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Antimicrobial potential of *Carica papaya*, *Ipomoea aquatica*, *Alpinia galanga* and *Piper betle* against the aquatic microbials

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Abstract. *Saptiani G, Hardi EH, Pebrianto CA, Agustina, Ardhani F. 2016. Antimicrobial potential of* Carica papaya, Ipomoea aquatica, Alpinia galanga *and* Piper betle *against the aquatic microbials. Nusantara Bioscience 8: 252-257.* This research was aimed to investigate the antimicrobial potential of the leaves extract from *Carica papaya*, *Ipomoea aquatica*, *Piper betle* and *Alpinia galanga* stem against *Aeromonas hydrophyla, Pseudomonas* sp., *Escherichia coli*, and *Saprolegnia* spp.; the pathogenic microbes in aquatics. These plants were dried and extracted with water and ethanol, separately. The extract were then tested for its inhibition effect to the microbial by *in vitro* agar disc diffusion (ADD) at the concentration of 100, 200, 400, 600, 800, and 1,000 ppm and minimal inhibitory concentration (MIC) at the concentration of 600, 800, and 1,000 ppm. The result showed that water or ethanol extract f of plants showed potent inhibitor against the aquatic microbial. The ethanol extract of *P. betle* showed the highest inhibition at 800 ppm, with inhibition zones to *A. hydrophyla*, and *Pseudomonas* sp. 12.3 mm, *E. coli*, and *Saprolegnia* spp. 12.7 mm. One thousand ppm ethanol extract of *P. betle* could inhibit the best microbes growth using MIC method to *A. hydrophila* 189.7 cfu/mL, *Pseudomonas* sp. 253.0 cfu/mL, *Saprolegnia* spp.169.3 cfu/mL, and 800 ppm to *E. coli* 172.7 cfu/mL.

Keywords: Antimicrobial, *Alpinia galanga, Carica papaya, Ipomoea aquatica, Piper betle*

INTRODUCTION

Plant and plant's products have been used as one of the most important sources of medicines and pharmacology's active substances since long time ago. The plants used for medicinal community are *Carica papaya*, *Ipomoea aquatica, Alpinia galanga* and *Piper betle*. These plants are a perennial herb and can be found commonly throughout the Indonesia. *C. papaya* leaf can be used for dengue fever, dressing wounds, injuries and anti inflammatory activity (Aravind et al. 2013; Owoyele et al. 2008). *I. aquatica* possesses antimicrobial and anti inflammatory effects and has no obvious acute toxicity, which advanced our understanding of the folk use of *I. aquatica* in treating various inflammatory disorders (Sivaraman et al. 2010). *A. galanga* rhizome and *P. betle* can be used as anti inflammatory, analgesic, antiallergic, antibacterial, antifungal and antioxidant (Chudiwal et al,. 2010; Pradhan et al. 2013; Subashkumar et al. 2013).

The sustainability and development of aquaculture are largely at stake as significant ecological and pathological problems. The microbial infections are one of the main factors responsible for diseases and mortality on fish culture. The microbial infections can also reduce fish culture production. In general, microbial diseases on freshwater fish culture are *Aeromonas hydrophyla, Pseudomonas* sp., and *Saprolegnia* spp. *Escherichia coli* is gastrointestinal bacteria on the intestine of ruminant or human. *E. coli* is in the water because its pollution from waste. In fish culture, the microbial infection caused stress, hemorrhagic, hyperemia, ulcer, reddish gills, ascites, destroyed of internal organs and mortality. The aquatic environment is a complex ecosystem which makes the distinction between health, performance and diseases. Fish culture still uses antibiotic and chemical substances to overcome the diseases. The use of uncontrolled antibiotic and chemical substances on fish culture will produce new problem like toxicity, residues, and resistance. The exploration of bioactive components from natural resources is an alternative way to overcome the disease on fish culture. Herbal products are cheaper source for therapeutics, and potential source of new drug compounds. However there hasn't been much research that suggests the usage of these four plants as antimicrobial pathogen on fish.

