Sambiloto (*Andrographis paniculata*) Extract Improves the Performance of Animal Model Infected with *Escherichia coli*

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Abstract

This study aims to understand the effect of natural antibiotics from sambiloto (*Andrographis paniculata*) extract to laying hens infected with Avian Pathogenic *Escherichia coli* (APEC). The activation test of sambiloto was performed by using a dilution method included a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC). Laying hens were distributed into five groups: normal group, APEC group with no treatment, and APEC group given 10%, 20%, 30% sambiloto extract for two weeks. The data were analyzed using ANOVA and continued with the F test. Based on the results, the 30% extract has improved the hen day production (HDP) of laying hens infected with APEC.

***Keywords**: Andrographis paniculata, Avian Pathogenic Escherichia coli, Antibiotic, Hen Day Production*

Introduction

Poultry farming has increased significantly every year, both in production and consumption. But its development has become difficult, one of which is caused by APEC. The decline in production caused by colibacillosis is quite alarming. This condition leads to high morbidity and mortality[1].

APEC attacks the reproductive system, affecting daily egg production or HDP. Laying hens exposed to APEC cause a 60% decrease in HDP. Antibiotic is commonly used as a treatment for colibacillosis[2]. The administration of synthetic antibiotics was performed as an effort to eliminate APEC. However, drug administration causes antibiotic residues in organs and bacterial resistance, creating a lower ability for the drug to tackle the disease. APEC has a good ability to adapt to drugs so that the continuous use of antibiotics, such as enrofloxacin, oxytetracycline, and sulfadimethoxine, would not overcome the APEC[3].

Antibiotic resistance affects the hen's performance. An alternative treatment is needed, that has a similar function as synthetic antibiotics but fewer side effects. One of which is by using medicinal plants. Indonesia has a very high diversity of medicinal plants[4,5]. A potential medicinal plant that can be used as a substitute for antibiotics is sambiloto. Sambiloto leaves possess analgesic, anti-inflammatory, antimalarial, antiviral, immunostimulator, antibacterial, hepatoprotective, anticancer performance.

Materials and Methods

Plant Identification

Taxonomic identification of the sambiloto (*Andrographis paniculata*) was carried out by the Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

Ethical Clearance: This study was approved by

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the Committee of Animal Care and Use, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

Sambilito Extract Preparation

This study conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Sambiloto extract was made into several doses, to know which dose gave the best effect against APEC. Around 1000 grams of sambiloto were smoothed into powder and two liters of 95% ethanol was added. The mixture was macerated for three days. The supernatant was evaporated and 50 grams of crude extract were collected.

Activity Test of Sambiloto Extract against APEC

The bacterial activation test was carried out using the diffusion method to determine the sensitivity of the sambiloto extract to APEC. The dilution method included a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC). The MIC method was done by adding 1 mL APEC suspension (3×10^8) to each concentration then incubated at 37 ºC for 24 hours. The MBC method was carried out using 6 plates of EMBA. One media was distributed into eight groups (7+1 controls) and then each was labeled. The MIC test results were planted in EMBA by streak method and incubated at 37 ºC for 24 hours.

Administration of Sambiloto Extract in APEC Laying Hens

The sample used in this study was 25 laying hens, strain Isa Brown, 20 weeks old. Animals were divided into five groups: normal group, APEC group with no treatment, and APEC group given 10%, 20%, 30% sambiloto extract for two weeks. All animals were acclimatized for one week. Laying hens aged 21 weeks were infected with 1 ml APEC (10^6 cells/kg BW) intramuscularly and then clinical symptoms were observed for three days. Treatment began at the age of 26 weeks and kept for four weeks until they are 30 weeks old. Data collected were feed consumption, egg production, feed conversion, and the amount of egg production.

Data Analysis

Data were analyzed by Anova (analysis of variance) and the F test. If there were significant differences ($p<0.05$), the statistical test was continued with the least significant difference (LSD) test.

Result and Discussion

The activity test result of sambiloto extract toward APEC shows the zone of inhibition at the concentration of 30%.

