Celery (Apium graviolens L.) extraction as the inhibition of pathogenic microorganism in broiler

Y F Nuningtyas1, O Sjofjan1, I H Djunaidi1 and M H Natsir1
1Faculty of Animal Science, Brawijaya University, Jl Veteran, Malang (65145), Indonesia
fritanuningtyas@ub.ac.id

Abstract. Pathogenic bacteria in the digestible tract can inhibit the optimization of the feed absorption on broiler chicken. The research aimed to determine the ability of the inhibition of bioactive compounds on celery as pathogenic bacteria such as Salmonella, Escherichia coli, and Clostridium perfringens was found in broiler digestive tracts. The stages of the study were the determination of celery plants and inhibitory test on Salmonella, Escherichia coli and Clostridium perfringens with P0 concentration: negative control (aquades), P1: positive control (5 µg / ml amoxicillin), P2: 2.5 mg / ml celery extraction, P3: 5 mg / ml celery extract, and P4: 10 mg / ml celery extraction. Variables observed were the inhibition zone diameter using the disc method. The data obtained were tabulated with the Microsoft Excel program, then analyzed by a completely randomized design (CRD) where the significant differences analyzed used Duncan's analysis. Based on the results study showed that plant determination was celery (Apium graveolens, L.). The addition of celery extraction with a concentration of 10 mg/ml showed a higher inhibitory power compared to the use of antibiotics against Salmonella and E Coli bacteria.

1. Introduction

In the last 40 years, antibiotics or antimicrobials were used as feed additives in the animal. Antibiotics are a growth promoter which relatively used in the small amount to improve feed efficiency and feed reproduction, so the use of these additives can improve the profit of farmers. According to the Regulation of the Minister of Agriculture No. 14/2017 concerning the classification of veterinary medicine stated that the use of antibiotics in Indonesia must be avoided, this is caused by two main factors. First, the possibility of residues from antibiotics will become toxic to consumers; second, antibiotics can create resistant microorganisms in the human body or livestock [5], especially pathogenic bacteria such as Salmonella, Escherichia coli and Clostridium perfringens.

Broilers are superior chicken crosses from chicken with high productivity, especially in meat production. The growth of broiler chicken approximately increases 3 % per day during the first age up to 20 % during five days, respectively [4]. Broiler has been widely consumed and developed because it has an economic value of meat. Currently, broiler is very popular in the community because it has a low price, also has soft, large and solid meat. The productivity of broiler chicken can not be optimal if there are no added antibiotics on feed. This is because in the broiler digestive tract is vulnerable as the growth of pathogenic bacteria such as Salmonella, Escherichia coli and Clostridium perfringens. However, the addition of antibiotics on feed continuously can be caused by the harmful effect on meat products.
The use of antibiotics in animal feed can replace with natural herbs that have potential as feed additives. Celery contains bioactive compound, namely flavo-glucoside (apiin and apigenin), malt, bitter substances, vitamins, choline, and lipase enzyme. Phenolic compounds in celery consist of flavonoids apiin, apigenin, and isokuersitrine. Furthermore, also contain tannin, selerin, bergapten, apiumosida, apiumetin, apigravrin, ostenol, isopimpinellin, isomperatorin, selereosida, and 8 hydroxy methoxyxypsoralen. Essential oils consist of limonen (60%), beta-selinen (10-15%), phthalide, apio1, sesquiterpen alcohol (1-3%) such as eusdemol, butyl phthalide and sedanelida[1]. In celery leaves contain high total phenolic and flavanoids content. The total phenolic is 403.84 mg/100 g and flavanoid is 156.89 mg/100 g [8]. Flavanoid is antibacterial with sixfold antibacterial activity, it shows that the antibacterial stronger than medicine or antibiotics [3] and also some synthetic flavonoids have the potential to against bacteria gram-negative and gram-positive.

Celery is one of the medicinal plants that have important properties for humans. Celery has been used for traditional medicine to improve digestion, cure fever, flu, and appetite enhancer [6] and also reduce high blood. Some studies said that the content of chemical compounds in celery herbs has activities as an antimicrobial, antihypertensive, antioxidant, antiplatelet, antidepressant, and anti-inflammatory. The bioactive substance in the form of flavonoids, saponins, and tannins in celery serves as an antimicrobial.

