

The Contents of Phenol and Flavonoid Compounds in Water Extract of *Gyrinops versteegii* Leaves have Potentially as Natural Antioxidants and Hypoglycemic in Hyperglycemic Wistar Rats

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Phenols and flavonoids compounds in their activities as antioxidants can reduce blood glucose levels (hypoglycemic) through a mechanism to inhibit cell damage at pancreatic Langerhans gland, inhibit glucose absorption in the intestine and cell resistance to insulin. Preliminary analysis shows that total phenol and flavonoid contents is 2099,5025 mg GAE / 100 g, and 8133,322 mg QE / 100 gram, Antioxidant capacity with $IC_{50} = 12.58$ mg / mL. $LD_{50} = 6,355,3536$ gram. This results revealed that the water extract of *Gyrinops versteegii* leaves can be developed as an alternative to natural antioxidant, safe for consumption and can reduce blood glucose in hyperglycemic Wistar rats. The results of the measurement of the effect of decreasing blood glucose content showed that the water extract of *Gyrinops versteegii* leaves at doses of 100, 200 and 400 mg / kgBW significantly ($p < 0.01$) could reduce blood glucose contents in hyperglycemic Wistar rats. The activity of reducing blood glucose levels (hypoglycemic) with oral doses of water extract of *Gyrinops versteegii* leaves was 100,200 and 400 mg / kgBW was 53.6% (53.6 ± 5.8), 58.9% (58.9 ± 8.6) and 75.8% (75.8 ± 1.3). High contents of phenol and flavonoids allegedly a factor that causes water extract of *Gyrinops versteegii* leaves to reduce blood glucose levels in hyperglycemic Wistar rats.

Keywords: Phenol, Flavonoids, *Gyrinops*.

Phenolic compounds such as flavonoids are antioxidant compounds through direct arrest mechanisms, giving one of the hydrogen atoms to free radicals, chelating metal catalysts and protecting endogenous antioxidants radical reactions that endanger health will not continue. Free radical balance with endogenous antioxidants in the body can prevent oxidative stress that can cause degenerative diseases such as cancer and Diabetes Mellitus. (Wong S.P., et al., 2006 and Adi Parwata, 2016). The content of phenol and flavonoid compounds and very strong antioxidant

activity in medicinal plant extracts can inhibit cell damage at pancreas because flavonoids can neutralize free radicals. Flavonoids also inhibit glucose absorption in the intestine and cell resistance to insulin. Flavonoids can also stimulate insulin production (Jack, 2012 and Ramaiah, 2013)

The in vivo preliminary test showed that water extracts of *Gyrinops versteegii* leaves with doses of 50, 100 and 200 mg / kgBW in Wistar rats that experienced oxidative stress were significantly ($p < 0.05$) lowering MDA levels with a mean of 6.37 ± 0 , $23.5.56 \pm 0.27$ and 4.32 ± 0.27 U / mL.



This dose is also able to increase the activity of the enzyme Superoxide Dismutase (SOD) with a mean of 12.15 ± 1.04 , 15.70 ± 2.02 , and 18.84 ± 1.51 and Catalase (CAT) enzymes with a mean of 6.68 ± 0.63 ; 8.20 ± 1.14 and 9.29 ± 0.79 U / mL. (Adi Parwata, 2016).

Referring the use as a medicinal plant and strong antioxidant activity in vivo test in Wistar rats with maximum activity, water extract of *Gyrinops versteegii* leaves potentially to be developed as hypoglycemic activity in hyperglycemic Wistar rats (Ramaiah, 2013).

MATERIALS AND METHODS

Material

Gyrinops versteegii leaves, Alloxan GR, methanol GR (E Merck), CMC-Na and Whatmann Filter Paper No.4 (E Merck), Mg crystal GR (E Merck), HCl GR (E Merck). Quercetin, Gallic Acid.

Instrument

UV-Visible double beam (Varian), analytic Digital Balance (Ohaus), Brand Memmert oven, (Colom 18), rotary vacuum evaporator Brand Buchii, Vortex, sonde and Glucometer.

