Proximate, Amino Acid Composition and Antibacterial Activity of Woton (*Sterculia sp.*) Leaves from Raja Ampat, West Papua, Indonesia

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ABSTRACT

Woton (*Sterculia sp*.) plant is an endemic plant of Papua and West Papua, Indonesia that has many benefits for the community. This research aims to examine the contents of proximate, aminoacid, and antibacterial activity from the leaves extract of two plants namely *Sterculia shilinglawii* and *Sterculia tragacantha*. The sample of *Sterculia sp*. leaves was cleaned, dried, and milled. Then, its chemical composition, amino acid, and bacterial activity were analyzed using a standard procedure. The chemical composition was analyzed using standard AOAC, the amino acid was examined using LCMS, and antibacterial activity was analyzed using a barrier zone against the bacteria that attack the fish. The research results showed that the chemical compositions of *Sterculia sp.* leaves are carbohydrates, protein, water, ash, and fat. The main amino acid contents of woton (*Sterculia sp.*) leaves are L-Arginine, L-Valine, L-Proline, L-Alanine. Bacterial Test Results showed that woton (*Sterculia sp.*) leaves extract has intermediate inhibition response against *V. algynolyticus*. It has been concluded that *Sterculia sp*. leaves have the potential chemical content.

Keywords: woton, *sterculia*, proximate, amino acid, antibacterial

INTRODUCTION

The development and sustainability of fish farming activities often face obstacles. One of them is when there is a disease attack either infection or non-infection. The attack of pathogens either viruses, bacteria, fungi, protozoa or parasites is a class of infectious diseases, while non-infectious diseases include the diseases that are caused by the environment, feed, genetics and tumors (Aryani, Henny, Iesje, & Morina, 2004). Disease and parasite infection can happen through several mechanisms such as direct contact between diseased fish and healthy fish, a carcass of diseased fish or through the water. The infection usually happens in one pool of cultivation. Other infection mechanisms are through tools and the transfer of fish from outbreak area to non-outbreak area (Sunarto, 2005). Bacteria that commonly attack fish farming are *Aeromonas hydrophila*, *Vibrio harveyi*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* (Nagawasa and Lecierda, 2004). The level of virulence of *A. hydrophila* that can cause death for the fish depending on the poison it creates. Gen Aero and hlyA are responsible for producing the aerolysin and hemolysin poisons on Aeromonas genus (Yousr, Napis, Rusul, & Son, 2007). Therefore, there should be an alternative solution to prevent or obstruct the pathogen bacteria.

Human and animal including fish can synthesize a number of essential amino acid. Hence, they need to acquire it from their diet. The essential amino acid is usually provided from the utilization oflivestock food (including meat, egg, and milk) as well as from various plants (especially cereal and nuts) that are provided together with optimal content of essential amino acid. Woton (*Sterculia sp*.) in Indonesia is spread in the Papua and West Papua area. The spreading of woton plant (*Sterculia sp*.) in West Papua is mainly located in Gag Island, Raja Ampat District that was usually used as additional food stuffs by the local community (Lekitoo et al., 2012). Woton (*Sterculia sp*.) leaves contain secondary metabolite compounds such as; flavonoids, tannins, tannins, phenolic, polyphenols (Sayuti, Supriatna, Hismayasari, Budiadnyani, & Yani, 2017). Phenolic and polyphenols are one of the biggest secondary metabolite groups that have shown antimicrobial activity, important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Bobbarala, 2012). Based on the secondary metabolite content of woton plant can be used as one of the alternatives in preventing or obstructing the pathogen bacteria in the fish farming activity.

This research is carried out to evaluate the contents of proximate content, amino acid, and activities of antibacterial from the leaves extract of *Sterculia shilinglawii* and *Sterculia tragacantha* plants.

