcuminoid content from Lumajang was higher compared to the other four regions, about 39.5 μg g^{-1}, whereas in the other four municipalities, the average curcuminoid content was 3.375 μg g^{-1} (Widodo *et al.*, 2023). These herbal ingredients have been tested in poultry and have shown good performance. Sensitivity tests against *E. coli)* and *Salmonella* spp. against turmeric and ginger showed the same MIC and MKC (1.56 % (MIC) and 3.13 % (respectively) (Ghasemzadeh *et al.*, 2016). The best treatment level in the *C. xanthorrhiza* study was 1 % feed additive. The recommendation was that chickens should be given *C. xanthorrhiza*. as a feed additive of 1 % because it produces the best carcass percentage (Rahayu *et al.*, 2019).

This study focused on testing the effectiveness of aromatic galangal extract containing high levels of curcumin from Lumajang municipality, which was applied to pathogenic bacteria in the digestive tract of chickens *in vitro*. Therefore, it was necessary to conduct an *in vitro* evaluation of aromatic galangal extracts containing high levels of curcumin as feed additives for pathogenic bacteria in the chicken digestive tract. The objective of this study was to analyze the *in vitro* evaluation of aromatic galangal extract with high curcumin content from the Lumajang district as a feed additive to reduce pathogenic bacteria in the digestive tract of the Super Kampong chicken.

Materials and Methods

This experimental study utilized primary laboratory data for its research. The research subject was placed on aromatic galangal extract. The research subject was selected through a deliberate process, based on observational results. The selection criteria focused on municipalities in East Java Province with aromatic galangal containing the highest active curcumin compounds. As a result, Lumajang Regency was selected as the research site.

Aromatic galangal from the Lumajang Regency had high curcumin content was used as the experimental subjects. Treatment was performed at the level of the aromatic galangal extract. Treatment was performed using duplicate methods. The mixing results were tested in a laboratory to determine the ability of aromatic galangal extract to reduce pathogenic bacteria. The bacteria used in this test were *Clostridium, Campylobacter, Pseudomonas multocida, Salmonella, Staphylococcus aureus, Streptococcus,* and *Escherichia coli*, isolated from the digestive tract of Super Kampong chickens. The variables observed were the inhibition zone, MIC, and MKC.

The inhibition zone test has used gelatine powder food grade 150 bloom diffusion from Mitra Jaya with the Kirby–Bauer method (Puspita *et al.*, 2021). Seven concentrations were used in this study: 0.78 %, 1.56 %, 3.12 %, 6.25 %, 12.50 %, 25.00 %, and 50.00 %. The inhibition zone test of aromatic galangal extract against pathogenic bacteria was carried out as follows: on a pure culture of rejuvenated pathogenic bacteria, it was collected and cultured in a sterile aquadest and then homogenized in a vortex machine. The gelatine nutrient medium in a Petri dish was smeared with a pathogenic bacterial culture on the surface of hardened gelatine media using sterile cotton swabs. The media that had been smeared with pathogenic bacteria

were placed on a 6 mm diameter disc paper that was soaked in a solution of aromatic galangal extract at concentrations of 0.78 %, 1.56 %, 3.12 %, 6.25 %, 12.50 %, 25.00 %, and 50.00 %, and each treatment was marked. The medium was incubated for 24 h at 37 ˚C. The inhibition zone or clear zone formed from each medium using disc paper was measured in mm using a Vernier calliper Caliper 300 mm Sigmat 12" 0 mm to 300 mm Steel made in China. The culture medium was examined after 24 h of incubation. The diameter of the inhibition zone or clear zone around the disc paper indicates bacterial sensitivity to the antibacterial materials used as test materials.

MIC method was conducted for aromatic galangal extract by levels of 0.125 %, 0.250 %, 1.500 %, 3.125 %, 6.250 %, 12.500 %, 25.000 % and 50.000 %. Each concentration was taken as much as 1 mL and then placed into a test tube and labelled according to the concentration. The previously prepared bacterial suspension (1 mL) was collected using a micropipette, placed into each labelled test tube, and vortexed until homogeneous. Next, the tubes were incubated at 37 ˚C for 24 h in an incubator, and the turbidity that occurred was observed by comparing the tubes with the control. The lowest concentration of sample solution that could inhibit bacterial growth (marked by the beginning of visual clarity by three independent observers) was determined as the MIC (Puspita *et al*., 2021).