This research was aimed to investigate the leaves extract from *C. papaya*, *I. aquatica*, *P. betle*, and *A. galanga* stem as a potential compound and also to find the optimal concentration to inhibit the microbia growth; *A. hydrophyla*, *Pseudomonas sp*., *E. coli*, and *Saprolegnia* spp. The results of this study are expected to be used as a basis for further research, as well as an alternative to prevent and reduce the risk of fish disease.

MATERIALS AND METHODS

Materials

Carica papaya, *I. aquatica*, *A. galanga* and *P. betle* were collected from Loa Janan, Kutai Kartanegara Regency, East Kalimantan Province, Indonesia. The microbes used for challenged test were *A. hydrophila*, *Pseudomonas* sp., *E. coli*, and *Saprolegnia* spp. The microbes procured from Aquatic Microbiology Laboratory of the Fisheries and Marine Science Faculty, Mulawarman University, isolated from fish and water. Bacterial media: Tryptone Soya Agar (Oxoid), Tryptone Soya Broth (Oxoid), Potato Dextrose Agar and Potato Dextrose Broth (Himedia India). Research tools were Rotavapor (Buchi RE 111 made in Switzerland), water bath (Jouan J15 Astel, France), Incubator (Memmert UNB 500, Germany), Electric Pressure Steam Sterilizer (NO 25 x, USA), analytical balance (Adventurer AR 2140 USA), micro pipette (Gilson France), and Hot plate (IKA RCT basic).

Methods

The leaves from *C. papaya*, *I. aquatica*, *P. betle*, and *A. galanga* stems were washed, drained and chopped, then dried without being exposed to direct sunlight, with a temperature of 30 ^{0}C for 15 days. Each plant was macerated in two different solvents, namely water and 80 % ethanol for 2 days, and then the extract was filtered 2 times. All concentrated were collected and then filtrated by Rotary evaporator and evaporated with water bath (Saptiani and Hartini 2008; Saptiani et al. 2013). So there were be eight kinds of extracts, namely water extract of *C. papaya*, *I. aquatica*, *A. galanga* and *P. betle*, ethanol extract of *C. papaya*, *I. aquatica*, *A. galanga* and *P. betle*. Each bacterium was cultured on tryptone soya agar (TSA) at 33^{0}C for 24 hours, and then cultured in tryptone soya broth (TSB) at 33^{0}C for 24 hours. *Saprolegnia* was cultured on Potato Dextrose Agar at 36^{0}C for 48 hours and then cultured in Potato Dextrose Broth at 36^{0}C for 48 hours. Total microbe used for the test was 10^6 cfu/mL.

The antimicrobial studies of the water and ethanol extract from these plants were carried out by the agar disc diffusion method (ADD) and Minimum Inhibitory Concentration (MIC). ADD is a common method of testing the sensitivity of bacteria to antibiotics. The treatments of concentration extract are starting from 100, 200, 400, 600, 800, and 1,000 ppm, negative control (H_2O) and positive control (tetracycline). In ADD, each concentration of extract was dropped onto paper dish 6 mm, then placed and arranged in petridisc which were cultured bacteria in the TSA, then incubated at 33^{0}C. Clear zone diameter was observed at the 20, 24, 36, 48 and 60 hours. In MIC, the bacteria were cultured in TSB medium at 33^{0}C for 24 hours. A half mL of bacteria containing 10^6 cfu/mL was mixed with each concentration of the extract. The mixture was cultured on TSA media and incubated for 24 hours at 33^{0}C. The growing colonies were calculated by total plate count (TPC).

