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NUTRITIONAL COMPOSITION AND SECONDARY METABOLITES OF WOTON LEAVES (STERCULIA SP.): ALTERNATIVE RAW MATERIAL FOR FISH FEED

Sayuti Mohammad*, **Supriatna Iman**, **Hismayasari Intanurfemi B.**, **Budiadyani I Gusti Ayu**, **Yani Ahmad**
Marine and Fishery Polytechnics Sorong, Indonesia
*E-mail: mohsayut@gmail.com

ABSTRACT
The woton leaves (*Sterculia sp.*) were collected from an island called Gag, Raja Ampat, West Papua, Indonesia in July, 2017. The objective of this study was to explore natural resources from Papua as an alternative raw materials for fish feed production. The leaves were cleaned, dried, grinded and finally analyzed to describe their chemical composition, vitamin C content and anti-nutritional content using the standard procedures. The findings showed that there were two types of woton plants (*Sterculia sp.*), namely *Sterculia sp*. and *Sterculia trigacantha*. The proximate composition for *Sterculia sp*. leaves was 4.8% protein, 0.5% fat, 64.32% water, 2.10% ash, 28.29% carbohydrate, and 81.79 mg/100 g vitamin C. The proximate composition for the *Sterculia trigacantha* leaves was 4.45% protein, 8.86% fat, 50.59% water, 1.4% ash, 34.22% carbohydrate, and 17.36 mg/100 g vitamin C. The woton leaves (*Sterculia sp.*) also contained secondary anti-nutrients/metabolites such as flavonoids, tannins, tannin errors, phenolic, and polyphenols. TLC Spectrophotometricometry test results showed that *Sterculia sp.* leaves contained hyperoside and routine flavonoids, whereas *Sterculia trigacantha* leaves contained compounds of quarcetin and routine flavonoids. The results of chemical and phytochemical screening tests showed that the leaves (*Sterculia sp.*) are potential and affordable raw material for fish feed production.

KEY WORDS
Sterculia, proximate, anti-nutritive, flavonoids.

Fish is the most affordable source of animal protein (Allison, 2001). Fish people consume is either caught by fishermen or obtained from fish farming (cultivation). Fish cultivation is the fastest food production in the world (Kureshy, et.al, 2000). Qualified fish feed is pivotal element in successful fish farming. Artificial feed is frequently used in fish farming business, especially intensive cultivation aiming to accelerate fish growth (Afriyanto and Evi, 2005). Qualified fish feed consists of qualified ingredients. Quality of fish food depends on how much nutrients such as proteins, fats, carbohydrates, vitamins and minerals it contains (Gopalan et al., 2004). Fish farming finds it difficult to get artificial fish feed since it is relatively expensive, even reaching 60-70% of total cost for production. Generally, price of fish feed on the market is relatively expensive (Nasution, 2006). Home-made fish feed produced using simple technique and inexpensive raw materials may become an alternative to overcome the problem. Raw materials used for fish feed should have good nutritional value, accessible, be easy to process, contain nutrients needed by fish, and be affordable (Nasution, 2006). One example of a potential plant for fish feed ingredient is woton (*Sterculia sp.*) leaves or peel. In Indonesia, woton (*Sterculia sp.*) can be found in Papua and West Papua. In West Papua, woton (Sterculia sp.) grows in Gag Island, Raja Ampat and people living in the island use the plant as alternative food (Lekitoo, et.al, 2012). Therefore, an analysis is needed to describe chemical composition, vitamins and secondary metabolites of woton (*Sterculia sp.*) because of its potential as raw ingredient for fish feed.

METHODS OF RESEARCH

Sample Collection. Woton leaves (*Sterculia sp*) were collected in July, 2017 from Gag Island, Raja Ampat, West Papua, Indonesia. The leaves were dried naturally for 4 days and

grinded using electric mill. Sample was filtered using size 40 mesh sieves. It was stored inside a plastic bag in room temperature for further analysis.

The Proximate Analysis. The proximate and vitamin C analysis were carried out in the Testing Laboratory of Food Quality and Food Safety, Brawijaya University, Indonesia. The chemical analysis describing percentage of crude protein, water, ash, fat and carbohydrate were carried out using methods described by AOAC (1999). All determinations were done in triplicates. Vitamin C was also analyzed according to the Methods of Vitamin Assay (FEFANA, 2006).

The Phytochemical Analysis. The respective anti-nutritive factors such as tannins, phenol, polyphenols and saponins were evaluated according to the standardized chemicals procedures (Harbornes, 1984). This analysis was carried out in *UPT. Materia Medica Batu*, located in Batu, East Java.