Determination of MKC was carried out at concentrations of 0.125 %, 0.250 %, 1.500 %, 3.125 %, 6.250 %, 12.500 %, 25.000 % and 50.000 %. The next stage was the calculating the number of colonies using the drop plate mill mesra method. The test material with the above concentration was 5 µL for each concentration and then dropped onto MHA with as many as six replications. The specimen was left for 15 min to 20 min until dry and then incubated at 37 ºC for 3 h to 6 h. The number of bacterial colonies was calculated based on the principle that one living bacterial cell, when cultured on solid media, grows into one bacterial colony. If the shape of the colony was wide, one colony as considered, and two colonies were considered if the shape was two colonies. The unit used was the colony-forming unit (CFU mL^{-1}) of liquid (suspension). In the next stage, the number of bacterial colonies in each drop was calculated, and the average was calculated and multiplied by dilution and multiplier factors. The concentration used to calculate the number of colonies was the initial concentration (before dilution) without a dilution factor. In addition, because the suspension of the test material and bacteria was dropped onto solid media at a volume of 5 µL, the calculation was multiplied by a multiplier factor of 200 to obtain results that met the standard (CFU mL^{-1}) (Puspita *et al*., 2021).

The data were processed in three stages: (i) open coding, (ii) axial coding, and (iii) selective coding (Creswel, 2007). The open coding stage was used to explore the effectiveness of the aromatic galangal extract against bacteria. This stage aimed to familiarize researchers with the environmental conditions of aromatic galangal extract subjects and obtain broader and deeper information to broaden the understanding of the effectiveness of the aromatic galangal extract being studied. The axial coding stage involved developing concepts and categories in research on the effectiveness of the inhibition zone, MIC, and MKC of aromatic galangal extracts produced by open coding. The selective coding stage was more comprehensive because it emphasized the relationship between categories as a whole. The selective coding stage ended after the conceptual descriptions of the effectiveness of the inhibition zone, MIC, and MKC of the aromatic galangal extract against bacteria underwent verification and modification.

The quantitative descriptive data analysis was presented in both formal and informal forms (Creswel, 2007). The formal form was a data description of the effectiveness of aromatic galangal extract in reducing the number of bacteria in the form of numbers. A simple statistical analysis in the form of averages was used to comprehensively display the resistance of the bacteria to aromatic galangal extract. The display is in the form of tables and graphs to illustrate the effectiveness of the aromatic galangal extract in reducing the presence of pathogenic bacteria. The informal form was in the narrative form, namely, a description of sentences that explained all aromatic galangal extract research activities in the form of chapters. The presentation of aromatic galangal extract data was made systematically and efficiently so that it was easy to understand and provided optimal clarity.

The first step was a quantitative descriptive analysis of the effectiveness of the inhibition zone, MIC, and MKC of the aromatic galangal extract against pathogenic bacteria. The second stage was to determine

the effectiveness of the inhibition zone, MIC, and MKC of the aromatic galangal extract against the pathogenic bacteria. The third stage involved building a concept regarding the effectiveness of the inhibition zone, MIC, and MKC of the aromatic galangal extract against pathogenic bacteria. The results of the analysis are presented in the form of a description of the following process stages: (i) data on the effectiveness of the aromatic galangal extract, (ii) reduction of aromatic galangal extract data problems, (iii) verification of aromatic galangal extract data problems, and (iv) conclusions.

Results and Discussion

Evaluation of inhibition zone of aromatic galangal ethanol extract to pathogenic bacteria

As shown in Table 1, the aromatic galangal extract exhibited antibacterial activity against all tested bacteria. The inhibition zone against bacteria has occurred at a concentration of 0.78 %, amounting to 6.00 mm ± 0.00 mm to 7.98 mm ± 1.77 mm, while at an extract concentration of 50.00 %, the inhibition zone reached 8.11 mm ± 0.27 mm to 19.67 mm ±1.71 mm. The inhibition zone of the aromatic galangal extract against all tested bacteria was included in the moderate-to-strong category, as stated by Puspita *et al.* (2021). The clear zone was categorized as very strong (diameter > 20 mm), strong (diameter 11 mm to 20 mm), moderate (diameter 6 mm to 10 mm), or weak (< 5 mm). If it was weak and no clear zone was formed, this indicated that the bacteria were resistant to the extract. The bacterial resistance was thought to be due to the presence of enzymes that could damage the active compounds in the aromatic galangal extract. According to Jubair *et al.* (2021), the enzymes that cause resistance are as follows.

