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PHYTOCHEMICAL POTENCY AND ANTIMICROBIAL ACTIVITY OF ARECA VESTIARIA GISEKE AS A CANDIDATE FEED ADDITIVES IN BROILER

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Abstract

Abstract o protect themselves, plants accumulate the secondary metabolites as a source of antioxidant and antimicrobial. The objectives of the present study were to investigate the potency of Areca vestiaria Giseke as a candidate for broiler feed additives. The screening methods were performed using phytochemical screening, antibacterial assay against Escherichia coli and Staphylococcus aureus, antioxidant assay, and proximate analysis. The result showed that plant material extracted with 50% ethanol in water, contain flavonoid, tannin, saponin, and quinone. This compound can play a role as an antioxidant agent. An antioxidant activity (% inhibition) of Areca vestiaria is 32.58 ppm. There is antimicrobial activity either by microdilution or diffusion method. Proximate analysis showed, Areca vestiaria contain 6.59% moisture, 3.71% ash, 5.33% crude protein, 4.7% extract ether, 1.1% crude fiber, 47.16% nitrogen free extract, and 4045 kcal kg^{-1} metabolizable energy. From this study can be concluded that based on content of nutrition, antioxidant activity, and antibacterial activity, Areca vestiaria Giseke can be used as feed additives in broiler ration.

Key words*: phytochemical, antimicrobial activity, feed additives, Areca vestiaria.*

INTRODUCTION

In Indonesia since January 2018, antibiotic growth promoter (AGP) is prohibited its use in chicken feed. AGP is an antibiotic that is added in the diet in small concentrations to help suppress the adverse microbes present in the gastrointestinal tract. Prohibition of use of AGP provides consequences of possible nutrient absorption disorders that will eventually interrupt the productivity of chickens. Exploration of natural materials that have double biological activity, both as antibiotics and antioxidants become one of the target researchers. Based on several studies that have been developed, the compounds that have potential as antimicrobial and antioxidants are generally tannins, saponins and alkaloids as well as phenol compounds such as flavonoids. One of the plants that potentially contain these compounds is *Areca vestiaria* (yaki betel nuts). Yaki betel nuts are a kind of wild palm, one of the ornamental plants, endemic to the eastern part of Indonesia. In the area of origin of North Sulawesi, this betel nut called "*pinang yaki*" (monkey nut) because it is a typical monkey of Sulawesi *Macaca nigra* (black monkey) like to dwell on the stem of betel nut tree and eating the fruit. Yaki betel nuts are known to contain flavonoids as quercetin, catechins and tannins in seeds that have antioxidant activity against 2.2-diphenil-1-picrylhydrazyl.The amount of antioxidant figures closely related to the content of flavonoids. The more flavonoids contained the greater the total antioxidant activity. Utilization of yaki betel nut, especially the seeds as one source of antioxidants and antimicrobial need to be studied for livestock.This study aims to explore and test the potential of *Areca vestiaria* Giseke as a broiler feed additive.

MATERIALS AND METHODS

The research material consisted of yaki betel nut (AV) flour obtained during March to August 2015 from Tomohon area of North Sulawesi. The preparation of AV flour sample begins by separating the flesh from the fruit in a fresh state. Seed part was dried, after dry

separated from seed leather. The seeds were then dried again using a 40°C oven, resulting in a water content of less than 10%. The dried seed sample was smoothed with a JZ7114 1400 rpm type milling machine to obtain a size of 65 mesh (Satolom et al., 2015). Proximate analysis of yaki betel nut flour followed AOAC method 2003.6 (2005). Qualitative analysis (phytochemical screen) and quantitative flavonoids, catechins, tannins, and saponins were performed according to Harborne (2006).

Quantitative antioxidant activity test of the sample was performed following the procedure applied by Laboratory of Research Center for Medicinal Plants and Herbs, Bogor, Indonesia. Determination of antioxidant activity was done by α method, α-diphenyl-β picrylhydrazil (DPPH) (Li *et al*., 2011). The process for obtaining resistance values in DPPH was used a series of residual volumes that have been dissolved in methanol p.a. into a test tube containing a volume of test sample added 1 mL of methanol and 1 mL of 0.002% DPPH solution (time recorded at the time of addition of DPPH solution). The solution was shaken and allowed to stand for 30 minutes in a dark room and measured uptake at a wavelength of 517 nm. The calibration curve is made by making a series of butylated hydroxyl toluene (BHT) solution as standard. The inhibitory strength is expressed in% inhibition (IC50) which can be determined by making the concentration graph as the x-axis and % inhibition as the y-axis to obtain linear linear equations y = ax + b. Then this value is converted into μmol-ek unit BHT.100^{-1} g fresh weight. Manufacture of BHT calibration curve. BHT stock solution was made with 1000 μg mL^{-1} in methanol. Then the stock solution was diluted to a working solution of 10 μg mL^{-1}. The standard series solution was made by piping the working solution so that the standard end result of the standard series was 2.27-227.27 μg mL^{-1}. The percentage of inhibition can be determined by drawing concentration graph as x-axis and% inhibition as y-axis, so that linear line equation y = ax + b. Then this value is converted into μmol-ek unit BHTx 100^{-1} g fresh weight. Percentage of DPPH radical inhibition was calculated by the formula:

% inhibition = (Abs. control - Abs. Sample)/(Control Abs) × 100

The percentage of inhibition states the antioxidant activity for each sample. All the analysis was carried out twice, and the taken is the average value. BHT (Sigma Chemical Co., St. Louis, MO), was used as an antioxidant reference.

