ANTIBACTERIAL TEST OF PANGIUM (*Pangium edule* Reinw) EXTRACT AGAINST THE GROWTH OF FISH SPOILAGE BACTERIA

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ABSTRACT

An antibacterial test was conducted on fresh and fermented *Pangium edule* Reinw. Seed extracts against gram positive and negative spoilage bacteria associated with fish. Seed extraction was carried out by maceration with water, water-ethanol (1:1), and n-hexane. Antibacterial activities were determined by an agar diffusion method. Water extract of fresh *Pangium edule* seed inhibited the growth of gram positive and negative bacteria at concentrations of 40-80 mg/mL, water-ethanol extract inhibited the growth of gram positive and negative bacteria at concentrations of 30-80 mg/mL, while n-hexane extract showed no inhibition. On the other hand, extract of fermented *Pangium edule* seed using water, water-ethanol, and n-hexane showed no antibacterial activity against gram positive and negative bacteria. The minimum inhibitory concentration values of fresh *Pangium edule* seed water extract were 3.49-11.73 mg/mL, mostly effective against *Pseudomonas fluorescens.* Meanwhile, the minimum inhibitory concentration of water-ethanol extract were 6.04-10.54 mg/mL, mostly effective against *Enterobacter aerogenes, Alcaligenes eutrophus,* and *Staphylococcus aureus.*

KEYWORDS: **antibacterial activity, agar diffusion method, gram positive and fish spoilage bacteria, minimum inhibitory concentration,** *Pangium edule* Reinw

INTRODUCTION

Many places in Indonesia are not properly facilitated by basic infrastructures so that in remote areas but fish is highly produced, problems of quantity and quality loss arise due to the lack of ice needed to preserve the fish. To overcome this, fishermen use fresh pangium *(Pangium edule* Reinw) seed, called *picung*, to preserve fish traditionally for many decades. Considering its biochemical properties, pangium has a great challenge as a natural preservative, especially for fish and fish products. The lack in quantity, the high price, and the low quality of ice, are known as reason in promoting the misuses of such illegal preservatives like formalin in fish handling in remote areas (Heruwati *et al.*, 2004; Sampurno, 2006). The benefits of pangium for fish handling are not only attributed to its anti-bacteria and antioxidant properties, but pangium is also believed in producing typical flavor on fish and fish product.

Some studies revealed that pangium contains many substances such as flavoring agent, cyanide acid (Heyne, 1959; Bishop, 1997), antioxidant (Anwar, 1992; Panghegar, 1990; Adidjaja, 1991; Romlah, 1992), tannin and antimicrobial agents including hidnocarpic acid, and chaulmogric acid (Hilditch & Williams, 1964). The cyanide acid is believed to be a derivative product of gynocardine glucosides by the role of gynocardase, while hidnocarpic acid (2 cyclopentene 1 undecanoic/$C_{16}H_{28}O_2$) and choulmogric acid (2 cyclopentene 1 tridecanoic/$C_{18}H_{32}O_2$) are unsaturated cyclic fatty acids as the acidification product of oleic and linoleic acid contained in the kernel. Pangium also contains polar and nonpolar antioxidants. The nonpolars were á-, ã-, and ä-tocotrienol, though dominated by ã-tocotrienol (Puspitasari-Nienaber *et al.*, 1994). The antioxidant content is reduced during initial phase of seed germination and some of ã-tocotrienol change to á- and ä-tocotrienol. However, when the hypocotyls synthetize the chlorophyl, the tocotrienols increased again and at the same time, the tocoferol is synthetized. On the other hand, the polar antioxidants are possibly carboxylic acid and sugars, which is believe to be a glycon of phenolic conjugate. The total phenolic contents as well as the antioxidant activity of pangium kernel are increased during seed germination. The antioxidant might protect the cell from potential oxidation-induces deterioration (Andarwulan *et al.*, 1999).