1. β-lactamase enzymes hydrolyse the lactam rings of penicillin, cephalosporins, and carbapenems. Approximately 300 β-lactamases have been identified, based on their structural and functional properties.
2. Chloramphenicol acetylatess its hydroxyl group, rendering it unable to bind properly to the target site. *Haemophilus influenza, Gram-negative bacilli, Enterobacteriaceae, Acinetobacter,* and *P. aeruginosa*, fight chloramphenicol using this mechanism.
3. Aminoglycoside modifying enzymes in *Staphylococcus aureus, Streptococcus pneumoniae*, and *Enterococcus faecalis* resist antibiotics by producing enzymes that modify the drug molecule and decrease its affinity for the 30S subunit.

Aromatic galangal ethanol extract of 96 % can inhibit growth against Streptococcus at a concentration of 0.78 % of 6.00 mm ± 0.00 mm and a concentration of 50.00 % of 9.99 mm ± 0.52 mm. These results are better than those reported in Fajeriyati and Andika (2018), which was carried out on *Streptococcus pyogenes* bacteria using 70 % ethanol solvent. The inhibition zone at a concentration of 0.78 % and 50 %, respectively, was 1.2 mm and 5.3 mm. The difference in the bacterial inhibition zones in the aromatic galangal extracts extracted with 70 % ethanol and 96 % ethanol was thought to be caused by several factors related to the physicochemical properties of the solvent and active components in the extract. Ethanol 70 % had a lower alcohol concentration than ethanol 96 %. Lower alcohol concentrations can affect the ability of the solvent to dissolve the active compounds responsible for the antibacterial activity. In contrast, 96 % ethanol may be more effective in dissolving certain active compounds because of its polar nature.

Table 1: *Average inhibition zone of aromatic galangal ethanol extract to bacteria pathogen (mm).*

No	Gut bacteria	Aromatic galangal ethanol extract concentration (%)						
		0.78	**1.56**	**3.12**	**6.25**	**12.50**	**25.00**	**50.00**
1.	*Clostridium*	6.85±0.48	6.97±0.36	7.86±0.31	8.16±0.17	8.51±0.31	9.12±0.58	9.12±0.58
2.	*Campylobacter*	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.85±0.90	8.68±1.62
3.	*P. multocida*	6.75±022	6.82±0.17	7.05±0.45	7.67±0.46	8.02±0.38	9.29±0.91	11.45±0.95
4.	*Salmonella*	6.65±0.38	6.70±0.35	6.75±0.08	6.61±0.21	7.12±0.25	7.38±0.35	8.11±0.27
5.	*S. aureus*	7.00±0.51	7.06±0.65	7.16±0.20	7.39±0.41	7.35±0.30	7.44±0.30	8.47±0.42
6.	*Streptococcus*	6.00±0.00	6.00±0.00	6.00±0.00	6.70±1.21	7.54±1.34	9.08±0.35	9.99±0.52
7.	*E. coli*	7.98±1.77	9.35±0.38	8.73±0.80	14.32±3.66	15.03±5.22	20.32±3.66	19.67±1.71

