Potential of atung seeds (*Parinarium glaberrimum* Hassk.) as a phytobiotic candidate in poultry ration based on nutrient composition, phytochemical and antibacterial properties

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Abstract. This study determined the nutrient composition, phytochemical, and antibacterial activity of atung seed (*Parinarium glaberrimum* Hassk.) as a phytobiotic candidate in poultry ration. Analysis of seed nutrient includes proximate analysis, calcium, phosphorus, and gross energy. Qualitative analysis of phytochemical compounds of atung seed extract using the thin layer chromatography method. Antibacterial test on pathogenic and non-pathogenic bacteria used agar diffusion method with eight concentrations of atung seed and tetracycline as a positive control. Data of nutrient composition and phytochemical components were analyzed descriptively. Data of antibacterial test was analyzed by analysis of variance using nine treatments and four replications. The results showed that the atung seed contains 75.08% nitrogen-free extract and 5847.78 kcal/kg gross energy. Atung seed contains phenol compounds, tannins, flavonoids, saponins, and alkaloids. Atung seed has a moderate to strong inhibition of pathogenic bacteria *Escherichia coli*, *Salmonella* sp., *Salmonella pullorum*, and *Staphylococcus aureus*. In contrast, the seed has a weak inhibition against *Bifidobacterium bifidum*. It can be concluded that atung seed has the potential as a phytobiotic candidate based on phytochemical components and their ability to inhibit the growth of pathogenic bacteria.

1. Introduction

More than half a century, the poultry farming industry in various countries has a dependence on antibiotic growth promoters (AGP) to increase the productivity. The role of AGP in increasing feed efficiency and animal productivity is an indirect influence by suppressing the growth of pathogenic bacteria in the digestive tract. AGPs reduce the toxins production from pathogenic bacteria, reduce nutrient use by bacteria, increase the production of vitamins and other growth factors, and increase nutrient absorption by livestock due to depletion of the intestinal mucosa [1,2]. Giving AGP is usually carried out over a long period and at subtherapeutic doses that cause the emergence of pathogenic bacteria that are resistant to these antibiotics, and leave residues on meat or eggs that endanger consumers [1].

Concern about the negative effects of the use of AGP has caused the ban of its use in several countries. A ban on AGP in animal nutrition has been in place in Sweden since 1986 and in Switzerland since 1999 [3] and later by other countries. Beginning in 2006, the European Union has
banned the use of AGP as feed additives [4]. Indonesia, since January 2018, has prohibited the use of AGP as feed additives based on Permentan No. 14/2017.

Phytobiotics, in addition to probiotics, prebiotics, synbiotics, and organic acids, are alternatives that have been developed as substitute for AGP [4,5]. Mode action of phytobiotic in improving livestock production performance is very diverse. Its stimulate the secretion of digestive enzymes, enhances immunity, coccidiostat, anti-inflammatory, antioxidant, anthelmintic, and as antibacterial [5]. Atung (Parinarium glaberrimum Hassk.) plant is a forest plant that is widely found in Maluku-Indonesia and has been used as a traditional medicine especially to treat diarrhea, stop the bleeding, and as food preservatives, particularly the marine fish [6]. The fruit seed was used for these purposes. This study was examined the potential of atung fruit seed as phytobiotic candidate based on nutrient composition, phytochemical content, and antibacterial activity.

2. Material and methods

2.1. Material
Atung fruit seeds were obtained from the village of Soya, Maluku, Indonesia. The isolates of pathogenic bacteria used were Escherichia coli, Staphylococcus aureus, Salmonella sp., and Salmonella pullorum. These bacteria are field isolates from poultry originating from the collection of the Microbiology Laboratory of the Faculty of Veterinary Medicine, Universitas Gadjah Mada. Non-pathogenic bacteria used as a comparison was Bifidobacterium bifidum, which is a collection of the Microbiology Laboratory of PAU - UGM. The medium agar used included Nutrient Agar and Nutrient Broth (Oxoid), Mueller Hinton Agar (Merck), De Man Rogosa, and Sharpe (MRS) agar and MRS broth (Merck). Tetracycline (Katitra, PT. Yekatria Farma, Solo-Indonesia) was used as a positive control.