MATERIALS AND METHODS

Sampling

Samples in this research were *S. shillinglawi* and *S. tragacantha* Lindl leaves. The leaf samples were taken from Gag Island, Raja Ampat, West Papua, Indonesia on July 2017. The leaves samples were sent to Genetika Science Indonesia and tested its taxonomy. The sample was then cleaned and dried naturally by aerating it for 7 days. The dried sample was smoothed/milledusing a machine, and it was then filtered using a mesh 65. After that, the sample was stored for further testing.

Proximate Analysis

Proximate analysis was carried out in Food Quality and Safety Testing Laboratory, Brawijaya University, Indonesia. Chemical analysis to describe the percentage of crude protein, water, ash, fat, and carbohydrates was carried using AOAC standard method (AOAC, 1999). All of the analysis was carried out in three repetitions.

Extraction

In this research, a methanol solvent wasused for the extraction. The extraction method used in this research is maceration extraction. The extraction was carried out with the ratio between sample and solvent 1: 3 for 48 hours at the room temperature (Sayuti, Putri, & Yunianta, 2016). The results of extraction were then filtered using filter paper with a vacuum filter. The filtrate generated from the filtration was evaporated at 40°C to get solid extract to be collected and stored at 0°C for further testing.

Amino Acid Test with LC-MS

Sample preparation: weighting the sample of ± 2.5 gram, putting it in the test tube test tube with screw of 50 mL, adding HCl 6N as many as 20 mL, hydrolyze in the autoclave at the temperature of 110°C for 12 hours, neutralize using NaOH 6N, adding up to 50 mL, filtering using filter of 0.22 µM, diluting for 10 times, injecting it to LC-MS 2 µL.

Mobile phase condition: (A) : 0.1% Pentadecafluorooctanoic Acid (PDFOA) 99.5% : 0.5% Water/CH3CN with 0.1% Formic Acid; (B): 0.1% PDFOA, 10% : 90% Water/CH3CN with 0,1% Formic Acid; Flow : 0.6 mL/min, Injection Volume : 2 uL, Capillery = 3,5 kV; Desolvation Temp = 500°C; Disolvation = 1000L/Hr; Collision Energy V = 15 V.

Antibacterial Activity testing:

a. Organisms and culture medium

Pathogenic bacterial strains were obtained from the Jepara BBPBAP laboratory. The bacteria used in this research is the one that attacks fish such as *Aeromonas* *hydrophila* bacteria that causes an ulcer to freshwater fish, *Vibrio harveyi* that causes diseases to shrimp, *Vibrio alginolyticus* that causes an ulcer to seawater fish especially grouper and *Vibrio parahaemolyticus* that causes white feces diseases and EMS in shrimp. Nutrient agar was entered into a petri dish that has been previously sterilized. Then, it was set a side for 5 minutes. Pure bacteria were implanted into the agar media and set aside for 15 minutes to be incubated for 24 hours.

b. The antibacterial activity of woton(*Sterculia sp.*)leaves extract

Each of extract leaves was entered into bacteria media with a concentration of 12.5%; 25%; 100% and then *enrofloxacin* with the concentration of 5µ added as the control. Checking the barrier zone was carried out for 24 hours by measuring the diameter of the formed clear zone.

RESULTS AND DISCUSSION

Proximate Test Results of Woton (*Sterculia sp.*) Leaves

Proximate analysis on dried woton (*Sterculia sp.)* leaves was carried out to know the outline of the number of nutrition such as water, ash, protein, carbohydrates, and fats in these leaves. Proximate Test Results of woton (*Sterculia sp.*) leaves presented in Table 1.

