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EVALUATION OF ELEUTHERINE (ELEUTHERINE AMERICANA) POTENTIAL AS FEED ADDITIVE FOR POULTRY

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ABSTRACT

In vitro studies were exploring eleutherine (Eleutherine americana) potential to be used as feed additive for poultry. The eleutherine bulbs were extracted using four different organic solvents: methanol, ethyl acetate, diethyl ether and hexane. The crude extracts were analyzed for their bioactive compounds, tested for anioxidant and antibacterial activity and tested for growth of non-pathogen bacteria. The results indicated that bioactives are indentified:phenol and tannin. Average of antioxidant activity were 33.74, 20.36, 15.14 and 1.98 ppm AEAC for methanol, ethylacetate, diethylether and hexane, respectively. Furthermore, average of inhibition growth for pathogen bacteria of Escherichia coli was 5.94, 4.75, 4.56 and 4.44 mm for methanol, ethylacetate, diethylether and hexane, respectively, while inhibition for Staphylococcus aureus was 5.37, 6.50, 5.37 and 5.18 mm for methanol, ethylacetate, diethylether and hexane, respectively. The extracts also have ability to enhance the gorwth of non-pathogen bacteria Lactobacillus acidophilus. Average of bacteria growth improvement was 21.12, 28.57, 28.16 and 19.59 NTU for methanol, ethylacetate, diethylether and hexane, respectively. The presence of antioxidant and antibacterial activity and growth enhancer activity could be attributed to the bioactive and non digested carbohydrate content of the bulbs extracts. Therefore, it could be concluded that the bulbs of eleutherine extract is potential source for "feed additive" as phytobiotics as well as prebiotics.

Key words: Eleutherine, Extract, Bioactive, Phytobiotics, Prebiotics

INTRODUCTION

Feed additive such as antibiotics, flavour, ionophore and growth hormone, in livestock production system is subjected to improve intake, liveweight gain, feed efficiency and health status. The use of antibiotics and growth hormone synthetics however produce negative effect on animal host by enhancing microbiota resistance in the digestive tract (Windisch et al., 2008) and may create environmental problems. Furthermore, some antibiotics have been found to have serious undesirable side effect which limit their application. Therefore, there is serious need to develop new antibacterial agents that are very effective with minimal undesirable side effects. Plants are representation of potential source for feed additive such as antibiotics.

Eleutherine has long been recognized as spicy food and herbal medicine (Ifesan et al., 2010). Bulbs of eleutherine have been reported to have antibacterial activity on gram-positive and gram-negative bacteria (Ifesan et al., 2009; 2010), as well as fungi (Ifesan et al., 2010). Recently, Phoem and Voravuthikunchai (2013) proposed that bulbs of eleutherine extracts could be used as growth media for nonpathogen bacteria. These indicated that the bulbs extract could be used as phytobiotics and prebiotics sources. Therefore, the objectives of present preliminary research are to explore the potential of bulbs extract to be used as phytobiotic and/or prebiotic in poultry diet through in vitro evaluation.

MATERIAL AND METHODS

Research materials: The bulbs of eleutherine were washed and cleaned with tap water and then chopped. Chopped bulbs were dried to about 10% of water content and then ground to powder. The powders were individually extracted using methanol, ethylacetate, diethylether and hexane, respectively. Extraction process was done for 7 days. Crude extracts were rotary evaporated until the extract became completely dry as pellet. The pellet were then subjected to the chemical analysis for its bioactive compunds. The pellet were also individually disolved in distilled water for further analysis and evaluation.

Antioxidant evaluation: Antioxidant activity was conducted using DPPH (diphenylpicrylhydrazyl) method and the concentration of tested extract was 0.25, 0.50, 0.75 and 1 mg pellet in the solution. Krings and Berger (2001) suggested that scavenge of free radical is assessed on the absorbance at the wave length of 517 nm and the unit is expressed as ppm of AEAC (ascorbic acid equivalent antioxidant capacity).

Antibacterial evaluation: Agar diffusion based on Ayad et al. (2000) was applied to evaluate antibacterial activity of the bulbs extract. Suspension of the tested bacteria (Escherichia coli and Staphylococcus aureus) were prepared to contain approximately 108cfu/mL and the disc containing solid agar were innoculted by spreading up 1 ose of bacteria suspension. A 100 µL of crude extract from individual solvent which was preparing at the level of 0, 0.25, 0.50, 0.75 and 1mg extract/mL were placed in the hole (4 mm depth and 8mm diameter). Inhibition zone diameter was measured four times after allowing 24 h at 37oC in the incubation equipment. A control positive was synthetic antibiotic of tetracycline

Growth promoting assay: Four types of eleutherine extract and two controls were subjected to the growth-promoting assay based on Phoem and Voravuthikunchai (2013), and tested bacteria was Lactobacillus acidophilus. Bacteria growth was assessed using turbidimeter (NTU; nephelometric turbidity unit). Test tubes were containing 9 ml of liquid growth media and 1 mL of extracts (1 mg/mL) and sterile distilled water, respectively and positive control was 10 mL of growth media. The test tubes were anaerobically incubated for 2, 6, 10, 14, 20, 24, 48 and 72 hour.

