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NUTRIENT COMPOSITION AND SECONDARY METABOLITE OF KEBAR'S GRASS (BIOPHYTUM PETERSIANUM KLOTZSCH)

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ABSTRACT
Kebar's grass *(Biophytum petersianum* Klotzsch*)* was collected from Central and East Kebar region, Manokwari, West Papua, Indonesia in April 2018. The objective of this research was to determine secondary metabolite content in Kebar's grass *(B. petersianum,* Klotzsch*)*. Kebar's grass sample was cleaned, dried, ground, and analyzed using a procedural standard to get chemical composition, vitamin C content, antioxidant activity, and anti-nutrient content. Based on the research result, the average score of proximate composition for Kebar's grass *(B. petersianium* Klotzsch*)* is 5.99% protein, 3.47% fat, 7.81% water, 14.25% ash, 68.47% carbohydrate, 418.20 mg/100g vitamin C, and 0.88 mg/ml antioxidant activity. Kebar's grass *(B. petersianium* Klotzsch*)* also contains anti-nutrient compounds/secondary metabolites; flavonoids, tannin, error tannin, phenolic, polyphenol. The test result of TLC-Spectrophotodensitometry shows that Kebar's grass *(B. petersianum* Klotzsch*)* contains rutin flavonoid, hyperoside, quercitrin, quercetin, and positively contains phenol and triterpenoid. This research reaches a conclusion that Kebar's grass *(B. petersianium* Klotzsch*)* contains essential secondary metabolite compounds for a human.

KEY WORDS
Kebar, biophytum, proximate, antinutrient, phenol, flavonoid.

Medicinal herbs can be processed into beneficial herbal remedies to boost body endurance, disease prevention, cure of disease, health recovery and also served as uterine fertilizers (Soedibyo, 1992). Kebar's grass (*Biophtyum petersianum* Klotzsch) is one of medicinal herbs types that grow wildly. Visually, the colour of Kebar's grass from Papua is light green while the one from Java is dark green but both of them have a yellow flower (Sembiring and Darwati, 2014). Kebar's grass known to have a potential for anti-bacterial, hypoglycemia, immunomodulator, chemoprotective, hypocholesterolemia, apoptosis, anti-inflammation, antitumor, and prostaglandin biosynthesis (Natarajan, Shivakumar and Srinivasan, 2010), impacts on the COX-2 expression (Guruvayoorappan and Kuttan, 2008), stimulates body immune system cells (Inngjerdingen *et al.*, 2008), and influences apoptosis cells B16F-10 and inhibits NO and cytokine production playing role in the formation of tumor (Guruvayoorappan and Kuttan, 2007).

Kebar's grass *(B. petersianum* Klotzch*)*, as an endemic plant, has been observed to have various benefits, among others, as anti-inflammatory, immunostimulant, and to be able to boost animal's reproduction performance (Grønhaug *et al.*, 2008). In Africa, this plant is utilized as a medicine for sting and snake bite, as well as for stomachache drug (Inngjerdingen *et al.*, 2004), (Inngjerdingen *et al.*, 2006) (Inngjerdingen *et al.*, 2008). Based on the People of Papua's experience, mainly mothers, Kebar's grass can be utilized as uterine fertilizers. Kebar's grass distribution is in Kebar Sub-district, Manokwari District, West Papua so that the people of Papua calls Papua land as a home of kebar's grass. The local name of this plant is banondiy meaning to have many children (Sembiring and Darwati, 2014).

N-hexane fraction of Kebar's grass extract contains saponin that is capable of declining cholesterol level in total blood serum in animal models of hyperlipidemia (Sambodo, Tethool and Rumetor, 2015). Water extract of Kebar's grass can constraint the development of nematode's ova (Baaka, Widayati and Novitanti, 2017). Concentrate formulation of ration consumption and ration efficiency at formula 10% and 15% of Kebar's grass show a

significant influence on the weight gain of rabbit body (Kayadoe, Faidiban and Nurhayati, 2012). Kebar's grass infuse is proven able to improve spermatogenesis' activity, but at a too high concentration, it can root the decline of spermatogenesis' activity (Lefaan, 2014). Kebar's grass has the potential to be utilized as one of the natural materials for controlling *Aspergillus flavus* and inhibiting aflatoxin production (Lisangan *et al.*, 2015). Water extract of *B.petersianium* has a potential for natural feedstuffs in manipulating rumen fermentation to increase N per unit, N is digested and decreases input of total N excretion per unit N so that it has a positive environmental impact (Santoso, Kilmaskossu and Sambodo, 2007). This research aims to determine the content of macro and micronutrient from Kebar's grass *(B. petersianum)* from Papua.