Table 1.Proximate Composition of Dried Woton (*Sterculia sp.*) Leaves

Nutrition	Leaves Content (Averagely±SD (%))	
	S. shillinglawii	*S.tragacantha*
Protein (%)	10.12±0.08	11.04±0.20
Fat (%)	3.14±0.35	3.09±0.05
Water (%)	8.25±0.09	7.91±0.06
Ash (%)	6.18±0.01	6.23±0.02
Carbohydrate (%)	72.50±0.44	71.75±0.31

The values are mean±standard deviation of triplicate determination expressed in dry weight basis

Based on the result of the proximate analysis, it was seen that the biggest component in the *S. shillinglawii* and *S.tragacantha* leaves is carbohydrate amounted to 72.5% and 71.75% respectively (Table 1). The second biggest component is protein. The protein content in *S. shillinglawii* and *S.tragacantha* leaves are 10.12% and 11.04% respectively. The lowest component from *S. shillinglawii* and *S.tragacantha* leaves is fat. Ash content is the parameter to show the value of mineral content (inorganic material) in the substance or product. The contents of inorganic material in a substance are calcium, potassium, phosphorus, iron, magnesium, and others. The ash content of *S. shillinglawii* and *S.tragacantha* leaves are 6.18% and 6.23% respectively. The fat content of *S. shillinglawii* and *S.tragacantha* leaves are 3.14% and 3.09%. It is lower compared to bay leaves (3.96%) used as antidiabetic drugs (Safithri, Fahma, & Marlina, 2012). *S. shillinglawii* leaves have a contents of 4.8% protein, 0.5% fat, 64.32% water, 2.10% ash, 28.29% carbohydrate, and 81.79 mg/100 g vitamin C, while *Sterculia tragacantha* leaves have a contents of 4.45% protein,8.86% fat, 50.59% water, 1.4% ash, 34.22% carbohydrate, and17.36 mg/100 g (wet base) vitamin C (Sayuti et al., 2017).

Amino Acid Content of WotonLeaves (*Sterculia sp.*)

LC-MS is used to examine the content of amino acid of woton leaves extract. The results of the LC-MS test for woton leaves presented in Table 2.

Table 2. Amino Acid of Woton Leaves (*Sterculia sp.*)

No	**Compound**	**Result (µg/gram)**	
		S. shillinglawii Leaves	*S.tragacantha* Leaves
1	L-Arginine	100.57	80.77
2	L-Valine	97.77	51.66
3	L-Proline	50.44	47.19
4	L-Alanine	42.04	20.70
5	L-Lycine	26.96	20.26
6	L-Aspartic acid	25.78	17.75
7	L-Glutamic acid	18.07	15.55
8	L-Threonine	14.34	14.68
9	L-Leucine	23.53	13.36
10	L-Isoleucine	22.76	12.48
11	L-Phenylalanine	14.22	8.23
12	L-Tyrosine	18.99	7.97
13	L-Serine	6.71	4.38
14	L-Glycine	6.47	4.19
15	L-Cysteine	0.01	3.52
16	L-Histidine	40.15	3.10
17	L-Methionine	0.07	0.00

The main contents of amino acid of woton (*S. Shillinglawii)*) leaves are L-Arginine, L-Valine, L-Proline, L-Alanine, L-Histidine, L-Lycine, L-Aspartic acid, L-Leucine, L-Isoleucine, L-Tyrosine, L-Glutamic acid, L-Threonine, L-Phenylalanine. While the main contents of amino acid of papaya (*S.tragacantha*) leaves are L-Arginine, L-Valine, L-Proline, L-Alanine, L-Lycine, L-Aspartic acid, L-Glutamic acid,L-Threonine, L-Leucine, L-Isoleucine. The main contents of amino acid of the woton papaya (*S. tragacantha*) skin are L-Arginine, L-Proline, L-Glutamic acid,L-Lycine, L-Valine, L-Alanine, L-Aspartic acid, L-Threonine, L-Serine.

The main contents of amino acid in *S. setigera* are aspartic acid, leucine, glutamic acid, and proline. Meanwhile, the main contents of amino acid in *S. urens* are aspartic acid, valine, leucine, proline, and serine. Furthermore, the main contents of amino acid in *S.villosa*are aspartic acid, valine, proline, glutamic acid and glycine (Anderson, Howlett, & McNab, 1985). The main contents of fat flour amino acid in cotyledon *S. urens* are glutamic acid, arginine, and aspartic acid and cysteine, ethionine, tyrosine, and histidine are observed in small amount (Galla, Pamidighantam, & Akula, 2012).