RESULTS AND DISCUSSION

All the eight extracts showed different degrees of activities against the bacterial and fungal pathogens. The growth inhibition zones were measured by using ADD methods presented in Table 1. In this study, ethanol extract of *P. betle* showed maximum inhibition against *Saprolegnia* spp., and *E. coli* with inhibition zone 12.67 mm, *A. hydrophyla* and *Pseudomonas* sp. 12.33 mm. The water extract from *P. betle* showed maximum inhibition against *Saprolegnia* spp., and *E. coli* with inhibition zone 12.33 mm, *A. hydrophyla* 12.00 mm, and *Pseudomonas* sp. 11.67 mm. The result showed that ethanol extract has higher inhibition effect than water extract. *P. betle* leaf has been used in Indonesia as traditional medicine for a long time.The leaf contains water, proteins, carbohydrates, minerals, fat, fiber, essential oil, tannin, and alkaloid. It also contains different vitamins like vitamin-C, nicotinic acid, vitamin-A, thiamine, riboflavin beside this it contains minerals such as calcium, iron, iodine, phosphorus, and potassium (Guha 2006; Pradhan et al. 2013).

The ethanol extract of *C. papaya* has inhibited *E. coli* with inhibition zone of 12.33 mm, *A. hydrophyla* and *Saprolegnia* spp. 12.00 mm, and *Pseudomonas* sp. 11.67 mm. The water extract has inhibited *E. coli* with inhibition zone of 12.00 mm, *A. hydrophyla*, and *Saprolegnia* Spp 11.67 mm, and *Pseudomonas* sp. 11.33 mm. The result showed that *C. papaya* ethanol extract has the highest inhibition power than its water extract. People use papaya leaf as food, also for fever, antidiarrhea and anthelmintic. The leaves from *C. papaya* are used variously for the treatment of fever, pyrexia, diabetes, and inflammation. Preliminary phytochemical analysis from the extract revealed the presence of alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthraquinones, reducing sugars, steroids, phenolics and cardenolides (Owoyele et al. 2008). Phytochemical screening of *C. papaya* leaf showed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins, and amino acids, phytosterol, phenolic compounds, flavonoids, terpinoids, and tannins. The presence of phytosterol in *C. papaya* was very prominent in all the extracts. The saponins, glycosides, proteins, and amino acids, flavinoids, terpinoids, showed greater intensity of their presence in ethanol, methanol, ethyl alcohol and acetone extraction than other (Baskaran et al. 2012).

The ethanol extract of *A. galanga* stems has inhibited against *E. coli* with inhibition zone of 12.33 mm, *Saprolegnia* spp. 12.00 mm, *A. hydrophyla*, and *Pseudomonas* sp. 11.67 mm. The water extract has inhibited against *E. coli* with inhibition zone of 12.00 mm, *Saprolegnia* spp. 11.67 mm, *A. hydrophyla*, and *Pseudomonas* sp. 11.33 mm. The ethanol extract of *A. galanga* rhizome has inhibited against *Saprolegnia* spp. with inhibition zone of 12.67 mm, *A. hydrophyla* 12.33 mm, and *Pseudomonas* sp. 12.00 mm, but *A. galanga* leaf has inhibited against *Saprolegnia* spp. with inhibition zone of 12.33 mm, *A. hydrophyla* 12.00 mm, and *Pseudomonas* sp. 11.67 mm (Saptiani et al. 2015).

Table 1. Inhibition zone of extract *C. papaya, I. aquatica, A. galanga,* and *P. betle* to microbes

