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Antidiarrheal Activity of *Apus* Bamboo (*Gigantochloa apus*) Leaf Extract and its Bioactive Compounds

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ABSTRACT

Most of every part of bamboo plants had economical value. The wood is used as building and furniture, the shoot is processed as health foods and medicines, the root and culms are used as traditional medicine. The leaf has been believed that it could heal diarrhea in piglets, rabbit, poultry and calves. This research was designed to investigate the inhibition activity of *apus* bamboo leaf extracts against four strains pathogenic *Escherichia coli*. The leaf of *apus* bamboo (*G. apus*) was extracted in methanol, ethanol and methanol-ethanol (1:1), subsequently dried and assayed for their antibacterial activity using diffusion and dilution. Among three solvents used in this study, ethanol was the best with a yield of 18.74% and its effectivity was about 0.44% compared to tetracycline. The bioactive compounds in the extract were fatty acids, esters and alcohols.

Keywords: Antidiarrheal Activity, *Escherichia Coli* O157:H7, Microdilution, Fatty Acid

1. INTRODUCTION

Plants have been used as the source of functional foods and drugs (Ciocan and Bara, 2007). Many modern drugs were initially used in crude form in traditional or folk healing practices. Many reports have been published about natural antimicrobial agents from plants and microbiota origin. Some of them are described below.

The aqueous extract of edible mushroom (*Dictyophora indusiata*) had antimicrobial activities against several pathogenic or putrefactive bacteria and fungi, such as *Escherichia coli*, *Alcaligenes faecalis*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *B. cereus*, *Aspergillus flavus*, *Candida albicans and Cryptococcus neoformans* and among all tested microbiota, only *A. niger* that was resistant (Oyetayo *et al*., 2009). The fruits of *Phyllanthus acidus*, *Punica granatum*, *Citrus aurantifolia* and *C. aurantium* had inhibitory effect against *E. coli.* The leaf of *Tamarindus indica* and *Bamboosa arundinaceae* had inhibitory effect against *E. coli* (Melendez and Capriles, 2006; Singh *et al*., 2010).

Elavazhagan and Arunachalam (2010) reported that chloroform extract of *Memecylon edule* seed was effective to inhibit *B. subtilis.* Kahriman *et al*. (2012) used hydro- and microwave distillations to obtain the essential oil of *Vicia dadianorum* which had antimicrobial and antifungal activities against *S. aureus*, *Enterococcus faecalis*, *B. cereus*, *Mycobacterium smegmatis* and *C. albicans*.

In the previous study, we found that ethanolic extract of the resin from *Shorea eximia*, an Indonesian forest plant, could inhibit *Streptococcus* sp., *S. aureus*, *S. epidermidis*, *B. cereus* and *Chromobacter violaceum* (Mulyono *et al*., 2012a). The affectivity of that resin was about 0.007-0.015% compared to tetracycline, depending on the strain of bacteria. The major components in the extract were δ-cadinene, valencene and spathulenol.

Bamboo plays important roles for Indonesian people. The wood is cheap and lightweighted, so it is often used for housing, bridge, piping, furniture, handicraft and prayer kit. *Apus* bamboo (*Gigantochloa apus*) is one of

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the common bamboo in Indonesia which wood has superior mechanical properties to black bamboo (*G. atroviolaceae*). The shoot can be processed into a wide variety of health foods and medicines after the natural toxin (cyanogen) has been removed (Pandey and Ojha, 2011). The total potential of bamboo worldwide is estimated at $10 billion with China and Taiwan as the leading countries in exporting edible bamboo shoots (Satya *et al*., 2012).

There were about 60 species of bamboo in Indonesia, such as *apus* bamboo, ater bamboo (*G. ater*) andong bamboo (*G. pseudoarundinacea)*, black bamboo and petung bamboo (*Dendrocalamus asper*) (Krisdianto and Ismanto, 2006). In Bali, the root of *apus* bamboo was used to cure some diseases such as cough, hepatitis, hypertension, breast cancer and diabetes mellitus, while the culms were used to cure heartburn and rejuvenate skin (Sujarwo *et al*., 2010).

According to the best knowledge of authors, the antibacterial activity of *apus* bamboo leaf has not been scientifically studied yet. Therefore, this research was aimed to evaluate the activity of *G. apus* leaf against *E. coli* strains which cause diarrhea in poultry, piglet and human and to identify the bioactive compounds in the extract.