Experimental design and statistical analysis: Experiment was designed as Block Design, in which blocks were types of solvent and four level of pellet conectration in the solution as treatments within 3 or 4 replicates. Treatment levels were 0.25, 0.5, 0.75 and 1 mg. Parameters were antioxidant activity, antibacterial activity and growth of bacteria. Data were analyzed using analysis variance and least significant different (LSD) for comparison means analysis (Steel and Torrie, 1990).

RESULTS AND DISCUSSION

Bioactive compounds

The bulbs of eleutherine were extracted using four different solvents: methanol, ethylacetate, diethylether and hexane. Extracted materials produce bioactive compounds as shown in Table 1. The absence of bioactive compounds in the extract is likely related to the lack of sensivity of equipment and method used, and also low concentration of extract in the solution. Bioactive compounds could therefore be detected when concentration of the eleutherine extract is elevated in the solution. Nonetheless, all extracts have tannin compounds.

Table 1. Bioactive composition of 1 mg pellet/ml solution

Solvents	Fenol (mg/kg)	Flavonoid (%)	Tannin (%)
Methanol	nd	1.29	0.09
Ethylacetate	nd	63.48	0.20
Diethylether	nd	nd	0.10
Hexane	nd	nd	0.04

nd=not detected

Antioxidant activity

Mean values of antioxidant activity of extract are presented in Table 2. All extracts produced antioxidant activity and tended to be different within types of organic solvent, in which methanol produced the highest value of antioxidant activity. This study revealed that antioxidant activity is elevated as concentration of extracts increased in the solution. This pattern agrees with the results of Rusdi et al. (2009 and 2014). In vivo situation, natural antioxidanta have been reported to improve nutrients digestibility, feed efficiency, egg production and egg quality (Radwan et al., 2008). Furthermore, inclusion of natural antioxidant during laying period significantly reduced melonaldehyde-egg yolk and had postive effect on oxidation stability of egg-shell and improved fertility as well as egg hatchability. Meanwhile, Abd El-Hakim et al. (2009) reported that antioxidant generated from plant materials significantly improved a daily liveweight gain of broiler for the first 3-week old.

Table 2. Antioxidant activity of exctracted eleutherine from different solvents at level of 0.25, 0.50, 0.75 and 1 mg of pellet in the solution (n=3)

Solvents	ppm of AEAC				SEM
	0.25	0.50	0.75	1.0	
Methanol	18.57^{a}	25.03^{b}	35.98^{c}	55.36^{d}	4.21
Ethylacetate	4.79^{a}	13.67^{b}	24.85^{c}	38.16^{d}	3.80
Diethylether	612.08^{a}	13.43^{b}	14.71^{c}	20.33^{d}	0.95
Hexane	0^{a}	0^{a}	0^{a}	7.92^{b}	1.07

SEM, standard error of the mean. Means in the same row with different superscript differ significantly ($P<0.01$)

Antibacterial activity

Antibacterial activity result is summarized in Table 3. The activity was firstly recorded when exctract is 0.5 mg in the solution and it increases as concentration increases. Similar pattern has been reported by Akiyama et al. (2001); Pereira et al. (2007); Sakunpak dan Panichayupakaranant (2012). They found antibacterial activity on polyphenol compounds. Previous studies reported that polyphenol, phenol, flavonoid and essential oil generated from plants reduce the growth of pathogen bacteria of E. coli, S.aureus, L. monocutogenes and Salmonella spp (Friedman et al., 2004; Oussalah et al., 2006). 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 replace synthetic antibiotics.

Table 3. The growth of bacteria Lactobacillus acidophilus (NTU) on media added with 1 mg of bulbs extract from methanol, ethylacetate, diethylether and hexane (n=4)

Solvent	Inbitition (mm)									
	Escherichia coli					*Staphylococcus aureus*				
	0.25	0.50	0.75	1.00	SEM	0.25	0.50	0.75	1.00	SEM
Methanol	nd	3.00^{a}	7.50^{b}	13.25^{c}	1.30	nd	4.00^{a}	7.25^{b}	10.25^{c}	0.99
Ethylacetate	nd	3.00^{a}	7.75^{b}	8.25^{c}	0.89	nd	7.25^{a}	8.75^{b}	10.00^{c}	1.01
Diethyether	nd	3.75^{a}	6.00^{b}	8.50^{c}	0.82	nd	4.75^{a}	6.00^{b}	10.75^{c}	1.01
Hexane	nd	4.25^{a}	5.75^{b}	7.75^{c}	0.75	nd	5.50^{a}	8.50^{b}	9.25^{c}	0.95
Tetracycline			26.00					25.50		

nd = not detected. SEM, standard error of the mean. Means in the same row within bacteria with different superscript differ significantly (P<0.01)