Concentration	Plant materials	Solvents	Zone of inhibition (mm)			
			A. hydrophyla	*Pseudomonas* sp.	*E. coli*	*Saprolegnia* spp.
1,000 ppm	*C. papaya*	Water	11.33±0.58	11.33±0.58	11.67±0.58	11.67±0.58
	C. papaya	Ethanol	11.67±0.58	11.67±0.58	12.00±0.00	11.67±0.58
	I. aquatica	Water	11.33±0.58	11.33±0.58	11.33±0.58	11.00±0,00
	I. aquatica	Ethanol	11.67±0.58	11.33±0.58	11.67±0.58	11.67±0.58
	A. galanga	Water	11.33±0.58	11.33±0.58	12.00±0.58	11.67±0.58
	A. galanga	Ethanol	11.67±0.58	11.67±0.58	12.33±0.58	12.00±0.58
	P. betle	Water	11.67±0.58	11.33±0.58	11.67±0.58	12,33±0.58
	P. betle	Ethanol	12.33±0.58	12.00±0.58	12.33±0.58	12,67±0.58
800 ppm	*C. papaya*	Water	11.67±0.58	11.33±0.58	12.00±0.00	11.33±0.58
	C. papaya	Ethanol	12.00±0.58	11.67±0.58	12.33±0.58	12.00±0.00
	I. aquatica	Water	11.33±0.58	11.33±0.58	11.33±0.58	11.33±0.58
	I. aquatica	Ethanol	11.67±0.58	11.67±0.58	11.67±0.58	11.67±0.58
	A. galanga	Water	11.33±0.58	11.33±0.58	12.00±0.00	11.67±0.58
	A. galanga	Ethanol	11.67±0.58	11.67±0.58	12.33±0.58	12.00±0.58
	P. betle	Water	12.00±0.58	11.67±0.58	12.33±0.58	12.00±0.58
	P. betle	Ethanol	12.33±0.58	12.33±0.58	12.67±0.58	12,67±0.58
600 ppm	*C. papaya*	Water	11.33±0.58	11.00±0.00	11.33±0.58	11.00±0.58
	C. papaya	Ethanol	11.67±0.58	11.33±0.58	11.33±0.58	11.33±0.58
	I. aquatica	Water	10.67±0.58	11.00±0.00	11.00±0.58	11.33±0.58
	I. aquatica	Ethanol	11.33±0.58	11.33±0.58	11.33±0.58	11.33±0.58
	A. galanga	Water	11.00±0.58	11.00±0.00	11.67±0.58	11.33±0.58
	A. galanga	Ethanol	11.67±0.58	11.67±0.58	12.00±0.00	11.67±0.58
	P. betle	Water	11.67±0.58	11.67±0.58	11.67±0.58	12.00±0.00
	P. betle	Ethanol	12.00±0.58	12.00±0.00	12.33±0.58	12,33±0.58
400 ppm	*C. papaya*	Water	10.67±0.58	10.67±0.58	10.67±0.58	10.67±0.58
	C. papaya	Ethanol	11.00±0.58	11.00±0.00	11.00±0.58	11.00±0.58
	I. aquatica	Water	10.33±0.58	10.67±0.58	10.33±0.58	10.67±0.58
	I. aquatica	Ethanol	10.67±0.58	11.00±0.00	11.00±0.58	11.00±0.58
	A. galanga	Water	10.67±0.58	10.67±0.58	11.33±0.58	11.33±0.00
	A. galanga	Ethanol	11.33±0.58	11.33±0.58	11.67±0.58	11.67±0.58
	P. betle	Water	11.00±0.58	11.33±0.58	11.33±0.58	11,67±0.58
	P. betle	Ethanol	11.67±0.58	11.67±0.58	12.00±0.00	12.00±0.58
200 ppm	*C. papaya*	Water	10.33±0.58	10.00±0.00	10.33±0.58	10.00±0.58
	C. papaya	Ethanol	10.67±0.58	10.33±0.58	10.33±0.58	10.67±0.58
	I. aquatica	Water	9.67±0.58	10.33±0.58	9.33±0.58	10.33±0.58
	I. aquatica	Ethanol	10.33±0.58	10.33±0.58	10.33±0.58	10.33±0.58
	A. galanga	Water	10.00±0.58	10.00±0.00	11.00±0.00	11.00±0.58
	A. galanga	Ethanol	11.00±0.00	10.67±0.58	11.00±0.00	11.00±0.58
	P. betle	Water	10.67±0.58	10.67±0.58	10.67±0.58	11.00±0.58
	P. betle	Ethanol	11.33±0.58	11.00±0.00	11.33±0.58	11,33±0.58
100 ppm	*C. papaya*	Water	9.67±0.58	9.33±0.58	9.67±0.58	9.33±0.58
	C. papaya	Ethanol	10.00±0.00	9.67±0.58	9.67±0.58	9.67±0.58
	I. aquatica	Water	9.67±0.58	9.00±0.58	9.67±0.58	9.67±0.58
	I. aquatica	Ethanol	9.67±0.58	9.67±0.58	10.00±0.00	10.00±0.00
	A. galanga	Water	9.67±0.58	9.67±0.58	10.33±0.58	10.33±0.58
	A. galanga	Ethanol	10.67±0.58	10.33±0.58	10.67±0.58	10.67±0.58
	P. betle	Water	10.33±0.58	10.00±0.00	10.00±0.58	11.00±0.00
	P. betle	Ethanol	11.00±0.00	10.67±0.58	11.00±0.58	11.33±0.58
		Control+	18.67±0.58	18.00±0.00	17.67±0.58	17.67±0.58
		Control-	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00