2. MATERIALS AND METHODS

2.1. Materials

The leaf of *apus* bamboo was freshly harvested from Baturaden (Central Java, Indonesia). Methanol, ethanol and tween-20 were purchased from Merck (Germany). The media for antibacterial assay, which were Nutrient Agar (NA), Nutrient Broth (NB) and Mueller Hinton Agar (MHA), as well as NaCl, were purchased from Oxoid (United Kingdom). Four strains of *E. coli* used as tested bacteria were two strains isolated from diarrheal piglet (Veterinary Research Institute, Bogor) and poultry (Gadjah Mada University, Yogyakarta) and *E. coli* O157:H7 isolated from well water and river water, obtained from Culture Collection, Microbiology and Fermentation Laboratory, Faculty of Biotechnology Atma Jaya Catholic University (Jakarta).

2.2. Sample Preparation and Extraction

The fresh bamboo leaf was cut into small pieces, oven dried at 50°C for 2 days and milled to become flour. Finally, the flour was sieved using 40 mesh siever to obtain fine powder. The powdered leaf of *G. apus* was extracted at concentration of 6% (w/v) using methanol and ethanol and the mixture thereof with a volume ratio 1:1. The mixture was filtered to remove the insoluble matter and the solventless extract was obtained by evaporating the solvent using vacuum rotary evaporator (Buchi R-215) and kept at 4°C for further use. The solventless extract density was measured using calibrated picnometer. All extraction were done triplicate and each dry extract was used for antibacterial activity assay and the assay were done duplo. The yield was calculated as follows:

$$\text{yield}\left(\%\frac{\text{w}}{\text{w}}\right)=\frac{\text{weight of solventless extract}}{\text{weight of fresh leaf}}\times 100\%$$

2.3. Antibacterial Activity Assay

E. coli from diarrheal poultry and swine were grown in NA at 37°C for 24 h, then 5 colonies was suspended in 5 mL NB and reincubated at 37°C so that the turbidity became 0.5 McFarland, using the protocol described by ASM (2005). The antibacterial activity assay was conducted using three methods, i.e. well and disc diffusions (Kusmiyati and Agustini, 2007) and microdilution (Tanaka *et al*., 2011). The first and second methods were conducted using MHA as the media and the tested bacteria were diluted in physiological saline solution. The last method was established by mixing 10-40 µL of extracts with 5000 µL of NB, then adding 10 µL *E. coli*. The concentration of extract (% v/v) could be calculated using the equation:

$$[\text{extract}]=\frac{V_{\text{extract}}}{V_{\text{extract}}+V_{\text{media}}+V_{\text{E.coli}}}\times 100\%$$

The absorbance of samples at 630 nm after being incubated at 37°C for 24 h was measured. The extracting solvents were used as negative controls. The percentage of inhibition was calculated using the equation:

$$\%\,\text{inhibition}=100=\left(\frac{A_{\text{E.coli+NB+extract}}-A_{\text{NB+extract}}}{A_{\text{E.coli+NB+solvent}}-A_{\text{NB+solvent}}}\times 100\right)$$

Tetracycline was used as positive control and its inhibition was determined using microdilution. Similar to the assay for sample, 5-10 µL of tetracycline (10 mg mL^{-1} in aquadest) was added to 5 µL of NB, subsequently 10 µL *E. coli* was added. The concentration of tetracycline (mg/mL) could be calculated using the equation:

$$[\text{Tetracycline}]=\frac{V_{\text{tetracycline}}}{V_{\text{tetracycline}}+V_{\text{media}}+V_{\text{E.coli}}}\times 10$$

Finally, the concentration of extract was converted to mg/mL unit and the effectivity of extract to inhibit *E. coli* growth compared to tetracycline could be determined using the following equation:

$$\text{Effectivity of extract}(\%) = \frac{[\text{tetracycline}]_{I=99.9}}{[\text{extract}]_{I=99.9}} \times 100\%$$

2.4. Identification of Bioactive Compounds

The selected extract with the highest antibacterial activity was identified by pyrolisis-GC/MS (QP 2010 Shimadzu) using the method reported by Mulyono *et al*., (2012b).

3. RESULTS

3.1. Yield of Extract

The yield of extracts depended on the polarity of solvents which were used for extraction. The results showed that the yield of methanolic, ethanolic and methanol-ethanolic extracts were 22.14±0.94, 18.74±4.99 and 20.19±1.93% (w/w), respectively. The density of all solventless extracts was 1.00, 0.86 and 0.83 g mL^{-1}, respectively.

