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Research Article
In vitro Evaluation of Coconut Husk Potential as Phytobiotics for Poultry

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Abstract

Background and Objective: The current *in vitro* study was carried out to evaluate the potential of coconut husks as phytobiotics for poultry. **Materials and Methods:** Coconut husks were collected from the local market and dried in the oven. The dried materials were finely ground and extracted using methanol, ethyl acetate or acetone. Crude extracts from the three types of solvents were rotary evaporated until dry. The dry extracts were then subjected to chemical analysis, evaluation of antioxidant and antibacterial activities and bacterial growth performance of *Lactobacillus acidophilus*. **Results:** The results indicated that the extracts consisted of bioactive compounds such as flavonoid, steroid, gallic acid and tannin and the content was affected by the type of solvent ($p<0.05$). The type of solvent also had a significant effect on antioxidant activity and antibacterial activity for *Escherichia coli* ($p<0.05$) but not for *Staphylococcus aureus* ($p>0.05$). Antioxidant activity (IC_{50}) was 85.15, 119.78 and 143.59 ppm ascorbic acid equivalent antioxidant capacity (AEAC) for methanol, ethyl acetate and acetone, respectively. The average inhibition for pathogenic *E. coli* was 11.82, 11.93 and 12.34 mm for methanol, ethyl acetate and acetone respectively, while inhibition for *S. aureus* was 11.99, 12.18 and 12.27 mm for methanol, ethyl acetate and acetone respectively. Interestingly, the extracts also produced a significant effect ($p<0.05$) on the growth of *Lactobacillus acidophilus*. The average growth improvement was 1.62 and 1.77 nephelometric turbidity units (NTUs) for methanol and acetone, respectively. **Conclusion:** Crude extracts of coconut husk have the potential to be used as phytobiotics for poultry production.

Key words: Antioxidant, coconut husk, *Lactobacillus acidophilus*, phytobiotics, poultry production, *Staphylococcus aureus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feed additives such as antibiotics, flavorings, ionophores or growth hormones are used to improve intake, liveweight gain, feed efficiency and health status of livestock. However, the excessive use of synthetic antibiotics as growth promoters and growth hormones had a negative effect on the animal by enhancing microbiota resistance in the digestive tract and environmental pollution[1,2]. Furthermore, some antibiotics have a serious undesirable side effect that limits their application. Therefore, there is an urgent need to develop feed additives that are very effective with minimal undesirable side effects on the animal as well as the environment. Plants or plant parts are potential sources for such feed additive as phytobiotics and can be delivered to livestock in solid, dried, ground or extract forms. According to Windisch *et al.*[1] and Gheisar and Kim[2] the positive effects of phytobiotics in poultry production are due to activation of feed intake, secretion of digestive enzymes, immune stimulation, antibacterial properties, anthelmintic, anti-inflammatory activities or antioxidant properties.

Many studies on alternative feed additives have focused on medicinal plants that are a rich source of pharmacologically active compounds and that have fewer side effects than synthetic antibiotics. The present study explored a nonmedical plant, the coconut (*Cocos nucifera*), that is commonly found in tropical areas. Coconut plants grow well in tropical region and are used as staple food crops and as sources of wood and handicrafts. They also show promise as botanical medicines. For instance, extracted material from dried coconut shells has antibacterial and antifungal activities because of the presence of phenolic compounds. The liquid extracted from the coconut husk fiber has activity against *Staphylococcus aureus* and the extract consisted of cathechin, epicatechin and condensed tannin[3], when it was extracted using water. Meanwhile, the *in vitro* study of Oliveira *et al.*[4] showed that ethyl acetate–coconut husk extract inhibited 100% of egg hatching and 99.77% of larval development of *Haemonchus contortus*. All of these beneficial effects of coconut husk extracts are due to the presence of many bioactive compounds[3-8] that can be used as a source of phytobiotics. The bioactive compounds and chemical composition of phytobiotics in the final product, however, may vary widely depending on the part of the plant used, geographical origins and maturity of the plant[1,9]. Therefore, coconut plants have the potential to be used medically, since they have bioactive compounds, antibacterial activity, antioxidant activity, a continuous supply, negligible side effects and a friendly environment. The objective of the present *in vitro* study was to explore the potential of coconut husk extracts as phytobiotics for poultry through the evaluation of bioactive compounds, antioxidant activity, antibacterial activity and bacterial growth promotion of the bacteria *Lactobacillus acidophilus*.