Experiment using chopped pangium seed mixed with granular salt, and overspread on the gutted mackerel fish (*Rastrelliger brachysoma*) at ratio of 2, 4, and 6% kernel and 2 and 3% salt (w/w to fish weight) revealed that pangium was significantly reduced the pH and TVB content of fish, although it had no effect on other parameter assessed. At the same time, salt also showed a significant effect on both TVB content and the number of H_2S producing bacteria. However, pH, TVB content as well as number

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of enteric and H_2S producing bacteria increased during fish storage. Sensory evaluation resulted that from the texture, odor and flavor point of view, treatments with 2% pangium (whether mixed with 2 or 3% salt) was rejected at day-6, while the one with pangium 6% (with 2 or 3% salt), and combination of 4% pangium with 3% salt was still accepted by the panelists at day-9. Nevertheless, treatments with high content of pangium were not preferred by the panelists due to the fish appearance which were rather brownish in color. This undesirable color may be produced by the reaction between tannin (tannic acid) with ferric compound from fish meat (myoglobine). The tannic acid contained in fish were between 16.7-23.7 ppm at initial and 7.7-11.0 ppm at the end of experiment; while for cyanide, the content in fish were between 20.6-44.4 ppm at initial and 2.5-19.2 ppm at the end of experiment depending on the amount of pangium added (Heruwati *et al.*, 2007).

Though it has been proved both by experiments and by commercial practices that pangium could prolong the shelflife of fish, yet, it still very limited information available on what active substances contained in pangium that responsible in fish preservation, and how long they could delay the spoilage of fish. For this purpose, a study was conducted on the antibacterial activity of water, water-ethanol, and n-hexane extracts of fresh and fermented pangium against some species of bacteria responsible in fish spoilage. It was expected that based on resulted information on extract that has highest preservative activity, experiment could be followed by isolation, characterization, and purification of the active substances. In the long run, the production of natural preservative for fresh fish could be realized.

MATERIALS AND METHODS

Ripe pangium (*Pangium edule* Reinw) fruits were collected from Pabuaran, Cileungsi, Bogor. The seeds then were drawn out and washed with water. Fermented seeds were made by boiling the seed for 6 hrs, followed by wrapping in plastic bag and keeping at room temperature for about 40 days.

Two species of gram positive bacteria i.e. *Micrococcus luteus*, and *Staphylococcus aureus*, six species of gram negative i.e. *Alcaligenes eutrophus*, *Enterobacter aerogenes*, *Flavobacterium gleum*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, and *Serratia marcescens* were used in this experiment. These cultures were obtained from Microbiological Laboratory of Research Institute for Marine and Fisheries Product Processing and Biotechnology, Jakarta and Microbiological Laboratory of the Bandung Institute of Technology, Bandung.

Chloramphenicol (Indofarma) at concentration of 10 mg/mL was used as a positive control, while water and water-ethanol (1:1) and n-hexane were used as negative control. Nutrient Agar (NA) (Difco) was used as the growth medium for the cultures, and Mueller Hinton Agar (MHA) (Difco) was used in antibacterial test.

Preparation of Pangium Extracts

Fresh and fermented pangium seeds were cleaned and washed, then the shells were cracked to get the kernel. The kernel was homogenized and freeze-dried at -40°C followed by grinding them into powder. Extraction was done by macerating 200 g of fresh or fermented pangium powder in 1,000 mL of distilled water by shaking at 30 rpm for 3x24 hrs. After filtration using Whatman paper no.42, the filtrate was dried using vacuum evaporator at 72 mbar to obtain crude water extract. After filtration, the residue was collected and remacerated using 1,000 mL of mixture of water-ethanol (1:1) with the same procedure as water extraction. Filtrate of the later extraction was dried by using vacuum evaporator at 175 mbar to get crude water-ethanol extract. The residue of second extraction was then remacerated using 1,000 mL n-hexane by the same procedure. In this last extraction, evaporation of the filtrate was done at 335 mbar to get crude n-hexane extract. All crude extracts were then freeze-dried, weighed, and kept in capped bottle until used (Harborne, 1998; Andarwulan *et al.*, 1999).

Preparation of Test Culture

All cultures were grown at slanted NA for 24 hrs at 37°C, an ose of each culture was then grown in 20 mL of nutrient broth, incubated in a shaking water bath at 160 rpm, for 24 hrs 37°C. The optical density was measured using spectrophotometer at ë 600 nm to get an absorbance of 0,5-0,6. To make sure that a total number of 10^6-10^8 cfu/mL had been achieved, counting of the total bacteria using Mc Farland method (DG of Drug & Food Control, 1995) was also conducted.