Preparation Extract

Samples of leaves that have been dried with air at room temperature (30°C) are made in powder form with a size of approximately 40 mesh then extracted. Maceration of *Gyrinops versteegii* leaves powder (simplicia) followed procedure or Harborn (1996), Biswas R. et.al.(2005) and Ashafa (2010). *Gyrinops versteegii* leaves powder with 40 mesh fineness and $<10\%$ water content macerated with warm distilled water ($70-80^{\circ}\text{C}$) for 24-48 hours. The extracts were filtered and the filtrate was evaporated to dryness using a rotary evaporator and then freeze dried. Condensed extract water collected is weighed and stored at a temperature of -20°C . This extract is used for test or further analysis.

Determination Total Phenol

Total phenol contents in the herbal extracts water of *Gyrinops versteegii* leaves were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003 and Almey, 2010). An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 10- 15 minutes and allowed

to stand for 90 minutes at room temperature for colour development. Absorbance was read at 760 or 765 nm using a spectrophotometer. Extracts were evaluated at a final concentration of 1 mg/ml. Total phenolic content was expressed as mg gallic acid equivalent/100 gram sample, using the equation obtained from a calibration curve of gallic acid or mg GAE /100 gram samples.

Determination Total Flavonoid

Total flavonoids were estimated using the method of Chang and Wen (2002), Ashafa (2010) and Ordon-ez et al. (2006). Half a ml of 2% AlCl_3 ethanolic solution was added to 0.5 ml of the extracts in 10 mL volumetric flask. After one hour at room temperature for 25 minutes, the absorbance was read at 415/420 nm. The development of yellow colour was taking as indication of the presence of flavonoids. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the equation $Y = ax + b$ obtained from the calibration curve or mg QE/ 100 gram sample.

Determination Antioxidant Capacity

Antioxidant Capacity Analysis followed procedure Almey (2010). The analysis begins with making of a standard solution of gallic acid 0-100 mg/L. Weighed 0.1 grams each extract, then diluted with methanol to a volume of 5 mL flask and then in the vortex so that a homogeneous solution. This homogeneous solution is centrifuged at 3000 rpm for 15 minutes. Each solution has been pipetted 0.5 mL of this homogeneous, then add 3.5 ml of 0.1 mM DPPH in methanol at a test tube and then in the vortex. This solution was incubated at 25°C for 30 minutes so DPPH reacts with the sample. Each solution was measured absorbance at $\lambda_{\text{max}} = 517$ nm. Antioxidant capacity was calculated using linear regression equation $Y = ax + b$. Antioxidant capacity can be seen from the results % peredamannya and IC_{50} . IC_{50} value is the value which is the concentration of test samples that provides damping DPPH oxidation by 50%. IC_{50} value can be calculated from the linear regression equation $y = ax + b$. Some of the extract concentration was measured percent of inhibition and included in the calibration curve. Extract concentration (ppm) as absis (x), while % inhibition as coordinates (y). The calculation result $y = 50$ included in the equation in order to obtain the value of x as the IC_{50} value of each sample. Contents of antioxidants can be seen from

the IC_{50} , $IC_{50} < 50$ ppm is said to be very powerful antioxidant, said to be strong IC_{50} 50-100 ppm, said moderate is IC_{50} 100-150 ppm and IC_{50} was > 151 is said to be weak as antioxidants.

Hypoglycemic analysis

Hypoglycemic analysis followed the procedure Szkuldelski (2001) and Nandhagopal (2013). Analysis of decreased blood glucose in rats (hypoglycemic) begins with the manufacture of hyperglycemic rats with Aloxane induction at a dose of 100-125 mg / kg BW. A total of 25 Wistar rats were adapted for 2 weeks. Wistar rats that have undergone this adaptation measured their blood glucose levels (day 0). Furthermore, alloxane induced at a dose of 100-125 mg / kg BW. On the 3rd day, these rats measured their blood glucose levels. After all were said to be hyperglycemic (fasting blood glucose levels exceeded 135 mg / dL or approximately 140 mg / dL (Carvalho, 2003, Lansan, 2008 and Manjusha, 2011).

