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In Vitro study of Antibacterial activity of Combination of Water extract of Turmeric and Garlic

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Abstract

This research was conducted to evaluate the level of the water extract of the combined aqueous extract of turmeric and garlic (TGE) as a feed additive that has a function as an antibacterial against *Lactobacillus*, *Salmonella*, and *E. coli* bacteria by the agar well diffusion method. Antibacterial activity test using a completely randomized design with 6 treatments unidirectional pattern 3 replications followed by Duncan's test 's New Multiple Range Test (DMRT). The results showed that TGE phytobiotic capable as antibacterial and significantly ($P<0.05$) against *Lactobacillus*, *Salmonella* and *E. coli*. TGE optimal concentration of 2.5% with ratio of 1TE : 3GE. The conclusion of this study that TGE capable as antibacterial and can be used as an alternative to feed additive antibiotic growth promoters (AGPs).

Key-Words: Phytobiotic, Feed additive, Antibacterial activity

Introduction

Treatment with antibiotics in addition to expensive can have a negative impact on the health of livestock if the resulting product containing residues[1]. Microbial resistance to antibiotics in human pathogens is a major public health problem. Livestock industry must reduce the use of antibiotics [2,3] in animal production and look for other alternatives in disease control to replace the use of antibiotics.

In the gastrointestinal tract of the day-old chickens contained a complex bacterial community[4]. Communities of bacteria (commensals and pathogens) in the digestive tract will interact intra bacterial communities and the host through a network of chicken digestive organs[5]. Bacterial pathogens such as *Salmonella* and *E. coli* be competing for nutrients with the commensal bacteria in the digestive tract of chicken. Utilization of phytobiotic as *Natural Growth Promoters* (NGPs) has been identified as an effective alternative to antibiotics. NGPs as a phytobiotic highly developed as a feed additive, immunity, improve performance and is highly effective in improving the health of the digestive tract[6] and stimulate livestock nutrition, antimicrobial, and antihelmintik coccidiostatic[7].

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Various herbal products were found to have beneficial properties that may be useful as an alternative to the previous *Antibiotic Growth Promoters* (AGPs) routinely used in poultry feed. The mechanism of action of phytobiotic as AGPs is associated with their ability to inhibit the growth of harmful intestinal microflora in the GIT [8,9] and stimulates the function of the digestive organs[10].

Curcumin and its biotransformastion compounds results belong to the class of compounds called polyphenols, believed to have the same ability that denature membrane proteins[11], also has the ability to attenuate and alter membrane fluidity[12]. Garlic and ginger have antibacterial power[13]. Anticancer effects[14] and its ability to induce apoptosis without showing cytotoxic effects on normal cells[15]. Turmeric and ginger spices one that has an active ingredient curcumin shaped form of phenolic compounds that can interfere with the formation of the cell membrane of some pathogenic bacteria such as *Salmonella* and *Escherichia coli*, may also increase the secretion of the salivary glands, bladder, stomach, pancreas and intestines. Biological response to garlic among others, include reduced risk of cancer and anti-tumor, immune function stimulation[16, 17, 18, 19, 20, 21, 22]. Allicin reported to be effective antibacterial activity against a large number of Gram positive and Gram negative including *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiela*, *Proteus*, *Bacillus*, *Clostridium*, *Lactobacillus* and *Coliform*, and some anaerobic bacteria[23, 24, 25, 26].

Resistance of pathogenic bacteria in humans is a problem faced today. Utilization TGE phytobiotic as a feed additive in one of its functions is as an antibacterial, can be used as an alternative to the use of antibiotics with regard to its use in optimal levels in the ration. This study was conducted to determine the potential TGE phytobiotic as an antibacterial *in vitro* against bacteria *Lactobacillus, Salmonella,* and *E. coli*. This study enhance previous research that has not been set when the ratio of the two is able phytobiotic combined synergistically to strengthen the function of each phytobiotic.

Material and Methods

Sample collection

Turmeric Turina-2 obtained from Balai Tanaman Obat Aromatik, Bogor, West Java of Indonesia, with 10-month maturity, garlic kathing of traditional markets. The collected turmeric samples were washed with seawater and then in fresh water and extraneous matters were removed. After that they were brought into the laboratory in sterile plastic bags.