Antibacterial Test

Each 800 g of freeze-dried crude extract was dissolved in its appropriate solvent (i.e. water, water-ethanol, n-hexane), added with phosphate buffer at pH 7 to make a stock solution containing 80 mg extract/mL. Each stock solution was then diluted into

test solution at concentration of 10, 20, 30, 40, 50, 60, 70, and 80 mg/mL (DG of Drug & Food Control, 1995).

Antibacterial test was conducted using agar plate diffusion method. Twenty µL of test bacteria were inoculated into test tubes containing 15 mL of MHA medium, homogenized, and poured into sterile plates. Sterile paper disks (6 mm in diameter) that had been dipped in 20 iL of pangium extract at concentration of 10, 20, 30, 40, 50, 60, 70, and 80 mg/mL were then put onto the surface of solid MHA plates which had been cultured by tested bacteria, and incubated at 37°C for 24 hrs. Six paper disks were put in each plate, two of them were positive control (chloramphenicol at concentration of 10 mg/mL) and negative control (appropriate solvent), respectively. The inhibition area were assessed by measuring the diameters of clear zone surrounding the paper disks (DG of Drug & Food Control, 1995). Experiment was conducted using 3 replications.

Antibacterial activity of the extract was determined based on the clear zone area. Low activity is the one having clear zone of less than 5 mm, medium activity is between 5-10 mm, while strong activity is between 11-20 mm, and very strong is the one having clear zone between 21-30 mm (Morales *et al.*, 2003 *in* Ismaini, 2007).

Minimum Inhibition Concentration

Minimum inhibition concentration was calculated using the linear regression between Mo (concentration of pangium extract test solution) and x^2 (square of inhibition area) which crossed the ordinat at MT. MIC was determined as anti-ln of ¼ x Mt (Bloomfield, 1991 *in* Nuraida *et al.*, 2000).

RESULTS AND DISCUSSIONS

Pangium Seed Composition and Yield of Pangium Extracts

Fresh pangium seed contains about 52% water, 16% fat, 18% carbohydrate, and 13% protein. There are also some minerals contained in the fresh seed (Heruwati *et al.*, 2007).

Out of 200 g of raw material, extraction of fresh pangium seed using water yielded 4.93 g powder, while extraction using water-ethanol and n-hexane yielded 5.45 and 1.08 g powder respectively. On the other hand, the yield of water, water-ethanol, and n-hexane extract of 200 g of fermented seed were 15.43, 20.53, and 1.13 g powder respectively. For both fresh and fermented seeds, extraction using water-ethanol gave the highest yield because the solvent effectively drawn out polar and semipolar substances contained in the pangium seed such as the flavonoids, phenol, tannin, and perhaps small amount of terpenoids, saponins, alcaloids, and steroids. These substances are produced as a biodegradation of fat and protein contained in the seed (Harborne, 1998). Claeson *et al.* (1998); Andarwulan *et al.* (1999) stated that water-ethanol is a good solvent to extract the polyphenols, phenols, glycocides, and flavonoids from plants. According to Dicko *et al.* (2006), phenolic substances are easily dissolved in polar solvent, due to its aromatic ring having one or more hydroxyl group (s).

Water extraction of fresh and fermented seed yielded less of extract because as a polar solvent, water could only pulled out polar substances such as flavonoids and tannin, as well as anionic substances, such as thiocianates, nitrates, chlorides, and sulphates. Wahyono (2004) found that extraction of *Scurrula atropurpurea* with water could drawn out the flavonoids. On the other hand, extraction of fresh and fermented pangium seed with n-hexan produced the least yield because n-hexane is a non-polar solvent so it only could extract the non polar substances especially those containing oil and fat such as triterpenoids (kamphor, linalool) and steroids.