Normal blood glucose levels in fasting rats are 50-135mg / dL (Carvalho, 2003). An 25 of these rats were divided into 5 groups of 5 rats. Then each group is given treatment. Group I was not given the water extract of *Gyrinops versteegii* leaves (negative control), Group II, III and IV

were given water extract of *Gyrinops versteegii* leaves respectively with doses of 100, 200 and 400 mg / kg BB (Reagen Shaw, 2007). Group IV is given Glibenclamide drug (positive control). Blood glucose measurements were carried out on days : 0,3,7 and 14. Day 0 was a measurement of blood glucose at the time before Aloxan was induced. Day 3 is 3 days after induced alloxane (hyperglycemic). Day 7 and 14 after being treated with oral of water extract of *Gyrinops versteegii* leaves. Blood glucose levels were measured using an easy touch glucometer (Gluko Dr. test). A drop of blood from the tip of the mouse's tail is dripped on the glucometer strip, wait 10 seconds to read the value or number on the device. The value stated on the tool is the value of blood glucose levels in units of mg / dL (Ramaiah *et al.*, 2013).

RESULT AND DISCUSSION

Preparation Extract

The preparation of the extract begins with the measurement of the water content simplicia of *Gyrinops versteegii* leaves. Water content of powder *Gyrinops versteegii* leaves is 5.98% w / (8,5805±0,0097) as explained in table 1 :

The maceration results of 1000 grams of *Gyrinops versteegii* leaves powder were obtained 100 grams dry extract.

Total Phenol Contents Analysis

The measurement of Total phenol contents from water extract *Gyrinops versteegii* preceded by making standard curve of gallic acid with concentration with Absorbance as explained in table 2 and described figure 1,

Table 1. Water contents of simplicia

Code	Initial sample weight (g)	Final sample weight (g)	Water content (%)
A	1,16990	1,06942	8,588768
B	1,46302	1,33745	8,582931
C	1,19851	1,09580	8,569808

Average± STDEV. 8,5805±0,0097

Table 2. Absorbance of Gallic Acid Standard

Concentration (mg/L)	Absorbance
0	0
10	0,040
20	0,077
40	0,180
60	0,249
80	0,322
100	0,397

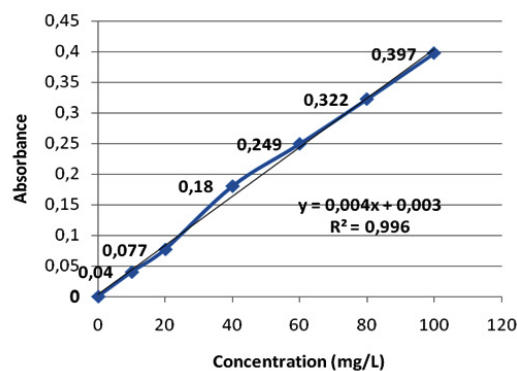


Fig. 1. The Standard curve of Gallic Acid

After absorbance the sample is included in the standard curve equation of gallic acid above obtained the total content of phenol Water Extract of *Gyrinops versteegii* leaves = 6020,4860 (6020,4860 ± 32,17) mg GAE/100 gram as explained in table 3:

Total Flavonoid Contents Analysis

The measurement of Total flavonoid contents from water extract *Gyrinops versteegii* leaves preceded by making standard curve of quercetine with some concentration as explained in table 4 and described figure 2 :

After absorbance the sample is included in the standard curve equation of Querceti above

obtained the total content of flavonoid Water Extract of *Gyrinops versteegii* leaves = 8133,322 mg QE / 100 gram as explained in table 5 :

Analysis of IC₅₀

Measurements or calculations of IC₅₀ preceded by the preparation or measurement of absorbance of some extract concentrations from the sample proceeded to make a graph between% inhibition (y axis) with concentration (x axis) then from the linear regression formula obtained can be calculated IC₅₀ as explained in table 6 and described figure 3 :