The ethanol extract of *I. aquatica* has inhibitory against *E. coli*, *Saprolegnia* spp., *A. hydrophyla*, and *Pseudomonas* sp. with inhibition zone of 11.67 mm, but the water extract has inhibition zone of 11.33 mm. *I. aquatica* has antimicrobial and antiinflammatory effects, which provide pharmacological evidence for folk uses of *I. aquatica* (Sivaraman et al. 2010). Humans use Ipomoea for their content of medical and psychoactive compounds, mainly alkaloids. The genus includes food crops, the tubers of sweet potatoes and the leaves of water spinach are commercially important food items (Aorora et al. 2013).

Table 2. Inhibition effect of extracts of *C. papaya*, *I. aquatica*, *A. galanga* and *P. betle* against microorganisms

Concentration	Plant materials	Solvents	Total plate count (Cfu/mL)			
			A. hydrophyla	*Pseudomonas* sp.	*E. coli*	*Saprolegnia* spp.
1,000 ppm	*C. papaya*	Water	228.00±7.55	281.33±8.08	243.67±8.14	250.67±7.57
	C. papaya	Ethanol	192.67±6.35	271.67±7.57	193.67±10.69	194.67±6.11
	I. aquatica	Water	280.67±6.66	297.00±3.00	296.00±7.21	298.67±5.03
	I. aquatica	Ethanol	205.67±5.86	260.33±8.50	271.00±7.81	278.00±4.36
	A. galanga	Water	275.67±5.51	295.00±4.58	280.67±4.62	275.67±5.86
	A. galanga	Ethanol	216.33±4.51	272.00±7.21	245.33±14.15	193.67±6.03
	P. betle	Water	217.00±11.27	277.67±7.09	199.33±5.69	198.00±7.21
	P. betle	Ethanol	189.67±5.03	253.00±9.17	173.00±9.54	169.33±6.03
800 ppm	*C. papaya*	Water	227.67±10.41	282.00±7.00	251.00±8.19	255.67±5.13
	C. papaya	Ethanol	196.33±3.51	276.33±4.73	200.00±10.54	199.67±6.66
	I. aquatica	Water	282.33±6.66	297.33±2.52	296.33±3.21	299.33±7.51
	I. aquatica	Ethanol	202.33±7.09	263.00±4.58	273.67±5.51	277.00±4.00
	A. galanga	Water	277.67±3.21	297.00±7.00	280.33±1.53	277.33±3.21
	A. galanga	Ethanol	214.67±4.16	271.67±3.06	248.00±7.21	196.67±4.16
	P. betle	Water	220.67±5.86	278.00±2.00	199.00±9.54	197.67±10.97
	P. betle	Ethanol	190.00±6.00	255.00±8.54	172.67±5.51	170.33±6.51
600 ppm	*C. papaya*	Water	282.00±12.17	307.00±14.73	292.33±14.50	279.00±11.36
	C. papaya	Ethanol	257.67±8.02	291.00±4.00	218.67±14.50	240.33±11.06
	I. aquatica	Water	298.00±9.17	324.67±10.69	308.00±10.39	306.00±12.17
	I. aquatica	Ethanol	257.00±6.24	269.67±8.50	285.33±11.93	282.67±7.02
	A. galanga	Water	291.00±8.54	312.67±26.39	285.33±4.04	291.00±3.61
	A. galanga	Ethanol	254.00±3.46	286.00±6.08	260.67±5.51	255.67±3.79
	P. betle	Water	250.00±7.55	291.33±10.02	253.00±6.08	239.67±9.50
	P. betle	Ethanol	236.67±6.51	278.33±4.93	219.67±9.50	210.33±5.86