Inhibition Rate of Each Extract Against the Growth of Gram Positive and Negative Bacteria

a. Water extract of fresh and fermented pangium seed

Water extract of fresh pangium seed could inhibit the growth of gram positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) at concentration of 40-80 mg/mL, with the diameter of inhibition zone of 5.00-8.50 mm for *S. aureus* and 0.33-1.33 mm for *M. luteus* (Appendix 1). This inhibition was possibly due to the antibacterial substances contained in the extract such as phenols, flavonoids, alkaloids, tannins, and anionic substances like thiocianates, nitrates, chlorides, and sulphates. The mechanism of bacterial inhibition by phenols is by bonding of hydroxyl group of phenol with bacterial protein membrane, leading to the disruption of membrane permeability, affecting the electron transport system, and the rupture of peptidoglycan layer of bacterial cell walls. The inhibition mechanism of anionic substances to bacterial growth is through the inhibition of extracellular enzyme during oxidation process of disulphide bonds (El Astal *et al.*, 2005). However, water extract at concentration of 10-30 mg/mL could not inhibit the growth of *S. aureus* and *M. luteus* due to low toxicity of the extract so it couldn't be able to

rupture the peptidoglycan layer of bacterial cell membrane.

Water extract of fresh seed could inhibit the growth of gram negative bacteria, *Alcaligenes eutrophus* and *Enterobacter aerogenes* at concentration of 30-80 mg/mL, with inhibition zone diameter of 3.33-2.00 mm for *A. eutrophus*, and 0.16-1.67 mm for *E. aerogenes*, while for *Serratia marcescens, Pseudomonas fluorescens, Flavobacterium gleum,* and *Salmonella typhimurium* the effective doses were 40-80 mg/mL, giving zone diameter of 0.83-2.67 mm, 1.00-1.17 mm, 0.50-1.17 mm, and 1.00-3.00 mm for *S. marcescens, P. fluorescens, F. gleum*, and *S. typhimurium* respectively (Appendix 2). The mechanism of gram negative bacteria inhibition by phenols was possibly because the diffusion of phenol into the surface of the bacterial cell, especially at hydrophobic lipid bilayer, therefore the hydrolytic enzyme in lipopolysaccharide and the phospholipid layer were inhibited resulting in the loss of the protection of bacterial cytoplasmic membrane.

For the fermented seed, water extract at concentration of 10-80 mg/mL didn't show any antibacterial activity (Appendix 1 and 2) against gram positive (*S. aureus* and *M. luteus*) and gram negative bacteria (*A. eutrophus, E. aerogenes, S. marcescens, P. fluorescens, F. gleum,* and *S. typhimurium*). This is presumably due to the decomposition of antibacterial substances or related enzymes during the boiling of the seed before fermentation process.

b. Water-ethanol extract of fresh and fermented pangium

Water-ethanol (1:1) extract of fresh pangium could inhibit the growth of gram positive *S. aureus* at concentration of 30-80 mg/mL with zone diameter of 7.00-4.67 mm (Figure 1) and *M. luteus* at concentration of 40-80 mg/mL with zone diameter of 1.00-3.00 mm (Appendix 1). The inhibition mechanisms were possibly similar to the inhibition of water extract because the polarity of both solvents were nearly similar, so that the same substances were also extracted. Dicko *et al.* (2006) reported that proantocyianidine (condensed tannin) is toxic to microorganism by inhibition of hydrolytic enzymes, and inactivation of protein transport in *envelope cell.* According to Gilbert (1984) biosynthetic disturbance of peptidoglycan is also due to the diffusion of toxic phenolic into the cell membrane, causing increase in permeability, thus resulted in the increase of intracellular pressure to peptidoglycan. The diameter of clear zones of water-ethanol extract treatments on *S. aureus* was wider than that of *M. luteus* i.e. 5.00-8.50 mm, 7.00-14.67 mm, and 0.33-1.33 mm, 1.00-3.00 mm presumably because *S. aureus* is more sensitive to phenols, alkaloids and flavonoids than *M. luteus*. El Astal *et al.* (2005) revealed that *S. aureus* was very sensitive to ethanol extract of *Thymus vulgaris* and *Salvia officinalis* at concentration between 2.5-40.0 mg/mL.