It result can be proved water extract of *Gyrinops versteegii* leaves had the highest

Table 3. Total Contents of Phenol

Code Sample	Weight of extract (g)	Abs.	Contents (mgGAE/100 gram)	Content (%)
E1	0,064	0,158	6039,0625	6,0391
E2	0,060	0,147	5983,3333	5,9833
E3	0,064	0,158	6039,0625	6,0361

Average ± STDEV 6020,4860±32,176, 0195±0,0313

GAE =Gallat acid Equivalent; Abs. = Absorbance ;STDEV = Standard Deviation

Table 4. Quercetine Standard Curve

Concentration (mg/L)	Absorbans
0	0
5	0,040
10	0,077
15	0,180
20	0,249
25	0,466

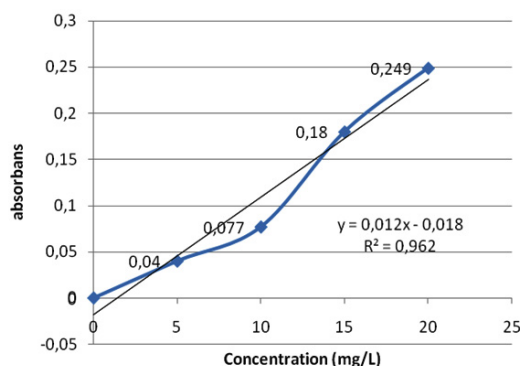


Fig. 2. Standard curve of Quercetine

Table 5. Total Content of Flavonoid

Code of Sample	The Weight of Extract (g)	Absorbans	Contents (mg QE/ 100 g)	Contents (% g/100 g)
D1	0,076	1,246	6929,825	6,929825
D2	0,055	1,256	9651,515	9,651515
D3	0,051	0,939	7818,627	7,818627
		Average	8133,322	8,133322

antioxidant capacity with $IC_{50} < 50$ ppm or very powerful antioxidant and potentially to be developed into a source of natural antioxidants.

Analysis Hypoglycemic

Blood glucose measurement results in the negative control group starting from the day before alloxan was induced (day 0), hyperglycemic (day 3 after alloxane induction) and after treatment (days 7 and 14) as explained table 7 :

Blood glucose measurement results in dose 100 group (Group II) starting from the day before alloxan was induced (day 0), hyperglycemic (day 3 after alloxane induction) and after treatment (days 7 and 14) as explained in Table 8 :

Blood glucose measurement results in dose 200 group (Group II) starting from the day before alloxan was induced (day 0), hyperglycemic (day 3 after alloxane induction) and after treatment (days 7 and 14) as explained in Table 9 :

Blood glucose measurement results in dose 400 group (Group IV) starting from the day before alloxan was induced (day 0), hyperglycemic (day 3 after alloxane induction) and after treatment (days 7 and 14) as explained in Table 10 :

Blood glucose measurement results in positive control /Glibenklamid (C+) starting from the day before alloxan was induced (day 0), hyperglycemic (day 3 after alloxane induction)

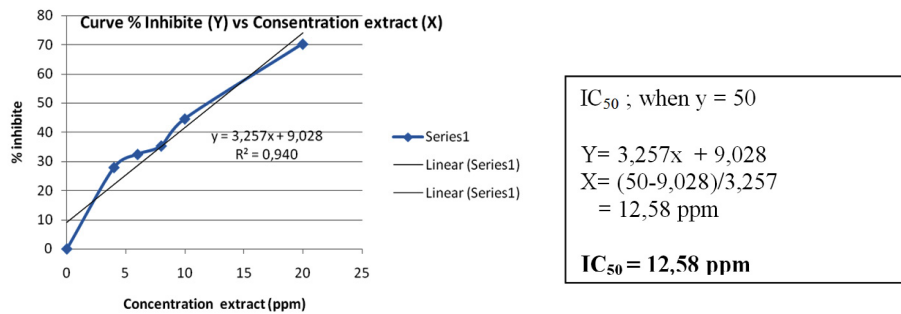


Fig. 3. Curve % Inhibit vs Concentration extract

Table 6. IC_{50} of *Gyrinops versteegii* leaf water extract

No.	Concentration extract (ppm)	Blanco	Absorbance		Persamaan regresi
			Extract	% inhibit	
1	0,00	0,222	0,00	0,00	$y = 3,257x + 9,028$ $R^2 = 0,940$
2	4		0,160	27,93	
3	6		0,150	32,43	
4	8		0,148	33,33	
5	10		0,123	44,59	
6	20		0,066	70,27	

