Inhibitory effect of tannin extract from Acacia bark (*Acacia mangium* Willd.) against gastro-intestinal pathogenic bacteria

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**Abstract.** Acacia (*Acacia mangium* Willd) is a fast growing tree species that widely found in Indonesia. Acacia bark contains a number of secondary metabolites such as tannin, saponin and flavonoid that may inhibit the growth of undesirable microbes including the pathogenic ones. The purpose of this research was to evaluate the antimicrobial activity of tannin extract obtained from Acacia bark against common pathogenic bacteria species present in the gastro-intestinal tract of livestock, i.e., *Salmonella typhimurium* and *Eschericia coli* as Gram negative bacteria. The disc diffusion method was employed for assessing the antimicrobial activity of Acacia tannin extract. Treatments consisted of negative control (NC), positive control by using chloramphenicol (PC), Acacia tannin extract at two concentrations, i.e., 1% (AT1) and 2% w/v (AT2), and commercial chestnut tannin at 1% (CT1) and 2% w/v (CT2). Data were analyzed by using analysis of variance and followed by a post-hoc test namely Duncan’s multiple range test. Results showed that the inhibitory diameters formed on the activity assay of AT in the form powder, liquid and CT with concentration of 2% against *Salmonella typhimurium* bacteria were 17.3, 7 and 16 mm, respectively. Inhibitory diameters of AT powder, AT liquid and CT against *Eschericia coli* bacteria were 17.3, 0 and 17 mm, respectively. The AT powder resulted in a higher inhibition of both pathogenic bacteria species than that of liquid form (P<0.05), whereas its inhibitory effect was similar to commercial CT. In conclusion, 2% AT can be used to inhibit the growth of *Salmonella typhimurium* and *Eschericia coli* bacteria in which its magnitude of inhibition is comparable with commercial tannin extract from chestnut.

1. **Introduction**

Nowadays livestock health is always be a concern, one of which is the health of the digestive tract of livestock. The WHO in 1948 defined the health of the digestive tract as a form of absence of disease or complaints regarding disorders of the digestive tract. Health of the digestive tract is more than the absence of disease, but the prevention or avoidance of diseases in the digestive tract is an integral part of animal health [1]. This definition usually links intestinal health with pathogens that cause clinical or subclinical disease, death and (or) morbidity to livestock, and economic losses.

For decades, control of pathogen in the gastrointestinal tract has been widely studied, the use antibiotic, antibacterial, or synthetic antifungal and prophylactic ingredients causes increased resistance. Antibiotics have been banned in routine animal production systems. Their use as growth promoters has led to an increased occurrence of antibiotic-resistant bacteria in farm animals and colonisation of human...
consumers by antibiotic-resistant bacteria [2]. In addition, there is currently increasing consumer demand for organic animal products. Global concern about development antimicrobial resistance and transference of resistance genes from animal to human is rising [3-5]. This triggers to look for potential alternative solutions to control pathogens in the gastrointestinal tract. Bioactive content in plants is a potential solution to use [6]. In fact, good active ingredients are derived from plants compared to those from animals. One of the plants that produce secondary metabolites (active ingredients) is Acacia (Acacia mangium Willd). Acacia contains secondary metabolites such as flavonoids, phenols, tannins and saponins.

Tannins, polyphenolic compounds which naturally occur in the bark, leaves, seeds and roots of many plants. Biological activity of tannin is related to its ability to interact with primarily protein [7], which present in the cell wall and enzyme system of pathogenic microbes. Therefore, the active ingredients will be used to inhibit the growth of pathogenic agents which are very detrimental for ruminants. Current scientific evidence suggests that there is significant potential in the use of tannins to improve animal nutrition and health [8]. In moderate concentrations in temperature forages containing Condensed Tannin (CT) have different effects, dependent on their concentration and chemical structure [9]. This study will use the Acacia bark as a source of tannin for inhibit the growth of pathogenic bacteria. Several studies have proven that tannins from acacia bark have a high enough condensed tannin content, so this can be used as feed additives to inhibit the growth of gram negative bacteria such as Eschericia coli and Salmonella typhimurium at the right dosage. Tannins are classified into two namely Hydrolisable Tannin (HT) and Condensed Tannin (CT). This compound has different metabolic effects in the rumen. CT compounds have a higher stability compared to HT [10]. Therefore, this study aims to evaluate the use of tannin extracts from acacia (Acacia mangium Willd) bark as candidate feed additive to inhibit the growth of pathogenic bacteria in the digestive tract of ruminants. Then, the use of tannins from Acacia bark will be compared with commercial tannins from Chestnut.

2. Methods
This study uses 40 kg acacia bark obtained from PT. Indonesia Fibreboard Industry, Mendis Jaya Village, Bayung Lencir District, Musi Banyuasin Regency, South Sumatra Province.