Water-ethanol extract of fresh pangium could inhibit the growth of gram negative *E. aerogenes* and *A. eutrophus* at concentration of 30-80 mg/mL with zone diameter of 7.00-16.67 mm for *E. aerogenes*, and 7.00-12.00 mm for *A. eutrophus*. This extract also inhibit *F. gleum, S. typhimurium, P. fluorescens,* and *S. marcescens* at concentration of 40-80 mg/mL, with zone diameter of 1.33-2.33 mm for *F. gleum*, 1.00-3.00 mm for *S. typhimurium*, 1.00-3.00 mm for *P. fluorescens*, and 1.00-2.00 mm for *S. marcescens* (Appendix 2). Pimia *et al.* (2001) found that ethanol extract of *Fragaria* sp. inhibited *Escherichia coli* and

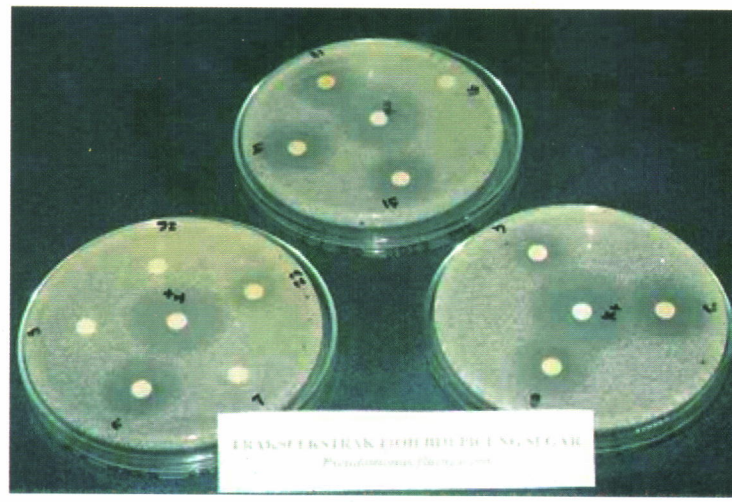

Figure 1. The inhibition zone of water-ethanol extract of pangium seed against *S. aureus*.

S. typhi, while El Astal *et al.* (2005) revealed that ethanol extract of *Thymus vulgaris* and *Salvia aucheri* inhibited *P. aeruginosa*.

Water-ethanol extract of fermented pangium seed could inhibit the growth of gram positive *S. aureus* and gram negative *S. marcescens* at concentration of 70-80 mg/mL with zone diameter of 5.33-6.00 mm, and 2.67-3.50 mm respectively, but not at concentration of 10-60 mg/mL (Appendix 1 and 2). The mechanism of inhibition was presumably because the fermented seed extract contains antioxidants which could perform as an antibacteria. Andarwulan *et al.* (1999); Anwar (1992) revealed that fermented pangium contained many antioxidants such as tocopherols, tocotrienols, and antioxidants originated from primary amine groups and aromatic (benzene) groups.

c. n-hexane extract of fresh and fermented pangium

It was noted from the experiment that n-hexane extract of fresh and fermented pangium at all concentration could not inhibit all bacteria tested. This probably because the extracts contain fats and fatty acid substances, that might hindered the diffusion of antibacterial agents into the peptidoglycan of the cell wall of gram positive bacteria or hydrophobic lipid bilayer of the gram negative bacteria. Moshi & Mbwambo (2005) also found that non polar extract of *Terminalia sericea* could not suppress the growth of *S. aureus, S. typhii,* and *P. aeruginosa*. Similar to this, Priyono (2004); Indriyanto (2004) reported that n-hexan extracts of *Parkia timoriana* seeds, *Ruta angustifolia* leaves, and *Parameria barbata* stem shell could not also inhibit *S. aureus*, *M. luteus*, and *S. typhosa* growth.

d. Antibacterial activity of water and water-ethanol extract of fresh pangium

Based on the categorization of antibacterial activity of the extracts (Morales 2003 *in* Ismaini, 2007), the antibacterial activity of water extract of fresh pangium were medium against *S. aureus*, low to medium against *E. aerogenes*, and low against *M. luteus*, *A. eutrophus, P. fluorescens, S. marcescens*, *F. gleum*, and *S. typhimurium*. Meanwhile, the antibacterial activity of water-ethanol extracts were medium to high activity against *S. aureus, A. eutrophus*, and *E. aerogenes*, and low against *M. luteus, P. fluorescens, S. marcescens*, *F. gleum*, and *S. typhimurium*. El Astal *et al.* (2005) showed that water extract of *Thymus vulgaris* had high antibacterial activity against *S. aureus*, and *Pseudomonas aeruginosa* while Digrak *et al.* (1999) showed that *Phlomis bourgei* extract had high activity against *Bacillus subtilis, S. aureus, E. coli, P. aeruginosa*, and *Proteus vulgaris*.