Table 7. Blood Glucose contents of negatif control group (C-)

No. Rats	Code (group)	Blood Glucose contents on the day(mg/dL)			
		0	3	7	14
1	C (-)	115	208	325	447
2	C (-)	122	210	345	462
3	C (-)	128	217	351	487
4	C (-)	134	224	356	498
5	C (-)	164	267	378	498
Average±STDEV		132,6 ±18,9	225,2±24,2	351±19,1	478,4±22,8

and after treatment (days 7 and 14) as explained in Table 11 :

DISCUSSION

The water content of the simplicia obtained in this study was 5,98 % w/w. This result

Table 8. Decrease of blood glucose contents on dose 100 mg / kg BB group (T1)

No. Rats	Code (Group)	Blood Glucose contents on the day(mg/dL)				Δ decrease	% Decrease
		0	3	7	14		
1	T1	89	240	187	101	139	57,9
2	T1	90	242	184	110	132	54,5
3	T1	106	242	184	112	130	53,7
4	T1	107	250	188	121	129	51,6
5	T1	117	250	193	131	119	47,6
Average \pm STDEV		101,8 \pm 12,0	244,8 \pm 4,8	187,2 \pm 3,7	115,0 \pm 11,4	129,8 \pm 7,2	53,6 \pm 5,8

Table 9. Decrease of blood glucose contents on dose 200 mg / kg BB group (T2)

No. Rats	Code (Group)	Blood Glucose contents on the day(mg/dL)				Δ decrease	% Decrease
		0	3	7	14		
1	T2	86	266	163	93	173	65
2	T2	90	267	178	100	167	62,5
3	T2	97	279	178	102	177	63,4
4	T2	108	286	191	115	171	59,8
5	T2	115	291	191	163	128	43,9
Average \pm STDEV		99,2 \pm 12,2	277,8 \pm 11,2	180,2 \pm 11,6	114,6 \pm 28,2	163,2 \pm 20	58,9 \pm 8,6

Tabel 10. Decrease of blood glucose contents on dose 400 mg / kg BB group (T3)

No. Rats	Code (Group)	Blood Glucose contents on the day(mg/dL)				Δ decrease	% Decrease
		0	3	7	14		
1	T3	98	356	144	89	267	75
2	T3	100	398	189	90	308	77
3	T3	107	408	204	95	313	77
4	T3	112	410	209	98	312	76
5	T3	120	424	210	111	313	74
Average \pm STDEV		107,4 \pm 8,9	399,2 \pm 25,9	191,2 \pm 27,7	96,6 \pm 8,9	302,6 \pm 20	75,8 \pm 1,3

Table 11. Decrease of blood glucose contents on positive control (C+)

No. Rats	Code (Group)	Blood Glucose contents on the day(mg/dL)				Δ decrease	% Decrease
		0	3	7	14		
1	C(+)	92	212	212	103	322	76
2	C(+)	97	209	215	112	326	74
3	C(+)	97	204	218	112	337	75
4	C(+)	100	201	221	119	349	75
5	C(+)	133	214	295	123	353	74
Average \pm STDEV		103,8 \pm 16,6	451,2 \pm 20,9	232,2 \pm 36,3	113,8 \pm 7,7	337,4 \pm 13,6	74,8 \pm 0,6

has been in accordance with the Regulation of the Head of the Food and Drug Inspection Agency (BPOM) No. 12 of 2014 on quality requirements of traditional medicine. This regulation states that the water content of a herbal simplicia of traditional medicine is less than or equal to 10% w/w or a maximum of 10% w/w.