This study aims to evaluate bioactive compounds in celery herbs, where bioactive compounds are expected to be able to inhibit the growth of pathogenic bacteria in the digestive tract of broilers. As long as these pathogenic bacteria inhibit the productivity of broilers.

2. Materials and Methods
This research was carried out on July – September 2018 in the Materia Medika Laboratory Batu Malang, Animal Food Technology Laboratory in the Faculty of Animal Science University of Brawijaya Malang, Plant Disease Laboratory Faculty of Agriculture University of Brawijaya Malang, and Animal Husbandry laboratory University Muhammadiyah Malang.

2.1. Herbs Determination
2.1.1. Dried of celery.
Dried the leaves, stems, and roots of celery begin with sorted to select fresh plants and washing celery. Furthermore, the clean celery was dried in the oven at 60 °C to remove the water content.

2.1.2. Extraction.
Dry celery plants were ground and weighed 10 g of celery powders to extract used 96% ethanol with a ratio between celery powder and ethanol (1: 20 w/v) for 24 hours at room temperature. Moreover, the maceration process used a rotary evaporator at 40 °C for 2 hours under vacuum pressure to obtain a concentrated extract of celery.

2.2. Inhibition of Microorganism
2.2.1. Rejuvenated of microorganism.
Pure culture of Salmonella, E. coli, and Clostridium perfringens were inoculated separately in a tube containing sterile nutrient broth (NB). Then the culture of microorganism was incubated at 37 °C for 24 hours.

2.2.2. Microorganism starter.
The rejuvenated Salmonella, E. coli, and Clostridium perfringens bacteria were inoculated into 100 mL containing 10 mL of sterile Nutrient Broth (NB). Then, Salmonella, E. coli, Clostridium perfringens cultures were incubated on Waterbath shat at 37 °C for 10 hours.
2.2.3. *Inhibition of microorganism.*

The paper disk was filled with celery extraction of 2.5 mg/mL, 5 mg/mL, and 10 mg/mL, negative control (aquades), and positive control (amoxicillin 5μg/mL). Then, the discs were placed on a petri dish that contained a different concentration of *Salmonella, E. coli,* and *Clostridium perfringens.* It was incubated at 37 °C for 24 hours. Then calculated the total of microorganism inhibition.

2.3 Treatment

The experimental design was used 5 treatments and 5 replications:

- **P0:** negative control (aquades)
- **P1:** positive control (5 μg / ml amoxicillin)
- **P2:** 2.5 mg / ml celery extraction
- **P3:** 5 mg / ml celery extract
- **P4:** 10 mg / ml celery extraction

2.4 Statistical analysis

Data were analyzed using a completely randomized design (CRD), if there were significant differences (P > 0.05) then tested by Duncan's multiple range test.

3. Results and Discussions

The results of this experiment consist of plant determination of celery plants and inhibitory effect of pathogenic bacteria such as *Salmonella, E. coli,* and *Clostridium perfringens.* In these experiments were used the leaves, stems, and roots of celery. Then it was cleaned and dried by 60 °C oven.

The purpose of the plant determination was to identity of the plant characterization, whether the plant is really the desired plant. Thus, the incompatibility of the plants collection for the research can be avoided. Celery plants (*Apium graveolens* L) for this experiment was determined at the Materia Medica Laboratory, Batu, Malang by Flora of Java [2]. Plants determination showed that the celery or *Apium graveolens* L as follows:1b-2b-3b-12b-13b-14b-15b-17b-18b-19b-20b-21b-22b-23b-24b-25b-26b-27a-28b-29b-30b-31a-32b-74a-75b-76a-77a-78b-103c-104b-106b-107a-108b-109a-115a-116a-117b-118b...148. Apiace 1a-2a-3b-4b-6b-7a-8b-10b. Apium 1b. *Apium graveolens* L. Based on the determination results can be obtained that the plants used in these experiments were *Apium graveolens* L species. It was accordance with the type of the plants that expected.