MATERIALS AND METHODS OF RESEARCH

Collection of sample. Kebar's grass *(B. petersianium* Klotzsch*)* was collected in April 2018 in Central and Eat Kebar, Manokwari, West Papua, Indonesia. It was aerated for 4 days and then mashed up using an electric mill. Plant sample was filtered using a filter with mesh size 40. The refined sample was then put into a plastic bag and kept in a room temperature for further analysis. Plant sample was filtered using a filter with mesh size 40.

The proximate, Vitamin C and Antioxidant analysis. The proximate and vitamin C analysis were carried out in the Testing Laboratory of Food Quality and Food Safety, Brawijaya University, Indonesia. Protein, carbohydrate, fat, water, and ash content were analyzed based one the standard of AOAC (AOAC, 1999). All the test were repeated three times. Vitamin Vitamin C was analyzed based on the standard of AOAC 967.21 (AOAC, 2000). Antioxidant activity test was conducted based on the method of antioxidant test (Sayuti, Putri and Yunianta, 2016)

The phytochemical analysis. The respective anti-nutritive factors such as tannins, fenol, polifenol dan saponins were evaluated according to the standard chemicals procedures (Departemen Kesehatan, 1995). This analysis were carried out in the UPT. Materia Medica Batu, Kota Batu, Jawa Timur.

Flavonoid TLC. Two plates with a size of 10 x 10 cm were cleaned and activated. The first spot was 10 mm from the left edge and 10 mm from the under plat, bandwidth is 3 mm, and the distance among spot is 6 mm. All stain are spotted on 2 separated plates. The first plate is eluted with TE system movement phase and the second plate with a TF system movement phase. Chamber was saturated before eluted for 30 minutes. Elution was done until the development distance of 8 cm, then the plat was dried at a temperature of 60°C for 10 minutes in an oven. Dried plate was scanned by spectrophotometer TLC-Scanner 3 (Camag-Mutenz-Switzerland) at the wavelength of 210 and the spectrum of each peak is read on the range of wavelength 190- 400 nm and tested its purity of the spectrum. Rf: 0.85-0.90 (Quercetin); Rf: 0.60-0.65 (Quercitrin); Rf: 0.45-0.50 (Hiperoside); Rf: 0.25-0.30 (Rutin)

RESULTS AND DISCUSSION

Table 1 shows that kebar's grass (*B. petersianum* Klotzsch) contains vitamin C quite high by 418.2 mg/100g, different from Sterculia leaf which one of the Papua endemic plants containing vitamin C by 81.79 mg/100g (Sayuti *et al.*, 2017). Gurame fish weight gain and feed supplementation give a good effect because of the content of vitamin C and flavonoid (Sulhi, Samsudin and Hendra, 2011). Aside from, Kebar's grass also contains very strong antioxidant activity IC_{50} by 0.88 mg/ml, different from the methanol extract of *S. tragacantha* which one of Papua endemic plants containing antioxidant by 2.25 ppm (Sayuti *et al.*, 2018) . The smaller the value of IC_{50}, the higher the activity of antioxidant (Molyneux, 2004).

Phytochemical Screening Test Result of Kebar's Grass (B. petersianum Klotsch). The result of the phytochemical analysis showed that some antinutritive factors such as flavonoids, tannin, triterpenoid, phenolic, saponin were detected (Table 2).

Table 1 – Vitamin C of *Kebar's Grass*

Nutrients	Mean composition ± SD (%)
Protein content	5.99±0.00
Fat content	3.47±0.08
Water content	7.81±0.02
Ash content	14.25±0.30
Carbohydrate	68.47±0.34
Vitamin C (mg/ 100 g)	418.20±33.88
Antioxidant IC_{50}(mg/ml)	0.88±0.04

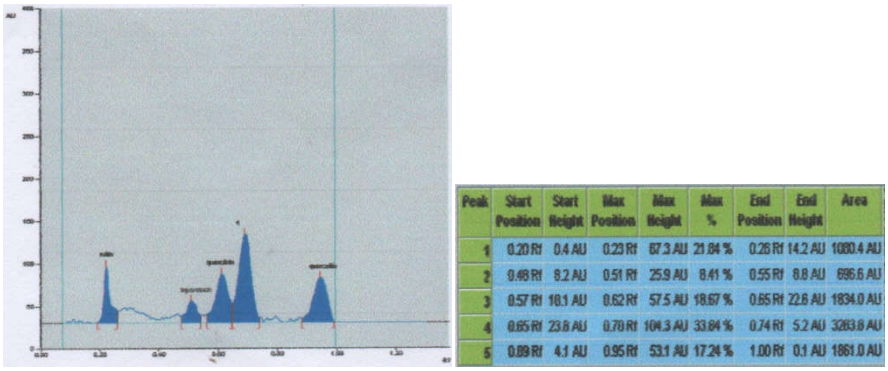
The values are mean ± standard deviation of triplicate determination expressed in dry weight basis.