Bioactive compounds in the particular media generally produce antioxidant and antibacterial activity on bacteria, fungi and even more it may reduce the growth of mosquito's larvae (Ferreira et al., 2008). The rate of 0.5 mg in the present study is not high enough to produce an antibacterial activity on all type of extracts. This is supported by the results of Banso and Adeyemo (2007). They found that inhibition growth is achieved when tannin concentration in the media is 4.0 to 5.5 mg/mL. Furthermore, Sakunpak and Panichayupakaranant (2012) reported the value of concentration of 10 mg/mL in the media to produce antibacterial activity. This means that tannin is the most important compound to create antibacterial activity in the substance.

Table 4. The growth of bacteria Lactobacillus acidophilus (NTU) on media added with 1 mg of bulbs extract from methanol, ethylacetate, diethylether and hexane (n=4)

Solvent	Incubation time (h)							
	2	6	10	14	20	24	48	72
Methanol	0	2.18^{b}	3.62^{b}	4.74^{c}	9.10^{ab}	12.25^{a}	39.63^{ac}	77.43^{a}
Ethylacetate	0	2.44^{b}	2.68^{c}	3.59^{d}	8.54^{bc}	11.87^{a}	76.53^{b}	94.35^{b}
Diethyether	0	3.07^{a}	6.92^{a}	8.09^{a}	9.57^{a}	11.84^{a}	60.20^{ab}	97.45^{b}
Hexane	0	2.18^{b}	2.42^{c}	3.50^{d}	9.42^{a}	11.83^{a}	26.25^{cd}	81.55^{a}
Control (+)	0	2.19^{b}	3.22^{b}	5.75^{b}	8.14^{c}	9.66^{b}	12.15^{d}	58.65^{c}
Control (-)	0	1.19^{c}	1.63^{d}	1.66^{e}	2.42^{d}	2.63^{c}	3.38^{d}	32.60^{d}
SEM		0.13	0.23	0.31	0.34	0.53	10.96	2.32

SEM, standard error of the mean. Means in the same colon with different superscript differ significantly (P<0.01)

Bacterial growth

Current study clearly revealed that eleutherine extract is enhancing the growth of Lactobacillus acidophilus bacteria (see Table 4). Oganic solvent types produced different growth pattern of bacteria, in which methanol solvent, in general, tended to performing better growth than the other solvents. The growth was linearly improved as incubation time increased. This trend agrees with the previous studies of Maligan et al. (2006) and Usmiati et al. (2011). Moreover, Maligan et al. (2006) reported alogaritmics phase growth was achieved in 35 h incubation time and

continuously increased until 70 h incubation time. Additionally, Lactobacillus bacteria was growing and improving within 21 days on yoghurt milk (Usmiati et al., 2011). Present study indicated that the growth rate of bacteria with eleutherine extract was significantly higher than those without eleutherine extract ($P<0.01$).

Bacterial lactate acid producer of Lactobacillus and Bifidobacterium have been reported to have benefit effect on the health (Bernet et al., 1993), and other effects are following: nutrition, physiology and antibacterial (Naidu and Clemens, 2000). In fact that all non digested carbohydrate that categorized as prebiotics may stimulate the growth of those bacteria and therefore enhance animal productivity. For example, isomalto-oligosakarida (IMOS), trans-galakto-oligosakarida (TGOS), mannan-oligosakarida (MOS) and pectin-oligosakarida are categorized as prebiotics. These prebiotics produce different mechanisms in stimulating the growth improvement of livestocks and IMOS was selectively fermented Bifidobacteria and Lactobacilli but not for Salmonella or E.coli (Chung and Day, 2004). The manno-oligosaccharida enhanced the population of Lactobacilli in the ileum (Yang et al., 2008). Moreover, the growth improvement of livestocks is ralated to the improvement of energy used (Yang et al., 2008).

The current in vitro results agree with the results of Gibson et al. (2004) stating that the non digested carbohydrate of eleutherine has positive effects on the particular non pathogenic bacteria in the colon and improved health status. Similarly, Phoem and Voravuthikunchai (2013) reported that eleutherine could be used as prebiotics to stimulate the growth of non pathogenic bacteria through enhancement of short-chain acids production.Furthermore, oligosaccharides extract from eleutherine elevated the growth of Bifidobacteria from 9.63 to 12.8 log cfu/ml and 5.80 to 8.85 log cfu/ml for mix- and pure culture media respectively. They concluded that extracted materials from eleutherine could be used as a functional food for human.

CONCLUSION

Bulbs of eleutherine extracts consisted of bioactive compounds and produced antioxidant, antibacterial activity as well as enhancer bacteria growth activity. Therefore, it could be concluded that extracted materials from eleutherine bulbs is potentially to be used as feed additive “phytobiotics and prebiotics.

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