3.2. Antibacterial Activity Assay

Antibacterial activity assay using well and disc diffusion methods could not result in definite clear zone. Therefore, we conducted the third method to validate the result, which was microdilution. The result of microdilution assay showed that antibacterial activity of *G. apus* leaf depended on the extracting solvent and the strain of *E. coli* (**Table 1**).

The data in **Table 1** were used to develop a linear regresion to determine the required dosage of extracts to inhibit the growth of *E. coli* by 99.9% so that the effectivity of extracts to inhibit the bacteria growth could be compared to that of tetracycline.

Table 3 showed the effectivity of *G. apus* leaf extracts to inhibit the growth of *E. coli* compared to tetracycline.

Table 1. Inhibition of the growth (%) of *E. coli* at several concentration of *G. apus* leaf extract

Extract		Me[1]	Et[2]	Mix[3]	Me[1]	Et[2]	Mix[3]
µL	% v/v	*E. coli* from diarrheal poultry			*E. coli* from diarrheal piglets		
10	0.199	6.8	2.8	0	19.4	27.8	0
20	0.398	9.8	48.1	0	57.2	42.3	1.1
40	0.792	31.2	90.4	0	67.9	40.7	56.7
		E. coli O157:H7 from well water			*E. coli* O157:H7 from river water		
10	0.199	0	0	0	0	18.6	6.1
20	0.398	14.9	3.6	0	0	44.1	24.3
40	0.792	26.8	7.6	0	0	29.1	24.1

Me[1] = methanolic extract, Et[2] = methanolic extract, Mix[3] = methanol-ethanolic extract

Table 2. Percentage inhibition of *E. coli* in the presence of tetracycline

Tetracycline		Me[1]	Et[2]	Mix[3]	Me[1]	Et[2]	Mix[3]
µL	mg/mL	*E. coli* from diarrheal poultry			*E. coli* from diarrheal piglets		
5	0.0100	44.4	39.3	37.1	8.1	36.4	6.3
8	0.0159	48.8	44.2	42.2	44.9	61.9	43.8
10	0.0199	50.7	46.2	44.3	62.0	73.7	61.2
		E. coli O157:H7 from well water			*E. coli* O157:H7 from river water		
5	0.0100	13.4	19.3	15.0	32.9	45.7	42.4
8	0.0159	15.4	21.2	17.0	49.7	59.3	56.8
10	0.0199	18.3	23.9	19.8	52.5	61.5	59.2

Me[1] = tetracycline dissolved in methanol, Et[2] = tetracycline dissolved in ethanol, Mix[3]= tetracycline dissolved in methanol-ethanol (1:1)

Table 3. Effectivity of *G. apus* leaf extracts to inhibit the growth (%) of *E. coli* compared to tetracycline

Sources of *E. coli*	Me[1] (%)	Et[2] (%)	Mix[3] (%)
Diarrheal poultry	0.40	1.34	0
Diarrheal piglets	0.23	0.08	0.26
E. coli O157:H7 from well water	0.78	0.27	0
E. coli O157:H7 from river water	0	0.07	0.14
Average	0.35	0.44	0.10

Me[1] = methanolic extract, Et[2] = ethanolic extract, Mix[3] = methanol-ethanolic extract