2.2. Methods
Analysis of nutrient composition, calcium and phosphorus content was based on the AOAC method [7]. Gross energy measurements using calorimeter bombs (basic IKA C2000, IKA-Works, Inc., Wilmington, NC, USA). The extraction of phytochemical compounds of atung seed was carried out by the maceration method using 96% of ethanol. Thin Layer Chromatography (Camag TLC Scanner 4, Germany) was used to qualitative analysis of phenols, tannins, flavonoids, alkaloids, saponins, and steroids compound. The antibacterial test used the agar diffusion method [8]. Atung seed flour is made as a solution with concentration of 0% (negative control), 0.5, 1, 2, 4, 8, 16 and 32% (w/v). The 50 ppm of tetracycline was used as a positive control. The strength of bacterial inhibition is categorized: very strong (clear zone> 20 mm), strong (clear zone 10-20 mm), medium (clear zone 5-10 mm), weak (clear zone <5 mm) [9]. Data of nutrient composition and phytochemical screening were analyzed descriptively. Data of antibacterial test was analyzed by analysis of variance in a Complete Randomized Design using nine treatments and four replications [10].

3. Result and discussion

3.1. Nutrient composition
Atung seeds contain relatively low protein, extract ether, ash, crude fiber, but high of non-protein nitrogen (NPN) and gross energy (Table 1). It is not yet known clearly what compounds in seeds caused high energy content, presumably because of the high nitrogen-free extract content. In addition to the ether extract content, publications about the chemical composition of atung seed have not been available. The ether extract content of the seed in this study is 2.0%, which was much lower than other studies that P. glaberrimum seeds contained 15% oil [11]. This difference is thought to be due to the different sources of P. glaberrimum and analytical methods.
Table 1. Nutrient composition of atung fruit seed

| Nutrient                      | Contents |
|-------------------------------|----------|
| Dry matter (%)                | 88.45    |
| Crude protein (% DM)          | 5.86     |
| Ether extract (% DM)          | 2.00     |
| Crude fiber (% DM)            | 3.14     |
| Ash (% DM)                    | 2.37     |
| Nitrogen free extract (% DM)  | 75.08    |
| Phosphorus (% DM)             | 0.15     |
| Calcium (% DM)                | 2.10     |
| Gross energy (Kcal/kg)        | 5847.78  |

3.2. Phytochemical screening

Phytochemical screening (Table 2) showed that the atung seed contains several secondary metabolites, and these compounds have biological activities such as antibacterial. Besides providing beneficial compounds such as flavonoids, atung seed also contains tannins, which are antinutrients for poultry. Although tannin has many biological activities, the presence of high tannin in poultry rations reduced the digestibility value of protein, crude fat, and organic matter [12].

Table 2. Screening of phytochemical compounds of ethanol extract of atung seed

| Phytochemical component | Qualitative Test       | Color Spot in the Visible |
|-------------------------|------------------------|---------------------------|
| Phenols                 | +                      | Ferric chloride           | Gray Black                |
| Flavonoids              | +                      | Ammonia                   | Yellow                    |
| Tannins                 | +                      | Ferric chloride           | Gray Blue                 |
| Saponins                | +                      | Anisaldehyde sulphuric acid | Bluish Green            |
| Alkaloids               | +                      | Dragendorff               | Orange                    |
| Steroids                | -                      | Liberman Bucard           | Red Brown                 |

+ Detected in atung seed extract; - Not Detected in atung seed extract

3.3. Antibacterial test

In general, atung seed can inhibit the growth of E. coli, Salmonella sp, S. pullorum, S. aureus, and B. bifidum (Table 3). The diameter of the inhibition zone increases (P<0.05) with an increasing concentration of atung seed flour. It is because of the higher concentration of atung seed flour, and it also increases the concentration of secondary metabolites such as flavonoids, tannins, saponins, and alkaloids that act as antibacterial. Higher antibacterial agent concentrations can inhibit more bacteria [13].

