Effectiveness of Ketapang (Terminalia cattapa L.) Extract Against Avian Pathogenic Escherichia coli (APEC) Infections in Layer Performance

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ABSTRACT
This study aimed to determine the activity of Ketapang extract (Terminalia cattapa L.) in layers infected with APEC. In vitro study was conducted using dilution methods to determine the concentration of Ketapang extract that inhibits bacterial growth. The data were analyzed using ANOVA and Duncan’s test. In vivo study was conducted by randomly dividing 20 layers into five treatment groups, with each group consisting of three layers. The chickens were infected with APEC using a 5% solution. The data were analyzed using ANOVA and Duncan’s test. The results showed that the Ketapang extract significantly inhibited the growth of APEC in both in vitro and in vivo studies.

INTRODUCTION
Collibacillosis is a disease that often infects chicken farms. It is an acute and chronic disease caused by Avian Pathogenic Escherichia coli (APEC). Collibacillosis disease in chickens shows several symptoms, such as colisepticemia, hemorrhagic septicemia, coligranuloma (Hjarre’s disease), swollen head syndrome, venereal colibacillosis, coliform cellulitis, peritonitis, salpingitis, orchitis, osteomyelitis/synovitis, panophthalmitis, omphalitis/yolk sac infection and enteritis.1-3 It has been observed that avian colibacillosis is a major infectious disease worldwide in birds of all ages, which imparts a significant economic impact on poultry production. Losses are economical, as a result of mortality and decreased productivity of affected birds, mainly around the peak egg production period and throughout the late lay period.4-6

Collibacillosis disease can be treated using antibiotics, including tetracycline, sulpha, neomycin, and fluoroquinolone. However, any treatment exploitation using antibiotics that do not follow the control dose can cause bacteria to become antibiotic-resistant; thus, it will complicate the process of preventing and treating the disease.1,4-6

Herbal medicine is an alternative used to prevent pathogenic bacterial infection. Ketapang (Terminalia cattapa L.) is an herbal plant often used for medicine. Ketapang extract contains antibacterial substances; therefore, it can be used to inhibit bacterial growth. Besides, this extract also consists of bioactive compounds that have antibacterial activity from tannins, saponins and flavonoids. Consequently, the provision of herbal plants can allegedly stabilize chickens’ health conditions and increase the efficiency of chicken feed.7,11

Specifically, the Ketapang herbal plant’s active substances inhibit and kill the bacteria, including flavonoids, tannins, and saponins. For example, flavonoids work by denaturing the proteins in the cell walls, consequently changing the structure and mechanism of permeability in the cell walls of bacteria. Furthermore, tannins serve to inactivate the enzymes and interfere with cell protein transport. As for saponins, its mechanism damages the bacteria’s cell membranes, releasing important components, such as proteins, nucleic acids, and nucleotides; thus, bacteria turn into lysed.12-16 Previous studies have discussed the antibacterial activity of Ketapang against various bacteria,17 but none has discussed its antibacterial activity against APEC. This study aimed to determine the activity of Ketapang extract (Terminalia cattapa L.) in layers infected with APEC.

MATERIALS AND METHOD
Ethical approval
This study was approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia (Approval No: 346/UN3/2020).

Study period and location
The study was conducted during March 2022 and April 2022 at several places in the Faculty of Veterinary Medicine, Universitas Airlangga, such as the Animal Cage Unit, the Bacteriology and Microbiology Laboratory of the Microbiology

Department, and the Pharmacology Laboratory of Basic Medical Science Department.

**Ketapang extract**

Ketapang (*Terminalia cattapa* L.) plants (10 kg) from Tawangmangu, Central Java, Indonesia, were determined and approved by Fauzi from the Center for Research and Development of Medicinal Plants and Traditional Medicines, Research and Development Agency, Ministry of Health of the Republic of Indonesia (voucher specimen: B2P2TO-OT). The plants were dried in an open and shady place for 7 days. Then, the dried Ketapang was ground and sieved to obtain 1 kg Ketapang powder form. Then, 1 kg Ketapang powder was soaked for 3 days with a maceration procedure using 3 L 96% methanol solution and stirred once daily for 3 days. Finally, the immersion of Ketapang powder was squeezed using a flannel cloth, and the extract was evaporated using a rotary evaporator at a temperature of 50°C at 0.14 kg speed.