Table 4. Organic compounds in methanolic extract of *G. apus* leaf

Groups	Chemical compounds	Formula	Me[1] %	Me[1] SI[3]	Et[2] %	Et[2] SI[3]
Phenol derivatives	Guaiacol	$C_7H_8O_2$	0.17	85	-	-
	3,4,5-Trimethoxybenzaldehyde	$C_{10}H_{12}O_4$	0.23	89	-	-
	Maltol	$C_6H_6O_3$	0.44	89	-	-
	Phenol	C_6H_6O	0.58	97	-	-
	Phenol, 4-ethenyl-2-methoxy-	$C_9H_{10}O_2$	0.22	93	-	-
	2,6-Dimethoxyphenol	$C_8H_{10}O_3$	0.38	88	-	-
	Butylated hydroxyanisole	$C_{11}H_{16}O_2$	0.21	66	-	-
Cyclic hydrocarbons	l-Limonene	$C_{10}H_{16}$	0.27	92	1.40	97
	Corylon	$C_6H_8O_2$	0.76	96	-	-
Aliphatic hydrocarbons	n-Pentadecane	$C_{15}H_{32}$	0.16	93	1.83	97
	n-Tridecane	$C_{13}H_{28}$	-	-	0.88	97
	n-Undecane	$C_{11}H_{24}$	-	-	2.29	97
	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]	$C_{20}H_{40}$	0.31	90	-	-
Fatty acids and esters	Methyl palmitate	$C_{17}H_{34}O_2$	9.07	97	-	-
	Palmitic acid	$C_{16}H_{32}O_2$	-	-	6.72	95
	Methyl eicosanoate	$C_{21}H_{42}O_2$	-	-	1.71	86-94
	8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	-	-	3.57	88
	Lauric acid	$C_{12}H_{24}O_2$	-	-	48.76	96
	Ethyl laurate	$C_{14}H_{28}O_2$	-	-	7.03	92
	Caprylic acid	$C_8H_{16}O_2$	-	-	1.35	97
	Stearic acid	$C_{18}H_{36}O_2$	-	-	0.81	79
	Methyl stearate	$C_{19}H_{38}O_2$	3.75	92	-	-
	Dioctyl adipate	$C_{22}H_{42}O_4$	-	-	1.16	92
	Myristic acid	$C_{14}H_{28}O_2$	-	-	4.53	95
	Capric acid	$C_{10}H_{20}O_2$	-	-	6.45	93
	Oleic acid	$C_{18}H_{34}O_2$	29.00	88-89	-	-
	Methyl heptadecanoate	$C_{18}H_{36}O_2$	0.95	90	-	-
	Citronellyl acetate	$C_{12}H_{22}O_2$	-	-	1.01	87
	Methyl oleate	$C_{19}H_{36}O_2$	14.21	93	-	-
	Methyl linolenate	$C_{19}H_{32}O_2$	-	-	1.90	95
	Linoleic acid	$C_{18}H_{32}O_2$	-	-	1.64	89
	Ethyl linoleate	$C_{20}H_{36}O_2$	12.13	92	-	-
Long chain ketone	Palmitone	$C_{31}H_{62}O$	1.22	89	-	-
Long chain alcohols	9,12,15-Octadecatrien-1-ol	$C_{18}H_{32}O$	0.40	90	-	-
	Phytol	$C_{20}H_{40}O$	3.62	93-95	5.30	90
Nitrogenous compounds	N,N-Dimethyl-3-methoxypropylamine	$C_6H_{15}NO$	2.48	91	-	-
	Hexadecanamide	$C_{16}H_{33}NO$	0.79	95	-	-
	1,3-Dimethylthymine	$C_7H_{10}N_2O_2$	0.28	85	-	-
	5-oxo-pyrrolidine-2-carboxylic acid methyl ester	$C_6H_9NO_3$	0.44	93	-	-
	1,3-Dimethyluracil	$C_6H_8N_2O_2$	0.36	95	-	-
Miscellaneous	Acetol	$C_3H_6O_2$	0.57	97	-	-
	Chloromethane	CH_3Cl	2.72	100	-	-
	Butanoyl chloride, 3-methyl-	C_5H_9ClO	0.54	74	-	-

[1]Me = methanolic extract, [2]Et = ethanolic extract, [3]SI = similarity index (%)

Table 5. Some physical characteristics of bioactive compounds in ethanolic extract predicted using ACDLABS 12.0

Chemical compounds	%	Formula Weight	Index of Refraction	Surface Tension (dyne/cm)	Density g/cm^3
Phytol ($C_{20}H_{40}O$)	5.30	296.53	1.459± 0.02	29.8± 3.0	0.845± 0.06
Palmitic acid ($C_{16}H_{32}O_2$)	6.72	256.42	1.453 ± 0.02	33.3 ± 3.0	0.892 ± 0.06
Methyl eicosanoate ($C_{21}H_{42}O_2$)	1.71	326.56	1.446 ± 0.02	30.8 ± 3.0	0.862 ± 0.06
8,11,14-Eicosatrienoic acid ($C_{20}H_{34}O_2$)	3.57	306.48	1.489 ± 0.02	34.7 ± 3.0	0.917 ± 0.06
Lauric acid ($C_{12}H_{24}O_2$)	48.76	200.32	1.447 ± 0.02	33.2 ± 3.0	0.905 ± 0.06
Ethyl laurate ($C_{14}H_{28}O_2$)	7.03	228.37	1.435 ± 0.02	29.6 ± 3.0	0.867 ± 0.06
Caprylic acid ($C_8H_{16}O_2$)	1.35	144.21	1.437 ± 0.02	33.0 ± 3.0	0.929 ± 0.06
Stearic acid ($C_{18}H_{36}O_2$)	0.81	284.48	1.455 ± 0.02	33.4 ± 3.0	0.888 ± 0.06
Dioctyl adipate ($C_{22}H_{42}O_4$)	1.16	370.57	1.451 ± 0.02	33.1 ± 3.0	0.929 ± 0.06
Myristic acid ($C_{14}H_{28}O_2$)	4.53	228.37	1.451 ± 0.02	33.3 ± 3.0	0.898 ± 0.06
Capric acid ($C_{10}H_{20}O_2$)	6.45	172.26	1.443 ± 0.02	33.1 ± 3.0	0.915 ± 0.06
Citronellyl acetate ($C_{12}H_{22}O_2$)	1.01	198.30	1.442 ± 0.02	28.0 ± 3.0	0.885 ± 0.06
Methyl linolenate ($C_{19}H_{32}O_2$)	1.90	292.46	1.475 ± 0.02	31.6 ± 3.0	0.895 ± 0.06
Linoleic acid ($C_{18}H_{32}O_2$)	1.64	280.45	1.478 ± 0.02	34.3 ± 3.0	0.911 ± 0.06
n-Pentadecane ($C_{15}H_{32}$)	1.83	212.41	1.431 ± 0.02	26.9 ± 3.0	0.769 ± 0.06