MATERIALS AND METHODS

Plant material: The young green coconut husk was obtained from the local market. The husk was finely chopped and dried to approximately 10% of its original water content. The dried husk was ground into powder and extracted using methanol, ethyl acetate and acetone, individaully. A 250 g of husk powder was mixed with 500 mL of solvent and the extraction process was performed for seven consecutive days based on Phoem and Voravuthikunchai[10] using automatic shaker (Pol-Eko Aparatura). The extract was filtered and rotary evaporated until the extracts became dried pellets. The pellets were subjected to chemical analysis for their bioactive compounds and were also individually dissolved in sterile aquabidest for further analysis and evaluation.

Bioactive compounds: The tannin content of the extract was measured using a spectrometer (T90+UV/VIS Spectrometer, PG Instruments Ltd.) and plant tannin was used as an internal standard based on Lokeswari and Sujatha[11]. Gallic acid, flavonoid and sterol were measured using thin layer chromatography (TLC) according to Striegel and Hill[12], in which quercetin and beta sitosterol were used as standards for flavonoid and steroid, respectively.

Antioxidant activity: Evaluation of the antioxidant activity was conducted using the diphenylpicrylhydrazyl (DPPH) method and the concentration of the tested extract was 2.5, 5 and 10 mg pellet in the solution. Krings and Berger[13] suggested that scavenging free radicals was assessed based on the absorbance at a wavelength of 517 nm using a spectrometer (T90+UV/VIS Spectrometer, PG Instruments Ltd.). Ascorbic acid was used as a standard of reference for antioxidant activity. The antioxidant activity of IC_{50} was calculated by linear regression and expressed as ppm of ascorbic acid equivalent antioxidant capacity (AEAC). Antioxidant activity evaluation was performed in three replicates per treatment.

Antibacterial activity: Agar diffusion based on Ayad *et al.*[14] was applied to evaluate the antibacterial activity of the coconut husk extract. Each extract was subjected to the antibacterial activity assessment for two species of bacteria. A suspension of the test bacteria *E. coli* and *S. Aureus* was

prepared to contain approximately 10^5 CFU mL^{-1} and the discs containing solid agar were inoculated by spreading up to 1 oz of bacterial suspension. A 100 µL aliquot of crude extract from individual solvents prepared at 6, 12, 25 and 50 mg extract mL^{-1} was placed in the hole (4 mm depth and 8 mm diameter). The discs were incubated for 24 h at 37°C to achieve maximum growth in the culture media. The diameter of the inhibition zone of growth was measured to estimate the degree of the antibacterial activity. Chloramphenicol antibiotic was used as a positive control. Assessment of the antibacterial activity was performed three times per treatment.

Growth-promoting assay: Due to technical problems, only two types (methanol and acetone) of coconut husk extract and two controls were subjected to the growth-promoting assay based on Phoem and Voravuthikunchai[10] and the tested bacteria was *L. acidophilus*. Indirect bacterial growth was assessed using a turbidity meter and unit of measurement was nephelometric turbidity unit (NTU). Nine milliliters of liquid growth media of MRS broth and 1 mL of extracts (1 mg mL^{-1}) or sterile aquabidest (negative control) was added to achieve 10 mL of the total volume. The positive control consisted of 10 mL of growth media only. All tubes were seeded with *L. acidophilus* by spreading up to 1 oz of bacteria at a cell density of 1.5×10^5 CFU mL^{-1}. The test tubes were anaerobically incubated for 2, 6, 10, 14, 20, 24, 48 and 72 h. After incubation, increased growth of the bacteria from each tube was measured using a turbidity meter (LaMotte 2020we, Maryland) in the three replicates.