L-Arginine is effective in improving endotheliosis glomerulus (Sulistyowati, Budiarta, & Soetrisno, 2017), it can heal the damage in endothelial cells of mouse (*Musmusculus*) (Riyadi, 2017), giving effect to hepatocyte cell damage in mouse (*Musmusculus*) (Nekso, 2017), can treat damage to the Spiral Artery in the mouse (*Musmusculus*) preeclampsia model (Wibowo, 2017). Thus, the metabolism of arginine cell immunity is fundamentally involved in cancer, inflammation, infection, fibrosis, pregnancy, and immune regulation in general (Kropf et al., 2007), (Müller, Munder, Kropf, & Hänsch, 2009), (Gabrilovich & Nagaraj, 2009), (Munder, 2009), (Raber, Ochoa, & Rodrı, 2012), (Noy & Pollard, 2014).

Antibacterial Activity of Woton Leaves Extract (*Sterculia sp.*)

Inhibition activity test of woton (*Sterculia sp.*) leaves extract against the pathogen bacteria namely on the *Aeromonas hydrophila*, *Vibrio harveyi*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* bacteria presented in Table 3.

Table 3. Test Results of the Antibacterial of Woton Leaves Extract (*Sterculia sp.*)

Bacteria Type	Material concentration (%)	*S. shillinglawii* Leaves		*S.tragacantha* Leaves	
		Barrier Zone (mm)	Response Inhibition	Barrier Zone (mm)	Response Inhibition
Vibrio alginolyticus	12.5	11	Intermediate	20	Intermediate
	25	7	Resistant	22	Intermediate
	100	14	Intermediate	25	Intermediate
	K (+)	12	Sensitive	11.5	Sensitive
Vibrio harveyi	12.5	0	Resistant	0	Resistant
	25	0	Resistant	0	Resistant
	100	7	Resistant	0	Resistant
	K (+)	19	Sensitive	17.5	Sensitive
Vibrio parahaemolyticus	12.5	0	Resistant	0	Resistant
	25	0	Resistant	0	Resistant
	100	7	Resistant	0	Resistant
	K (+)	15	Sensitive	15	Sensitive
Aeromonas hydrophila	12.5	0	Resistant	0	Resistant
	25	0	Resistant	0	Resistant
	100	8	Resistant	0	Resistant
	K (+)	23	Sensitive	25	Sensitive

Table 3 shows the test results of the antibacterial of *S. shillinglawii* leaf extract against *Vibrio alginolyticus* with intermediate response inhibition at the concentration of 12.5% and 100%. *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila* bacteria are unaffected. Table 3 also shows the test results of antibacterial of *S. tragacantha* leaf extract against *Vibrio alginolyticus* with intermediate response inhibition at the concentration of 12.5%, 25%, and 100%. Meanwhile, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Aeromonas hydrophila* bacteria are unaffected. Ammonia in the water causes depression to the immune response and improvement in the mortality of *L. vannamei* from the *V. alginolyticus* infection (Liu & Chen, 2004). Some *V. alginolyticus* strains are closely related to the toxicity of puffer, and possibly from other species (Noguchi et al., 1987). Protease 34 kDa plays an important role in the pathology of vibriosis (Lee, Yu, Chen, Yang, & Liu, 1996).

CONCLUSION

The biggest proximate compositions of woton (*Sterculia sp.*) leaves in dry condition were carbohydrate, protein, water, ash, and fat. The main amino acid contents of woton leaves (*Sterculia sp.*) are L-Arginine, L-Valine, L-Proline, L-Alanine. Bacterial Test Results showed that woton leaves extract (*Sterculia sp.*) has inhibition response against *V. alginolyticus* at the intermediate level.

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