Sys Rev Pharm 2020;11(7):246-252
A multifaceted review journal in the field of pharmacy

Antioxidant and Antimicrobial Activities of Methanolic Extracts of *Scorodocarpus borneensis* Becc

Yohana Sutiknyawati Kusuma Dewi[1]*, Cico Jhon Karunia Simamora[2], Dzul Fadly[1]

[1]Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia

***Corresponding author**: Yohana Sutiknyawati Kusuma Dewi, email: yohana@ps-itp.untan.ac.id

ABSTRACT

Local communities in Borneo island usually use *Scorodocarpus borneensis* Becc. (known as Kulim tree) to prevent a deterioration of oil and meat. This study aims to find out the preservatives mechanism by identifying the total phenolic, antioxidant, and antimicrobial capabilities of *S. borneensis*. In this study, the phenolic compounds of the leaf, stem bark, and fruit were extracted by 70 % methanol before subjected to the antioxidant activity determined by the DPPH method. The antimicrobial activities were determined toward a sample extract possessed the highest antioxidant activities, by the well diffusion method against bacterial cultures and inhibitory proteolytic bacteria method in red tilapia fillet. The results showed that the phenolic compounds' value varied from 30.83 ± 3.3 to 52.93 ± 4.0 mg GAE/g. The highest antioxidant activity was identified from the leaf, followed by stem bark and fruit, with an IC_{50} value of 36.88 ppm, 52.45 ppm, and 86.20 ppm, respectively. This methanol extract of *S. borneensis* leaf played a role as an inhibitory against MRSA bacteria as a Gram-positive bacterium. While also inhibit the growth of *Salmonella typhii* and *Candida albicans*. The extract of Kulim's leaf contained many bacteriostatic bioactive compounds, which can be used to maintain bacteria's proteolytic population in tilapia fillets. The study suggests that the methanolic extract of *Scorodocarpus borneensis* Becc exhibits an excellent potential for antioxidant and antimicrobial activities and may be useful as a natural preservative.

Keywords: *Scorodocarpus borneensis* Becc., *phenol, antioxidant activity*, antimicrobial activity, natural preservative

Correspondence:
Yohana Sutiknyawati Kusuma Dewi
[1]Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia
***Corresponding author**: email: yohana@ps-itp.untan.ac.id

INTRODUCTION

Scorodocarpus borneensis Becc. belongs to the Olacaceae family and is commonly known as Kulim tree or Garlic tree. It is one of the indigenous trees which grow naturally in South Thailand, Sumatra Island, Lingga Island, Peninsular Malaysia, and Borneo/Kalimantan (Lim et al., 1999). Local communities in those areas consume different parts of the Kulim tree, such as old leaves, stem bark, and seeds as a traditional spice. It smells of garlic and popular to inhibit oil rancidity (Dewi et al., 2018). Natural compounds usually are found ubiquitously in fruits, nuts, seeds, flowers, vegetables, barks, and herbs (Andersen and Markham, 2006). These commonly utilized as coloring agents, flavoring, aromatizes, and antioxidants (Farhan et al., 2013).

An antioxidant can be defined as a compound that inhibits or delay, but do not entirely prevent the oxidation. Two types of antioxidants are natural antioxidants and synthetic antioxidants. The synthetic antioxidants consist of phenolics structure with various degrees of alkyl substitutions, while the natural antioxidants can be phenolic compounds, quinones, or lactones, as well as polyphenolics. This phenolic commonly identified in plants, and they possess antibacterial and antioxidant activities (Fawole et al., 2012; Mokbel and Hashinaga, 2005). Phenolic contents have correlated to their antioxidant activities (Butsat and Siriamornpun, 2016; Zielinski et al., 2014; Xu and Chang, 2008; Souri et al., 2008).

Study on the *Scorodocarpus borneensis* Becc. has been carried out by several researchers. (Sudrajat et al., 2016) observed that ethyl acetate extract of the Kulim's stem bark had a high antioxidant activity and expressed as IC_{50} valued about 55.224 ppm by 2,2-diphenyl-1-pycrylhidrazyl (DPPH) radicals assay. (Dewi and Mayasari, 2017) found that the extraction using 70% methanol toward the leaf of *S. borneesis* results in the highest phenolic substance and antioxidant activity.

The aims of this study were to understand the preservatives mechanism by determining the phenolic contents, antioxidant activities, and antimicrobial activities of the methanolic extracts obtained from the leaf, stem bark, and fruit of *Scrodocarpus borneesis* Becc.

MATERIALS AND METHODS

Materials

The primary materials of the study were the leaf, stem barks, and fruit of *Scrodocarpus borneesis* Becc. Those samples were collected from a local forest in Sanggau Regency, Kalimantan Barat Province, Indonesia (0°23'16.7"N and 110°43'24.8"E). DPPH (2,2-diphenyl-1-picrylhydrazyl), folin ciocalteou, and gallic acid were purchased from Sigma-Aldrich (MO, USA). Methanol was purchased from E-Merck. All other reagents were analytical grades.