Statistical analysis: Data were presented as the mean±standard deviation and were analyzed using the analysis of variance and Duncan's multiple range test was used for comparison means[15]. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Bioactive compounds: The coconut husk was extracted using three different solvents: methanol, ethyl acetate and acetone. Extracted materials contained bioactive compounds of gallic acid, flavonoid, steroid and tannin, as shown in Table 1. Gallic acid content was statistically similar for three different solvents but the other three components performed different values ($p<0.05$). The crude extracts were rich in tannin content and the highest levels were found in the methanol extract ($p<0.05$). The current finding is consistent with the results of Esquenazi *et al.*[3] and Oliveira *et al.*[4], who found tannin as a significant compound in the extract. This is supported by the fact that methanol and acetone are commonly used to extract tannin from the plant[16]. Meanwhile, the study by Sung *et al.*[7] reported that tannin was extracted from agricultural by-products using distilled water, ethanol and acetone and the highest content of tannin was achieved when extracted by acetone. Many more compounds have been found in coconut husk fiber extracts such as cathechin and epicatechin, using the water-coconut husk extraction method[3].

The chemical composition of the extracts in the present study is supported by previous studies[3,4,6,8]. Crude extracts of coconut husk may contain thousands of compounds including flavonoids, proanthocyanidins and even soluble polysaccharides[6]. Coconut husk yielded flavonoids, steroids and tannins when extracted using ethyl acetate[4]. Therefore, chemical compounds of extract varied widely due to the difference in the extraction/analysis method and/or due to the presence of other constituents. Consequently, this phenomenon leads to different performances in the efficacy of antioxidant and antibacterial activities.

Antioxidant activity: Polyphenol compounds are well documented as scavengers of reactive oxygen species, peroxide decomposers, electron donors and inhibitors of lipoxygenase[17]. Tannins have been reported to have the scavenging activity[18]. The mean values of the antioxidant activity of the extract are presented in Table 2. The types of solvents produced a significant difference ($p<0.05$) in the antioxidant activity. The antioxidant activity of the methanol extract was higher than that of the other two solvents ($p<0.05$). The values in Table 2 show that the lowest IC_{50} value of oxidation was achieved in the methanol extracts. This means that IC_{50} for methanol require only 85.15 ppm AEAC which is lower than 119.78 or 143.59 ppm AEAC, for ethyl acetate and acetone, respectively. Similarly, Sung *et al.*[7] revealed that the antioxidant activity of agricultural

Table 1: Bioactive compounds of coconut husk extracts from methanol, ethyl acetate and acetone

Solvents	Gallic acid (%)	Flavonoid (%)	Steroid (%)	Tannin (%)
Methanol	1.82±0.44	5.42±0.42^a	4.18±0.47^a	27.58±0.08^a
Ethyl acetate	2.11±0.21	4.51±0.47^a	9.89±0.34^b	24.64±0.04^b
Acetone	2.55±0.14	9.32±0.16^b	5.19±0.33^c	22.83±0.06^c

Values within the column that do not share a letter are significantly different ($p<0.05$)

by-products was higher in ethanol than in acetone or distilled water. Therefore, the present results indicated that the methanol-extract of coconut husk has effective and powerful antioxidant activity. Extracts from leaves of *Camelia tallensis* containing abundant hydrolyzable tannin produced remarkable antioxidant activity *in vitro*[5]. Moreover, tannins were extracted from persimmon leaves using chloroform and ethyl acetate has strong antioxidant activity[19]. The current study also revealed that the antioxidant activity was elevated as the concentration of extracts increased in the solution. The pattern agrees with our previous findings on legume leaves and bulbs of *Eleutherine americana* extracts[20,21].

The present study is also supported by the results of previous studies by Singla *et al*.[6] and Rodiah *et al*.[8], which demonstrated strong antioxidant activity of coconut husk extracts. *In vivo*, natural antioxidants improved nutrient digestibility, feed efficiency, egg production and egg quality[22]. Furthermore, the inclusion of natural antioxidants during the laying period significantly reduced malonaldehyde-egg yolk and had a positive effect on the oxidation stability of the egg-shell and improved fertility as well as egg hatchability. Abd El-Hakim *et al*.[23] reported that antioxidants generated from plant materials significantly improved the daily liveweight gain of broilers for the first 3 weeks.

The presence of an antioxidant in the diet protected for further fat oxidation, allowing improvement of broiler performance through an elevation in the intake, body weight and bodyweight gain[24,25]. The inclusion of an antioxidant, however, produced no effects on carcass weight, carcass percentage and physical characteristics of the meat[25]. Antioxidants also increased the antioxidant capacity of broiler chicken tissue[24] and reduced the lipid oxidation of breast meat[25]. These findings strongly suggest that the application of antioxidants in the broiler diet leads to improved broiler performance and quality of the meat.