Based on the results of the research on the inhibitory test of pathogenic microorganisms in the digestive tract of broilers consist of *Salmonella, E. coli,* and *Clostridium perfringens* showed in the table below:

| Treatment | *Salmonella* (mm) | *E. Coli* (mm) | *Clostridium Perfringens* (mm) |
|-----------|------------------|----------------|-------------------------------|
| P0        | 0±0              | 0±0            | 0±0                           |
| P1        | 10.91±1.67       | 13.03±1.67     | 9.86±1.67                     |
| P2        | 7.99bc±1.28      | 10.64b±1.28    | 7.38bc±1.28                   |
| P3        | 10.44bc±0.77     | 13.69b±0.77    | 8.35bc±0.77                   |
| P4        | 12.24d±1.15      | 14.37d±1.15    | 9.14cd±0.15                   |

Note: 1) P0: negative control (aquades), P1: positive control (amoxicillin 5μg / ml), P2: 2.5 mg / ml of celery extraction, P3: 5 mg / ml of celery extraction, P4: 10 mg / ml of celery extraction
2) a-d Menas within column with different superscripts were significantly different (P < 0.05)
The use of celery extraction can inhibit the growth of pathogenic bacteria. The use of celery extraction can inhibit (P > 0.01) the growth of Salmonella bacteria. Based on the table above the use of celery extraction on the inhibition of salmonella bacteria showed sequentially P0: 0, P1: 10.91 mm, P2: 7.99 mm, P3: 10.44 mm, and P4: 12.24 mm. PO is a negative control that not given treatment in the form of antibiotics or celery extraction so the inhibitory is 0 mm. P1 as a positive control was added amoxicillin as much as 5µg / ml increase the inhibition of 10.91 mm. In P4 using the addition of celery extract with a concentration of 10 mg/ml can increase the inhibition of Salmonella bacteria higher than the addition of amoxicillin. It showed that bioactive compounds (flavanoids) in celery extraction increased the inhibition zone of salmonella bacteria. Bioactive compounds on celery such as flavonoids can inhibit pathogenic bacteria gram-negative such as Salmonella [3]. Pathogenic bacteria are bacteria that can inhibit the digestion of food which occurs in the small intestine of broiler chickens.

The use of celery extraction has increased (P > 0.01) inhibitory zone to the growth of E. coli bacteria. Based on the table above the use of celery extracts on the inhibition of E. coli bacteria can be seen respectively P0: 0, P1: 13.03 mm, P2: 10.64 mm, P3: 13.69 mm, and P4: 14.37 mm. PO is a control that is not given treatment in the form of antibiotics or celery extracts so that the inhibitory power is 0 mm. P1 was used amoxicillin as much as 5µg / ml increased the inhibition power by 13.03 mm. In P3 and P4 were used the addition of celery extract with a concentration of 5 mg/ml and 10 mg/ml increased the inhibition of salmonella bacteria higher than the addition of amoxicillin. These results showed that flavanoids in celery extract can increase the inhibition zone of E. coli bacteria. It was agreed with the statement of [8] that total flavonoid content on the leaves of celery can inhibit pathogenic bacteria such as E.Coli.

The use of celery extraction increased (P > 0.01) inhibitory zone to the growth of Clostridium bacteria. Based on the table above the use of celery extracts on the inhibitory power of Clostridium bacteria can be seen in succession P0: 0, P1: 9.86 mm, P2: 7.38 mm, P3: 8.35 mm, and P4: 9.14 mm. PO is a control that was not given treatment in the form of antibiotics or celery extract so the inhibitory power 0 mm. P1 used amoxicillin as a positive control as much as 5µg / ml increased the inhibition of 9.86 mm. The higher the concentration of the treatments has the better the inhibition of Clostridium bacteria. However, the addition of 5 mg/amoxicillin has better than giving celery extract as much as 10 mg/ml.

4. Conclusions
10 % concentration of celery (apium graveolens L) extraction can inhibit Salmonella and E.Coli compared antibiotics.

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6. References
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