The Methanolic Extract Preparation

All samples collected were shortage and cleaned before cutting into pieces and then dried at room temperature for about 14 days. Samples were milled and sieved with a sieve sized of 80 mesh. The powdered samples were kept at constant room temperature until the moisture content reached approximately 8-11%. The dried powder samples were macerated by 70% methanol in a glass container with a ratio of 10:1 at room temperature for 24 hours then filtered by using Whatman No. 1. The extraction was repeated three times. The combined filtrates were decolorized with cartridges (Sep-Pak C_{18}) and concentrated to dryness by a rotary evaporator. They were concentrated by an evaporation process under a reduced

pressure at 40 °C until viscous then immersed in liquid nitrogen until dry (Dewi, 2006). Extracts were stored in sealed glass bottles at -4 °C for further analysis.

Determination of Yield

The total yield of methanolic extracts was measured before stored and expressed in percentage yields. Those percentage yields of those three samples (leaf, stem bark, and fruit) were calculated by a fresh weight basis (Dewi, 2002).

Determination of Total Phenolic Content

The total phenolic content evaluation was performed by using Folin-Ciolcalteu reagent (Lister and Wilson, 2001) with a minor modification toward the methanolic extracts derived from leaf, stem bark, and fruit of *S. borneesis* Becc. The absorbance was measured at a wavelength of 765 nm. Further, the analysis was performed in triplicate for each extract. Gallic acid standard solution (0-400 µg/mL) was used to obtain a calibration curve. Total phenolic content was presented as a percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Determination of Antioxidant Activities

The antioxidant activities were examined by a DPPH radical scavenging activity that referred to Blois's method (Blois, 1958) with a minor modification. Various concentrations of each extract were pipetted into the DPPH solution by 50 µg/mL (volume 1:1) to obtained the calibration curve. After incubation for 30 minutes, the absorbance was read at a λ of 515 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Furthermore, methanol was used as a blank, DPPH solution 50 µg/mL as control and ascorbic acid as standard. Antioxidant activity of each extract was then determined by calculating the percentage of oxidation inhibitory capability through the reduction of DPPH absorbance (Bedawey et al., 2010). IC_{50} of DPPH scavenging activity of each extract can be calculated by using the calibration curve.

Antimicrobial Activity Analysis by Well Diffusion Methods

The antimicrobial was conducted on the samples with the highest antioxidant activities. It was initially started by microbial culture propagation and preparation (Brooks et al., 2005).

The microbial culture propagation and preparation was carried out by preparing three pure culture of microbes, i.e., *Methyl Resistant Staphylococcus aureus* (MRSA), *Salmonella typhii*, and *Candida albicans* acquired from the Institut Pertanian Bogor Culture Collection (IPBCC). MRSA and *S. typhii* were refined by a quadrant streak from one pure loop colony into each Nutrient Agar medium (Beef Extract 3 g, NaCl 5 g, Pepton 5 g, Aquades 1000 mL, Bacto Agar 18 g), and *C. albicans* to Potato Dextrose Agar (Potato 200 g, Dextrose 20 g, Aquades 100 mL, Bacto Agar 18 g). They were then incubated at room temperature for 24 hours. After, a starter culture of each microbial pathogen was made by inoculating one loop of MRSA and *S. typhii* into Mueller Hinton Broth medium (3 g beef extract, 8.75 g casein, 0.75 g starch, 1000 mL aquades, 18 g bacto agar) and *C. albicans* into Sabouraud Dextrose Yeast Broth + Chloramphenicol medium (10 g peptone, 40 g glucose, 2 g yeast extract, 200 ppm Chloramphenicol, 10000 mL aquades). Then, they were incubated in incubator shaker for 48 hours at room temperature, 200 rpm. Starter cultures that started to turn white and sour-smelling indicated a good microbial growth.

Antimicrobial activity analysis was conducted on the methanolic extract of the Kulim's leaf, which possessed the highest antioxidant activity. Antimicrobial was observed by well diffusion methods (Garriga et al., 1993). Initially, microbial culture was used on colonies density 10^7 - 10^8 CFU/mL. Furthermore, inoculation of 0.2% starter culture into 20 mL Mueler Hinton Agar (*MRSA* and *S. typhii*) and Sabouraud Dextrose Yeast Agar + Chloramphenicol *(C. albicans)* media, and the number of colonies on each plate was calculated, 10^5 - 10^6 CFU / mL. Then, pour the medium to sterilized Petri dishes to solidify and make a well hole (5 wells/Petri dishes) by using sterile Cock Borer with a diameter of 6 mm and a drop of 60 µL extract (0% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 40% w/v, and 50% w/v). Two other wells were used as positive controls for *MRSA* and *S. typhii* (Chloramphenicol 0.02 % w/v), *C. albicans* (Ketoconazole 0.02 % w/v) and negative controls (sterile Aquades). Petri dish was incubated at room temperature for 48 hours, and then the inhibition zone was measured based on the diameter of the clear zone (mm).