Antibacterial activity: Antibacterial activity results are summarized in Table 3. The activity was first recorded when there was 6 mg exctract in the solution and the activity increased as the concentration increased. This result is consistent with the findings of Akiyama *et al*.[26] and Sakunpak and Panichayupakaranant[27]. They found antibacterial activity on polyphenol compounds. Other studies also reported that polyphenol, phenol, flavonoid and essential oil generated from plants reduce the growth of pathogen bacteria of *E. coli*, *S.aureus*, *Listeria monocytogenes* and *Salmonella* spp[28,29]. The type of solvent produced a difference in the average antibacterial activity on *E. coli* ($p<0.05$) and *S. aureus* ($p>0.05$). Acetone was higher than two other solvents. Different bioactive compounds of the extracts (Table 1) can explain the difference in the antibacterial activity. Although the extracts showed an efficacy of inhibition of 10.43-11.53 mm at the concentration of 6 mg extracts mL^{-1}, the value of inhibition was lower compared with chloramphenicol antibiotic inhibition 34.22 mm at the level of 0.01 mg mL^{-1} (data not shown) as a positive control. This fact can be explained by the presence of small concentrations of the active ingredients in the coconut husk extracts, unlike synthetic antibiotics, where the chemical compounds are isolated pure form. The presence of antibacterial activity in both gram-positive and gram-negative bacteria in the current study proved that these extracts could be categorized as a broad spectrum antibiotic to potentially replace synthetic antibiotics. *Escherichia coli* and *S. aureus* are well known as pathogenic bacteria in poultry and may cause mortality.

Table 2: Antioxidant activity (IC_{50}) of coconut husk extracts from different solvents at the concentration of 2.5, 5 and 10 mg of pellet mL^{-1} in the solution

	ppm AEAC			
Solvents	2.5	5	10	Average
Methanol	94.04±0.23[a]	88.08±0.59[a]	73.32±0.16[a]	85.15±0.27[a]
Ethyl acetate	128.99±1.75[b]	118.75±0.72[b]	111.56±0.62[b]	119.78±7.13[b]
Acetone	160.61±0.73[c]	141.61±0.19[c]	128.57±0.48[c]	143.59±0.44[c]

Values within the column that do not share a letter are significantly different ($p<0.05$)

Table 3: Bacterial growth inhibition of extracts from different solvents on *Escherichia coli* and *Staphylococcus aureus* at concentration of 6, 12, 25 and 50 mg of the pellet mL^{-1} in the solution

	Inhibition (mm)									
	Escherichia coli					*Staphylococcus aureus*				
Solvents	6	12	25	50	Average	6	12	25	50	Average
Methanol	10.97±0.23[a]	11.77±0.23	12.10±0.17[a]	12.47±0.23[a]	11.82±0.22[a]	11.10±0.69	11.77±0.23	12.23±0.06	12.87±0.23	11.99±0.27
Ethyl acetate	10.43±0.21[b]	11.77±0.23	12.33±0.23[ab]	13.20±0.17[b]	11.93±0.14[a]	11.23±0.46	11.97±0.40	12.47±0.23	13.07±0.40	12.18±0.38
Acetone	11.53±0.06[c]	12.17±0.06	12.63±0.06[b]	13.03±0.06[b]	12.34±0.03[b]	11.53±0.06	12.00±0.17	12.33±0.23	13.20±0.17	12.27±0.05

Values within the column that do not share a letter are significantly different ($p<0.05$)

Table 4: The growth of bacteria *Lactobacillus acidophilus* (NTU) on media with 1 mg of coconut extract mL^{-1} from methanol, acetone and control

	Incubation time (h)								
Solvents	2	6	10	14	20	24	48	72	Growth of the bacteria
Methanol	3.89	3.95	4.08	4.24	4.47	4.66	5.45	5.57	1.62 ± 0.02^{a}
Acetone	4.59	4.66	4.71	4.89	5.02	5.31	5.59	6.17	1.77 ± 0.04^{b}
Control (+)	3.48	3.66	3.87	3.95	4.14	4.34	5.50	5.94	2.64 ± 0.02^{c}
Control (-)	3.18	3.24	3.30	3.37	3.47	3.53	3.96	4.15	0.99 ± 0.03^{d}

Values within the column that do not share a letter are significantly different ($p<0.05$)