The chemical constituents in ethanolic and methanolic extracts were identified using pyrolisis-GC/MS (**Table 4**) so that the important bioctive compounds which play important role in inhibiting *E. coli* could be estimated.

Py-GC/MS analysis showed that ethanolic extract of *G. apus* leaf had 18 organic compounds and 13 of them could be identified with similarity index ≥90%, but there might be some other compounds in the extract which could not be detected by this particular technique. They could be grouped as fatty acids and esters (86.61%), long chain alcohol (5.30%), aliphatic hydrocarbons (5.00%) and cyclic hydrocarbon (1.40%). Meanwhile, methanolic extract contained more various organic compounds, it had 28 organic compounds and 19 of them could be identified with similarity index ≥90%. They could be grouped as fatty acid and esters (69.11%), nitrogenous compounds (4.35%), long chain alcohols (4.02%), derivatives of phenol (2.23%), cyclic hydrocarbons (1.03%), long chain ketone (1.22%), aliphatic hydrocarbon (0.47%) and miscellaneous (3.83%).

Some important physical characteristics of chemical constituents in ethanolic extract have been predicted using ACDLABS 12.0 so that either purification or fractionation to increase the efficacy strategy could be considered (**Table 5**).

4. DISCUSSION

Antibacterial activity which was evaluated by well diffusion and disc diffusion methods resulted in ambiguous data. The clear zone around the well or the disc could not be seen obviously. There were two possibilities that cause those negative results: (1) the extract did not have antibacterial activity against all tested *E. coli* so no clear zone appeared and (2) the extract had antibacterial activity, but while diffusing to the media, the extract caused the turbid area because colloid was formed. According to Dominguez *et al.* (2001), broth microdilution was faster, easier and more reproducible than diffusion.

The result of microdilution assay showed that antibacterial activity of *G. apus* leaf depended on the extracting solvent and the strain of *E. coli* (**Table 1**). Most of the extracts showed that the higher the extract concentration, the lower the absorbance was. It meant that the extract inhibited *E. coli* growth. However, some extracts caused a turbid phase when mixed with the media before the bacteria was added and the absorbance was too high. Altman (2003) stated that the spectrophotometer will not give accurate results if the absorbance is either too high (>1.5) or too low (<0.02).

Table 1 indicated that ethanolic extract of *G. apus* leaf could inhibit all tested pathogenic *E. coli*, but the methanolic and methanol-ethanolic extracts only inhibited three and two of four tested *E.coli*, respectively. The ethanolic extract seemed to be more effective to inhibit the growth of *E. coli* from diarrheal poultry and *E. coli* O157:H7 from well water than methanolic extract, however *E. coli* from diarrheal piglets and *E. coli* O157:H7 from well water seemed to be more sensitive to methanolic than ethanolic extracts.

Table 2 showed that the percentage inhibition of *E. coli* by tetracycline depended on the strain of *E. coli* but not on the solvent used to dissolve the antibiotic. It seemed that *E. coli* isolated from diarrheal piglets was the most sensitive to tetracycline.

Table 3 indicated that ethanolic extract had the broadest spectrum to inhibit the growth of all tested pathogenic *E. coli* and its average effectivity was the highest among all strains of tested *E. coli*. Therefore, ethanolic extract was suggested as the best extract to obtain antidiarrheal agent from *G. apus* leaf.

