In vitro evaluation of phytogenic potential of seed from mango (*Mangifera indica*), moringa (*Moringa oleifera)* and sweet apple (*Annona squamosa*) for poultry

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ABSTRACT: A series *in vitro* works have been conducted to evaluate phytogenic potential of three seeds from tropical plants for poultry production. Local variety of mango (*Mangifera indica*), moringa (*Moringa oleifera)* and sweet apple (*Annona squamosa*) were locally collected from plantation. All types of seeds were dried and ground for further extraction process using organic solvent of methanol. Bioactive compounds from seed extraction process are phenol, tannin and fatty acid of solid crude fat. *In vitro* evaluation was carried out through evaluation of antimicrobial and antioxidants activity. The analysis methods were diffusion agar method and DPPH assay for antimicrobial and antioxidant activity respectively. The results indicated that methanol extraction from three types of seed have an antioxidant activity. Its activity tends to increase with increase in concentration of extract materials in the solution. Mean values of antioxidant activity were 17,75, 85.28 and 91.61% for moringa, sweet-apple and mango seed respectively. Extracted materials of mango and moringa seeds had antimicrobial activity on the tested bacteria of *Escherichia coli*, however, moringa seed did not produce an antimicrobial activity on *Salmonella typhi* as mango seed kernel did. Additionally, seeds from sweet apple had no antimicrobial activity on both *Escherichia coli* and *Salmonella typhi* bacteria. In conclusion, extract materials of mango and moringa seed in methanol had an antioxidant and an antimicrobial activity. Sweet apple seed is, however, only had an antioxidant activity.

Key words: mango, moringa, sweet apple seeds, antimicrobial and antioxidant

INTRODUCTION

Phytogenic as feed additive are derivated from plant or part of the plant such as leaves, seed or root used in animal feeding to improve animal performance. This class of feeding has gained increasing interest, especially for use in pig and poultry production system. This appears to be strongly driven by the fact that the use of synthetic feed additives or antibiotics has been reported to produce a risk as generating antibiotic-resistance in phytogenic micro biota and also may create environmental problems of antibiotic residues. As a consequence, new commercial additive of plant origin, considered to be natural products that the consumer would accept. However, complication arise of phytogenic feed additives may vary widely with respect to botanical origin, processing and composition.

The mango (*Mangifera indica*), moringa (*Moringa oleifera)* and sweet apple (*Annona squamosa*) are a tree-type plant, grows naturally in tropical region and is important plants for food materials. The fruits are partly consumed as vitamin C sources. The flower and leaf are commonly eaten as vegetable. Other parts of plant reasonably present antioxidant and antimicrobial. The current study has explored extract seeds of these three tropical-plants. Methanol-extract of the seeds were evaluated through *in vitro* study of their antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Experimental materials of mango (*Mangifera indica*), moringa (*Moringa oleifera)* and sweet apple (*Annona squamosa*) were collected from local plantation. The seeds were dried to about 10% of moisture. All seed ground individually to pass through 1 mm screen. Seed samples were analysed for

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crude protein, fat and crude fibre content (AOAC, 1985). Total phenol and tannin content were analysed using a procedure of Senter *et al.* (1989). All seed samples were individually excrtacted using methanol solvent. One part of sample was mixed througly with two parts of methanol (w/v). The mixtures were shaking for about 16 h at room temperature. The mixtures were then filtered using three layers nylon screen to remove a coarse particle and subsequently centrifuzed to get a clear extract. These clear extracts were rotary evaporated at 40^{o}C to reduce 50% volume and were kept for antioxidant and antimicrobial activity evaluation.

Antimicrobial Activity Evaluation. Antimicrobial activity was determined in agar well-diffusion assay against selected pathogen bacteria (Ayad *et al.*, 2000). An approximately 10^8 cfu of tested bacteria in 10 mL nutrient broth was incubated for 24 h at 37^{o}C. 100 µL of culture bacteria were added to 20 mL agar at 45^{o}C on the dishes. The dish-agar was rounded 8 mm diameter with a steril pippete after solidification. Subsequently, 100 µL of extracts in different concentration (0,1,3,5 and 7%) were dispensed in individual-dish wells. The dishes were incubated for 24 h at 37^{o}C and after which the diameter of the inhibition zones was measured.

Antioxidant Activity Evaluation. Antioxidant activity was evaluated using a method of DPPH assay. The free radical scavening activity of the seed extracts were measured by measuring the decrease in absorbance of methanolic DPPH solution at 517 nm of absorbance in the presence of the extracts (Krings and Berger, 2001). The initial concentration of DPPH was 0.1 mM and then the reading of absorbance was done after allowing the solution to stand for 30 min period. In the case of the absorbance decrease too much before 30 min period, the sample was appropriately diluted. The antioxidant activity was expressed as %= $\{(A_{control}-A_{sample})/(A_{control})\}* 100\%$.