e. Minimum inhibitory concentration of water and water-ethanol extract of fresh pangium

The minimum inhibitory concentration of fresh pangium water extract were 3.49 mg/mL against *P. fluorescens* and between 8-9 mg/mL against *S. aureus*, *M. luteus*, and *E. aerogenes*. The minimum inhibitory concentration against *A. eutrophus, F. gleum, S. marcescens*, and *S. typhimurium* were somewhat higher, reaching more than 10 mg/mL (Table 1). Based on the minimum inhibitory concentration values, water extract of fresh pangium at concentration of about 4 mg/mL was effective enough to inhibit *P. fluorescens*, but for other species, higher extract concentration is needed. El Astal *et al.* (2005) reported that water extract of *Thymus vulgaris* could inhibit *S. aureus* at minimum inhibitory concentration value of 2.5 mg/mL, *E. coli of* 20 mg/mL, *S. typhi* of 10 mg/mL, and *P. aeruginosa* at minimum inhibitory concentration value of 20 mg/mL.

The minimum inhibitory concentration of fresh pangium water-ethanol extract were 6-7 mg/mL against S. *aureus, A. eutrophus,* and *E. aerogenes,* and 8-10 mg/mL against *F. gleum* and *S. marcescens,* and more than 10 mg/mL against *P. fluorescens, M. luteus,* and *S. typhimurium* (Table 1). Salvat *et al.* (2001) stated that water-ethanol extract of *Vassobia breviflora* was effectively suppress *S. aureus* at minimum inhibitory concentration of 0.25 mg/mL, while for *P. aeruginosa* and *S. typhimurium,* the inhibition was at minimum inhibitory concentration of 1 mg/mL respectively.

Table 1. Minimum inhibitory concentration of water and water-ethanol extract of fresh pangium seed against various species of bacteria

Kinds of bacteria	Water extract (mg/mL)	Water-ethanol extract (mg/mL)	Kinds of bacteria	Water extract (mg/mL)	Water-ethanol extract (mg/mL)
M. luteus	9.57	10.35	*P. fluorescens*	3.49	10.54
S. aureus	8.39	7.17	*E. aerogenes*	9.70	6.04
S. marcescens	10.87	9.83	*S. typhimurium*	11.73	10.11
F. gleum	10.51	8.08	*A. eutrophus*	10.44	7.14

CONCLUSION

1. Water-ethanol extract of fresh and fermented pangium seed produced the higher yield (5,45 and 20,53 g respectively) than that produced by other kinds of solvent, i.e. water and n-hexane.

2. Water and water-ethanol extracts of fresh seed showed an effective antibacterial activity against gram positive and negative bacteria at concentration of 40-80 mg/mL, but n-hexane extract didn't show any antibacterial activity at all concentration (10-80 mg/mL).

3. Water, water-ethanol, as well as n-hexane extract of fermented seed didn't show any antibacterial activity against gram positive or negative at all concentration (10-80 mg/mL).

4. Water extract of fresh seed showed high antibacterial activity against *P. fluorescens* with the minimum inhibitory concentration of 3.49 mg/mL, while the water-ethanol extract showed medium to high antibacterial activity against *S. aureus* and *E. aerogenes* with the minimum inhibitory concentration of 7.17 and 6.04 mg/mL respectively.

RECOMMENDATIONS

Though traditionally, pangium kernel has been widely employed as spices, yet, to be accepted in preservation of fish and fish product in areas where pangium has not been popular is not easy. Further studies are important to provide the maximum benefit of pangium as preservative, especially by characterizing the active substances of pangium that responsible to prolong the shelf life of wet fish as well as developing method of industrialization, whether in crude or pure basis.