The extract of *C. papaya*, *I. aquatica*, *A. galanga* and *P. betle* can inhibit the growth of microbes in MIC method. The results of minimum inhibitory concentrations are showed in Table 2. The microbe content of ethanol extract of *P. betle* against *Saprolegnia* spp. is 169.33 cfu/mL, *E. coli* 172.67 cfu/mL, *A. hydrophyla* 189.67 cfu/mL., and *Pseudomonas* sp. 253.00 cfu/mL. Water extract of *P. betle* against *Saprolegnia* spp. is 197.67 cfu/mL, *E. coli* 199.00 cfu/mL, *A. hydrophyla* 217.00 cfu/mL and *Pseudomonas* sp. 277.67 cfu/mL. Ethanol extract of *P. betle* can suppress the growth of microbes than water extracts, and *P. betle* can suppress the growth of fungi than bacteria. The microbe content of ethanol extract of *C. papaya* against *A. hydrophyla* is 192.67 cfu/mL, *E. coli* 193.67 cfu/mL, *Saprolegnia* spp. 194.67 cfu/mL, and *Pseudomonas* sp. 271.67 cfu/mL. Water extract of *C. papaya* against *A. hydrophyla* is 227.67 cfu/mL, *E. coli* 243.67 cfu/mL, *Saprolegnia* spp. 250.67 cfu/mL, and *Pseudomonas* sp. 281.33 cfu/mL. Ethanol extract of *C. papaya* can suppress the growth of microbes than water extracts, and *C. papaya* can suppress the growth of *A. hydrophyla* than another microbe.

The microbe content of ethanol extract of *A. galanga* against *Saprolegnia* spp. is 193.67 cfu/mL, *A. hydrophyla* 214.67 cfu/mL, *E. coli* 245.33 cfu/mL, and *Pseudomonas* sp. 271.67 cfu/mL. Water extract of *A. galanga* against *Saprolegnia* spp., and *A. hydrophyla* are 275.67 cfu/mL, *E. coli* 280.33 cfu/mL, and *Pseudomonas* sp. 295.00 cfu/mL. Ethanol extract of *A. galanga* stem can suppress the growth of microbes than water extracts, and *A. galanga* stem can suppress the growth of fungi than bacteria. The microbe content of ethanol extract of *I. aquatica* against *A. hydrophyla* is 202.33 cfu/mL, *Pseudomonas* sp. 260.33 cfu/mL, *E. coli* 271.00 cfu/mL, and *Saprolegnia* spp. 277.00 cfu/mL. Water extract of *I. aquatica* against *A. hydrophyla* is 280.67 cfu/mL, *E. coli* 296.00 cfu/mL, *Pseudomonas* sp. 297.00 cfu/mL, and *Saprolegnia* spp. 298.67 cfu/mL. Ethanol extract of *I. aquatica* can suppress the growth of microbes compared with water extracts, and *I. aquatica* can more suppress the growth of bacteria than fungi.

The screening of plant's extracts and bioactive product for antimicrobial activity has shown that plants represent a potential source of medical and pharmacological substance. Antimicrobial activities of the plant and plant product can be done by *in vitro* methods before tested in the organism (Saptiani et al. 2015). Antimicrobials with ADD method were based on the diameter of inhibition zone. Inhibition zone was formed on 14 hours after incubation, and further enlarged until the 48 hours. In generally inhibition zone showed no increase after the 48 hours.