Table 2 – Phytochemical Screening Test Result of Kebar's Grass

No.	Compound Identification	Result	Standard
1	Flavonoid	+	A dark red/pink color is formed
2	Alkaloid:		
	Meyer Alkaloid	-	White sediment is formed
	Alkaloid dragendroff	-	Orange sediment is formed
	Alkaloid Bouchardat	-	Brown sediment is formed
3	Tannin	+	Blackish blue/blackish green colour is formed
4	Steroid	-	Bluish green colour is formed
5	Triterpenoid	+	Orange or brwonish orange color is formed
6	Phenol	+	Blackish blue or blackish green colour is formed
7	Saponen	+	Permanent foam is formed

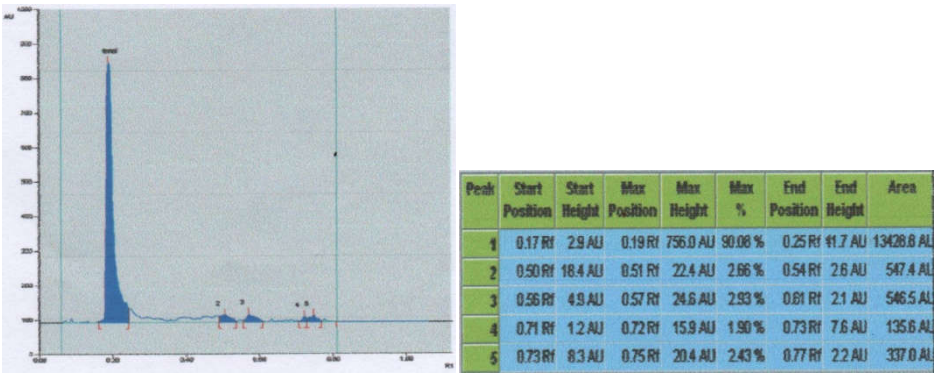
-, absent; +, present

Flavonoid Test Result with TLC-Spectrophotodensitometry. Flavonoid, phenol, triterpenoid test result of Kebar's grass *(B. petersianum* Klotsch*)* with TLC-Spectrophotodensitometry is presented in Figure 1, 2, and 3.



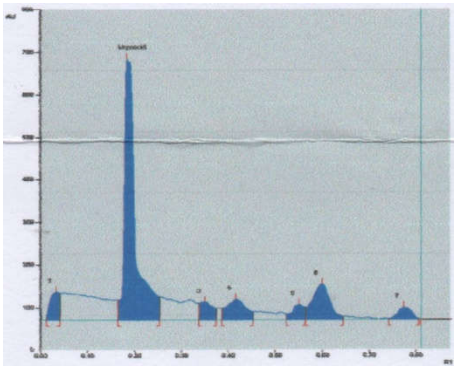
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area
1	0.20 Rf	0.4 AU	0.23 Rf	67.3 AU	21.84 %	0.26 Rf	14.2 AU	1080.4 AU
2	0.48 Rf	8.2 AU	0.51 Rf	25.9 AU	8.41 %	0.55 Rf	8.8 AU	696.6 AU
3	0.57 Rf	10.1 AU	0.62 Rf	57.5 AU	18.67 %	0.65 Rf	22.6 AU	1834.0 AU
4	0.65 Rf	23.8 AU	0.70 Rf	104.3 AU	33.84 %	0.74 Rf	5.2 AU	3263.8 AU
5	0.89 Rf	4.1 AU	0.95 Rf	53.1 AU	17.24 %	1.00 Rf	0.1 AU	1861.0 AU