This study implemented Ketapang extract at doses of 5%, 10%, 15%, 20%, 25% and 30%, in relation to the dosage of the extract. The extract’s diluting process was performed from high to low concentrations. A 30% dose was made using 30 g concentrated Ketapang extract, which was evaporated and dissolved in a diluent solution (1% Na CMC solution) until the solution reached 100 mL. Then, a total of 60 mL extract solution was put into a bottle and labeled with a 30% dosage, while the remaining 40 mL extract solution was dissolved again by adding 20 mL 1% CMC Na solution. Furthermore, the new solution was divided into three parts; each part was a different concentration (mg/mL) of the Ketapang extract resulting in clear broth incubated for 24 h at 37°C. MIC was determined and then incubated at 37°C for 24 h. If there was no bacterial growth, it indicated that Ketapang extract inhibited APEC growth indicated by forming a clear area on the bacterial colonies on the nutrient agar media.

**RESULTS AND DISCUSSION**

The antibacterial activity of Ketapang extract against APEC could be displayed using the dilution method, which included MIC and MBC. The results of these examinations are shown in Figure 1. The MIC test results could be presented by observing the clarity or turbidity in the test tube of the Ketapang extract before and after being incubated for 24 h at 37°C. MIC was defined by the result of the lowest concentration (mg/mL) of the Ketapang extract resulting in a clear broth media. The MIC test results could be presented by observing the clarity or turbidity in the test tube of the Ketapang extract before and after being incubated for 24 h at 37°C. MIC was confirmed by looking at the minimum bactericidal concentration (MBC) results.

**Ketapang extract antibacterial activity against APEC in vivo**

This study had a completely randomized design. Twenty layers were reared from the age of 24 weeks in battery cages; fed, and watered using ad libitum. Before treatment period, the chicken was adapted in 7 days. The layers were divided into five equal groups consisting of four layers per group. This division was based on the Federer formula. Next, those chickens (except in the P0− and P0+ groups) were administered orally with Ketapang extract by group from day 8 to day 14. Then, in day 15th the layers infected with APEC with a concentration of 10^8 cells/mL as much as 1 mL/head intramuscularly (except in the P0- group). They were observed on which clinical symptoms were caused by APEC infection. Furthermore, Ketapang extract was administered orally from day 15 to day 21 along with the calculation of their daily feed consumption and hen day production. At the same time, their feed conversion was calculated after obtaining the data of feed consumption and egg production. The treatment groups were as follows: P0−: A group of four layers that were not administered with any Ketapang extract nor APEC but given distilled water as a substitute for the extract. P0+: A group of four layers that were unadministered with any Ketapang extract but given with APEC as a substitute for the extract and infected with APEC intramuscularly with a concentration of 10^8 cells/mL as much as 1 mL/head.

**DILUTION METHOD**

**Determination of minimum inhibitory concentration (MIC)**

MIC was used to determine the minimum concentration of an antibacterial solution that could inhibit bacterial growth. MIC was performed by preparing 15 test tubes; tubes 1-3 were added with 3 mL 5% Ketapang extract, tubes 4-6 with 3 mL 10% Ketapang extract, tubes 7-9 with 3 mL 15% Ketapang extract, tubes 10-12 with 3 mL 20% Ketapang extract, and tubes 13-15 with 3 mL 25% Ketapang extract, tubes 16-19 with 3 mL 30% Ketapang extract. Then, each concentration was combined with APEC suspension (1x10^8 CFU/mL) as much as 3 mL per concentration, then incubated at 37°C for 24 h. Consequently, the MIC results could be presented by observing the clarity or turbidity in the test tube of the Ketapang extract before and after being incubated for 24 h at 37°C. MIC was defined by the result of the lowest concentration (mg/mL) of the Ketapang extract resulting in clear broth media. The MIC test results could be presented by observing the clarity or turbidity in the test tube of the Ketapang extract before and after being incubated for 24 h at 37°C. MIC was confirmed by looking at the minimum bactericidal concentration (MBC) results.

