Potency of several local phytogenic feed additives as antioxidant and antimicrobial sources for non-ruminant animals

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Abstract. The purpose of this study is to evaluate the potency of several local phytogenic (Vernonia amygdalina Del., Calotropis gigantean, Syzygium oleana, Syzygium cumini, L) as antioxidant and antimicrobial sources in feed additives for non-ruminant animals. Antibiotic as feed additive has been banned since 2006 due to harmful effect of antibiotic not only for animals but for consumers. Phytogenic as suitable natural alternatives has been intensively studied and had positively effect on animal health and productivity. Five extract phytogenic that massively grow in Aceh were extracted by using maceration method with the comparison of material and ethanol solvent 1:4. Antioxidant testing by using DPPH method and antimicrobial pathogen testing (Salmonella and E. coli) by using Kirby-Bauer disk diffusion method with gradient doses of extract (250 mg/mL, 500 mg/mL, 750 mg/mL), chloramphenicol as positive control and aquadest as negative control. The results of study indicated that Syzygium cumini, L had antioxidant activity (IC50 19.91) and inhibition zone diameter of 4.80 and 12.78 for E.coli and Salmonella respectively with the dose of 750 mg/mL. In conclusion, all phytogenic feed additives can be utilized as feed additives for non-ruminant animals with S. cumini, L as the best phytogenic feed additives.

1. Introduction

Escherichia coli and Salmonella spp infection in poultry production had a serious problem due to the effect of colibacillosis and salmonellosis and finally resulted high mortality in chickens up to 3 weeks [1]. Administration of antibiotics as animal feed without controlling in animal feed caused resistance of animal on antibiotics to pathogen bacteria both animals and humans. Antibiotic residues in animal product such as meat, egg and milk was harmful to humans consuming antibiotic residue products. Therefore, Indonesia government banned to utilize antibiotics as feed additives since 1 January 2018. Environmental factors such as high temperature and humidity caused oxidative stress to animals and reduced animal health and performances.

As a tropical country, Indonesia has abundant herbal medicines containing various bioactive components and metabolite compounds such as phenolic compounds, flavonoids, polyphenols, carotenoids, polypeptides flavones, alkaloids, and essential oils. These compounds encompass antifungal, anti-bacterial, antioxidant, and functional properties that function to modify animal metabolism for animal health and animal performance. Several plants that are source phytogenic feed additives such as Vernonia amygdalina Del., Calotropis gigantean, Syzygium oleana, Syzygium
cumini, L can be found easily in our surroundings. Phyto- genic feed additives contained various bioactive compound such as for V. amygdalina [2], alkaloid, triterpenoid, steroid, saponin, phenolic dan flavonoid for S. olea [3], alkaloid, phenol, flavonoid, tannin, and essential oil for Syzygium cumini, L [4] and flavonoid, saponin, steroid, and triterpenoid for Calotropis gigantean [5].

Research conducted by [6] indicated that administration up to 75ml/l of V. amygdalina Del improved broiler performance. C. gigantean is well known containing potency of anti-microbial [7] and its twigs had anti-microbial pathogen [8]. However, other research [8] reported that sap, juice, ethanol and n-hexane from C. gigantean was not shown inhibition activities of S. mutans, E. coli, S. aureus, and P. aeruginosa growth at the concentration 10000, 50000, 100000, dan 200000 μg/mL. Syzygium oleana had a relatively high antioxidant activity at IC50 with 26.68 ppm and was able to function as anti-mutagenic in mice [9] and Syzygium oleana had potency as anti-bacterial [3]. Syzygium cumini was extracted by water containing high antioxidant close to vitamin C with the value of IC50 : 6.98[10]. However researches relating to the ability of those bioactive compounds containing in phyto- genetic feed additives as sources of antimicrobial mainly Escherchia coli dan Salmonella spp were still limited. Therefore, it was considered important to study the potency of several local phyto- genic (Vernonia amygdalina Del., Calotropis gigantean, Syzygium oleana, Syzygium cumini L.) as antioxidant and antimicrobial sources in feed additives for non-ruminant animals.