Determination of water content of sample is done by heating method (AOAC 2005) using oven. Dry the empty cup covered in oven 105°C for 3 hours then cooled in desiccator, then weighed (W1). The sample is weighed as much as ± 3 grams and fed into the porcelain plate evenly, place the cup containing the sample and closed into the oven and dry at 105°C for 3 hours then cooled in desiccator, then the sample weighed. Then dried again with oven and cooled in a desiccator until it reaches a constant weight (W2).Calculation of water content as follows:

Water content (%) = $(W_1-W_2)/(W_1) \times 100$

where:

W_1 = weight (g) sample before being dried

W_2 = weight (g) of sample after being dried

The antibacterial test performed on this experiment was by agar diffusion method. Preparation of test solution with various dilution of ethanol extract 70% of yaki betel nut was 10%, 15%, 20%, 25% and 30%. The medium used is NA (nutrient agar) and TSA (tryptone soya agar). The sterilized NA and TSA media were added each with *S. aureus* bacteria for NA and E. coli for TSA of 0.1 mL 100 mL^{-1} and then the media poured into different petri dishes already marked using markers for each extract concentration of 20 ml. Each bacterium was made of 5 Petri each. After the NA and TSA media hardened then the paper discs were placed on the already labelled medium and dripped with ethanol extract 70% *Kalanchoe pinnata* (Lam.) leaf as much as 10 μL with concentration of 10%; 15%; 20%; 25% and 30%. For positive control, on other paper discs dripped with 10μL of chloramphenicol solution and for negative control only containing DMSO-treated dessy paper (dimethyl sufoxide). The Petri dish was then wrapped in wrap paper and incubated at 37°C for 24 hours in an upside position. The resistor zone formed on each disc was measured using a sliding range.

The other antibacterial test performed on this experiment was by microdilution method on 96

well plates. The media used are TSB (Typtic Soy Broth) and NB (Nutrient Broth). A number of *S. aureus* and *E. coli* made and determined its optical density. The extract was diluted in DMSO with a concentration of 10000-78.12 µg mL^{-1} in the well. A total of 100 µL TSB and 10 µL inoculant solutions of *S. aureus* bacteria were added, as did 100 µL NB and 10 µL inoculant solution of E. coli bacteria. The process was continued with incubation at 37°C for 24 hours. MIC (minimum inhibitory concentration) is the lowest concentration of the well clear after incubation. The clear wells were piped 100 µL to 96 new well plates and TSB media added to *S. aureus* and NB media for *E. coli* of 100 µL. Incubation was continued for 24 hours at 37°C. Continued with incubation for 24 hours at 37°C. The clear well after incubation is MKC (minimum kill concentration). DMSO 20% was used as a negative control whereas positive control was used chloramphenicol.

RESULTS AND DISCUSSIONS

A qualitative test of phytochemical AV flour is conducted to determine the chemical compounds contained. Determination of this class of chemical compounds by looking at the presence or absence of color changes by reagents used. Furthermore, a quantitative test of flavonoids, catechins, tannins and saponins was performed. Phytochemical test results obtained from this study can be seen in Tables 1 and 2.

The results of qualitative phytochemical tests show that yaki nut seeds contain saponin, tannin, alkaloid, phenolic, flavonoid, triterpenoid and glycoside compounds which are likely to be bioactive potential compounds. It is known that phytochemical compounds are produced with the ability to protect these plants against environmental attacks (Gonzales-Lamothe et al., 2009).

Tannins, and flavonoids have antitumor, antiallergic, antihepatotoxic, and antioxidant activity. The triterpenoid group can be used as an antibacterial, anticancer, and to treat wounds and inflammation (Simbala and Tallei, 2010).

Feed ingredients generally contain anti-nutritional substances that can inhibit the efficiency of its nutrient utilization (Farrel, 2005).