Figure 1 – The result of Flavonoid TLC of *B. petersianum* Klotzsch



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area
1	0.17 Rf	2.9 AU	0.19 Rf	756.0 AU	90.08 %	0.25 Rf	11.7 AU	13428.8 AU
2	0.50 Rf	18.4 AU	0.51 Rf	22.4 AU	2.66 %	0.54 Rf	2.6 AU	547.4 AU
3	0.56 Rf	4.9 AU	0.57 Rf	24.6 AU	2.93 %	0.61 Rf	2.1 AU	546.5 AU
4	0.71 Rf	1.2 AU	0.72 Rf	15.9 AU	1.90 %	0.73 Rf	7.6 AU	135.6 AU
5	0.73 Rf	8.3 AU	0.75 Rf	20.4 AU	2.43 %	0.77 Rf	2.2 AU	337.0 AU

Figure 2 – The Result of Fenol TLC of *B. petersianum* Klotzsch



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area
1	0.01 Rf	4.2 AU	0.04 Rf	66.0 AU	7.21 %	0.04 Rf	33.3 AU	1221.4 AU
2	0.17 Rf	46.7 AU	0.19 Rf	613.0 AU	66.91 %	0.26 Rf	54.1 AU	13262.4 AU
3	0.34 Rf	38.4 AU	0.35 Rf	42.0 AU	4.58 %	0.38 Rf	27.0 AU	1119.7 AU
4	0.39 Rf	27.2 AU	0.42 Rf	48.4 AU	5.28 %	0.46 Rf	18.0 AU	1968.2 AU
5	0.53 Rf	13.3 AU	0.55 Rf	35.5 AU	3.88 %	0.57 Rf	29.0 AU	864.1 AU
6	0.57 Rf	28.8 AU	0.60 Rf	82.9 AU	9.04 %	0.65 Rf	6.2 AU	2853.7 AU
7	0.75 Rf	1.7 AU	0.78 Rf	28.4 AU	3.10 %	0.81 Rf	1.8 AU	776.0 AU

Figure 3 – The Result of Triterpenoid TLC of *B. petersianum* Klotzsch

Table 2 indicates that Kebar's grass (*B. petersianum* Klotzsch) has very potential content to be utilized as medicinal ingredients because containing flavonoid, tannin, triterpenoid, phenol, and saponin. Figure 1 indicates that Kebar's grass plant (*B. petersianum* Klotzsch) contains rutin flavonoid, hyperoside, quercitrin, whilst figure 2 and 3 indicates that Kebar's grass plant (*B. petersianum* Klotzsch) positively contains phenol and triterpenoid.

Flavonoid compound serves as antioxidant, antibacterial, immunomodulator, and anti-inflammation (Middleton, Kandaswami and Theoharides, 2000) and saponin compound plays a role as permeabilizing membrane and can influence growth and increase animal's feed respond (Das *et al.*, 2012). Flavinoid and tannin commonly contained in fruits and vegetables, as well as beverages, are able to inhibit nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through ACE inhibition, eNOS-specific increase, and also change cyclooxygenase-2 (COX-2) expression(Kizhakekuttu and Widlansky, 2010); (Beg *et al.*, 2011) (Sharifi *et al.*, 2013). Flavonoid and tanin constraint ACE activity which plays an important role in blood pressure control. Phenol activity derives from total hydroxyl group on the benzene ring. Docking's research shows that phenolic acid and flavonoid inhibits ACE through an interaction with ion zink and this interaction is stabilized by another interaction with amino acid at an active side (Guerrero *et al.*, 2012).

Saponin is a compound having biological activity, among others, as hypoglycemic, virucidal, antimicrobial, and impacts on cholesterol metabolism (Desai, Desai and Kaur, 2009). Saponin compound content in this plant is predicted to be a factor that determines its benefit (Santoso, Kilmaskossu and Sambodo, 2007). Various studies of saponin effect on animal reproduction have been many conducted. Saponin extract from ginseng given in vitro can boost sperm motility (Chen *et al.*, 1998). Aside from, saponin extract from Turnera diffusa and Pfaffia paniculata can boost copulation perform sexually from a mouse conditioned to be not effective in a sexual ability (Arletti *et al.*, 1999).

CONCLUSION

Kebar grass (*B. petersianum* Klotzsch) contains proten 5.99%, fat 3.47%, water 7.81%, ash 14.25%, carbohydrate 68.47%. In addition, Kebar's grass (*B. petersianum* Klotzsch) contains vitamin C and high activity of antioxidant and antinutrient compounds; flavonoids, tannins, triterpenoids, phenols, and saponins. TLC test result shows that Kebar's grass (B. petersianum Klotzsch) contains rutin flavonoids, hyperoside, quercitrin, quercetin.

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