Ethanol 70 % is a mixture of ethanol and water that can affect the solubility of various bioactive compounds present in aromatic galangal. Some compounds may be more soluble in solutions with lower alcohol concentrations, whereas others may be more soluble in pure alcohol. Therefore, the type of solvent can affect the composition of the active compounds in the extract, and in turn, the antibacterial activity. Differences in the concentrations of the active compounds in the extract could lead to differences in the size of the inhibition zone. If 96 % ethanol is more effective in extracting active compounds from aromatic galangal, this extract may produce a larger inhibition zone than the 70 % ethanol extract. Several researchers have reported that the diameter of the inhibition zone of red chili ethanol extract against *S. aureus* increases with increasing ethanol concentrations. Ethanol concentrations of 45 %, 60 %, 75 %, and 90 %, respectively produced inhibition zones of 8.7 mm, 10.8 mm, 13.56 mm, and 16.53 mm, while *E. coli* produced inhibition zones of 9.3 mm, 10.4 mm, 12.1 mm, and 14.5 mm (Pratiwi, 2006). Alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* have strong antibacterial properties of MIC was 0.025 mg mL^{-1} and 0.21 mg mL^{-1} against *S. aureus* and *P. aeruginosa,* respectively. The MKC of *Callistemon citrinus* extract was 0.835 mg mL^{-1} against *S. aureus* (Belgis *et al.*, 2021). The results of other studies showed that the combination of moringa leaf extract and basil leaf extract at a concentration of 55 % + 25 % had the highest inhibition zone diameter (20.92 mm) against *S. aureus* compared to the other combinations (50 % + 30 %, 45 % + 35 %, and 40 % + 40 %) (Rahayu *et al.*, 2020).

There was a tendency for the increasing concentration of aromatic galangal extract to increase the bacterial inhibition zone, which was related to several factors, namely (i) Active Compound Content: The antimicrobial compounds were available to interfere with bacterial activity, leading to a larger inhibition zone. (ii) Concentration Effectiveness: At higher extract concentrations, more active compounds were dissolved in the test medium, and bacterial growth was inhibited more effectively. This resulted in a wider inhibition zone around the extracted source. (iii) Diffusion Method: In gelatin diffusion test methods or Kirby-Bauer diffusion tests (Puspita *et al.*, 2021), antimicrobial compounds contained in the aromatic galangal extract spread from the extracted area to the surrounding media. At higher extract concentrations, the spread of the antimicrobial compounds was greater, creating a larger inhibition zone. According to several researchers, using the diffusion method, a higher concentration of ethanol extract from *Aloe vera* leaves and roots (15 µL, 20 µL, 25 µL, and 30 µL) provided a larger inhibition zone against Gram-negative, Gram-positive, and fungal pathogenic bacteria (Danish *et al.*, 2020). (iv) Penetration into Bacterial Cells: The active compounds in the aromatic galangal extract may be more efficient at penetrating bacterial cell membranes and disrupting vital cellular biological processes at higher concentrations. This increased the ability of the extract to inhibit bacterial growth. This has been supported by several studies; antimicrobial activity depends on the type of solvent used for extraction and the concentration of the plant material (Ibrahim and Kabede, 2020). Phytochemical compounds, such as alkaloids, steroids, saponins, flavonoids, tannins, terpenes, phenolic compounds, and cardiac glycosides, have been observed in various *Ocimum americanum* extracts. Alkaloids, saponins, and terpenes were found in the acetone extracts. Steroids and cardiac glycosides are present in the methanol extract, whereas saponins, flavonoids, tannins, and phenolics are present in the ethyl acetate extract (Widodo *et al.*, 2021).

E. coli has the highest sensitivity to aromatic galangal extract compared to other bacteria. At the same extract concentration, namely 0.75 %, the inhibition zone was the highest, namely 7.98 mm ± 1.77 mm, at a concentration of 50.00 %, the inhibition zone was 19.67 mm ± 1.71 mm. Several factors that influence this condition are (i) Cell Membrane Composition: *E. coli* is a gram-negative bacterium that has an outer membrane consisting of lipopolysaccharides, whereas *Staphylococcus aureus, Clostridium,* and *Streptococcus* were Gram-positive bacteria that have thick cell walls consisting of peptidoglycan. These components can affect the active compounds in the aromatic galangal extract that interact with the bacteria. Active compounds may penetrate the outer membrane of *E. coli* more easily than the cell walls of *Staphylococcus aureus, Clostridium, and Streptococcus*. (ii) Enzyme structure and function: Enzymes involved in bacterial cell metabolism and defence may differ between *E. coli* and other bacteria. These differences may affect the effectiveness of the active compounds in aromatic galangal against each bacterium. (iii) Active Compound Content: Aromatic galangal extract contains active compounds such as alkaloids,

flavonoids, monoterpenoids, diterpenoids, and essential oils with antimicrobial activity (Pham *et al*., 2021). These compounds may be more effective in inhibiting the growth of *E. coli* than other bacteria. Phytochemical screening has revealed several secondary metabolites, such as alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, saponins, tannins, triterpenes, and steroids. Several molecules are known to be active against various pathogens. The differences in the antibacterial activities of the extracts may be due to the differences in their chemical compositions and bioactive mechanisms (Hemeg *et al*., 2020). The ethanol extract of aromatic galangal contains several active compounds including alkaloids, flavonoids, tannins, and phenolics, but not saponins. Galangal rhizomes contain alkaloids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins, flavonoids, steroids, and terpenoids (Susanto *et al*., 2020).

