In vitro study: optimization of antimicrobial activity of the kapok leaves extract (*Ceiba pentandra* (L.) Gaertn.) using nanoencapsulation technology

Muhammad Hidayat, Zuprizal*, and Bambang Ariyadi

[1] Faculty of Animal Science, Universitas Gadjah Mada, Indonesia

Corresponding author: zuprizal@ugm.ac.id

Abstract. This research aims to identify the antimicrobial activity of the kapok leaves extract (*Ceiba pentandra* (L.) Gaertn) that were used as a feed additive for broiler chicken ration. The research was conducted with a directional pattern completely randomized design. The method applied was agar well diffusion method. The treatment was divided into positive control/ tetracycline 100 mg/L antibiotic (P1); negative control/aquadest (P2); kitosan 2,5 mg/ml (P3); Sodium tripolyphospate/STPP 7,5 mg/ml (P4); kapok leaves extract (KLE) 20 mg/ml (P5); and kapok leaves nanoencapsulation extract (KLNE) 20 mg/ml (P6). Each treatment was divided into three replications. The inhibition test was conducted to *Salmonella typhimurium, Escherichia coli, Staphylococcus aureus,* and *Lactobacillus acidophilus* bacteria. The research result showed that the KLNE application improved the antimicrobial activity ($P<0.05$) better than that of KLE and negative control. However, it was still lower ($P<0,05$) than the positive control, except *Escherichia coli* that has higher antimicrobial activity ($P<0.05$) than the positive control. Based on the result, it was identified that the nanoencapsulation technology can optimize the antimicrobial activity of kapok leaves extract.

1. Introduction

The use of antibiotics as a feed additive for broiler chicken extensively will bring negative effect caused by the accumulation of antibiotic residue that cannot be absorbed by the broiler chicken, which also results in environmental pollution [1]. The residue accumulation also exists in the broiler chicken meat [2] that can cause bacterial resistance for the human who consumes those broiler chicken meats. Bacterial resistance will harm the human body as if these resistant bacteria infected the human, the dosage of antibiotic given will increase from second until the third line and it will cause the length of the health recovery [3]. Based on this occurrence, the use of antibiotics has been prohibited in certain areas, including Indonesia. This prohibition has been stated in Indonesian government by Permentan No. 14 Year 2017. It needs to seek alternatives to replace antibiotics as a feed additive for broiler chicken which is safe from the pathogenic bacterial resistance.

Various kinds of plants in the world are acknowledged to contain various active compounds. The active compounds such as flavonoid, saponin, tannin, terpenoid, and alkaloid are identified to have activities as antimicrobial [4]. The existence of active compounds in these plants has similar function as antibiotics as a feed additive for broiler chicken. The antimicrobial activities of the active compounds will benefit to broiler chicken as it can condition the digestive tract profile of broiler chicken, especially in the intestine. The protected intestine will absorb the nutrient to the broiler chicken's body optimally. A fulfilled nutrient will give effect for a better broiler chicken growth.

Kapok plant (*Ceiba pentandra* L.) is one of the plants that contain many active compounds on its roots [5]; stem bark [6-7] and leaves [6,8]. It is also known that the largest amount of active compounds is in the leaves [7]. Some researches show that kapok leaves contain active compounds like flavonoid, tannin, alkaloid, and saponin [8-9]. The research result showed that with an in vitro test, the extract of kapok leaves had presented an antimicrobial activity on the dosage 7.5 mg/ml and showed an inhibition effect of bacteria *Klebsiella pneumonia, Pseudomonas aeuroginosa, Salmonella thypimurium, Escherichia coli, Staphylococcus aureus,* dan *Bacillus subtilis* [7,10].

One of the weakest points of the phytochemical active compound is the low bioavailability, stability, absorption, and specific target [11] when it enters the digestive tract. It because the condition of the digestive tract is very complex with a quick alter of pH within digestive organs. Besides, the macro phytochemical compound has not been dissolved well in the water [12] and there is no specific receptor for the absorption process of this phytochemical in the intestine [13].

The nanoencapsulation technology with ionic gelation method is one of the applications that can be a solution to maximize the role of active compounds in the digestive tract. The benefit of nanoparticle is its ability to penetrate higher cell wall, either through diffusion or opsonification, and its flexibility to be combined with other various technologies, so it can open a wide potency to be developed on various needs and targets [14]. It becomes a benefit as the encapsulated extract of kapok leaves will maximize the antimicrobial function on the digestive tract of broiler chicken. In addition, there will be more optimum absorption of active compounds in the lumen of the small intestine, which will be beneficial for the metabolism of broiler chicken. The objective of the present study is to compare the antimicrobial activity of the kapok leaves extract with and without nanoencapsulation technology to that of antibiotic use, aquades as positive and negative control and also chitosan and STPP as the nanoencapsulation ingredients.