Preparation of turmeric and garlic extract

Both garlic and turmeric herbal washed with destilled water thoroughly. Five hundred gram of garlic in a blender with water added as many as 2500 ml of distilled water. Turmeric herbs blended with 100 g of fresh turmeric ratio plus 800 ml of water. Old herbal blend of approximately 30 minutes. Furthermore, the filtration process is carried out for 1,5 hours. Furthermore, filrat obtained approximately 4 hours evaporated to obtain the extract. Extracts stored in the refrigerator at 4°C.

Test organisms

Extracts were tested against three bacterial stains *Lactobacillus, Salmonella* and *Escherichia coli. E. coli* and *Salmonella* cultures obtained from a culture collection portion of Microbiology, Faculty of Veterinary Medicine Gadjah Mada University enteritik isolated from cases that occurred, and *Lactobacillus sp.* is a private collection that were isolated from the digestive tract of broilers.

Preparation of bacterial media

Before the specimens cultured, made the media the way cooked NA, NA as much as 2 g dissolved in 100 ml of distilled water, and then heated over a medium magnetic stove to boil so that the NA medium is completely dissolved. Then using autoclave sterilization for 15 minutes at a pressure of 2 atm, temperature 121°C. Media MHB was made to test the antibacterial activity. MHB media used to see the MIC, prepared by dissolving 4.2 grams of powdered media MHB in distilled water to 200 ml. The solution was heated until the powder is completely dissolved, then put into a test tube and sterilization using autoclave for 15 minutes at a pressure of 2 atm, 115°C. Once sterilized, the media stored in the refrigerator. If it will be used again, the media reheated to boiling and then poured into each petri and allowed to cool.

Preparation of specimens

Breeding specimens was performed in an aerobic. Further breeding on NA media that have been provided previously in a petri dish. Bacterial cultures were incubated in an aerobic atmosphere at a temperature of 37^0C for 24 hours, then observed whether the bacteria have flourished and obtained pure. If the bacterial growth occurs infertile and other bacterial or fungal contamination, bacterial culturing and observation procedure is repeated until cultures are obtained completely pure. Then as many as 1-2 ose from a pure culture of the test bacteria were suspended in 0.9% NaCl solution in a sterile test tube. Then the bacterial suspension homoginazed with a vortex. Mc Farland turbidity standards-compliant or proportional to the number of bacteria $1x10^8$ CFU/ml.

Making concentrations of sample

Materials made treatment TGE with comparison (1TE: 3GE; 2TE: 2GE; 3TE: 1GE) at a concentration (1.00; 1.50; 2.00, and 2.50%) and tetracycline 10 ppm and 0.015% Zinc basitracin as a positive control in the inhibition of bacterial pathogens (*Salmonella* and *E. coli*) and non-pathogenic bacteria (*Lactobacillus* sp.).

Antibacterial activity test materials

BHA media as much as 20 ml was poured in a petri dish and allowed to stand at room temperature until hardened/solid. The media then inoculated bacteria will be inhibited (*E.coli*, *Salmonella* and *Lactobacillus*), then make the wells by using a ring punching holes (modification). A total of 50 ml of each concentration of the media trying to put into the hole and incubated for 24 hours at 37°C. The amount of antibacterial activity was measured from a rather clear zones formed around the hole samples using calipers. Unit diameter measurement in millimeters (mm).

Statistical analysis

Data for antibacterial activity test were analyzed using analysis of variance (ANOVA) with a completely randomized design (CRD) unidirectional pattern with 6 treatments and 3 replications and processed with the help of (software SPSS ver.18). Results presented are means (3 replicates ± SD). *Duncan's New Multiple Range Test* (DMRT) was used for the separation of means. A probability level of $P<0.05$ was chosen to indicate the significant differences[27].

Results and Discussion

Antibacterial Activity Test of combination of the water extract of turmeric and garlic (TGE)

Diameter of zone of inhibition testing TGE antibacterial activity against bacteria *Lactobacillus, E. coli* and *Salmonella* is shown in Table 1. Applicability combination of 2 or 3 types of herbs are already some researchers are using both the water extract, methanol and methanol. *In vitro* antibacterial activity against *S. aureus, S. typhi* and *E. coli* from some kind of herb (*Allium sativum, Zingiber officinale, Curcuma longa, Azadirachta indica*) either singly or in combination in the form of aqueous extracts and extract ethanol. The results showed that the water extract of *Allium sativum* combinations and *C. longa* has the highest inhibition zone against all types of bacteria[28].

Among other combinations of types of herbal extracts showed water gave the best results. It is estimated that the content of the active compounds contained in *Allium sativum* and *C. longa* has a synergistic effect as an antibacterial. This means that the water extract of garlic and turmeric phytobiotic potential which indicates that water is a better extractant to take an active compound of this type phytobiotic. Water is a solvent mostly used for the preparation of infusions of herbs.