Antimicrobial Activity Analysis in Red Tilapia Fillets.

The antimicrobial against proteolytic bacteria of methanols extract derived from *S. borneesis* Becc leaf in Tilapia fillet was referred to (Raeisi et al., 2016). Equal to the previous observation over the microbial activity, the observation in this analysis was executed on the sample with the highest antioxidant activities. It was started by preparing the solution. The methanolic extract of the leaf was weighed according to the five required concentration level (0% w/v, 5% w/v, 10% w/v, 20% w/v, 30% w/v, 4% w/v, and 50% w/v). Then it was dissolved into 3 mL sterile distilled water with the addition of 2% emulsifier Tween 20 (0.06 g). Next, the extract solution was shaken with vortex for 2 minutes and homogeneous.

The observation then initialized by cleaning fresh Tilapia fish. Then, the fish filleted 5 g for each and soaked at five levels of methanolic extract that have been prepared for 15 minutes. Then, the fillet was drained from the soaking water and stored in a sterile plastic zipper at 15 °C.

The next step was the microbial calculation. Calculation of proteolytic bacteria was executed at different storages duration of fish fillets (0, 6, 12, 18, and 24 hours). At each level of the incubation time, 1 g of fillet was taken and mashed with a sterile mortar, which was given 9 mL of physiological water (0.85% NaCl). Then, they were crushed to shreds. Next, 1 mL was taken from the fillet suspension and put into a series of dilution tubes of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} contained physiological water. Dilution results were taken 1 mL of suspension then proteolytic bacteria were isolated by Pour Plate method by using skim milk agar with modification which was a mixture of 150 mL skim milk agar (3 g casein milk powder, 2% agar, 150 mL aquades) and 150 mL nutrient agar (0/3 g beef extract, 0.5 g Peptone, 0.5 g NaCl, 150 mL aquades, and 2% agar) (Zahiroh, 2013). Then, incubation for 24 - 48 hours at room temperature. The total proteolytic bacteria colonies calculated after incubation form clear zones on the tested media.

The inhibition capability of the extract was then identified by calculating the bacterial inhibition percentage, according to (Zahiroh, 2013). It was calculated through the following formula:

$$\text{Inhibition Percentage} = \frac{Total\ Colony\ of\ Control - Total\ Colony\ of\ Treatment}{Total\ Colony\ of\ Control} x\ 100\%$$

Data Analyzes

The data on the total phenolic contents were analyzed by the Analysis of Variance (ANOVA) and Tukey's test for comparison of means at a significance level of 5% by using SPSS for windows.

RESULT AND DISCUSSION

The Yield of Methanolic Extract of *Scorodocarpus borneensis* Becc.

The yield extracts of *S. borneesis* Becc plant were calculated as a percentage of the weight of dryness after evaporation toward a fresh basis (Dewi, 2002). The yield of a methanolic extract derived from leaf, stem barks, and fruit was 5.06 ± 0.12%, 8.84 ± 0.14%, and 12.60 ± 0.57%, respectively. All of the extracts contained phenolic compounds. However, the highest yield after extraction was the fruit, which was then followed by stem bark and leaf. Thus, it might be due to the side effect of drying, size reduction, and extraction. The extraction process followed the principles of like-dissolves-like (Chew et al., 2011). The solvent that used to extract the phenolic compound of the leaf, stem bark, and fruit of this Garlic Tree was 70 % methanol.

Total Phenolic Content

Total Phenolic contents of the methanolic extract obtained from leaf, stem bark, and fruit of *S. borneesis* Becc are displayed in Table 1.

Table 1. Total phenolic content of leaf, stem bark, and fruit of *Scorodocapus borneensis* Becc. at different concentrations

Parts of Plants	Concentrations (ppm)			
	100	200	300	400
Leaf	162.89 ± 3.3^{b}	174.35 ± 2.9^{c}	191.61 ± 2.3^{e}	252.77 ± 1.2^{i}
Stem Bark	165.76 ± 3.3^{b}	198.75 ± 3.3^{f}	211.46 ± 2.9^{g}	251.75 ± 4.0^{i}
Fruit	155.83 ± 3.1^{a}	187.79 ± 0.9^{d}	210.80 ± 1.6^{g}	238.48 ± 3.5^{h}

*Expressed as mg GAE/g of dry plant material. The data displayed as a mean ± standard deviation of five replications. Mean values followed by different superscript in a column are significantly different ($p<0.05$).

The results obtained showed that the total phenolic content varied from 155.83 ± 3.3 to 252.77 ± 4.0 mg GAE/g. The highest contents of the total phenolic were obtained in 400 ppm of the leaf then follow stem bark and fruit. Different parts of plant and concentration influenced the total phenolic content. This result confirmed a previous study on an aqueous *Nepeta nepetella* and methanolic extracts, which was conducted in Algeria (Seladji et al., 2014).

This essential compound might found in the plants. This phenolic substance correlated to several health impacts. It is contributed as an antioxidant against free radical inhibition and also lowering the glycemic response of the food (Fadly et al., 2020b).