**Determination of MBC**

MBC was aimed to decide the minimum concentration of an antibacterial solution that could kill bacteria in the media. MBC was conducted by implementing 8 plates of nutrient agar media. Each plate was divided into three parts; each part was a different concentration of 30%, 25%, 20%; for one plate, and 15%, 10%, 5% in other plate. The MIC test results were planted on nutrient agar media by scratching on each part of the media whose concentration had been determined and then incubated at 37°C for 24 h. Furthermore, the results could be observed by the existence or absence of the growth from APEC colonies on the nutrient agar media.
upper dose of Ketapang extract. The antibacterial ability of Ketapang extract is from the antibacterial compounds containing flavonoids, saponins, and tannins.\textsuperscript{15-17}

Moreover, the antibacterial working mechanism of Ketapang extract began with the dismantling of the bacterial cell wall by flavonoids. Flavonoids that penetrated the cell wall caused damage to the permeability of bacterial cells as impaired permeability would cause the destroyed microsomes and lysosomes. Meanwhile, the mechanism of flavonoids as antibacterial was achieved by inhibiting nucleic acid synthesis, cytoplasmic membrane function, and bacterial energy metabolism.\textsuperscript{18-20}

Correspondingly, flavonoids are considered to possess antibacterial and antioxidant features that increase the work of the immune system because they can accelerate lymphoid and immune systems' activation by producing leukocyte cells as antigen eaters. Besides, the working mechanism of flavonoids is to denature the proteins contained in the cell wall; therefore, they can transform the structure and mechanism of bacterial cell wall permeability.\textsuperscript{21,22}

Furthermore, tannins contained in Ketapang extract operate by impeding bacterial growth through the bacterial protoplasm coagulation. Tannins are useful for preventing microorganisms' growth by precipitating the proteins from enzymes produced by microorganisms. Consequently, they become inactive and bacterial growth is hindered. Besides, tannins work with the mechanism related to their ability to inactivate the adhesion of bacterial cells and enzymes, along with interfering with the transport of protein in the inner layers of cells' inner layer. Moreover, tannins also have a target on the cell wall's polypeptides. Therefore, cell wall formation is imperfect. This affects the bacterial cell to lyse due to the osmotic pressure; thus, the bacterial cell will die. Tannins are antibacterial by precipitating the proteins.\textsuperscript{23,25}

Other chemical substances useful in Ketapang are saponins. As a mechanism, saponins harm the bacterial cell membranes by lowering the surface tension, resulting in increased cell membrane permeability and releasing important components, such as proteins, nucleic acids, and nucleotides, leading the bacteria to turn into lyses. Furthermore, saponins work by lysing the bacterial cell walls and disrupting the cell metabolism until death. In addition, saponins contained in Ketapang extract will interact with the bacterial cell wall and affect the bacterial cell wall into lyses.\textsuperscript{26,27}

Ketapang extract's antibacterial activity against APEC in vivo

Feed conversion Rasio (FCR)

According to the data analysis results using ANOVA shown in Table 1 explained a significant difference (p<0.05) between treatments related to the administered Ketapang extract on the feed conversion of layers. Duncan’s test results revealed that P0− feed conversion displayed no significant difference. P0− was significantly different from P0+, P1, and P3. Meanwhile, P2 was significantly different from P0+, P1, and P3.

The P2 treatment group with the dose of 10% Ketapang extract was insignificantly different from the P0− treatment which was not given any Ketapang extract and was uninfected with APEC. This indicates that Ketapang extract administration with a concentration of 10% has similar feed efficiency as the P0− treatment, which is the layers without any Ketapang extract administration and APEC infection.

Furthermore, P2 with 10% Ketapang extract concentration had a lower conversion rate than P1 with 5% Ketapang extract and P3 with 15% Ketapang extract. This means that P2 can suppress the feed conversion rate of layers infected with APEC. Accordingly, the concentration 10% of Ketapang extract leads to an effective content of active substances; therefore, the antibacterial activity will be efficient. The concentration 10% of Ketapang extract leads to the more contained secondary metabolites and the more bioactive compounds owned by Ketapang, which have antibacterial activity, including flavonoids, saponins, and tannins. Furthermore, the herbal Ketapang leaf extract has an antibacterial effect in restraining Salmonella bacterial growth. Consequently, layers become healthier and their digestion gets better; thus, the consumed feed can be absorbed better, leading to increased growth while the feed conversion gets low.