In general, the best inhibitory zone to microbial is ethanol extract from *P. betle*, followed by water extract from *P. betle* and ethanol extract from *A. galanga* stem, ethanol extract from *C. papaya*, water extract from *A. galanga*, water extract from *C. papaya*, ethanol extract from *I. aquatica*, and water extract from *I. aquatica*. The best microbial growth inhibitor is ethanol extract from *P. betle*, followed by ethanol extract from *C. papaya*, ethanol extract from *A. galanga* stem, water extract from *P. betle*, ethanol extract from *I. aquatica*, water extract from *C. papaya*, water extract from *A. galanga* stem, and water extract from *I. aquatica*. Although ethanol extracts is better than the water extracts, the water extracts is still applicable. The production cost for water extracts is cheaper and easier so it is applicable for fish cultures.

P. betle has antimicrobial activity towards microbial in aquatica i.e. *A. hydrophila*, *Pseudomonas* sp., *E. coli*, and *Saprolegnia* spp. *A. hydrophila*, *Pseudomonas* sp., and *Saprolegnia* spp. cause diseases on fish. Essential oils of the *Piper betle* contained phenolic compounds such as cavicol, cavibetol, carvacrol, eugenol and allilpyrocatechol (Subashkumar et al. 2013). The leaf from *P. betle* poses the broad spectrum antimicrobial activity against various bacterial strains including *Bacillus cereus, Enterococcus faecalis, Listeria monocytogenes, Micrococcus luteus, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa, Salmonella Enteritidis, Streptococcus mutans, Streptococcus pyogenes, Enterococcus faecium, Actinomycetes viscosus, Streptococcus sanguis, Fusobacterium*. Beside this, the *P. betle* also poses the antifungal and antiprotozoal activity against pathogen, which causing typhoid, cholera, tuberculosis, etc. (Guha 2006). Methanol and ethanol extract from *P. betle* exhibited antibacterial activity against various gram positive and gram negative bacterial pathogens (Datta et al. 2011). In the present study extract from *P. betle* can inhibit bacteria and fungi.

Ethanol extract from *A. galanga* stem can inhibit the growth of microbes, and more inhibit fungi than bacteria. *A. galanga* is known to having antimicrobial, antioxidant, antifungal, anti cancer, and gastro protective activities (Matsuda and Morikawa 2005). The *A. galanga* contains alkaloids, saponin, glycosides, terpenoids, phenolics, flavanols, flavanoids, phytosterols, and carbohydrates (Jadu et al. 2009). The rhizome of *A. galanga* contains flavonoids, some of which have been identified as kaemperol, kaempferide, galangin, and alpinin. Galangin is a flavonoid with multiple biological activities (Chudiwal et al. 2010).

Many parts of the *C. papaya* are employed in the treatment of several ailments; for example the fruit juice for lowering blood pressure, the seed is used for expelling worms, and the leaves are used variously for the treatment of fever, pyrexia, diabetes, inflammation and as dressing for foul wounds. In the present study, ethanol extract from *P. betle* and *C. papaya* showed the best growth inhibition against *A. hydrophila* compared to another extract. *C. papaya* leaf extract was found containing alkaloids, flavornoids, glycosides, cardiac glycosides, tannins, saponins and anthraquinones (Imaga et al. 2009). Leaves and stems from *I. aquatica* can be used as antioxidant, cytotoxic as emetic, purgative and antidote to arsenic (Yasmin et al. 2009). *I. aquatica* can be used as an easy accessible source of natural antioxidants, as a food supplement, or in the pharmaceutical and medical industry (Huang et al. 2005). In the present study, *I. aquatica* had the lowest inhibition compared to other plants, but the ethanol extract from *I. aquatica* can be used to inhibit microbial, particularly *A. hydrophila* and *Pseudomonas* sp.

In conclusion, water or ethanol extract from *C. papaya*, *I. aquatica*, *A. galanga* and *P. betle* can inhibit microbial aquatic. This study shows that the plants are antimicrobial, and can be used as an alternative to eradicate pathogens in aquatic. They could be the alternatives for the antibiotics against the pathogen. Eight hundred ppm ethanol extract for *P. betle* had the best inhibition zones, and 1,000 ppm had the best inhibit the growth of microbes in MIC method. Antimicrobial study using plants is cheap and does not contaminate the environment.