Antioxidant Activities

The antioxidant activity of Garlic Tree could be attributed to their hydrogen donating ability (Dewi, 2006; Kuspradini et al., 2016). They suggest that antioxidant substances become fundamental in suppressing the free radicals both in the biological system and also foods. The potential antioxidant activity of different parts of this tree was observed by the inhibitory activity of the free radical DPPH. DPPH scavenging activity is one of the well-known methods in identifying the antioxidant activity. Antioxidant may work as scavengers against DPPH free radical by donating $H^{+,}$ so the DPPH become a stable diamagnetic molecule (Fadly et al., 2020a).

The DPPH radical-scavenging activities of all parts of the plant extract are illustrated in Table 2. The IC_{50} value of the DPPH test in various parts of the tree is presented in Table 2.

Table 2. Free radicals scavenge of a methanolic extract derived from leaf, stem bark, and the seed of *Scorodocarpus borneensis* Becc.

Sources	Concentrations (ppm)	Radical Scavenging Activity (%)	IC_{50} (ppm)
Leaf	15	37.21 ± 0.84	36.88
	30	53.55 ± 1.10	
	45	66.28 ± 0.86	
	60	75.79 ± 1.14	
	75	80.58 ± 0.82	
	90	86.47 ± 1.04	
Stem Bark	15	9.40 ± 0.31	52.45
	30	38.71 ± 1.80	
	45	43.47 ± 0.91	
	60	68.86 ± 1.04	
	75	61.05 ± 0.94	
	90	81.56 ± 0.96	
Seed	15	12.61 ± 0.92	86.20

	30	19.90± 1.04	
	45	22.12 ± 0.88	
	60	35.62 ± 0.96	
	75	40.49 ± 1.02	
	90	55.64 ± 0.64	

*The data displayed as mean ± standard deviation of five replications.

Table 2 shows that all methanolic extract from *S. borneensis* had antioxidant activities. The extracts from leaves possessed the highest DPPH radical inhibitory activity with an IC_{50} value of about 36.88 ppm. It was followed by a stem bark with an IC_{50} value of 52.45 ppm and fruit with an IC_{50} value of 86.20 ppm, respectively. This result supported previous research in *Moringa oleifera* methanolic extract in which the leaf extract has higher antioxidant activities than the seeds (Fitriana et al., 2018). Besides, a study works on ethanolic extracts obtained from the leaves and stems of *Dillenia suffruticosa* reveals that the leaves possessed more potent antioxidant activity than the stem also exceeds the vitamin C as a standard antioxidant (Masriani et al., 2020).

According to this study, the highest phenolics contents identified in the extract of Kulim's leaf that has antioxidant activity. The phenolics compound of the plants was recognized by the donation hydrogen atom exhibiting its essential key as a potential antioxidant (Proestos et al., 2006; Kähkönen et al., 1999).

Antimicrobial Activities by Well Diffusion Agar Test

Antimicrobial activities of methanolic extracts from the leaf of *S. borneensis* were observed by the well diffusion method indicated that the overall concentration used in this research might inhibit all microbial pathogens. This is indicated by the formation of clear zones around the well. The higher level of extract used was directly proportional to inhibition percentage of microbial cell growth, expressed as a larger inhibitory zone, which indicated that the compound applied has potential as an antimicrobial agent (Warsidah et al., 2020).

The test results evaluated the different antimicrobial abilities of plant extracts against MRSA, *Salmonella typii*, and *Candida albicans* in Table 3. Low concentration of extract by 5% revealed an inhibitory capability which was classified as a weak response because the percentage was lower than the positive control patent antibiotics used in this research, *MRSA* (115.33 ± 78%), *Salmonella typhii* (108.18 ± 25%), and *Candida albicans* (100.44 ± 73%). The results showed 20% and 30% extract concentrations could inhibit stable categories based on the increase of percentage inhibition exceeding 10% of positive controls (Table 3)

Table 3. Determination of antimicrobial agents of the methanolic extract obtained from the leaf of *Scorodocarpus borneensis* Becc

Concentration (%)	Inhibition (%)			Inhibition Responses
	MRSA	***Salmonella thypii***	***Candida albican***	
Control (+)	197.98 ± 19	201.46 ± 42	136.90 ± 27	-
Control (-)	0	0	0	-
5	115.33 ± 78	108.18 ± 25	100.44 ±73	Weak
10	143.53 ± 4	120.17 ± 40	129.16 ± 69	Weak
20	161.20 ± 13	238.59 ± 58	154.46 ± 32	Strong
30	210.63 ± 14	254.38 ± 30	164.58 ± 25	Strong
40	245.68 ± 38	279.38 ± 40	176.48 ± 41	Very Strong
50	287.93 ± 7	286.40 ± 19	204.46 ± 65	Very Strong

The analysis of the several variant concentration levels of methanolic extract from Kulim's leaf continued to increase up to 40%, and 50% can produce a very strong inhibitory response category because increasing the inhibition value over 25% from the inhibition of Chloramphenicol and Ketoconazole as positive controls.