Polyphenols, including *Streptococcus, Staphylococcus,* and *E. coli*, exhibit antibacterial activities against pathogenic bacteria in the intestine. Polyphenols can damage bacterial cells through various pathways, including reactions with proteins, inhibition of nucleic acid synthesis by bacterial cells or DNA damage, interaction with bacterial cell walls or inhibition of cell wall formation, and changes in the function of the cytoplasmic membrane, such as modification of membrane permeability or fluidity, damage to the cytoplasmic membrane and consequent membrane disruption, inhibition of energy metabolism, changes in cell attachment, inhibition of biofilm formation, and the seizure of substrates and metals (Nanasombat *et al*., 2018).

Flavonoids are a group of phytochemical compounds found in abundance in plants and are known to have a variety of biological activities, including antimicrobial activity. The mechanism by which flavonoids kill bacteria involves several processes, including inhibition of bacterial cell wall synthesis, damage to cell membranes, inhibition of enzymes, stimulation of the formation of free radicals, inhibition of biofilm formation, interaction with bacterial DNA, and disruption of genetic replication and transcription. This explanation is based on several reports (Hotimah and Herdianty, 2024) that aromatic galangal rhizomes contain flavonoids, which are the most abundant phenolic compounds found and inhibit many enzymatic and non-enzymatic oxidation reactions. The mechanism of action of flavonoids as antibacterial agents involves the formation of complex compounds with extracellular proteins and the disruption of the integrity of bacterial cell membranes, thereby inhibiting the function of microbial cells.

Alkaloids are organic compounds often found in various herbal plants and have significant biological effects, including antibacterial activity. The mechanism of action of alkaloids in damaging bacterial cells generally involves several mechanisms depending on the type of alkaloid and the bacteria affected. The common mechanisms include cell membrane disruption, inhibition of protein synthesis, disruption of nucleic acid synthesis, and inhibition of bacterial metabolic enzymes. This description is based on a report stating that alkaloids represent a new type of potential natural antibiotic with broad-spectrum antibacterial activity. The main antibacterial mechanisms include the inhibition of bacterial cell wall synthesis, changes in cell membrane permeability, inhibition of bacterial metabolism, and inhibition of nucleic acid and protein synthesis. Alkaloids are effective against methicillin-resistant *S. aureus* (MRSA); therefore, they can be developed as new antibacterial Hafsan *et al*. (2021). Among the antibacterial agents, berberine (BER), produced by plants, is one of the main components of alkaloids and is known to exhibit broad-spectrum antimicrobial activity against various microorganisms, including MRSA. BER has been reported to exhibit antimicrobial activity against almost all MRSA strains tested, with minimum inhibitory concentrations of MIC ranging from 32 μg mL^{-1} to 128 μg mL^{-1} (Zhang *et al*., 2020).

Evaluation of MIC and MKC of aromatic galangal ethanol extract to pathogenic bacteria

Table 2 shows the best sensitivity of *E. coli* to aromatic galangal extract because, at the lowest extract concentration (0.125 %), the bacteria experienced growth inhibition. However, up to a concentration of 50.00 %, the bacteria did not reach the MKC, indicating that up to a concentration of 50.00 %, the *E. coli* bacteria were not killed. *E. coli* was the cause of colibacillosis which often attacks poultry and is detrimental to the economy and public health. Several studies have reported that colibacillosis can result in high morbidity and mortality (up to 20 %), reduced meat and egg production, and increased contamination (up to 43 %) of carcasses in chicken slaughterhouses, causing major economic losses in all

Table 2: *Average minimum inhibitory concentration (MIC) and minimum kill concentration (MKC) of aromatic galangal ethanol extract to pathogenic bacteria.*