2. Materials and Methods
2.1. Sample Collection and Preparing Ethanol Extract by Maceration
Leaves of V. amygdalina Del., C. gigantean, S. olea, S. cumini L. (Figure 1) as samples were collected and then dried up to 2-3 d to reach dry matter about 15%. Samples were ground by hammer mill and then soaked with 96% ethanol for 2-3 d with comparison of samples and ethanol (1:4). After being soaked, samples were evaporated to produce in the form of paste that were used to test antioxidant and antibacterial.

![Figure 1](image-url) : Herbal (phytogenic) as feed additives used to replace antibiotics in animal feed: (1&2 ) Vernonia amygdalina-Daun Afrika, (3 ) Syzygium cumini-Jemblang (4 ) Calotropis gigantea-Biduri (5) Syzygium oleana – Pucuk merah

2.2. Testing of antioxidant and antibacterial
Quantitative test of antioxidant was carried out by using soaked free radical method 1,1-difenil-2-pikrilhidrazil (DPPH). Antioxidant acts as “free radical scavenger” by using DPPH to reach absorbance of 516 nm. Samples and standard solved in methanol and DPPH (1:1), then incubated in dark room and sealed with aluminium foil for 30 minutes at room temperature. Absorbent wavelength was measured at the wavelength of 516 nm. The percentage of DPPH absorbent was calculated by using the following equation:
I(%) = \frac{A_o - A_s}{A_o} \times 100\% \quad (1)

in which:
I = the percentage of DPPH absorbent reduction, Ao = absorbent of DPPH solvent stock, As = sample absorbent added with DPPH.

Antioxidant activities stated in the value of IC50 which was calculated by using linear regression of extract concentration (bpj) on inhibition (%). The value of IC50 was determined as concentration as result of 50% inhabitation (y=50).

Antimicrobial pathogen testing (Salmonella and E. coli) by using Kirby-Bauer disk diffusion method with three gradient doses of extract (250 mg/mL, 500 mg/mL, 750 mg/mL) [11] chloramphenicol as positive control and aquadest as negative control. Each extracted leaves sample based on gradient doses was tested on the disk paper which was inserted pathogen bacteria E. coli and Salmonella and incubated for 24 h. Diameter of inhabitation zone around the discs were measured from disk edge of paper disk to the end of clear zone in millimetre.

2.3. Data Analysis
All data including antioxidant and antimicrobial activities were not calculated based on analysis of variance and the data were descriptively explained. The value of parameter is based on the average of two replication. The antioxidant activity was calculated based on linear regression

3. Results and Discussion
3.1. Antioxidant Activity
Antioxidant was considered as inhibition of oxidative reaction as result of free radical destroying unsaturated fatty acid, cell wall membrane, blood vessels, DNA bases and fat tissues [12]. Table 1 shows quantitative antioxidant test of several phytogenic feed additives by using DPPH methods.

The value of R^2 for all phytogenic feed additives were close to 1 (Table 1). It was indicated that inhabitation percentage has correlation to samples extract concentration. The value of IC50 from all tested samples indicated that the value less than 50. It means that all tested samples had a very strong antioxidant activities. The less of IC50 values means the highest of anti-oxidant activities. Specifically, compound had a very active antioxidant, if the value of IC50 was less than 50 ppm. The value from 50-100 ppm categorized active, the value from 101-250 ppm categorized middle, the value from 250-500 ppm categorized weak and the value from 250-500 ppm categorized not active [13].

Table 1. The antioxidant activity test for several phytogenic feed additives for non-ruminant animals

| Samples         | Inhibition (%) | Regression     | R^2  | IC 50 (ppm) |
|-----------------|---------------|----------------|------|-------------|
| V. amygdalina (leaf) | 72.02         | y = 1.923x + 3.998 | 0.974 | 23.922      |
| V. amygdalina (bud)  | 54.92         | y = 1.424+16.79   | 0.980 | 23.322      |
| S. oleana        | 66.32         | y = 0.289x+40     | 0.966 | 34.602      |
| S. cumini        | 39.92         | y = 1.265x+24.81  | 0.922 | 19.913      |
| C. gigantea      | 44.04         | y = 1.560x+16.36  | 0.987 | 21.652      |

Note: IC50 = required concentration to reduce 50% of DPPH absorbent and R^2 = correlation coefficient of regression.