Bioactive compounds in specific media generally display antioxidant and antibacterial activities against bacteria and fungi, may even reduce the growth of mosquito larvae[30] and larval development of *Haemonchus contortus*[4]. The concentration of 6 mg extract/mL in the present study was high enough to produce antibacterial activity in all types of extracts. Referring to Table 1 and 3, there was only 0.15 mg gallic acid mL^{-1} or 2.5 mg tannin mL^{-1} in the solution. These values are far too low compared to 4-5.5 mg tannin mL^{-1} [31], 8 mg tannin mL^{-1} [32] or 10 mg tannin mL^{-1} [27] in the media needed to produce antibacterial activity. Another study reported that the minimum inhibitory concentration of tannin for *Pseudomonas fluorescence*, *S.aureus*, *E.coli*, *K. pneumoniae* was 5 mg mL^{-1} [33]. This discrepancy could be attributed to the phenolic compounds, the source of tannin and the nature of the tannin of the extracts. For instance, high affinity tannin will produce a strong cross-link between bacteria and tannin, leading to stronger disruption of membrane integrity. Studies have reported that the mechanism of inhibitory bacterial growth of tannin is the disruption of membrane integrity[34,35] or the reduction of iron availability for the microbe[36]. The antibacterial activity of chestnut tannins was quantitatively higher than that of mimosa tannins[37]. Additionally, the site and the number of hydroxyl groups on the tannin are also likely contributing to the toxicity level, as proved by Min *et al.*[37]. Alternatively, the antibacterial activity of tannin is related to its ability to inactivate microbial adhesion and inhibit hydrolytic enzymes[38,39].

Bacterial growth: Interestingly, the current study also revealed the ability of coconut husk extract to stimulate the growth of *L. acidophilus* bacteria (Table 4). This evidence is supported by the findings of Ahn *et al.*[40,41]. They reported bacterial growth-inhibiting activity for *E. coli* and *S. aureus* but not for *Bifidobacterium adolescentis* and *L. acidophilus*. The rate of the growth change was affected by the type of organic solvents, in which acetone solvent, in general, had better growth ($p<0.05$) than methanol solvent. Extracts from methanol and acetone solvents were able to stimulate growth better than the negative control ($p<0.05$), even though it was lower than the positive control ($p<0.05$).

Furthermore, the growth was linearly improved as the incubation time increased. Maligan *et al.*[42] reported that logaritmic phase growth was achieved at 35 h of incubation and continuously increased until 70 h of incubation. Additionally, *Lactobacillus* bacteria grew and improved within 21 days on yogurt milk[43]. The results of the current study indicated that the presence of coconut husk extract produces no harmful effects on the important groups of intestinal microbiota. Therefore, the inclusion of such extracts may improve the health status of livestock, leading to increased production level.

Bacteria of *Lactobacillus* and *Bifidobacterium* have been reported to contribute to beneficial effect on health[44], nutrition, physiology and antibacterial[45]. In fact, all nondigested carbohydrate that are categorized as prebiotics may stimulate the growth of those bacteria and therefore enhance animal productivity. For example, isomalto-oligosaccharida (IMOS), trans-galacto-oligosaccharida (TGOS), mannan-oligosaccharida (MOS) and pectin- oligosaccharida are categorized as prebiotics. These prebiotics have different mechanisms of stimulating the growth improvement of livestock and IMOS was selectively fermented for *Bifidobacteria* and *Lactobacilli* but not for *Salmonella* or *E. coli*[46]. However, manno-oligosaccharida enhanced the population of *Lactobacilli* in the ileum[47]. Moreover, the growth improvement of livestock was related to the improvement of energy used[47].

CONCLUSION

Extracted materials from the coconut husk consisted of bioactive compounds and demonstrated antioxidant and antibacterial activities. The extracts, to some extent, also have the ability to stimulate the growth of bacteria *L. acidophilus*. Therefore, coconut husk extracts have potential to be used as phytobiotics for poultry.

SIGNIFICANCE STATEMENT

Coconut husk extracts produced bioactive compounds that depended on the type of solvents used. The antioxidant

and antibacterial activities were affected by the type of organic solvent. The growth of bacteria *Lactobacillus acidophilus* can be accelerated by adding a specific amount of coconut husk extract to the growth media.

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