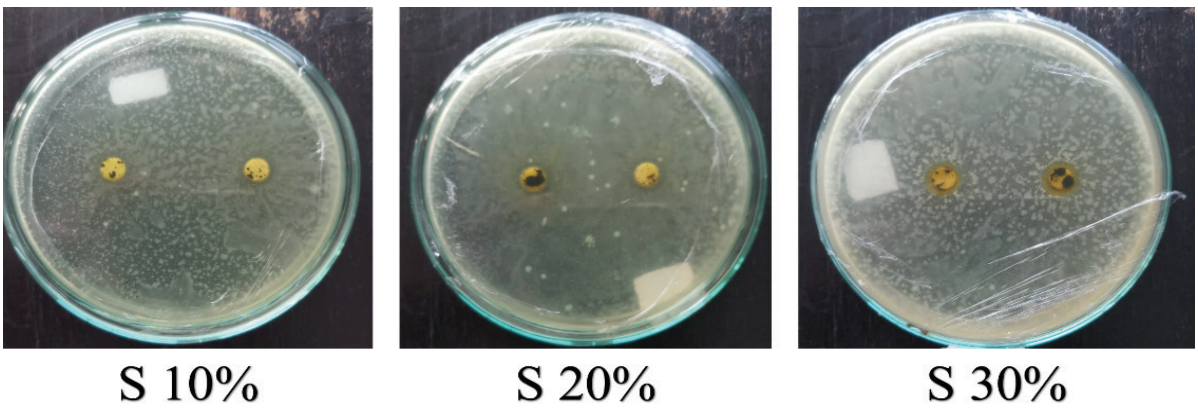

Figure 1. The MIC results using the diffusion method of sambiloto extract to APEC in different concentrations.

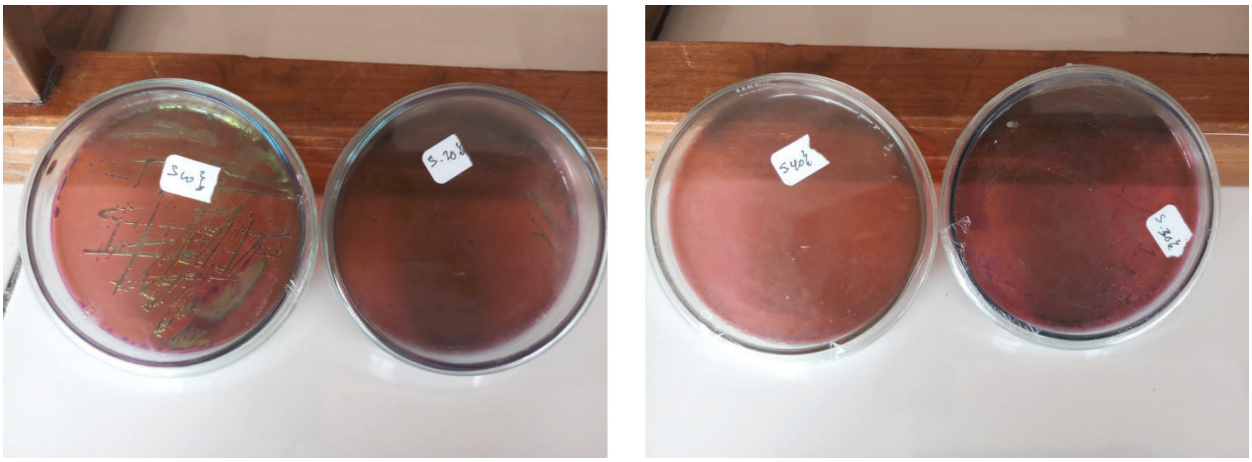

Figure 2: The MBC results of sambiloto extract to APEC in EMBA.

There was no APEC growth at 30% concentration in the MIC method. The MBC test is the continuation test of MIC to prove the MIC test visually. The growth or absence of bacteria at each concentration can be caused by different antibacterial levels in each concentration. The more dilute the MIC test means that the amount of solvent is greater than the amount of the sambiloto extract.

Based on the results of this study, the higher the concentration of sambiloto extract, the better its ability to inhibit APEC development. This was due to the higher the concentration, the higher the active substance contained in the extract. At the opposite, the lower the concentration, the antibacterial ability of the extract will also be lower. In general, high concentrations of antibacterial agents are considered as bactericidal while at low concentrations it is considered as bacteriostatic[6].

Table 1. The Performance of Laying Hens: Food Consumption, Egg Production, Food Conversion, and Total HDP.

Treatments	Food Consumption (X±SD)	Egg Production (X±SD)	Food Conversion (X±SD)	HDP (X±SD)
P0-	92.42b ± 3.64	65.40d ± 2.33	1.41b ± 0.07	97.5c±5
P0+	115.85e ± 2.74	52.40a ± 1.87	2.21e ± 0.09	82.5a±5
P1	108.30d ± 2.77	56.50b ± 1.19	1.91d ± 0.07	87.5ab±5
P2	100.32c ± 2.59	62.20c ± 1.96	1.61c ± 0.06	95bc±5.77
P3	85.60a ± 3.79	65.87d ± 2.00	1.29a ± 0.05	97.5c±5

Indonesia has a high biodiversity with may natural medicine sources. It has around 30,000 different plant species, of which 7,000 are known to possess medicinal properties[7,8,9,10]. Sambiloto is a medicinal herb that is cultivated in Southeast Asia. This plant is widely used as a traditional medicine in Taiwan, China, India, and Thailand to treat infections, colds, fevers, inflammation, and diarrhea. Andrographolide is the major lactone diterpene from sambiloto which contains 1.7% of the dried leaves and 0.8% of the stem. Sambiloto and andrographolide have recently got considerable interest because of their diverse physiological functions and therapeutic potential, including antioxidants, anti-inflammatory, antiapoptotic, antiatherosclerotic, anticancer, antiviral, antiviral and hypoglycemic effects[11].

