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Evaluation of Gooseberry (*Phyllanthus acidus L.* Skeels) Leaf Extract Based on Phytochemical, Total Flavonoid, and Antibacterial Activity as Potential Feed Additive in Broiler

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Abstract**—** *The aim of this research was to identify the phytochemical, quantify total flavonoid and antibacterial activity of Phyllanthus acidus leaf extract (PALE). In vitro antibacterial properties were determined by disc diffusion method against Escherichia coli and Lacic Acid Bacteria. The collected quantitative data were analyzed by using the analysis of variance, then continued with Duncan's multiple range test (DMRT) if there were significant differences. The result showed that PALE has bio-active compounds includes alkaloid, flavonoid, tannin, and steroid. The total flavonoid was 663,92 µg/ml QE. PALE had statistically highly significant effect (P<0.01) on inhibition zone against between pathogenic and non-pathogenic bacterial species compared to the standard antibiotic zinc bacitracin. PALE 2% (P3) produced the greatest clear zones than PALE 1.5% (P2) and PALE 1% (P1), but significantly lower than control antibiotic zinc bacitracin containing group. It may be concluded that PALE could potentially be used as antimicrobial feed additive in broiler.*

Keywords**—** *Phyllanthus acidus, phytochemical, lavonoid, antibacterial activity.*

I. INTRODUCTION

Antibiotics in the poultry production are used to prevent and treat diseases, as well as antibiotic growth promoters (AGPs). Currently, it has been changed considerably by implementing antibiotic-free poultry feed because of public health concern. In addition, the use of inappropriate antibiotic is associated with the resistance of microorganisms and residue on food product [1].

There are several studies attempted to find the natural alternative to antibiotic use. Some herbs have been elaborated to be used as feed growth promoter alternative to improve broiler performance. The herbs contained a variety of secondary metabolites which classified as phytobiotic. Flavonoid is a common phytobiotic with a good potent source and antimicrobial substances.

Phyllanthus acidus L. Skeels is commonly known as gooseberry is an underutilized botanical plant. *Phyllanthus acidus* has bio-active compounds that serve as antimicrobial, antioxidant, immunomodulator, acidifier which is a potential phytobiotic [2]. *Phyllanthus acidus* leaf extract have antibacterial activity evaluated in fish [3] *Phyllanthus acidus* leaf also has a pharmacologycal effect as anti-cholesterol [4]. Considering that *Phyllanthus acidus* leaf contained high flavonoid, it might be expected to improve performance of broiler. The current research aims to identify the phytochemical, quantify total flavonoid and antibacterial activities of *Phyllanthus*. *acidus* leaf extract as candidate for broiler antimicrobial feed additive.

II. MATERIALS AND METHODS

A. Materials

The materials used in this research were *Phyllanthus acidus* fresh leaf, soxhlet extractor, microwave, incubator, autoclave, spectrophotometer, vacuum drying oven, phytochemical's reagent, MHA medium, and bacteria isolate.

Extraction

The extraction of *Phyllanthus acidus* leaf was carried out according the previous method [5]. *Phyllanthus acidus* leaf was macerated for 48 hours with ethanol 96% (ratio 1:4 b/w). Then it is extracted in the microwave oven for 10-15 minutes at controlled temperature 40-50°C. Each extract was filtered through muslin cloth. All of the extracts were kept at 4°C before further analysis.

Phytochemical screening

Leaves extract was qualitatively tested for the presence of bio-active compounds as alkaloids, terpenoid, saponins, flavonoids, tannins and steroid. It identified by the characteristic colour changes by using standard procedures [6].

1. Alkaloids: The extract was tested for the presence of alkaloids with Mayer and Wagner's test. Formation of either a yellow cream precipitate with Mayer's reagent or brown/reddish brown precipitate with Wagner's test indicates the presence of alkaloids.
2. Terpenoids: The extract was tested for the presence of terpenoids with Salkowski's test.
3. Saponins: A small quantity of the methanol extract was boiled. The mixture was filtered and 2.5 ml of the extract was added to 10 ml of the distilled water in a test tube and shake well for about 30 seconds and observed for frothing.
4. Flavonoids: 1.5 ml of 50% methanol was added to 4 ml of the extracts. After warming add magnesium fillings

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followed by a few drops of concentrated hydrochloric acid. A pink or red color indicates the presence of flavonoid.

5. Tannins: A portion of the extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution were added. A blue or green color indicates the presence of tannins.

Total flavonoid

Total flavonoid content (TFC) was measured with the aluminum chloride colorimetric assay. The calibration curve was established by using quercetin (QE) to estimate concentration of flavonoid.

Antibacterial activity

The well diffusion test used as antibacterial activity and performed using MHA medium. The medium was autoclaved at 121ºC for 15 minute. It was cooled down until 50-55 ºC in the water bath. The medium was poured into sterile petriplates. Once the medium was solidified, then the culture was inoculated on the medium. Within 15 minute of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacterial suspension. The sterile swab was used to streak on the surface of the MHA containing plates.