Flavonoid Test with TLC Spectrophotometerometry. Two 10x10 cm plates were washed and activated. The initial bottle was 10 mm from the left edge and 10 mm from the bottom of the plate, the band width was 3 mm, and the distance between the bottles was 6 mm. All stains were bottled on 2 separate plates. The first plate was eluted with a TE system motion phase and the second plate with a TF system motion phase. The chamber was saturated for 30 minutes before elution. The elution was carried out until 8 centimeters, and then the plates were dried in the oven at 60°C for 10 minutes. The dried plates were examined using TLC-Scanner 3 (Camag-Mutenz-Switzerland) spectrophotometer at a 210 nm wavelength. The spectra of each peak were read between 190 and 400 nm wavelength and tested for their spectral purity. Rf: 0.85-0.90 (Quercetin); Rf: 0.60-0.65 (Quercitrin); Rf: 0.45-0.50 (Hyperoside); Rf: 0.25-0.30 (Routine).

RESULTS AND DISCUSSION

Results of DNA testing conducted in Genetics Laboratory using Genomic DNA extraction methods using ZR Plants and Seed DNA MiniPrep ™ Kit (Zymo Research), PCR amplification using KOD FX Neo (Toyobo), PCR products purification with Zymoclean ™ Gel DNA Recovery Kit (Zymo Research), and Bi-directional Sequencing showed that there were 2 types of woton (*Sterculia sp.*) in Gag Island, Raja Ampat, *Sterculia tragacantha* and *Sterculia sp*.

Proximate Test Result of Woton (Sterculia sp.) Leaves. Table 1 described the result of the proximate composition test towards woton leaves (*Sterculia sp.*) The composition of woton (*Sterculia sp.*) leaves was not much different from that of woton (Sterculia sp.) fruits. It consisted of 4.48% protein, 8.27% fat, 70.01% water content and 2.31% ash content 2.31%. Vitamin C the leaves contained was 178.94%, far different from vitamin C the fruit (*Sterculia sp.*) (Lekitoo, et.al, 2012).

Table 1 – Proximate composition of Woton (*Sterculia sp*) Leaves

Nutrients	Mean composition ± SD (%)	
	Sterculia sp	*Sterculia tragacantha*
Protein content	4.80±0.21	4.45±0.20
Fat content	0.50±0.01	8.86±0.25
Water content	64.32±0.12	50.59±0.16
Ash content	2.10±0.12	1.40±0.05
Carbohydrate	28.29±0.02	34.22±0.33
Vitamin C (mg/ 100 g)	81.79±0.31	17.36±2.66

The scores were mean ± standard deviation of triplicate determination expressed in wet weight basis.

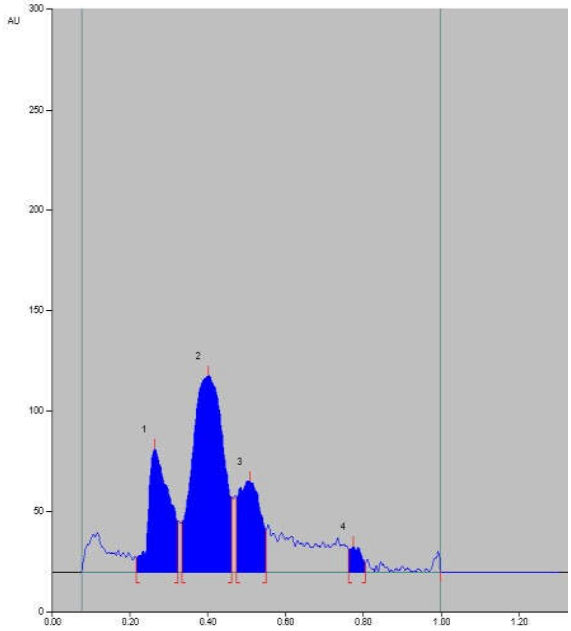
Photochemical Screening Test Result of Woton (Sterculia sp.) Leaves. Result of phytochemical analysis showed that some anti-nutritive factors such as flavonoids, tannins, tannins error, phenolic, polyphenol were detected (Table 2). Based on the phytochemical content, woton (*Sterculia sp.*) was potential ingredient for fish feed production. Flavonoid may function as antioxidants, antibacterials, immunomitators, and anti-inflammatory (Milddlenton, et. Al, 2000) and saponin compounds served as membrane permeabilising and

could affect growth and increase fish feeding response (Das, et.al., 2012). In addition, the findings of research on the increase of carp weights after the addition of green diet indicated that vitamin C and flavonoid content helped increasing the fish growth (Sulhi, et.al, 2011).

Table 2 – Phytochemical Content Test Result of Sterculia sp. Leaf

No.	Phytochemical Test	Type of Woton Leaf		Standard
		Sterculia sp	*Sterculia tragacantha*	
1	Flavonoid	+	+	Red/ pink was developed
2	Terpenoid	+	-	Greenish blue was developed
3	Steroid	-	-	Orange or brownish orange was developed
4	Alkaloid Meyer	-	-	White sediment was developed
5	Alkaloid dragendroff	-	-	Orange sediment was developed
6	Tanin	+	+	Blackish brown, blackish blue, and blackish green were developed
7	Tanin error	+	+	Blackish brown, blackish blue, and blackish green were developed
8	Tanin Catechol	-	-	Red sediment was developed
9	Phenolic	+	+	Blackish brown, blackish blue, and blackish green were developed
10	Polyphenols	+	+	Blackish brown, blackish blue, and blackish green were developed
11	Saponen	+	-	Permanent foam was developed

- absent; + present.