Table 1. Phytochemical screening of ethanol extracts of *Areca vestiaria* Giseke[1]

Sample code	Secondary metabolites	Result[2]	Note
AVB red	Saponins	+	Produce stable foam after shaking
	Tannins	+	Produces a greenish black after droping 1% FeCl
	Alkaloids	+	Produces red sediment color after added Dragendrof reagent, and brownish color after added Wagner reagent
	Phenolic	+	There is a change in color to blackish blue after dropping FeCl3 5%
	Flavonoids	+	Produces orange in the amylalchohol layer
	Triterpenoid	+	Does not produce red colour after adding anhydrous acetic acid and concentrated sulphuric acid
	Steroid	-	Does not produce light blue after adding anhydrous acid and concentrated sulphuric acid

Note: [1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015); [2]+ = present; - = not present

Table 2. Secondary metabolites content of *Areca vestiaria* Giseke

Secondary metabolites	%
Flavonoids as quercetine	0.33
Catekin	3.5
Tannins	8.41
Saponins	0.92

Note: [1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015).

To anticipate that we would analyzed the nutritional and anti-nutritional content of AV (Table 2 and 3). AV seeds contain flavonoids, catechins, tannins, and saponins. The compounds are classified as compounds of bioactive potential or secondary metabolites. Secondary metabolites are non-nutritional chemicals that play an important role in the process of joint existence and evaluation among the different types in the environment (Mursyidi, 1989). It is known that these secondary metabolic compounds are produced by the ability to protect the plants against environmental attacks. It also has antioxidant activity that can neutralize the instability that occurs due to the existence of reactive molecules called free radicals. These compounds classified as secondary antioxidants (Winarsi, 2007), which serves to capture the oxidant compounds and prevent the occurrence of chain reactions.

In several studies that have been conducted on the activity of the three types of flavonoids it

turns out that quersetin studied in broiler to see its activity as immunostimulan with a dose up to 100 mg kg^{-1} BW (Zulnaidi, 2000). Another study of quercetin extracted with methanol from guava leaf, to a dose of 21.0 mg has not demonstrated contraceptive antifertility activity in white rats (Ariani et al., 2008). Catechins have inhibitory activity of converting histidine into histamine in the presence of histidine decarboxylase enzyme (associated with immune response), inhibiting cytokines TNF-α and IL-1β proteins. Catechins of green tea at doses of 800 mg kg^{-1} BW per day administered to mice can inhibit the growth of mammary gland tumors by 57.14% (Gunawijaya et al., 1999). The antioxidant activity of catechins and epicatechins was studied on the *Acacia catechu* tree and the leaves and stems of pale catechu (Duangyod et al., 2014). Antibacterial activity of Gambir has performed by Amos (2009) with catechin content of 25-35% and tannin of 60-65%. Inhibition of the action of digestive enzymes by tannins was generally indicated by the ability to binding proteins. Tannin inhibits pectinase, cellulase, amylase, protease, β-galatosidase, lipase and other enzymes that play a role in microbial fermentation (Fahey and Jung, 2000). The greatest inhibitory affinity of tannins was greater in protein than carbohydrates, due to the strength of the affinity of hydrogen binding to carboxyl oxygen in the peptide group. The use of tannins in the feed is limited to 2.6 g per kilogram of ration (Pour-Reza and Edriss, 1997).

Table 3. Nutrient composition of *Areca vestiaria* (as fed)[1]

Chemical composition	
Dry mater (%)	93.41
Ash (%)	3.71
Crude Protein (%)	5.33
Crude Fat (%)	4.7
Crude Fiber (%)	1.1
Nitrogen Free Extract (%)	47.16
[2]ME (kcal/kg)	4045

Note: [1]The results of the analysis of the Feed Science and Technology Laboratory, Fapet IPB, Bogor; [2]ME - metabolisable energy.

AV seeds contain dietary substances in the form of fat, protein, crude fiber and energy as listed in Table 3. Carbohydrates are components according to proximate analysis i.e. nitrogen free extract (NFE) and crude fiber. The easily digestible fraction and used as an energy source are NFE, whereas the hard-to-digest fractions are classified as crude fibers (Tilman et al., 1994). NFE contains soluble compounds in acidic and alkaline solutions and has high digestibility such as mono, di, tri and polysaccharide substances, especially starch. The content of food substances in the AV shows that this material can be used as a source of feed.

AV antioxidant activity compared with vitamin E can be seen in Table 4. This antioxidant activity test to know the value of resistance activity against free radical by DPPH method (2,2-diphenyl-1-picrylhydrazil).The parameters used are IC50 which was defined as the concentration of antioxidant compounds which causes 50% loss of DPPH activity (Molyneux, 2004). A substance has antioxidant properties if the IC_{50} value was less than 200 ppm. The smaller value of IC_{50} was then the compound effectiveness as a better radical catcher. The results of this study indicate that vitamin E antioxidant activity was better than AV.

Table 4. Antioxidant activity of *Areca vestiaria* and vitamin E

Test material	% inhibition (ppm)
Biji *Areca vestiaria*[1]	32.58
Vitamin-E[2]	10.43

Note:[1]Result of laboratory Analisis of Research Center for Medicinal Plants and Herbs. Bogor (2015);[2]Kurniawati (2011)

CONCLUSIONS

Areva vestiaria Giseke (AV) seed meal can be used as a feed additivesin broiler chickens ration in terms of phytochemical and antimicrobial activity.

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