The result of the inhibition percentage also showed that *S. borneensis* leaf contained a large spectrum of antimicrobial compounds. Regarding the inhibitory activities obtained, there were no significantly different from both analyses on various microbes, such as *Candida albicans* (unicellular fungi), *Methicillin-Resistant Staphylococcus aureus* (Gram-positive bacteria), and *Salmonella typhii* (Gram-negative bacteria). Nevertheless, the highest antimicrobial activity of methanolic extract from the leaf was seen in *Salmonella typhii.* That indicates *S. typhii was* more sensitive to Kulim's leaf methanolic extracts. It appears that the leaf possessed many bioactive compounds like phenol that is very effective in interfering lipopolysaccharide. It is the outermost membrane of the appendix of gram-negative bacteria such as *Salmonella typhii* and *Pseudomonas* spp (Lay and Hastowo, 1992).

The substantial finding regarding antibacterial ability, the methanol extract of *S. borneensis* leaf showed significant inhibition against *Methicillin-Resistant Staphylococcus aureus* as Gram-positive bacterium. MRSA bacterium is a pathogen that can inactivate the class of antibiotics Methicillin, which is a class of β-Lactam, as well as Penicillin. Those antibiotics have an essential role in interfering with PBPs crosslink glycopeptides in the formation of peptidoglycan (Gladwin and Tratler, 2003). However, this extract can be used to suppress in vitro growth and have an exceeded ability more than the antibiotic control inhibition. Results of the study revealed

that the structure of the chemical components of this Garlic Tree's leaf extract contained the highest number of novel compounds of the Methyl derivative and high amounts of methylthiomethyl disulfide (Kubota et al., 1994). It was inversely proportional to the MRSA, which is known to have the mecA gene complex. It plays a vital role in the binding of penicillin proteins, which should be able to defend the toxicity of the methyl compound found in *S. borneensis* Becc extracts. It indicates that there are many other compounds more than the methyl and methicillin groups which have not been identified as potential antibiotics. Research suggests that these other compounds indeed serve as antimicrobials yet do not belong to the methyl, ethane, chloroform, and ethanal groups. They are considered as the unknown compound of Kulim Tree, where most of the novel components have not been identified yet (Kubota et al., 1994).

Antibacterial against Proteolytic Bacteria in Red Tilapia Fillets

This analysis used to identify the inhibition capability of the methanolic extract obtained from the leaf of *S. borneensis* Becc against proteolytic bacteria growth. The observation showed different results depending on the concentration levels used. The increase of extract concentrations showed a higher ability to inhibit the proteolytic bacteria. The lowest concentration extract, i.e., 5%, was able to reduce the proteolytic bacterial population compared to the control. Meanwhile, the highest inhibitory percentage was obtained at the concentration level reached 50% (Table 4).

Table 4. Total of proteolytic bacterial colonies from red tilapia fillets treated with the methanolic extract obtained from the leaf of *Scorodocarpus borneensis* Becc.

Extract Concentrations (%)	Total of Bacterial Colony (CFU/mL)				
	0 Hour	6 Hour	12 Hour	18 Hour	24 Hour
Control	$1{,}14 \times 10^5$	$4{,}5 \times 10^6$	$1{,}617 \times 10^7$	$1{,}003 \times 10^7$	$2{,}2 \times 10^5$
5	5×10^4	$4{,}3 \times 10^6$	$7{,}3 \times 10^6$	$7{,}43 \times 10^6$	$1{,}8 \times 10^5$
10	$3{,}3 \times 10^4$	$3{,}8 \times 10^6$	$6{,}8 \times 10^6$	$5{,}5 \times 10^6$	1×10^5
20	1×10^4	$3{,}3 \times 10^6$	$5{,}127 \times 10^6$	$1{,}69 \times 10^6$	3×10^4
30	1×10^4	$8{,}1 \times 10^5$	$5{,}0 \times 10^6$	$1{,}673 \times 10^6$	$2{,}5 \times 10^4$
40	1×10^4	$5{,}9 \times 10^5$	$4{,}6 \times 10^6$	$1{,}47 \times 10^6$	7×10^4
50	1×10^3	$5{,}8 \times 10^5$	$1{,}857 \times 10^6$	$9{,}33 \times 10^5$	3×10^3

This study provides a new interesting finding of the potential of the extract derived from *S. borneensis*, especially regarding its effect on the leaf to suppress the population of proteolytic bacteria as food spoilage. As noted earlier, proteolytic bacteria are a class of microorganism which can produce extracellular protease, widely used in the fermentation process (Poza et al., 2001). This bacteria widely explores and develops as potential enzyme-producing microorganisms. However, this is different from the abundance of Proteolytic bacteria found in tilapia fillets. The Increased population of Proteolytic harms the nutritional quality and nutrition of stored fish fillets. Proteolytic bacteria are known as the main food spoilage in various livestock by-products such as milk and cheese, which have an impact on sour taste, even contain high levels of toxins (Mossel and De Bruin, 1957).