B. *Experimental Design*

For measuring the effect of PALE antibacterial activity, an *in vitro* experiment was carried out. There were three treatments, and each treatment had three replications. The experimental design was completely randomized design with the treatments given were:

P0 = zinc bacitracin (control)
P1 = 1% *Phyllanthus acidus* leaf extract
P2 = 1,5% *Phyllanthus acidus* leaf extract
P3 = 2% *Phyllanthus acidus* leaf extract

C. *Variable Observed*

The variables observed in this study were:

a) Phytochemical constituent.
b) Total flavonoid.
c) Zone inhibition of *Escherichia coli* and *Lactic Acid Bacteria* (LAB).

D. *Statistical Analysis*

Data on phytochemical constituent and total flavonoid were analyzed descriptively. While, data on diameter of zone of inhibition were analyzed with one way ANOVA and continued with Duncan's Multiple Range Test (DMRT) if there were significant differences.

III. Results and Discussion

A. *Qualitative Analysis of Phytochemical Constituents of Leaf Extracts of Phyllanthus Acidus*

TABLE 1. Phytochemical constituents of leaf extracts of *Phyllanthus acidus* L. Skeels.

Phytochemical constituent	Result
Alkaloid	+
Saponin	-
Tannin	+
Flavonoid	+
Terpenoid	-
Steroid	+

Note: + = Present; - = Absent.

The result showed that *Phyllanthus acidus* leaf extract (PALE) contain of alkaloid, tannin, flavonoid and steroid. These results were in agreement with previous studies that *P. acidus* leaves extracts contain some important phytochemical constituent i.e. flavonoids, phenolic compounds, alkaloids, steroids and glycosides are present in leaf extract [6, 7] who found flavonoids in *P. acidus* leaf extract.

B. *Quantitative Analysis of Flavonoid Constituents of Leaf Extracts of Phyllanthus Acidus*

TABLE 2. Flavonoid content

Flavonoid Content	Result (µg/mL) QE
Total Flavonoid	663,92

Flavonoids are the low molecular weight polyphenolic secondary metabolic compound. Polyphenols are often extracted in more polar solvents. Ethanol extract in this research showed total flavonoid content was 663,92 µg/mL QE. Previous study, PALE extraction with different solvent and concentration showed that PALE 70% had total flavonoid 0,50 mg/g QE and 0,51 mg/g QE for PALE with absoulte ethanol [8]. On the other study showed the total flavonoid content of methanol extract was found to be 61.28 mg/g QE [9]. There were many factors affecting different amount of total flavonoid i.e. type of solvent, extraction methods, concentration, etc. Total flavonoid content of PALE with ethanol solvent showed that ethanol had polarity level that resembles and is more effective in dissolving flavonoid compounds in ceremai leaves, so that PALE by using ethanol solvent produces high flavonoid compounds.

C. *Evaluation Antibacterial Activity of Phyllanthus Acidus Leaf Extract*

TABLE 3. Effect of treatment on diameter of inhibition

Group	Diameter of Inhibition Zone (mm)	
	Lactic Acid Bacteria	*Escherichia coli*
P0	$3,34 \pm 0,06^{d}$	$4,60 \pm 0,35^{d}$
P1	$0,33 \pm 0,10^{a}$	$2,59 \pm 0,28^{a}$
P2	$1,30 \pm 0,23^{b}$	$3,20 \pm 0,11^{b}$
P3	$2,28 \pm 0,23^{c}$	$3,74 \pm 0,2^{c}$

Notes: The different superscripts in the same column showed highly significant difference (P<0.01).

Antibiotic zinc bacitracin and PALE as antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones were measure. The quantitative estimation of antibacterial activity of PALE against pathogenic and non pathogenic bacteria compared with standard antibiotic *zinc bacitracin* by measuring the zone of inhibition diameter and expressed in mm are shown in table 3.

Phyllanthus acidus leaves extracts (PALE) showed highly significant difference (P<0.01) effect on diameter of inhibition zone in the disc diffusion assay. Though the diameter of inhibition zone obtained by the use of antibiotic is the largest, the use of highest level of PALE having the closest response with antibiotic group.

Narrow inhibition activity was noticed both antibiotic and PALE with the averages zone of inhibition ranges from 0,3-

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4,6 mm (table 3). It means weak antimicrobial (<5mm). Earlier study about methanol **e**xtract of *P. acidus* showed the average zone of inhibition from 8-12 mm respectively [10].

PALE with various concentration used were lower than antibiotic (P0). Highest inhibitory activity was observed against the growth of *E. coli* with the zone of inhibition 4,6 mm (P0). Moreover, PALE in inhibiting the growth of Gram positive bacteria than gram-negative. It is thought to be due to differences in the structure of the cell wall between the two groups of the bacteria. Higher concentration of PALE (P3) had zone inhibition widest between P2 and P1. Flavonoids are antibacterial through 3 mechanisms, those are inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism.

IV. CONCLUSSION

Based on research results, it could be concluded that *Phyllanthus acidus* L. Skeels leaf had bio-active compounds which could be a source of antibacterial as potential natural feed additive for broiler.

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