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REFERENCES

Aravind G, Bhowmik D, Duraivel S, Harish G. 2013. Traditional of medicinal uses of *Carica papaya*. J Med Plants Stud 1 (1): 7-15.

Arora S, Kumar D, Shiba. 2013. Phytochemical, antimicrobial and antioxidant activities of ethanol extract of leaves and flowers of *Ipomoea cairica*. Int J Pharm Sci 5 (1): 198-202.

Baskaran C, Rathabai V, Velu S, Kumaran K. 2012. The efficacy of *Carica papaya* leaf extract on some bacterial and a fungal strain by well diffusion method. Asian Pacific J Trop Dis 1: S658-S662.

Chudiwal AK, Jain DP, Somani RS. 2010. *Alpinia galanga* Wild.-an overview on phyto-pharmacological properties. Indian J Nat Prod Resour 1 (2): 143-149.

Datta R, Hoshdastikar S, Singh M. 2011. Antibacterial activity of *Piper betle* leaf against isolates of bacteria. Intl J Pharma Sci Res 2 (8): 104-109.

Guha P. 2006. Betel leaf: the neglected green gold of India. J Hum Ecol 19 (2): 87-93.

Huang DJ, Chen HJ, Lin CD, Lin YH. 2005. Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. Bot Bull Acad Sinica 46: 99-106.

Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kehinde MO, Bamiro SB. 2009. Antisickling property of *Carica papaya* leaf extract. African J Biochem Res 3 (4): 102-106.

Jadu SB, Indurwade NH, Sakar DM, Fuloria NK, Ali MD, Das S, Basu SP. 2009. Galango flavonoid isolated from rhizome of *Alpinia galanga* (L) swartz (zingeberaceae). Trop J Pharma Res 8 (6): 545-550.

Matsuda H, Morikawa T. 2005. Gastro protective effects of phenyl propanoids from the rhizomes of *Alpinia galanga* in rats: structural requirements and mode of action. European J Pharma 47 (1): 59-67.

Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. 2008. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. Inflammopharmacology 16: 168-173.

Pradhan D, Suri DKA, Pradhan DDK, Biswasroy P. 2013. Golden heart of the nature: *Piper betle* L. J Pharmacog Phytochem 1 (6): 147-167.

Saptiani G, Hartini. 2008. The inhibition of bettle leaf extracts on the in vitro growth of the *Vibrio harveyi* bacteria and the protective to tiger prawn larva. Paper. Conference of Indonesian Aquaculture. Indoaqua. Yogyakarta 17-20 November 2008.

Saptiani G, Prayitno SB, Anggoro S. 2013. Antibacterial potential of jeruju (*Acanthus ilicifolius*) leaf extracts on the in vitro growth of the *Vibrio harveyi*. J Kedokteran Hewan 7 (1): 17-20.
Saptiani G, Pebrianto CA, Hardi EH. 2015. Anti-microbial of *Alpinia galanga* extracts against the pathogen of *Clarias batrachus*. In: Isnansetyo et al. (eds) Proceeding The 1st Inter Symp on Marine and Fisheries Research (ISMFR), Gadjah Mada University, Yogyakarta, August 7th 2015. 99-104.
Sivaraman D, Muralidaran P, Kumar SS. 2010. Evaluation of anti-microbial and anti- inflammatory activity of methanol leaf extract of *Ipomoea aquatica* Forsk. Res J Pharma Biol Chem Sci 1 (2): 258-264.
Subashkumar R, Sureshkumar M, Babu S, Thayumanavan T. 2013. Antibacterial effect of crude aqueous extract of *Piper betle* L. against pathogenic bacteria. Intl J Res Pharma Biomed Sci 4 (1): 42-46.
Yasmin H, Kaisar MA, Sarker MMR, Rahman MS, Rashid MA. 2009. Preliminary anti-bacterial activity of some indigenous plants of Bangladesh. J Pharm Sci 8 (1): 61-65.

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