Proteins molecule can be decomposed by proteolytic bacteria to produce a simple protein structure, such as amino acids, intermediate compounds, and some toxins secreted into the environment. This research identified a correlation between the duration of storage time and the population of proteolytic bacteria. In the control treatment, the significant increase of bacteria population in the fillet was from 11.4 x 104 CFU / mL (0 hours) to 2.2 x 105 CFU/mL (24 hours). The increased bacterial population has a direct impact on the nutritional quality of the stored fish fillets, where more decomposed fish protein would be added.

Furthermore, this research revealed an interesting phenomenon in which an extract treatment with 0-hour storage had an inhibition effect of proteolytic growth. The control treatment with 0-hour storage had a 1.14×10^5 CFU/mL proteolytic population. However, when given 5% of methanol extract of the leaf, the population had a significant decline. It directly decreased when treated by 50% extract concentration at 0 hours storage of 1×10^3 CFU/mL bacteria. It phenomenon raises an assumption of bioactive compounds content in methanolic extract of Kulim's leaf has potential broad-spectrum bactericide (Kubota et al., 1994).

These results illustrated that methanol leaf extract contains many bacteriostatic bioactive compounds, which have been able to reduce some proteolytic contaminants in red tilapia fillets. Still, it has a potency to maintain the Proteolytic population more than the control treatment population. This antimicrobial activity can increase with the fractionation method to get pure materials and become a more stable inhibitory capability.

This research showed an interesting phenomenon regarding the treatment of several levels of methanol concentrations with various storage times. Each extract concentration used had different stability. Extract concentrations by 5%, 10%, 20% had an inhibitory effectiveness ability with a fluctuated trend line. They had a high inhibitory percentage at 0 hours, but decreased at 6 hours of storage time and rise again after 12 hours of storage time (Figure 1). This result was different from 30%, 40%, and 50% of extract concentrations, which have a more stable inhibition percentage ranging from 70-99%. Generally, the longer storage time of plant extract as a preservative can reduce antimicrobial activities. At 50% of

concentration, its inhibitory capability increases. *S. borneensis* leaf extract used in this study was a crude extract that combines many broad-spectrum compounds. This affected compound stability to inhibit proteolytic growth. A study about the phytochemical compound of this Garlic Tree extracts discovered 14 phytochemical compounds based on GC-MS analysis (Sudrajat et al., 2016). Fractionation of crude extracts into a single fraction was needed to increase the stability contact of active sites bioactive compounds with microbial targets that will be inhibited (Rao et al., 1998).

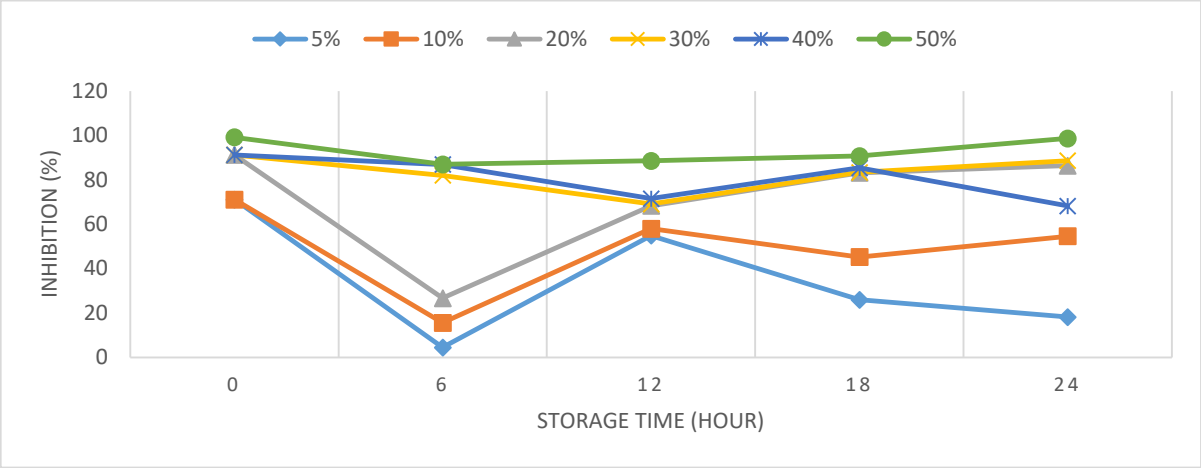

Figure 1. The effects of different lengths of storage of fish fillets with a methanolic extract from the leaf of *S. borneensis* on the inhibitory capacity against proteolytic bacteria

The potential of Kulim's leaf extract to inhibit the bacterial growth in the Well Diffusion Method and tilapia fillets describe important information. The higher inhibitory capability against the proteolytic population will give a quality impact of fish protein, which will not decrease due to a low decomposition by proteolytic groups (Mossel and De Bruin, 1957). In addition, besides serving as a fish fillet preservative, this *Scorodocarpus borneensis* Becc. plant extracts may also provide a distinctive flavor of garlic spices, which can increase the flavor quality of this fishery product.