Table 3. Inhibitory zone (mm) of atung seed at different concentrations against pathogenic and non-pathogenic bacteria.

| Treatments | E. coli (Inhibition Zone mm) | S. pullorum (Inhibition Zone mm) | S. aureus (Inhibition Zone mm) | B. bifidum (Inhibition Zone mm) |
|------------|------------------------------|----------------------------------|-------------------------------|---------------------------------|
| TA 0%      | 0.00 ± 0.000a                | 0.00 ± 0.000a                   | 0.00 ± 0.000a                 | 0.00 ± 0.000a                   |
| TA 0.5%    | 0.00 ± 0.000a                | 5.26 ± 0.409b                   | 5.25 ± 0.767b                 | 0.00 ± 0.000a                   |
| TA 1%      | 4.64 ± 0.315b                | 6.39 ± 0.239c                   | 5.88 ± 0.674b                 | 4.23 ± 0.029b                   |
| TA 2%      | 5.51 ± 0.676b                | 7.91 ± 1.165d                   | 6.38 ± 1.038b                 | 4.50 ± 0.000b                   |
| TA 4%      | 5.59 ± 0.312b                | 10.93 ± 0.876c                  | 10.63 ± 1.161c                | 8.83 ± 0.723c                   |
| TA 8%      | 8.14 ± 1.080c                | 13.28 ± 0.936f                  | 13.16 ± 0.910d                | 10.53 ± 0.699d                  |
| TA 16%     | 11.41 ± 1.135d               | 16.64 ± 0.259f                  | 16.69 ± 0.229c                | 11.09 ± 0.779e                  |
| TA 32%     | 14.10 ± 0.647e               | 18.98 ± 0.119b                  | 18.85 ± 0.319f                | 12.50 ± 0.292f                  |
| T50        | 0.00 ± 0.000a                 | 11.89 ± 0.229d                  | 12.41 ± 0.713d                | 11.38 ± 0.550e                  |

Means in the same column with different letters are significantly different at P < 0.05

TA = atung seed flour (% w/v); T50 = 50 ppm of tetracycline
The ability of atung seeds to inhibit *E. coli* is categorized as weak at a concentration of 1%, medium category at concentrations of 2, 4, and 8%, while at concentrations 16 and 32% belong to the strong. Fifty ppm of tetracycline cannot inhibit the growth of *E. coli*. Probably, this isolate has resistance. The antibacterial activity of atung seed against *Salmonella* sp. and *S. pullorum* respectively ranged from 5.26 to 18.98 and 5.25 to 18.85 mm, respectively, where concentrations of 0.5-2% had moderate activity, and concentrations of 4 - 32% had strong activity.

The eight percent of atung seed concentrations generated the same inhibition zone with 50 ppm of tetracycline against *S. pullorum*, while *Salmonella* sp. provides a higher inhibition zone. Atung seed flour at concentration of 0.5% did not inhibit the growth of *S. aureus*, concentrations of 1 and 2% provide a weak inhibition, and at concentrations of 8, 16 and 32% provide strong inhibition. The level of 16% seed flour and 50 ppm of tetracycline resulted similar inhibitory effect on *S. aureus*. The results showed that the atung seed until the concentration of 2% had not been able to inhibit the growth of *B. bifidum*, and at a concentration of 4%, the inhibiting ability was still relatively weak (<5 mm). Compared to the pathogenic bacteria, *B. bifidum* was less susceptible to atung seed at low concentrations. It is because of each type of microorganism has a different sensitivity to antibacterial agents [13].

4. Conclusion
Atung seed contains phenols, tannins, flavonoids, saponins, and alkaloids compounds and can inhibit pathogenic bacteria. Thus, it is potential as a phytobiotic candidate in poultry ration.

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