No.	Gut bacteria	Test	Aromatic galangal ethanol extract concentration (%)							
			0.125	0.250	1.500	3.125	6.250	12.500	25.000	50.000
1.	Clostridium	1.		MIC	MKC					
		2.		MIC	MKC					
		3.		MIC	MKC					
2.	Campylobacter	1.		MIC	MKC					
		2.		MIC	MKC					
		3.		MIC	MKC					
3.	P. multocida	1.							MIC	
		2.							MIC	
		3.							MIC	
4.	Salmonella	1.					MIC			
		2.				MIC				
		3.				MIC				
5.	S. aureus	1.		MIC	MKC					
		2.		MIC	MKC					
		3.		MIC	MKC					
6.	Streptococcus	1.			MIC					
		2.			MIC					
		3.			MIC					
7.	E. coli	1.	MIC							
		2.	MIC							
		3.	MIC							

aspects of the global poultry industry (Lokaewmanee and Sirival., 2022). The aromatic galangal extract inhibited *E. coli* at a very low concentration (0.125 %), which was higher than that of the neem leaf extract. Ali *et al.* (2021) reported that the MIC of neem leaf extract against *E. coli* was only achieved at a concentration of 100 mg $mL^{-1.}$

Based on Table 2 shows that MKC occurs at a concentration of 1.50 % aromatic galangal extract. The bacteria that can be killed at low concentrations are *Clostridium, Campylobacter*, and *S. aureus.* The *bacteria P. multocida, Salmonella, Streptococcus,* and *E. coli* were not killed by aromatic galangal extract. This shows that there were pathogenic bacteria that could not only be inhibited by aromatic galangal extract but also pathogenic bacteria that could be killed.

The results of this study showed that the sensitivity of *Salmonella* to aromatic galangal extract was higher than that of neem leaf extract (*Azadirachta indica A. Juss*) because the inhibition of bacterial growth occurred in the aromatic galangal extract at concentrations ranging from 3.125 % to 6.25 %, whereas it did not occur in the neem extract. Ali *et al.* (2021) stated that the growth of *S. pullorum* was not inhibited at a concentration of 6.25 mg mL^{-1} and 3.125 mg mL^{-1} neem leaf extract; likewise, the growth of *Salmonella gallinarum* was only achieved at a concentration of 50 mg mL^{-1}. Other researchers have reported that the ethyl acetate extract from *Anopheles arabaiensis* leaves and the methanol extract from the leaves showed the highest antibacterial activity against *Salmonella gallinarum*, with an MIC value of 0.309 mg mL^{-1} However, 12 extracts (70.59 %) showed moderate antibacterial activity, with MIC ranging from 0.781 mg mL^{-1} to 12.5 mg mL^{-1} (Rahmi *et al.*, 2016).

Conclusions and Recommendations

E. coli has the highest sensitivity to aromatic galangal extract compared to other bacteria. At the same extract concentration, which was 0.75 %, the inhibition zone was the highest, which was 7.98 mm ±1.77 mm, at a concentration of 50.00 %, the inhibition zone is 19.67mm ± 1.71 mm. The aromatic galangal extract inhibited *E. coli* at a concentration of 0.125 %, although it was not sufficient to kill the bacteria. As conclusion demonstrated that aromatic galangal extract is only associated with the inhibition of pathogenic bacteria but also have the ability to kill theses pathogenic bacteria isolated from the digestive tract of Super Kampong chickens.

Links Researchers

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Novelty Statement

Previous studies have not specifically examined aromatic galangal extracts as feed additives for Super Kampong chickens. The studies that have been conducted are generally not on the extract of aromatic galangal but on the use of aromatic galangal in poultry in general, such as broiler chickens and laying hens. Therefore, the novelty of this study is confirmed.

Author's Contribution

Wahyu Widodo: Conceptualized and designed the study, elaborated the intellectual content, performed literature search, manuscript preparation, and manuscript revision.
Adi Sutanto: Performed literature search and manuscript review
Imbang Dwi Rahayu: Research supervision, and elaborated the intellectual content
Apriliani Devi Anggraini: Statistical analysis.
Trisakti Handayani, Ivar Zekker, and Bayu Agung Prahardika: Performed a literature search and reviewed the manuscript.
Yuanara Augusta Rahmat Adikara: Administration, turnitin check, grammar check, rewrite.
All authors have read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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