From tested samples, the highest of antioxidant activity was found in S. cumini, followed by C. gigantea, V. amygdalina, and S. oleana respectively. The highest of anti-oxidant in Syzygium oleana was high correlated the chemical compound in the extract of Syzygium oleana such as
flavonoid and phenol compounds [14]. It has been reported that there was a strong positive correlation (0.998) between phenol total and antioxidant activity in herbs [15].

3.2. Antimicrobial Activities

The positive results of antimicrobial test were indicated by the forming of clear zone around disk paper containing identified extract which was known as inhibition power. The results of antibacterial activity test for several phytopgenic feed additives by using Kirby-Bauer disk diffusion method [11] are presented in the Table 2.

Table 2. The antibacterial activity test for several phytopgenic feed additives for non-ruminant

| Bacterial Test | Concentration (mg/mL) | V.amygda lina (leaf bud) | V.amygd alina | S. oleana | S. cumini | C.gigantea |
|----------------|-----------------------|--------------------------|---------------|-----------|----------|-----------|
| Escherchia coli | 250                   | 7.02                     | 6.17          | 10.01     | 8.77     | 6.18      |
|                | 500                   | 8.09                     | 7.25          | 11.86     | 13.09    | 7.23      |
|                | 750                   | 8.60                     | 7.31          | 14.40     | 14.80    | 8.16      |
| Control (+)    | 26.03                 | 25.27                    | 25.47         | 25.85     | 26.33    |
| Control (-)    | 0                     | 0                        | 0             | 0         | 0        |
| Salmonella spp. | 250                   | 7.45                     | 6.12          | 9.54      | 10.53    | 6.09      |
|                | 500                   | 8.71                     | 6.52          | 11.35     | 11.65    | 6.90      |
|                | 750                   | 10.12                    | 7.05          | 12.28     | 12.78    | 7.79      |
| Control (+)    | 25.68                 | 26.76                    | 25.96         | 25.26     | 26.27    |
| Control (-)    | 0                     | 0                        | 0             | 0         | 0        |

Note: Control (-) : Aquadest; Control (+) : Chloramphenicol 30 µg

The criteria of antimicrobial activities was divided into 4 categories based on inhibition zone: weak, middle, strong and very strong for ≤ 5mm, 5-10 mm, 10-20 mm, and ≥ 20 mm respectively [16]. Based on this criteria, all tested sample indicated bacterial activities from middle to strong with the lowest concentration of 250 mg/mL for E. coli dan Salmonella. The highest of microbial activity was at the concentration of 250 mg/mL on E. coli bacteria for S. oleana, etc. followed by S. cumini, V. amygdalina and C. gigantean. However, at the concentration of 500 mg/mL, S. cumini was higher antimicrobial activities compared to S. oleana. When tested with Salmonella, at the concentration of 250 mg/mL, S. cumini was the highest antimicrobial activity and categorized as strong category and the other phytopgenic feed additives were middle category (Figure 2).
Vernonia amygdalina

Vernonia amygdalina (young leaves)

Syzygium oleana

Syzygium cumini

Calotropis gigantea

Figure 2. Testing for Inhibition activities of several phytogenic feed additives by using two bacteria; *Escherchia coli* (A) and *Salmonella sp.* (B) at the concentration of 250 mg/mL; 500 mg/mL; and 750 mg/mL.

It was reported that ethanol-water fraction of *S. oleana* inhibited *E. coli* bacteria at the concentration of 1% with diameter of inhibition zone 6.97 mm [3]. Meanwhile, inhibition zone ethanol extract of *S. cumini* for *E. coli* was 10.22 mm with the concentration of 1000 ppm [17]. Extract of *S. cumini* with n-hexane inhibited the growth of *S. typhi*; middle at the concentration of 30% and strong at the concentration of 50%. Flavonoid compound was synthesized by plants as response of microbial infection. Therefore, it was effective for antimicrobial function. The mechanism action of this process might be caused by the ability of flavonoid to form complex of extracellular protein and soluble protein to form bacterial cell walls. Lipolytic flavonoid disturbed microbial membrane and destroy bacterial cell wall permeability [19].

4. Conclusions

Based on antioxidant and antimicrobial test for 5 ethanol extract phytogenic, it was concluded that all phytogenic feed additives in this study can be utilized as feed additives as alternative to antibiotics for non-ruminant animals with *S. cumini* as the best phytogenic feed additives.

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