The results of *in vitro* analysis evidently that water extract of *Gyrinops versteegii* leaves have the highest antioxidant capacity ie $IC_{50} = 12.58 \text{ mg/mL}$ ($< 50 \text{ ppm}$) Based on these results that the water extract of *Gyrinops versteegii* leaves can be regarded as a natural antioxidant and developed further testing in *in vivo* antioxidant activity. The *in vivo* preliminary test showed that oral administration of water extracts of *Gyrinops versteegii* leaves with doses of 50, 100 and 200 mg / kgBW in Wistar rats that experienced oxidative stress were significantly ($p < 0.05$) lowering MDA levels with a mean of 6.37 ± 0 , $23.5.56 \pm 0.27$ and $4.32 \pm 0.27 \text{ U / mL}$. This dose is also able to

increase the activity of the enzyme Superoxide Dismutase (SOD) with a mean of 12.15 ± 1.04 , 15.70 ± 2.02 , and 18.84 ± 1.51 and Catalase (CAT) enzymes with a mean of 6.68 ± 0.63 ; 8.20 ± 1.14 and $9.29 \pm 0.79 \text{ U / mL}$ (Adi Parwata, 2016). Acute Toxicity with $LD_{50} = 6,355,3536 \text{ gram}$. According to Weils table in Harmita (2006), this value is at the threshold value that is not toxic so that water extract of *Gyrinops versteegii* leaves safe for consumption both as herbal drink and other herbal medicinal materials (Jiang *et al.*, 2011 and Gunawan *et al.*, 2016).

High antioxidant capacity is $C_{50} = 12.58 \text{ mg/mL}$ ($< 50 \text{ ppm}$), high total content of phenolic and flavonoids the water extract of leaves *Gyrinops versteegii* that is 2099,5025 mg GAE/ 100 g and 8133,322 mg QE/100 g able to inhibit and neutralize free radicals (DPPH) (Mathew *et al.*, 2006 and Maisuthisakul *et al.*, 2007). Flavonoids can