The major components in *G. apus* leaf were fatty acids and esters. According to the peak area, their content in ethanolic and methanolic extracts were about 87% and 69%, respectively. This identification was relevant with Sujarwo *et al*. (2010) who used GC/MS for identification and reported that *apus* bamboo contained fatty acids and some aromatic compounds.

According to Desbois and Smith (2010), free fatty acids had antibacterial properties. Free fatty acids not only disrupt the electron transport chain and oxidative phosphorylation but also interfere with cellular energy production, enzyme activity and nutrient uptake, generate toxic peroxidation, direct lysis of bacterial cells and prevent initial bacterial adhesion and subsequent biofilm formation. Many organisms use those natural compounds to defend against parasitic or pathogenic bacteria. Esters also had antibacterial activity because they could be metabolized to yield fatty acids by lipase enzyme in the body.

The most abundant fatty acid in ethanolic extract was lauric acid and its ethyl ester, with total 55.79%. Khaliq-Uz-Zaman *et al.* (1998) reported that methanolic extract of *Chara coralline* var wallichii (A. Br.) R.D. Wood (Charophyta) could inhibit bacteria and fungi, includes *E. coli*. The major components in the extract were reported as Fatty Acid Methyl Ester (FAME)s, in the forms of saturated (36.45%), dienoic (35.17%), trienoic (15.06%) and monoenoic (13.24%). Some of the fatty acids present in the extract were lauric, heptadecanoic, myristic, oleic, linoleic and linolenic.

Lauric acid, together with its monoglyceride, capric, myristic and linoleic acids, were reported as the most potent inhibitors across MRSA, but has not been tested against *E. coli*. The antimicrobial activity of liposomal lauric acids against *Propionibacterium acnes* had also been proved (Yang *et al*., 2009).

There were some similar fatty acids in blind-your-eye mangrove (*Excoecaria agallocha*) and *apus* bamboo leaf, such as palmitic acid (56.02%), linoleic (3.13%), stearic (2.80%), oleic (1.71%) and heptadecanoic (1.04%). The first three were present in ethanolic extract of *apus* bamboo leaf and the last two were present in methanolic extract (Agoramoorthy *et al*., 2007). Those FAME could inhibit all tested bacteria and fungi, which were represented by Gram-positive bacteria (*B. subtilis*, *B. pumilus*, *Micrococcus luteus* and *S. aureus*), Gram-negative bacteria (*P. aeruginosa*, *Klebsiella pneumoniae and E. coli*) and yeasts (*C. albicans*, *C. krusei*, *C. tropicalis* and *C. parapsilosis*).

Other fatty acid present in both ethanolic and methanolic extracts of *G. apus* leaf was linoleic acid, either as acid or ester. Zheng *et al*. (2005) also reported that linoleic acid inhibited bacterial enoyl-acyl carrier protein reductase (FabI), which was essential for cell membrane synthesis. Pubmed (CID 5282184) reported that this acid was one of fatty acids with the most potent inhibitors against Methicillin-Resistant *S. Aureus* (MRSA), but has not been tested against *E. coli* yet.

Antibacterial activity of oleic acid, the major compound in methanolic extract, was reported by some previous research. Buteica *et al*. (2010) proved that magnetic nanofluid containing oleic acid could inhibit *S. aureus* and *E. coli.* Zheng *et al*. (2005) reported that this acid had similar mechanism to linoleic acid in inhibiting the bacterial growth. Chen *et al*. (2011) reported that oleic acid was an important component of the innate immune system. It is safe and did not show any toxicity to human sebocytes.

Phytol was other bioactive compound in ethanolic and methanolic extracts with the concentration of 5.30 and 3.62%, respectively. This alcohol could be oxidized to become fatty acid which had antibacterial activity. This alcohol, together with pentadecane and other organic compounds in essential oil of air-dried *Minuartia meyeri* (Boiss.) Bornm possessed moderate antibacterial activity against *Yersinia pseudotuberculosis*, *Enterococcus faecalis* and *S. aureus*, but the activity of *E. coli*, *K. pneumoniae*, *Serratia marcescens*, *B. subtilis*, *C. albicans* and *C. tropicalis* was not significantly inhibited by the presence of extract (Yayli *et al*., 2006).