Tannin is one of the polyphenol compounds which has antibacterial properties. Tannins can inhibit the adhesion of bacterial cells attached to the host cell, inhibit enzymes and interfere with protein transport in the cell layer. Tannins cause lysis in bacterial cells through osmotic pressure and physical stress. Flavonoids in sambiloto can act as antioxidants, antibacterial, anti-inflammatory, anti-allergic, and anti-thrombosis[12]. Our previous study found that *Phyllanthus niruri* L. extract can increase the immune response in laying hens infected by *E. coli*[13].

Food consumption in P0+ treatment was the highest, with a value of 115.85 grams/hen/day. While the lowest was P3 treatment with a value of 85.60 grams/hen/day. Increased food consumption in the APEC group with no treatment was presumably because the feed could not be absorbed properly by the infected intestine and caused much-wasted food. The food consumption in P1 and P2 groups was decreased because the infected hens had been treated with sambiloto extract with concentrations of 10% and 20%, respectively. Sambiloto contains tannin compounds that can coat the intestinal mucosa so that food absorption is inhibited. Tannin compounds that are contained in sambiloto plants are able to reduce food absorption by depositing mucous protein in the intestine, making it possible to reduce food consumption. Food consumption in the P0- group shows higher results than the P3 group, this is due to the presence of APEC in the intestine.

The Anova test, followed by the Duncan test, showed that there was a significant difference ($p<0.05$) on egg production among groups. The P0- group was not significantly different from P3 but it was significantly different from P0+, P1, and P2. The P1 group was significantly different from all groups. The P2 group was significantly different from all groups. The P3 group was not significantly different from P0- but significantly different from P0+, P1, and P2. The egg production of P0- and P3 groups showed similar results which were 65.40 grams/hen/day and 65.87 grams/hen/day, respectively. The egg production of P1 group (56.50 grams / hen / day) was lower compared to P2 (62.20 grams/hen/day). The lowest egg production was experienced by the P0+ group with 52.40 grams/hen/day. This was caused by the presence of APEC that attacks the reproductive system in laying hens, especially in the infundibulum. Increased egg production is also affected by andrographolide contained in sambiloto extract which functioned as antibacterial. Andrographolide is the most abundant substance in sambiloto. Besides its antibacterial property, andrographolide can also activate B lymphocyte cells to produce antibodies[11]. As an antibacterial, andrographolide can inhibit *E. coli* that have been infected in these laying hens. The number of doses of sambiloto extract also affects the amount of andrographolide content in the extract and influences the bacterial inhibition.

The food conversion data shows that the P0+ group was significantly different from P0-, P1, P2, and P3. The P0- group was significantly different from P0+, P1, P2, and P3. The P1 group was significantly different from P0+, P0, P2, and P3. The P2 group was significantly different from P0 +, P0-, P1, and P3. The P3 group was significantly different from P0+, P0-, P1, and P2. The P0+ and P1 groups had the highest food conversion with a value of 1.66 while P3 showed the lowest results with a value of 1.62. The P0 was not significantly different from the P2 group, with a conversion value of 1.63. Based on these results, the administration of sambiloto extract can reduce food conversion rates on laying hens infected with APEC. The higher the sambiloto extract dose, the lower the feed conversion rate. This is due to the flavonoid content in the extract. Flavonoids can inhibit bacterial growth by damaging the permeability of bacterial cell walls, microsomes, and lysosomes. It is the result of interactions between flavonoids and bacterial DNA[6]. The flavonoid content in sambiloto extract is what caused the inhibition of *E. coli* and the hens continued to form good antibodies and produce high egg production and low food consumption. Food conversion is closely related to food consumption and egg production. Low food consumption and high egg production can reduce food conversion while high food consumption and low egg production can increase food conversion which will be detrimental to farmers.

Duncan test results on HDP showed that the P0 group was significantly different from P0+ and P1 whereas it was not significant from P2 and P3. The P0+ group was significantly different from P0, P2 and P3 groups whereas it was not significant from P1. The P1 group was significantly different from P0 and P3 groups whereas it was not significant from P0+ and P2 groups.

The P2 group was significantly different from P0+, whereas it was not significant from P0, P1, and P3. The P3 group was significantly different from P1 and P0+ whereas it was not significant from P0 and P2. Based on these results, it was confirmed that the administration of sambiloto extract can increase the HDP of laying hens. It was due to the antibacterial and the antidiarrheal properties of the extract so that egg production is not inhibited by *E. coli*. The administration of sambiloto extract healed the hen's infundibulum infected with *E. coli*. This healing process normalizes the egg formation and production[14].

Conclusion

In sum, the study present that the administration of sambiloto extract at a 30% dose can improve the performance of laying hens infected with APEC.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding : This study supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

Acknowledgements : We thank EJA – Professional Translation Services for editing the manuscript.

Ethical Approval: This study was approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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