CONCLUSION

All the parts of *Scorodocarpus borneensis* Becc. possessed phenolic antioxidant properties, but the methanolic extracts of the leaf were more effective than the other parts. The phenolic extract of the leaf also contributed to an antimicrobial agent and a natural preservative of tilapia fillets. The study suggests that the methanolic extract derived from the leaf exhibited great potential for antioxidant and antibacterial activities and might be useful for their nutritional and medicinal functions.

REFERENCES

1. Andersen, Ø.M., Markham, K.R. (Eds.), (2006). Flavonoids: chemistry, biochemistry, and applications. CRC, Taylor & Francis, Boca Raton, FL.
2. Bedawey, A.A.E., Mansour, E.H., Zaky, M.S., Hassan, A.A., (2010). Characteristics of Antioxidant Isolated from Some Plant Sources. Food Nutr. Sci. 1, 5–12. https://doi.org/10.4236/fns.2010.11002
3. Blois, M.S., (1958). Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181, 1199–1200. https://doi.org/10.1038/1811199a0
4. Brooks, G.F., Butel, J.S., Morse, S.A., (2005). Medical Microbiology. Mc GrawHill, New York. Mc GrawHill, New York.
5. Butsat, S., Siriamornpun, S., (2016). Effect of solvent types and extraction times on phenolic and flavonoid contents and antioxidant activity in leaf extracts of Amomum chinense C 23, 180–187.
6. Chew, K.K., Ng, S.Y., Thoo, Y.Y., Khoo, M.Z., Wan mustapha, W., Chun Wai, H., (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Centella asiatica extracts. Int. Food Res. J. 18, 571–578.
7. Dewi, Y.S.K., (2006). Identifikasi dan karakterisasi antioksidan dari jus aloe chinensis dan evaluasi potensi aloe emodin sebagai antifotooksidan dalam sistem asam linoleat. Pasca Sarjana UGM. Universitas Gajahmada, Yogyakarta. https://repository.ugm.ac.id/id/eprint/70673
8. Dewi, Y.S.K., (2002). Karakteristik mikroenkapsulasi Aloe chinensis hasil ekstraksi karbon aktif pada pengering semprot. Pengembangan produk lidah buaya. SOFTCODE, Singapore.
9. Dewi, Y.S.K., Mayasari, E., (2017). Potensi ekstrak daun Sindu (Scorodocarpus borneensis Becc.) sebagai antioksidan alami indogenous Borneo. Presented at the Seminar National Perhimpunan Ahli Teknologi Pangan Indoesia (PATPI), Universitas Lampung, Lampung, pp. 700–705.
10. Dewi, Y.S.K., Purwayantie, S., Mayasari, E., (2018). Bahan tambahan alami: perisa bawang putih dari Pohon Bawang Hutan (Scorodocarpus borneensis Becc.), First. ed. IAIN Pontianak Press, Pontianak.
11. Fadly, D., Kusharto, C.M., Kustiyah, L., Suptijah, P., Muttalib, Y.S., Bohari, (2020). In Vitro Study of Antioxidant Activity of Carboxymethyl Chitosan derived from Silkworm (Bombyx mori L.) Pupa against Human Plasma Lipid Peroxidation. Syst. Rev. Pharm. 11, 76–81. http://dx.doi.org/10.31838/srp.2020.7.13
12. Fadly, D., Purwayantie, S., Arundhana, A.I., (2020). Total Phenolic Content, Antioxidant Activity and Glycemic Values of Non-Meat Burger Patties. Canrea J. Food Technol. Nutr. Culin. J. 1–9. https://doi.org/10.20956/canrea.v3i1.246