2.1. Tannin extraction
Acacia bark is cut and dried, after that the bark is ground into smaller particles. Bark is ready for extraction using the Water Hot Extraction method, with water as a solvent. Comparison between water and material is 1:3, the extraction process uses pressure 2 Bar and temperature 110 °C for 4 hours. Then tannin extract was concentrated using a rotary evaporator 65 °C for 4 hours. Then partially liquid tannin extract will be converted into powder / flour using spray dryer, this process uses maltodextrin as a solvent for tannin extract to powder.

2.2. Phytochemical screening
Phytochemical screening was carried out to determine the content of metabolite compounds contained in tannin extracts. The compound that will be carried out consist of steroids/triterpenoids, flavonoids, saponins, phenols, and tannins.

2.3. Antibacterial activity
The bacteria used in the test were rejuvenated first and microbial suspensions were made. The media to be used is also sterilized first. Then, acacia tannin extracts and chestnuts made a solution with a concentration of 1% and 2% w/v using sterile distilled water. The bacterial suspension is mixed in the TSA (Tryptic Soy Agar) medium then homogenized. Work is carried out aseptically on Laminar Air Flow. Then the mixture of bacteria and media is poured into each petri dish, allowed to stand until solidified. After the media solidifies, the disc paper is placed on the media. Test solutions with each concentration were taken as much as 20 µL and then dropped on disc paper. Incubated for 24 hours at 37 °C for bacteria. Because since 12 hours after incubation the bacteria have grown. Activity
antibacterial is positive if clear zone formed around the paper disc. The clear zone formed around the disc measured using calipers. As a comparison, empty disc with 20 µL sterile distilled water as a negative control and chloramphenicol antibiotics as a positive control were used. Then, the data were collected and subjected to analysis of variance and followed by post hoc test using Duncan’s multiple range test.

3. Results and discussion

3.1. Phytochemical screening
The results of phytochemical screening analysis of acacia and chestnut extracts can be seen in Table 1. That the metabolites contained between Acacia bark and Chestnut are the same, namely flavonoids, tannins, and saponins.

Table 1. The results of phytochemical screenings of Acacia bark and Chestnut.

| Material Test       | Secondary Metabolites | Result |
|---------------------|-----------------------|--------|
| Acasia Bark Extract | Flavonoid             | +      |
|                     | Alkaloid              | -      |
|                     | Tanin                 | +      |
|                     | Saponin               | +      |
|                     | Steroid               | -      |
|                     | Triterpenoid          | -      |
| Chestnut Extract    | Flavonoid             | +      |
|                     | Alkaloid              | -      |
|                     | Tanin                 | +      |
|                     | Saponin               | +      |
|                     | Steroid               | -      |
|                     | Triterpenoid          | -      |

After the compound content is known through phytochemical screening the following in Table 2. is the result of total tannin content, total phenol, and condensed tannin in Acacia bark and Chestnut in the through spectrophotometric test.

Table 2. The results of analysis tannin content by spectrophotometric methods.

| Item                                      | Tannin Content     | Total Phenol     | Condensed Tannin |
|-------------------------------------------|--------------------|-----------------|-----------------|
| Acacia Bark Tannin Extract (powder)       | 12.08 g / 100 g    | 25.97 g / 100 g | 1.45 g / 100 g  |
| Chestnut Tannin Extract                   | 19.08 g / 100 g    | 41.48 g / 100 g | 9.69 g / 100 g  |
| Acacia Bark Tannin Extract (Liquid)       | 16786 µg / ml      | 19587 µg / ml   |                 |

3.2. Antibacterial activity
Tannins compound has good activity against bacterial growth because it contains titanic acid against some genus of diseased bacteria through the ability to dissolve the fatty layer of bacteria wall that causes leakage of cells to fluid out of cells and destroy it [11]. The presence of antibacterial activity is indicated by the formation of clear zones around the disc paper as a form of inhibition. The results showed that the treatment of tannin extract was able to inhibit bacterial growth (P<0.05). This can be seen based on the clear zone formed in Figure 1.
Figure 1. Inhibitory zone formed around paper disc by 1% and 2% Acacia tannin, Chessnut tannin, Chloramphenicol (antibiotic) as positive control and paper disc with sterile water as negative control treatment.

The results of inhibition diameter of tannin extract (AT) at a concentration of 1% and 2% with two types of pathogenic gram negative bacteria, *Eschericia coli* and *Salmonella Thypimurium* shown in Table 3. Extracts of tannins from Acacia bark (AT) in liquid and powder form and commercial tannins from Chestnut (CT) concentrations 1% and 2% show antibacterial activity in the *Salmonella typhimurium* test bacteria. seen from the value of the inhibitory diameter. The results of the diameter of inhibition of liquid AT at a concentration of 1% and 2% against *Salmonella typhi* showed the same result which is 7 mm. Meanwhile, AT powder concentrations of 1% and 2% of *Salmonella typhi* had higher yields compared to CT 1% and 2% namely 16 mm, 17.33 mm, 13 mm, and 16 mm respectively. Van Parys found that Chestnut derived tannins were able to inhibit the growth of *Salmonella typhimurium* by in vitro test [12]. However, the test for inhibition of liquid AT against *E. coli* bacteria did not show antibacterial activity, as seen from the absence of clear zone in Figure 1 (0 mm). Meanwhile, for AT powder concentrations of 1% and 2% showed the presence of antibacterial activity against *E. coli* with a diameter of inhibition formed higher than CT, namely 15 mm, 17.33 mm, 13.67 mm, and 17 mm. Based on these results, AT powder at a concentration of 2% had significantly different results compared to liquid AT (P<0.05). The fact that tannins beneficial or detrimental properties depend on their chemical structure (associated with plant origin) and dosage [13]. Encapsulation on Acacia bark tannin extract enhance the stability and may be reduce the negative effects associated with tannins especially in feed industry, with tendency to control the release of the active compound for optimum effectiveness [14]. The fact antibacterial activity Acacia bark tannin extract in form powder (spray drying encapsulation) is better than the liquid form.
Table 3. Antibacterial test results for Acacia bark tannin extract and Chestnut tannin extract.