The combination of garlic and cloves, the combination of garlic, turmeric, and black pepper on antimicrobial test against *E. coli, S. ratti, S. aureus* and *B. cereus* showed positive results[29]. The combination of *Citrus limon, Allium sativum* and *Ocimum basilicum* showed strong antibacterial activity against Gram negative bacteria (*K. pneumoniae, P. aeruginosa, P. vulgaris, S. boydii, S. paratyphi* A and *S. paratyphi* B) and Gram positive (*S. epidermidis* and *S. faecalis*)[30]. The inhibition zone combination of garlic and turmeric (ratio 1:1) with 2 kinds of solvents, namely methanol and water against bacteria *Xanthomonas campestris*, showed the combination of the water solvent gave higher inhibition zone (21.90 mm), while the methanol (20.90 mm)[31].

The hypothesis has been expressed by different researchers reported that the mechanism of action of the antibacterial herbs involving hydrophobic and hydrogen bonding of the phenolic compounds of membrane proteins, membrane disruption and destruction of the electron transport system and cell wall disruption[32]. Antimicrobial activity of aqueous extracts can be derived anionic components such as thiocyanate, nitrate, chloride and sulfate in addition to other water-soluble components that occur naturally in plant material[33]. TGE combination with the best concentration results of this study of the zone of inhibition against *Lactobacillus, Salmonella* and *E. coli* bacteria is a combination TGE (1TE:3GE) at a concentration of 2.5%. Considerations for selecting a concentration of 2.5% was the absence of inhibition zone on *Lactobacillus* bacteria as bacteria and bacteria that are expected not inhibited and the bacteria *Salmonella* and *E. coli* have the highest inhibitory concentrations than the other.

Conclusion

Combination of turmeric and garlic water extrcat phytobiotic have antibacterial activity against bacteria *Lactobacillus, Salmonella* and *E. coli* as well as the optimal concentration of 2.5% with ratio 1TE : 3GE.

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Table 1: Antibacterial activity of different concentration of TGE against *Lactobacillus, S. pullorum* and *E. coli* by diffusion method

Concentration (%)	Diameter of inhibition zone (mm)		
	Lactobacillus	*S. pullorum*	*E. coli*
		1TE: 3GE	
1.0	$4.88^{c} \pm 0.25$	$0.00^{a} \pm 0.00$	$9.25^{b} \pm 0.29$
1.5	$4.13^{b} \pm 0.25$	$4.38^{b} \pm 0.25$	$9.75^{c} \pm 0.50$
2.0	$5.38^{d} \pm 0.25$	$9{,}63^{c} \pm 0.48$	$9.75^{c} \pm 0.29$
2.5	$0.00^{a} \pm 0.00$	$10.13^{d} \pm 0.48$	$11.56^{d} \pm 0.13$
		2TE: 2GE	
1.0	$5.00^{b} \pm 0.41$	$0.00^{a} \pm 0.00$	$9.38^{b} \pm 0.48$
1.5	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$10.00^{b} \pm 0.71$
2.0	$5.00^{b} \pm 0.00$	$10.00^{b} \pm 0.41$	$9.63^{b} \pm 0.48$
2.5	$0.00^{a} \pm 0.00$	$11.25^{c} \pm 0.50$	$9.75^{b} \pm 0.29$
		3TE: 1GE	
1.0	$4.63^{b} \pm 0.48$	$0.00^{a} \pm 0.00$	$9.25^{b} \pm 0.50$
1.5	$6.00^{d} \pm 0.00$	$0.00^{a} \pm 0.00$	$10.50^{c} \pm 0.41$
2.0	$5.13^{c} \pm 0.25$	$9.63^{b} \pm 0.48$	$9.63^{b} \pm 0.25$
2.5	$4.38^{b} \pm 0.48$	$10.13^{b} \pm 0.48$	$10.88^{c} \pm 0.25$

^{abcd} Different superscript at the same colom indicate significantly different ($P<0.05$).

Table 2: Antibacterial activity of standar antibiotics

Bacterial strains	Antibiotics	
	Tetracycline	Zinc bacitracin
Lactobacillus	26.50 ± 0.41	14.13 ± 0.25
Salmonella	27.75 ± 0.29	14.50 ± 0.41
E. coli	25.38 ± 0.25	13.38 ± 0.25

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