13. Farhan, H., Rammal, H., Hijazi, A., Daher, A., Reda, M., Annan, H., Chokr, A., Bassal, A., Badran, B., Ghaloub, A.N., (2013). Chemical composition and antioxidant activity of a Lebanese plant Euphorbia macroclada schyzoceras. Asian Pac. J. Trop. Biomed. 3, 542–548. https://doi.org/10.1016/S2221-1691(13)60110-0
14. Fawole, O.A., Makunga, N.P., Opara, U.L., (2012). Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. BMC Complement. Altern. Med. 12, 200. https://doi.org/10.1186/1472-6882-12-200
15. Fitriana, W.D., Ersam, T., Shimizu, K., Fatmawati, S., (2018). Antioxidant Activity of Moringa oleifera Extracts. Indones. J. Chem. 16, 297–301. https://doi.org/10.22146/ijc.21145
16. Gladwin, M., Tratler, B., (2003). Clinically Microbiology, Third. ed. MedMaster Inc, Miami.
17. Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.-P., Pihlaja, K., Kujala, T.S., Heinonen, M., (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J. Agric. Food Chem. 47, 3954–3962. https://doi.org/10.1021/jf990146l
18. Kubota, K., Ohhira, S., Kobayashi, A., (1994). Identification and Antimicrobial Activity of the Volatile Flavor Constituents from Scorodocarpus borneensis Becc. Biosci. Biotechnol. Biochem. 58, 644–646. https://doi.org/10.1271/bbb.58.644
19. Kuspradini, H., Rosiarto, A.M., Putri, A.S., Kusuma, I.W., (2016). Antioxidant and toxicity properties of anthocyanin extracted from red flower of four tropical shrubs. Nusant. Biosci. 8. https://doi.org/10.13057/nusbiosci/n080201
20. Lay, B.W., Hastowo, S., (1992). Mikrobiologi. Rajawali Press, Jakarta.
21. Lim, H., Kubota, K., Kobayashi, A., Seki, T., Ariga, T., (1999). Inhibitory Effect of Sulfur-containing Compounds in Scorodocarpus borneensis Becc. on the Aggregation of Rabbit Platelets. Biosci. Biotechnol. Biochem. 63, 298–301. https://doi.org/10.1271/bbb.63.298
22. Lister, F., Wilson, P., (2001). Measurement of total phenolic and ABTS assay for antioxidant activity. Crop Research Institute Lincoln, New Zeland.
23. Masriani, Fadly, D., Bohari, B., (2020). α-Glucosidase Inhibitory Activity of Ethanol Extract Obtained from Dillenia suffruticosa and Pycnarrhena cauliflora. J. Glob. Pharma Technol. 12, 881–887.
24. Mokbel, M.S., Hashinaga, F., (2005). Antibacterial and Antioxidant Activities of Banana (Musa, AAA cv. Cavendish) Fruits Peel. Am. J. Biochem. Biotechnol. 1, 125–131. https://doi.org/10.3844/ajbbsp.2005.125.131
25. Mossel, D.A.A., De Bruin, A.S., (1957). The enumeration of proteolytic bacteria in foods. Antonie Van Leeuwenhoek 23, 218–224. https://doi.org/10.1007/BF02545873
26. Poza, M., de Miguel, T., Sieiro, C., Villa, T.G., (2001). Characterization of a broad pH range protease of Candida caseinolytica. J. Appl. Microbiol. 91, 916–921. https://doi.org/10.1046/j.1365-2672.2001.01458.x
27. Proestos, C., Boziaris, I.S., Nychas, G.-J.E., Komaitis, M., (2006). Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. Food Chem. 95, 664–671. https://doi.org/10.1016/j.foodchem.2005.01.049
28. Raeisi, S., Sharifi-Rad, M., Quek, S.Y., Shabanpour, B., Sharifi-Rad, J., (2016). Evaluation of antioxidant and antimicrobial effects of shallot (Allium ascalonicum L.) fruit and ajwain (Trachyspermum ammi (L.) Sprague) seed extracts in semi-fried coated rainbow trout (Oncorhynchus mykiss) fillets for shelf-life extension. LWT - Food Sci. Technol. 65, 112–121. https://doi.org/10.1016/j.lwt.2015.07.064
29. Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V., (1998). Molecular and Biotechnological Aspects of Microbial Proteases. Microbiol. Mol. Biol. Rev. 62, 597–635.
30. Seladji, M., Bekhechi, C., Beddou, F., Dib, H., Bendimerad, N., (2014). Antioxidant activity and phytochemical screening of Nepeta nepetella aqueous and methanolic extracts from Algeria. J. Appl. Pharm. Sci. 4, 12–16. https://doi.org/10.7324/JAPS.2014.40203
31. Souri, E., Amin, G., Farsam, H., Barazandeh, T.M., (2008). Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. DARU J. Pharm. Sci. 16, 83–87.
32. Sudrajat, S., Susanto, D., Sudiastuti, S., (2016). Phytochemicals Analysis, Antioxidant Capacities and Antimicrobial Properties of Ethyl Acetate Extract of Stem Bark of the Garlic Tree Scorodocarpus borneensis Becc. J. Agric. Sci. Technol. A 6. https://doi.org/10.17265/2161-6256/2016.06.005
33. Warsidah, Fadly, D., Bohari, (2020). Antibacterial and Anti-inflammatory Activities of Ethanol Extract Obtained from The Hooks of Uncaria tomentosa (Wild. Ex Schult) DC Originated Kalimantan, Indonesia. Syst. Rev. Pharm. 11, 65–70.
34. Xu, B.J., Chang, S.K.C., (2008). Total phenolic content and antioxidant properties of eclipse black beans (Phaseolus vulgaris L.) as affected by processing methods. J. Food Sci. 73, H19-27. https://doi.org/10.1111/j.1750-3841.2007.00625.x
35. Zahiroh, S., (2013). Fermentasi biji kopi menggunakan bakteri selulolitik, xilanolitik, dan proteolitik asal Luwak. Institut Pertanian Bogor, Bogor.
36. Zielinski, A.A.F., Haminiuk, C.W.I., Alberti, A., Nogueira, A., Demiate, I.M., Granato, D., (2014). A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. Food Res. Int., Authenticity, Typicality, Traceability and Intrinsic Quality of Food Products 60, 246–254. https://doi.org/10.1016/j.foodres.2013.09.010