| Bacterial Test | Treatments | Inhibitory Diameter (mm) | Means ± SD |
|----------------|------------|--------------------------|------------|
|                |            | R1 | R2 | R3 |                   |
| NC -           | 0          | 0  | 0  | 0  | 0f                |
| PC 1%          | 20         | 20 | 22 | 20.667 ± 1.155a   |
| PC 2%          | 20         | 20 | 21 | 20.333 ± 0.577a   |
| AT liquid 1%   | 7          | 7  | 7  | 7 ± 0a            |
| AT liquid 2%   | 7          | 7  | 7  | 7 ± 0b            |
| AT powder 1%   | 16         | 16 | 16 | 16 ± 0c           |
| AT powder 2%   | 17         | 18 | 17 | 17.333 ± 0.577b   |
| CT 1%          | 13         | 13 | 13 | 13 ± 0d           |
| CT 2%          | 15         | 16 | 17 | 16 ± 0.577c       |

Note: NC = Negative control; PC = Positive control; AT = Acacia tannin; CT = Chestnut tannin; SD = Standard Deviation

a-f within a column, means different letter without common superscript

The results also showed that the higher the concentration of tannin extract, the higher the diameter of the clear zone formed. For comparative treatments negative controls (without the addition of tannin extract) showed no clear zone formed around the disc paper (0 mm) (Table 3). Positive control results (PC) have the highest inhibitory diameter in *E. coli* and *Salmonella typhi*. in concentrations 1% and 2%, between the treatment of 1% PC and 2% PC in each pathogenic bacteria were not significantly different (P>0.05).

4. Conclusions

The use of tannin extract significantly affected the activity of pathogenic bacteria (*E. coli* and *Salmonella typhi*). Acacia tannin extract (AT) in form powder concentration 2% can be used as an inhibitor of gastro-intestinal pathogenic bacteria such as *E. coli* and *Salmonella Typhi*, and it is comparable with commercial tannin extract from Chestnut.

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References

[1] Bischoff SC. 2011. ‘Gut health’: a new objective in medicine. BMC Med. 9 24
[2] Van den Bogaard and Stobberingh E 2000 *J. Antimicrobial Agents* 14 327-335
[3] Salyers A, Gupta A and Wang Y 2004 *Trends Microbiol*. 12 412–416
[4] Mathur S and Singh R 2005 *Int. J. Food Microbiol* 105 281–295
[5] Devirgiliis C, Zinno P and Perozzi G 2013 *Front. Microbiol* 4 301
[6] Waller PJ and Thamsborg SM 2004 *Trends Parasitol.* **20** 493–497
[7] Kondo M, Hirano Y, Ikai N, Kita K, Jayanegara A, Yokota H.-O 2014 *Asian-Australasian Journal of Animal Sciences* **27** 11 1571-1576
[8] Frutos P, Hervás G, Giráldez FJ and Mantecón A 2004 *Span. J. Agric. Res.* **2** 191–202
[9] Min B, Barry T, Attwood G and McNabb W 2003 *Animal Feed Science and Technology* **106** 3 19
[10] Makkar H 2003 *Quantification of Tannins in Tree and Shrub Foliage*: A Laboratory Manual. (Netherlands: Kluwer Academic Press)
[11] Al-ANI R, Mohammed N, Alhameed A, Mohammed S 2008 *Antibacterial Activity of Tannins Extracted from Some Medicinal Plants in vivo* Department of Biochemistry, Al-Anbar University, Ramadi, Iraq 61
[12] Van Parys A, Boyen F, Dewulf J, Haesebrouck F and Pasmans F 2010 *Zoon. Publ. Health* **57** 423–428
[13] Rendondo M, Chacana P, Dominguez J and Miyakawa M 2014 *Front.Microbiol.* **5** 1-6
[14] Adejoro F, Hassen A, Thantsha M 2018. Preparation of acacia tannin loaded lipid microparticles by solid-in-oil-in-water and melt dispersion methods, their characterization and evaluation of their effect on ruminal gas production In Vitro. PLoS ONE **13** e0206241