Some other minor compounds in methanolic extract of *G. apus* leaf which might inhibit the growth of *E. coli* were guaiacol (0.17%), phenol (0.58%), 9,12,15-octadecatrien-1-ol (0.40%) and 3,4,5-trimethoxybenzaldehyde. Guaiacol and phenol could inactivate the spores and vegetative forms of harmful microorganisms (Pubmed CID 460; Pubmed CID 996). The antimicrobial activity of 3,4,5-trimethoxybenzaldehyde had not been reported yet, but

this compound was used as precursor to synthesize 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one, which could inhibit all tested bacteria and fungi, such as *S. aureus K. pneumoniae P. aeruginosa*, *E. coli*, *A. flavus and A. niger* (Prakasha and Gouda, 2011). According to Carballo *et al*. (2010), palmitone was also a bioactive compound with antinociceptive activity. Because this activity is not our topic, therefore we count palmitone as impurities. The extract also contained butylated hydroxyanisole which is known as food antioxidant.

Total percentage of bioactive compounds in ethanolic extract of *apus* bamboo leaf was 93.77%. This amount was higher than that in methanolic extract, which was 73.29%.

Substituting synthetic antimicrobial agents to fatty acids potentially give some benefits (Desbois and Smith, 2010). Free fatty acids had broad spectrum of activity and non-specific mode of action. Their safety aspect makes them attractive as antibacterial agents for various applications in medicine, agriculture and food preservation, especially where the use of synthetic antimicrobials is undesirable or prohibited. In addition, Salehi and Bonab (2006) reported that avian pathogenic *E. coli* from broiler chickens with colisepticemia showed their high resistance against tetracycline (94%) and other synthetic antibiotics. Fatty acids are also active against methanogens in the guts of ruminants, so the emissions of methane could be reduced. Piglets treated with lipids and a lipolytic enzyme showed improved weight gain and feed conversion.

5. CONCLUSION

The sensitivity of diarrheagenic *E. coli* against *G. apus* leaf extract depended on the strain of *E. coli* as well as the bioactive compounds in the extract. *E. coli* isolated from diarrheal poultry was the most sensitive strain against ethanolic extract of *G. apus* leaf, while methanolic extract was better to inhibit the growth of *E. coli* from other sources. The major bioactive compounds in both extracts are fatty acids and their relatives, but their concentration depended on the extracting solvent. This promising result should be followed up by applying the selected extract using testing animals.

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7. REFERENCES

Agoramoorthy, G., M. Chandrasekaran, V. Venkatesalu and M.J. Hsu, 2007. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Braz. J. Microbiol., 38: 739-742. DOI: 10.1590/S1517-83822007000400028

Altman, J., 2003. Using the cary spectrophotometer. Protocols Altman Laboratory, Emory Vaccine Center.

ASM, 2005. Manual of antimicrobial susceptibility testing. American Society for Microbiology American Society for Microbiology.

Buteica, A.S., D.E. Mihaiescu, A.M. Grumezescu, B.S. Vasile and A. Popescu *et al*., 2010. The antibacterial activity of magnetic nanofluid: Fe3O4/oleic acid/chepalosporins core/shell/adsorption-shell proved on *S. aureus* and *E. coli* and possible applications as drug delivery systems. Digest J. Nanomater. Biostruct., 5: 927-932.

Carballo, A.I., A.L. Martinez, M.E. Gonzalez-Trujano, F. Pellicer and R. Ventura-Martinez *et al*., 2010. Antinociceptive activity of *Annona diversifolia* Saff. leaf extracts and palmitone as a bioactive compound. Pharmacol. Biochem. Behav., 95: 6-12. PMID: 19969018

Chen, C.H., Y. Wang, T. Nakatsuji, Y.T. Liu and C.C. Zouboulis *et al*., 2011. An innate bactericidal oleic acid effective against skin infection of methicillin-resistant *Staphylococcus aureus*: A therapy concordant with evolutionary medicine. J. Microbiol. Biotechnol., 21: 391-399. DOI: 10.4014/jmb.1011.11014

Ciocan, I.D. and I.I. Bara, 2007. Plant products as antimicrobial agents. Genetica si Biol. Mol., 8: 51-156.

Desbois, A.P. and V.J. Smith, 2010. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Applied Microbiol. Biotechnol., 85: 1629-1642. PMID: 19956944

Dominguez, M.C., M.D.L. Rosa and M.V. Borobio, 2001. Application of a spectrophotometric method for the determination of post-antibiotic effect and comparison with viable counts in agar. J. Antimicrobial Chemotherapy, 47: 391-398. PMID: 11266409

Elavazhagan, T. and K.D. Arunachalam, 2010. Phytochemical and antibacterial studies of seed extracts of *Memecylon edule*. Int. J. Eng. Sci. Technol., 2: 498-503.