2. Material and Methods

2.1. The extraction of kapok leaves

Fresh kapok leaves were drained using an oven with temperature 55°C for 3 days. Dry leaves were ground by using the Willey mill. Kapok leaves powder were macerated by using ethanol 70% [15] with the ratio of powder: ethanol is 1:5 (g/ml) [16] for 3 days, and stir it once a day. The solution was then distilled by using coarse filter paper so the pulp and the filtrate were separated. The filtrate was soaked in the water bath with temperature 55°C for being evaporated with ethanol until resulted in kapok leaves extract. The kapok leaves extract was dissolved in the aquadest with ratio extract:aquadest as much as 2:100 (g/ml) to result in kapok leaves extract (KLE) 20mg/ml.

2.2. Preparation of the Nanoencapsulation Kapok Leaves Extract.

KLE as much as 20mg/ml was mixed with Chitosan 0.25% (2.5 mg/ml) that had already dissolved before with a citric acid solution 2.5% for 30 minutes. The next, dissolve the mixture of KLE-chitosan in the aquadest for 30 minutes. Then, add this dissolved mixture with Sodium Tripolyphosphate (STPP) 0.75% (7.5 mg/ml). The mixing process used blender Philips HN-2116 with ratio KLE:Chitosan: STPP was 1:9:1/60 to result in kapok leaves nanoencapsulation extract (KLNE).

2.3. Bacteria Inhibition Test

The bacteria inhibition test was conducted through agar well diffusion method by growing the bacteria culture on the Mueller-Hinton agar media (MHA). It was designed into four treatments with four replications in which each of them was tested with four bacteria such as *Escherichia coli, Staphylococcus aureus,* dan *Salmonella typhimurium*, and *Lactobacillus acidophilus*. The treatment was designed with Completely Randomized Design (CRD) with one way ANOVA as follows: 1) positive control/ tetracycline antibiotic 100 mg/L; 2) negative control/aquadest; 3) khitosan 2,5mg/ml; 4) sodium tripolyphospate (STPP) 7,5 mg/ml; 5) kapok leaves extract with dosage 20 mg/ml; 6) kapok leaves nanoencapsulation extract with dosage 20mg/ml.

3. Result and Discussion

The result of in vitro bacteria inhibition test analysis can be seen in Table 1 below:

Table 1. The result of in vitro bacteria inhibition test analysis

Treatment	The width of bacteria inhibition zone (mm)			
	*Escherichia coli**	*Salmonella thypimurium**	*Staphylococcus aureus**	*Lactobacillus acidophillus**
Tetracycline 100 mg/L	$5{,}17 \pm 0{,}8^{b}$	$16 \pm 0{,}94^{c}$	$10{,}58 \pm 1{,}84^{d}$	$10{,}19 \pm 1{,}17^{c}$
Aquades	-	-	-	-
Kitosan 2,5 mg/ml	$8{,}6 \pm 1{,}39^{c}$	$5{,}65 \pm 0{,}97^{b}$	$7{,}23 \pm 1{,}75^{c}$	$7{,}48 \pm 1{,}1^{b}$
STPP 7,5 mg/ml	-	-	-	$3{,}04 \pm 0{,}78^{a}$
LNE 20 mg/ml	$3{,}88 \pm 0{,}74^{a}$	$2{,}67 \pm 0{,}63^{a}$	$3{,}1 \pm 0{,}36^{b}$	-
KLNE 20 mg/ml	$8{,}08 \pm 1{,}12^{c}$	$5{,}98 \pm 0{,}82^{b}$	$6{,}63 \pm 1{,}41^{c}$	$6{,}44 \pm 0{,}77^{b}$

* : significant (P<0,05)

abc : different superscript on the same column shows a real difference (P<0.05)

The above table shows the increase of bacteria inhibition activity in general when the extract was available in the form of nanoencapsulation (KLNE) than KLE. This increase can be seen based on the width of the inhibition area of bacterial growth on the dish media. The difference of the particles' size in the smaller nanometer scale can be a cause of optimization of the active substance in the kapok leaves extract that can inhibit the bacterial growth. Flavonoid as the active compound is much available in the kapok leaves extract [7,10] in this case, it will be easier to do penetration to damage the cell membrane of bacteria. The other factors that influence the inhibition are the content of chitosan and STPP that also have antibacterial activities [15] and also showed on the Table 1 above. It indicated that Chitosan and STPP also have antibacterial activities. In addition, the Chitosan activity showed a real difference (P>0,05) to KLNE. According to this result, it can be identified that chitosan as nanoencapsulation ingredient is potential for the intensification of antimicrobial activity of KLNE.