Meanwhile, P0+ had the highest feed conversion rate because the amount of feed consumed was imbalanced with the eggs production. The feed conversion value is expressed as a measure of feed efficiency, describing the livestock's ability to convert feed into a certain amount of production in certain units, both for meat and egg production. Although in P0+, the feed consumption and body weight gain were undisturbed, it can be seen in the feed conversion that the ability of layers to digest the feed to be converted into meat was poor because the feed conversion value remained high.

Similarly, the high and low feed conversion rates are due to the larger or lower difference in feed consumption ratio and body weight gain. The lower feed conversion rate contributes to a better condition because it
shows that the use of feed becomes more efficient. The more efficient chicken is in converting its food into meat drives, the better conversion value will occur. If the conversion rate is high enough, the feed consumption will not be balanced with the resulting egg production.1-5

**Hen Day Production (HDP)**

According to the data analysis results using ANOVA shown in Table 1 and Figure 3 explained a significant difference (p<0.05) between treatments related to the administered Ketapang extract on the HDP of layers. Duncan’s test results revealed that P0− and P2 HDP displayed no significant difference. P0− was significantly different from P0+, P1, and P3. Meanwhile, P2 was significantly different from P0+, P1, and P3.

The P2 treatment group with the dose of 10% Ketapang extract was insignificantly different from the P0− treatment which was not given any Ketapang extract and was uninfected with APEC. This indicates that Ketapang extract administration with a concentration of 10% has similar HDP as the P0− treatment, which is the layers without any Ketapang extract administration and APEC infection.

Furthermore, P2 with 10% Ketapang extract concentration had a higher HDP than P1 with 5% Ketapang extract and P3 with 15% Ketapang extract. This means that P2 can increase the HDP rate of layers infected with APEC. Accordingly, the concentration 10% of Ketapang extract leads to an effective content of active substances; therefore, the antibacterial activity will be efficient. The concentration 10% of Ketapang extract leads to the more contained secondary metabolites and the more bioactive compounds owned by Ketapang, which have antibacterial activity, including flavonoids, saponins, and tannins. Furthermore, the herbal Ketapang leaf extract has an antibacterial effect in restraining Salmonella bacterial growth. Consequently, layers become healthier and their digestion gets better; thus, the consumed feed can be absorbed better, leading to increased growth while the feed conversion gets low.19-21,28,29

Meanwhile, P0+ had the lowest HDP because the number of active layers was imbalanced with the eggs production. The HDP value is expressed as a measure of eggs production, describing the livestock’s ability to convert feed into a certain amount of production in certain units, both for meat and egg production.

**CONCLUSION**

In summary, Ketapang extract has shown to contain tannins, flavonoids and saponins. Ketapang extract possesses an antibacterial feature against APEC with an MBC of 50% concentration. Finally, administering Ketapang extract to layers infected with APEC can maintain the HDP, and reduce the layers’ value of feed conversion ratio. The best results are in the treatment using Ketapang extract with 10% concentration. This study shows that Ketapang extract can be used to antagonize APEC on layers and improve the performance of layers infected with the bacteria. The data of this study can be used as a reference for future studies on the use of Ketapang as an antibacterial against APEC on other poultry.

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**REFERENCES**


**Table 1: Performance of layers after giving Ketapang extract and infected APEC.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FCR±SD</th>
<th>HDP±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0−</td>
<td>1.99±0.177</td>
<td>89.28±7.14</td>
</tr>
<tr>
<td>P0+</td>
<td>2.07±0.037</td>
<td>85.71±0.00</td>
</tr>
<tr>
<td>P1</td>
<td>1.99±0.131</td>
<td>89.28±7.14</td>
</tr>
<tr>
<td>P2</td>
<td>2.23±0.178</td>
<td>78.57±8.24</td>
</tr>
<tr>
<td>P3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Means in the same row with different superscripts differ significantly (p < 0.05), SD = Standard Deviation, P0− = Negative control treatment, P0+ = Positive control treatment, P1 = Treatment 1 (Group that was administrated with Ketapang extract at 5% concentration), P2 = Treatment 2 (Group that was administrated with Ketapang extract at 10% concentration), P3 = Treatment 3 (Group that was administrated with Ketapang extract at 15% concentration).*


Rachmawati K, et al.: Effectiveness of Ketapang (*Terminalia cattapa* L.) Extract Against Avian Pathogenic *Eschericia coli* (APEC) Infections in Layer Performance

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