Kahriman, N., B. Yayli, M. Yucel, S.A. Karaoglu and N. Yayli, 2012. Chemical constituents and antimicrobial activity of the essential oil from *Vicia dadianorum* extracted by hydro and microwave distillations. Records Natural Products, 6: 49-56.

Khaliq-Uz-Zaman, S.M., S. Shameel, M. Shameel, S.M. Leghari and V.U. Ahmad, 1998. Bioactive compounds in *Chara coralline* var wallichii (A. Br.) R.D. Wood (Charophyta). Pak. J. Botany, 30: 19-31.

Krisdianto and G.S.D.A. Ismanto, 2007. Sari hasil penelitian bambu. Kementerian Kehutanan Republik Indonesia Spacer.

Kusmiyati and N.W.S. Agustini, 2007. Antibacterial activity assay from *Porphyridium cruentum* microalgae. Biodiversitas, 8: 48-53.

Melendez, P.A. and V.A. Capriles, 2006. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine, 13: 272-276. DOI: 10.1016/j.phymed.2004.11.009

Mulyono, N., B.W. Laya and S.S. Rusli, 2012a. The antibacterial activity of the Indonesian stone dammar (*Shorea eximia*). Biota, 17: 15-20.

Mulyono, N., C.H. Wijaya, D. Fardiaz and D.W.S. Rahayu, 2012b. Identifikasi Komponen Kimia Damar Mata Kucing (*Shorea Javanica*) dengan Metode Pirolisis-GC/MS. J. Natur. Indonesia, 14: 155-159.

Oyetayo, V.O., C.H. Dong and Y.J. Yao, 2009. Antioxidant and antimicrobial properties of aqueous extract from *Dictyophora indusiata*. Open Mycol. J., 3: 20-26.

Pandey, A.K. and V. Ojha, 2011. Precooking processing of bamboo shoots for removal of anti-nutrients. J. Food Sci. Technol. DOI 10.1007/s13197-011-0463-4

Prakasha, K.C. and D.C. Gowda, 2011. Synthesis of 5-arylidine-2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl)-thiazolidin-4-one derivatives as a novel class of antimicrobial agents. Int. J. Chem. Res., 2: 10-14

Salehi, T.Z. and S.F. Bonab, 2006. Antibiotics susceptibility pattern of *Escherichia coli* strains isolated from chickens with colisepticemia in Tabriz Province, Iran. Int. J. Poultry Sci., 5: 677-684.

Satya, S., P. Singhal, L.M. Bal and P. Sudhakar, 2012. Bamboo shoot: A potential source of food security. Mediterranean J. Nutr. Metab., 5: 1-10. DOI 10.1007/s12349-011-0086-3

Singh, V.K., R. Shukla, V. Satish, S. Kumar and S. Gupta *et al*., 2010. Antibacterial activity of leaves of bamboo. Int. J. Pharmaceutical Biosci., 6: 1-5.

Sujarwo, W., I.B.K. Arinasa and I.N. Peneng, 2010. Potensi bambu tali (*Gigantochloa apus* J.A. & J.H. Schult. Kurz) sebagai obat di Bali. Bul. Littro., 21: 129-137.

Tanaka, A., J.K. Hyo, S. Oda, K. Shimizu and R. Kondo, 2011. Antibacterial activity of moso bamboo shoot skin (*Phyllostachys pubescens*) against *Staphylococcus aureus*. J. Wood Sci., 57: 542-544. DOI: 10.1007/s10086-011-1207-9

Yang, D., D. Pornpattananangkul, T. Nakatsuji, M. Chan and D. Carson *et al*., 2009. The antimicrobial activity of liposomal lauric acids against *Propionibacterium acnes*. Biomaterials, 30: 6035-6040. DOI: 10.1016/j.biomaterials.2009.07.033

Yayli, N., C. Gulec, O. Ucuncu, A. Yasar and S. Ulker *et al*., 2006. Composition and antimicrobial activities of volatile components of *Minuartia meyeri*. Turkish J. Chem., 30: 71-76.

Zheng, C.J., J.S. Yoo, T.G. Lee, H.Y. Cho and Y.H. Kim *et al*., 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. FEBS Lett., 579: 5157-5162. DOI: 10.1016/j.febslet.2005.08.028