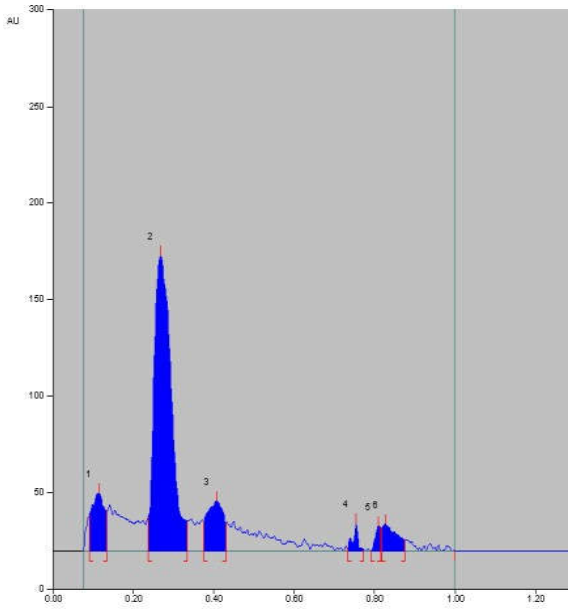
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height
1	0.22 Rf	7.4 AU	0.26 Rf	61.4 AU	28.21 %	0.32 Rf	25.4 AU
2	0.34 Rf	25.4 AU	0.40 Rf	97.9 AU	45.01 %	0.46 Rf	37.7 AU
3	0.48 Rf	37.2 AU	0.51 Rf	45.3 AU	20.84 %	0.55 Rf	20.8 AU
4	0.76 Rf	11.5 AU	0.78 Rf	12.9 AU	5.94 %	0.81 Rf	4.7 AU

Figure 1 – TLC Flavonoid Test Result on *Sterculia sp* Leaves

Several polyphenol compounds also had anti-hypertension activities. Some previous studies also showed that flavanoids and tannin, generally found in fruits, vegetables and beverage, were able to inhibit nicotinamida adenine dinucleotida phosphat (NADPH) oxidation by inhibiting ACE, increasing eNOS-specific, as well as changing cyclooxygenase-2 (COX-2) (Baradaran, et.al., 2014; Sulastri and Liputo, 2011; Kizhakekuttu and Widlansky, 2010; Beg, et.al., 2011; Sharifi, et. al., 2013; Mensah, et.al., 2010). Flavonoids and tannin inhibited ACE activities, which is vital in arterial blood pressure regulation. Phenol compound activities were the result of a number of hydroxyl groups in benzene rings. Docking studies

showed that phenolic and flavanoid acid inhibited ACE through interaction with zinc ions and the interaction was stabilized using another interaction with amino acid in the active side (Guerrero, et.al., 2012).

Flavonoid Test Using TLC Spectrophotodensitometer. Figure 1 described the result of flavanoid test using TLC spectrophotodensitometer for *Sterculia sp*. leaves and Figure 2 describe the result of the same test for *Sterculia trigacantha*.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position
1	0.09 Rf	18.2 AU	0.12 Rf	30.3 AU	12.06 %	0.14 Rf
2	0.24 Rf	17.1 AU	0.27 Rf	153.1 AU	60.98 %	0.33 Rf
3	0.38 Rf	15.3 AU	0.41 Rf	25.8 AU	10.29 %	0.43 Rf
4	0.73 Rf	1.4 AU	0.76 Rf	14.7 AU	5.87 %	0.77 Rf
5	0.79 Rf	0.0 AU	0.81 Rf	13.2 AU	5.28 %	0.82 Rf
6	0.82 Rf	11.9 AU	0.83 Rf	13.9 AU	5.52 %	0.88 Rf

Figure 2 – TLC Flavonoid Test Result on *Sterculia trigaganta* Leaves

Based on Figure 1, the TLC result showed that *Sterculia* sp. leaves contained quarcetin and routine flavanoids.

CONCLUSION

There are two types of woton (*Sterculia sp*.) grown in Gag Island, Raja Ampat, namely *Sterculia sp.* and *Sterculia trigacantha.* The proximate composition of *Sterculia sp.* leaves is 4.8 % protein, 0.5 % fat, 64.32 % water, 2.10 % ash, 28.29% carbohydrate, and 81.79 mg/ 100 g vitamin C. The proximate composition of *Sterculia trigacntha.* leaves is 4.45% protein, 8.86% fat, 50.59% water, 1.4% ash, 34.22% carbohydrate, and 17.36mg/100g vitamin C. *Sterculia sp* leaves contain anti-nutrient /secondary metabolite compounds such as flavonoids, terpenoid, tannin, tannin error, phenolic, polyphenols and saponin while *Sterculia trigacantha* leaves contain anti-nutrient /secondary metabolite compounds such as flavonoids, tannin, tannin error, phenolic and polyphenols. TLC test results showed that *Sterculia* sp. leaves contain hyperocide and routine flavanoids while *Sterculia trigacantha* leaves contain quarcetin and routine flavanoids.

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