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Appendix 1. Diameter of bacterial colony of varied extracts of fresh pangium (*Pangium edule* Reinw) seed treatment against fish spoilage gram positive bacteria

No.	Kinds of pangium extract	Kinds of bacteria	Diameter of inhibition zone(mm)*)									
			Concentration (mg/mL)								Control	
			10	20	30	40	50	60	70	80	Chl (+) 10 mg/mL	K (-)
1.	Water extract of fresh seed	*S. aureus*	nd	nd	nd	5.00±0.00	5.83±0.29	7.00±1.00	7.67±1.04	8.50±0.50	20.00	nd
2.	Water-ethanol extract of fresh seed	*S. aureus*	nd	nd	7.00±0.00	8.33±1.15	10.33±0.57	12.33±0.57	12.67±0.57	14.67±0.57	18.00	nd
1.	Water extract of fresh seed	*M. luteus*	nd	nd	nd	1.33±0.57	0.33±0.57	nd	1.33±0.57	1.33±0.57	8.00	nd
2.	Water-ethanol extract of fresh seed	*M. luteus*	nd	nd	nd	1.00±0.00	0.66±0.57	2.00±0.00	1.67±0.57	3.00±1.00	10.00	nd

Remarks: nd = not detected; *) = diameter of inhibition zone after subtracted by diameter of paperdisk; Chl (+) = positive control (chloramphenicol); K (-) = negative control (extraction medium: water or water-ethanol)

Appendix 2. Diameter of bacterial colony of varied extracts of fresh pangium (*Pangium edule* Reinw) seed treatments against fish spoilage gram negative bacteria

No.	Kinds of pangium extract	Kinds of bacteria	Diameter of inhibition zone(mm)*) Concentration (mg/mL)								Control	
			10	20	30	40	50	60	70	80	Chl (+) 10 mg/ml	K (-)
1.	Water extract of fresh seed	*A. eutrophus*	nd	nd	nd	3.33±0.57	4.67±1.15	6.67±0.57	10.67±0.57	12.00±0.00	18.00	nd
2.	Water-ethanol extract of fresh seed		nd	nd	5.67±0.57	7,00±0,00	8.67±0,57	11.00±1.73	11.67 ±1.53	12.00±2.00	16.33	nd
1.	Water extract of fresh seed	*E. aerogenes*	nd	nd	0.16±0.28	4.00±1.00	6.00±0.00	7.67±1.15	9.33±0,57	11.67±1.53	18.33	nd
2.	Water-ethanol extract of fresh seed		nd	nd	7.00±0.00	11.00±1.73	12.33±1.53	13.00±1.00	14.67±0,57	16.67±1.15	19.67	nd
1.	Water extract of fresh seed	*F. gleum*	nd	nd	nd	nd	0.50±0.50	0.67±0.57	1.00±0.00	1.17±0.76	9.00	nd
2.	Water-ethanol extract of fresh seed		nd	nd	nd	1.33±0.57	1.00±0.00	2.00±0.00	1.67±0.57	2.33±0.57	9.00	nd
1.	Water extract of fresh seed	*P. fluorescens*	nd	nd	nd	1.00±0.00	0.50±0.00	0.66±0.50	1.17±0.28	1.00±0.86	9.67	nd
2.	Water-ethanol extract of fresh seed		nd	nd	nd	1.00±0.00	1.00±0.00	1.33±1.15	1.33±0.57	3.00±1.00	7.00	nd
1.	Water extract of fresh seed	*S. marcescens*	nd	nd	nd	0.83±0.28	0.67±0.57	1.00±0.00	1.67±0.57	2.67±0.57	18.33	nd
2.	Water-ethanol extract of fresh seed		nd	nd	nd	1.00±0.00	0.67±0.57	1.00±0.00	2.00±0.00	2.00±1.00	7.00	nd
1.	Water extract of fresh seed	*S. typhimurium*	nd	nd	nd	nd	0.16±0.28	nd	0.66±1.15	1.17±0.76	10.33	nd
2.	Water-ethanol extract of fresh seed		nd	nd	nd	1.00±0.00	1.00±0.00	2.00±0.00	1.33±0.57	3.00±1.00	9.33	nd

Remarks: nd = not detected; *) = diameter of inhibition zone after subtracted by diameter of paperdisk; Chl (+) = Positive control (chloramphenicol); K (-) = negative control (extraction medium: water or water-ethanol)