The inhibition activity of *Escherichia coli* with KLNE was bigger (P<0.05) than the other addition. The value of kapok leaves extract inhibition was 3.88 ± 0.74 with dosage extract 2% or 20 mg/ml has relatively the same result compared to the other research that shows inhibition 2 mm for dosage 15 mg/ml [7] also 6.5 mm and 8 mm for dosage 30 mg/ml [7,10]. *Escherichia coli* is one of the pathogenic bacteria [16] and it exists in the digestive tract of chicken especially in its intestine [17]. There is also *Escherichia coli* species that is not pathogenic whose role is as decay bacteria and many exist in the caecum of digestive tract [18]. The previous study showed broiler chicken that is challenged with *Escherichia coli* results in negative effect by the decrease of weight gain, villi's height and width, the ratio of villi's height:crypta, and also the decrease of immunity response [19]. The existence of inhibition to bacterial growth caused by KLE or KLNE can be beneficial to maximize the process of nutrient absorption in small intestine.

The inhibition activity of *Salmonella typhimurium* resulted in the best activity for the administration of tetracycline antibiotic 100 mg/L. This bacteria species is also pathogenic with salmonellosis as the main disease and can easily attack a human who consume infected broiler chicken meats [16]. The other research shows that broiler chicken that is infected by *Salmonella typhimurium* showing a decrease in the body weight gain and feed conversion [20]. KLNE supplementation, in fact, had not given better inhibition than tetracycline 100 mg/L, but at least activity increase happened, compared to plain kapok leaves extract. This result was relatively same with the previous research that showed inhibition zone of kapok leaves extract to *Salmonella typhimurium* as large as 1 mm for dosage 15 mg/ml and 7 mm for dosage 30 mg/ml [7].

The administration of tetracycline antibiotic 100 mg/L also showed the best result of inhibition activity to *Staphylococcus aureus*. The value of KLE was 3.1 $\pm$ 0.36 mm. Compared with previous research, the value of inhibition for the studied KLE was relatively lower that showed inhibition zone 3 mm for dosage 15 mg/ml [7] and 6 mm and 8 mm for dosage 30 mg/ml [7,10]. However, this inhibition value was better after formed into nanoencapsulation as large as 6.63 $\pm$ 1.41 mm.

Staphylococcus aureus is pathogenic gram-positive bacteria that grow on the broilers' feet [21], skin [22] to respiratory cavity [23] and is included of facultative bacteria species. This bacteria also exist in the broiler chicken digestive tract [24]. It can also inhibit the growth of receptor on the skin epidermis that can cause skin inflammation [22]. A more detailed explanation mentions that *Staphylococcus aureus* infection is not only on the skin tissue, it also infects osteoarticular pleuropulmonary, and ureter. This infection will harm the broiler chicken during the cultivation andwill bother the chicken performance [25]. Therefore, inhibition activity to *Staphylococcus aureus* by KLE or KLNE will be a good investment to constrain the growth of these bacteria in the broiler chicken body.

The inhibition activity of *Lactobacillus acidophilus* was identified resulted in the best activity for the administration of tetracycline antibiotic 100 mg/L. While for KLNE also higher 6,44 $\pm$ 0,77 than KLE that was no inhibition zone. It became an exception as it is unfavorable because these bacteria are good to stimulate the optimization of nutrient absorption in the digestive tract of broiler chicken. The other research explains that *Lactobacillus acidophilus* added to broiler chicken diet, in fact, increase the population of bacteria in the small intestine [26]. *Lactobacillus acidophilus* addition also can increase the population of *Lactobacillus* and also decrease the population of pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens* [27]. This feeding treatment gives an impact on the increase of body weight and feed conversion compared to control treatment. The other positive impact of *Lactobacillus acidophilus* provides an improvement on the morphology of small intestine by the increase of villi's height and excluded the increase of crypta's depth [24].

4. Conclusion

Based on the research that has been conducted, it shows that the nanoencapsulation technology can result in the increase of antimicrobial activity from kapok leaves extract to *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus,* and *Lactobacillus acidophilus* bacteria.

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