Experimental design was a Completely Randomized Desing with 5 treatments within 3 replicates. Treaments were 0,1,3,5 and 7% of level extracts in the solution. The treatments were done for antioxidant and antimicrobial activity. Data were analysed using analysis variances of Steel and Torrie (1980). Treatment means were tested for significant treatment effects by Least Significant Different (LSD) analysis.

RESULTS AND DISCUSSION

Chemical Composition

All seeds used in this study were locally collected from plantation. Proximat analysis results and the profile of active components of methanol extracted materials from seeds of mango, moringa and sweet apple are presented in Table 1. Nutrients composition of experimental seeds were analyzed namely crude protein, lipid and crude fibre. Crude protein values of seed materials were 4.40, 29.62 and 17.06% for mango, moringa and sweet apple, respectively. Lipid values were 1.32, 27.41 and 20.32% for mango, moringa and sweet apple, respectively. The chemical composition of expereminetal seed in present study is to some extent not comparable to the previous studies (eg. Abdalla *et al*., 2007a,b; Anwar and Rashid, 2007; Kimbonguila *et al*., 2010). Nonetheless, the nutrients profile of the seed in present study indicated the potential used as supplement feeding for animal production.

Table1. Chemical composition of mango, moringa and sweet apple seed materials

	Protein	Lipid	Crude fiber	Phenol[1]	Tannin[1]
Seeds			%		
Mango	4.40	1.32	2.35	2,86	0.95
Moringa	29.62	27.41	14.59	0.13	0.04
Sweet apple	17.06	20.32	35.68	1.71	0,41

[1]Analysis procedure based on Senter *et al*. (1989)

Active component of the seeds, mango in particular was simlir to the previous results of Arogba (1997) and Abdalla *et al*. (2007a,b). Concentration of active materials and chemical composition was slightly lower than previous results (for instance Arogba, 1997; Abdalla *et al*., 2007a,b). This is possibly due to the diffrent in raw materials quality, solvent and/or analysis method. However, the previous results indicated that active compounds of extract from plant materials could enhanced

broiler performance (Hernandez *et al*., 2004; Aengwanich *et al*., 2009). Furthemore, the active components content present study produced an antioxidant and antimicrobial activity as discussed in this paper.

Antioxidant Activity

All methanol extract materials from mango, moringa and sweet apple seeds produced an antioxidant activity. Antioxidant capacity is based on the ability of media to neutralize free radical through absorbance (Krings and Berger, 2001). The presence of antioxidant activity is related to existence of polyphenol and ascorbic acid in the tested materials. Antioxidant activity for moringa seed significantly increases as concentration of active component incraeses in the solution from 3 % to 5%, but this trend was statistically nonsignificant for mango seed (Table 2). The extract materials of current study had antioxidant activity, which agree with previous study of Abdalla *et al.* (2007b). Furthermore, they reported that extract materials (including fat or fatty acid) from mango seed reduced sunflower-oil oxidation and therefore oil quality has been maintained for 12 months when it stored at dark place. Inclusion 5% of mango oil had stabilized oil oxidation at the same extend for inclusion 300 ppm tertiary butylhydroxyquinone (TBHQ). DPPH assay (1-Diphenyl-2-pycrrylhydrozy) is measurement to assess ability of the extract to donate hydrogen to the radical and reducing power measures ability of the extract to donate electron to Fe (III). Therefore, the existence of antioxidant activity of the extract is a strong indication its ability of substance to possibly have antioxidant ability *in vitro* as well as *in vivo* condition. The effectivness *in vivo* application however needs further study.

In general, inclusion of natural antioxidant in the diet produces a positive response in poultry performance. Radwan *et al*. (2008), for instance, found that supplemetation with natural antioxidant in the hens diet improved nutrients digestibility, feed efficiency, egg production and egg quality. Furthermore, natural antioxidant supplementation during laying period significantly decreased melonaldehyde formation in egg yolk and had a positive effect on oxidative stability of shell egg and also improved fertility and hatchability. While, Abd El-Hakim *et al.* (2009) reported that antioxidant function of plants or plant-extracts improved significantly live weight gain at the first 3 weeks of age and this trend is not significant at the 42 days of age. Extracts from plant generally improved broiler preformance (Hernandez *et al*., 2004; Aengwanich *et al*., 2009). Exctracts from seed coat of tamarind were used as antibiotic replacement to which improved broiler liweweight gain but did not influence the feed intake and feed conversion (Aengwanich *et al*., 2009).

Table 2. Antioxidant activity of extracts from mango, moringa and sweet apple seed (%) based on DPPH assay (n=3)

	(%)					
Extracts[1]	*0%*	*1%*	*3%*	*5%*	*7%*	Mean
MS	0	89.29[a]	91.66[a]	92.73[a]	92.75[a]	91.61
MO	0	16.87[a]	17.33[a]	24.82[b]	24.91[b]	17.75
SA	0	82.06	83.72	86.40	88.96	85.28

[1]MS=mango, MO=moringa oleifera and SA=sweet apple.
[a,b]Different superscript letters on the row are significantly different (P<0.01)

Antimicrobial Activity

Antimicrobial activity of extract seed for pathogen bacteria of *Escherichia coli* and *Salmonella typhi* is summarized in Table 3. Antimicrobial activity is indicated by growth ability of tested bacteria in agar media. The results indicated that sweet apple seed had no antimicrobial activity for tested bacteria and mango seed had an antimicrobial activity for both tested bacteria of *Escherichia coli* and *Salmonella typhi.* Antimicrobial activity increases as a concentration of bioactive increases in the solution. The current results of antimicrobial activity of mango seed agree with the previous studies of Kabuki *et al*. (2000) and Abdalla *et al*. (2007b). They reported that mango seed reduced growth bacteria of *Escherichia coli* and *Salmonella typhi.* Meanwhile, the absence the antimicrobial activity

of moringa and sweet apple seeds on *Salmonella typhi* could not be explained in the present study since these two seed extracts consisted of bioactive compounds as mango had (Table 1). Phenol compounds in mango seed have ability to reduce growth of both bacteria *Escherichia coli* and *Salmonella* spp (Kabuki *et al*., 2000).

Table 3. Inhibition growth of methanol extract on *Escherichia coli* and *Salmonella typhi* at the level 0,1,3,5 and 7% of extract in solution (n=3)

	Inhibition (mm)									
	Escherichia coli					*Salmonella typhi*				
Extracts[1]	*0%*	*1%*	*3%*	*5%*	*7%*	*0%*	*1%*	*3%*	*5%*	*7%*
MS	0^a	0.5^b	2^c	6^d	8^e	0^a	1.9^b	7.9^c	10^d	13.9^e
MO	0^a	4.3^b	6.7^c	7.3^d	8.3^e	n	n	n	n	n
SA	n	n	n	n	n	n	n	n	n	n
Antibiotic			30					30		

[1]MS=mango; MO= moringa oleifera and SA= sweet apple. antibiotic of chloramphenicol (C30).
[a,b,c,d,e]Different superscript, on the row are significantly different ($P<0.01$). n = not detected activity

In general, moringa plant consists of biocative compound of alkaloid, quercetin and kaempferol. Quercetin and kaempferol are flavanoids that have activity as antioxidant and antimicrobe on bacteria and funges, evenmore reduced the growth of mosquito larvae (Ferreira *et al*., 2008). Sweet apple extracts did not produce antimicrobial activity. This potential ability as antimicorbial could not be fully performed in present study (Table 3), therefore further study need to be done to clarify on this inconsistency.

The present study indicated that antimicrobial activity of methanol extracts increased as bioactive compound concentration increases in the solution. This could be explained that the increased of extract concentration leads to elevate the amount of bioactive compound to reduce the growth of microbe. Abdalla *et al*. (2007b) reported that there was a positive relationship between concentration of bioactive compounds in the solution and antimicrobial acitivity for pathogen bacteria of *Escherichia coli.* However, the excessive amount in feed formulation leads an increase in cost and may cause a toxicity in animals or human. This is due to hydrolisable tannin content (about 75% from total tannin) in the mango seed (Arogba, 1997). Metabolism process of hydrolisable tannin has a potential as toxin in the body depend on its ability to neutralize hydrolisable tannin as non toxin compound.

Interestingly, moringa seed extracts were more effective as antimicrobial for *Escherichia coli* compared to mango seed extracts (Table 3). This fenomenon could not be related to phenol and tannin content of extract as the phenol and tannin concentration of moringa were lower than mango seed. Phenol and tannin content were 13.100 and 4.218 mg/mL and 95.257 and 286.501 mg/mL for moringa and mango seed respectively. However, this evidence requires further investigation.

CONCLUSIONS

Methanol extract materials from mango, moringa and sweet apple seeds had an antioxidant activity. Mango and moringa seed had antimicrobial activity for *Escherichia coli.* Additionally, mango seed also produced antimicrobial activity for *Salmonella typhi.* The others two seed did not produce any antimicrobial activity for *Salmonella typhi.* In general, mango, moringa and sweet apple extract have potential to be used as natural antibiotic sources ”phytogenic” to improve poultry performance.

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