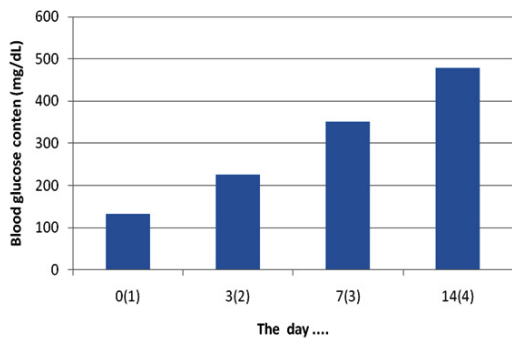


Fig. 4. Blood Glucose content on negatif control

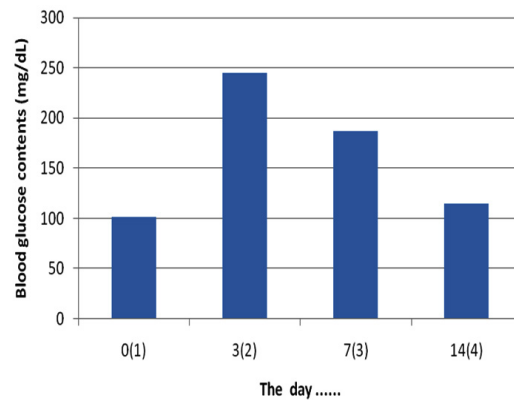


Fig. 5. Blood Glucose content in dose 100 mg/dL

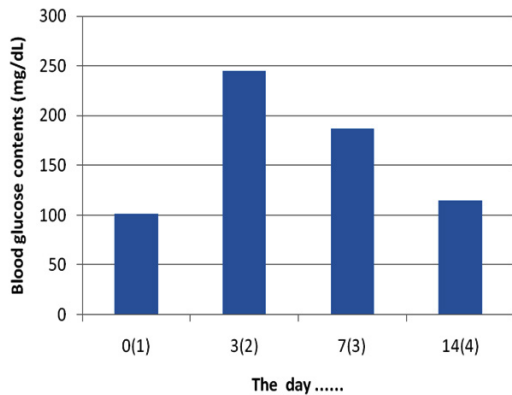


Fig. 6. Blood Glucose content in dose 200 mg/dL

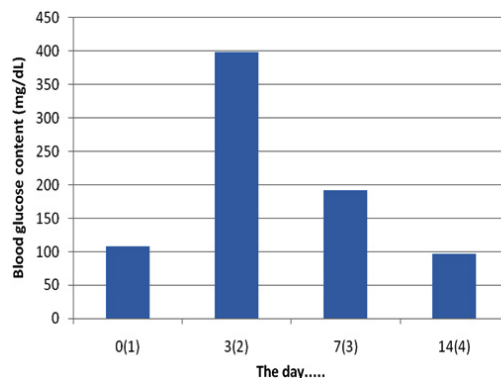


Fig. 7. Blood Glucose content in dose 400 mg/dL

be direct scavenging of Reactive Oxygen Species (ROS). Flavonoids can be prevention of ROS formation through metal chelation so prevention of redox reactions, inhibition of xanthine oxidase and inhibition of NADPH oxidase. Flavonoids can be protection of lipophilic antioxidants and induction of antioxidant enzymes (Akhlaghi, 2009). This resulted in a reaction barrier between ROS lipid peroxidation reaction with unsaturated fatty acids long-chain (PUFAs) can be suppressed and the results peroxidation MDA will decrease (Kaneto, 1999, Lecumberri, 2011; Akhlaghi, 2009).

The magnitude of hypoglycemic activity or decrease in blood glucose (\bar{A}) from the water extract of *Gyrinops versteegii* leaves on hyperglycemic mice can be calculated from the results of deviation (\bar{A}) of blood glucose levels on the last day of measurement (day 14) with blood glucose levels when hyperglycemic (day 3) and % reduction. The results showed that the negative control without oral induction of water extracts of *Gyrinops versteegii* leaves increased blood glucose levels starting from before hyperglycemia (day 0) until the last measurement of day 14, as described in figure 4 :

These results indicate that intraperitoneal induction of Alloxane at a dose of 100-125 mg / kgBB without treatment or treatment with certain drugs both syntheses and herbs will cause damage to Lagerhans gland of the pancreas but can still produce insulin (Nandhagopal, 2013).

The results showed that the treatment by giving orally extracts of water from *Gyrinops versteegii* leaves dose of 100 mg / kg after hyperglycemia or blood glucose content > 135 mg/dL or >140 mg/dL (Manjusha et al, 2011) blood glucose levels continued to decline from the 7th day until the last measurement of the 14th day, as described in figure 5 :

The results showed that the treatment by giving orally extracts of water from *Gyrinops versteegii* leaves dose of 200 mg / kg after hyperglycemia (blood glucose content > 135 mg/dL) blood glucose levels continued to decline from the 7th day until the last measurement of the 14th day, as described in figure 6 :

The results showed that the treatment by giving orally extracts of water from *Gyrinops versteegii* leaves dose of 400 mg / kg after

hyperglycemia (blood glucose content > 135 mg/dL) blood glucose levels continued to decline from the 7th day until the last measurement of the 14th day, as described in figure 7 :

Based on the results shown in the graph and table above shows that the dose of 100, 200 and 400 mg / kgBW can reduce blood glucose levels in hyperglycemic mice with a consecutive decrease of 53.6% (53.6 ± 5.8), 58.9% (58.9 ± 8.6) and 75% (75.8 ± 1.3). The highest dose can affect the decrease in blood glucose is a dose of 400 mg / kgBW.

High contents of total flavonoids and very strong antioxidant activity, the water extract of *Gyrinops versteegii* leaves is thought to have activity in lowering blood glucose in hyperglycemic wistar rats. Flavonoids in their capacity as antioxidants can inhibit cell damage at pancreas, inhibit glucose absorption in the intestine and cell resistance to insulin (Kaneto, 1999, Ruha et.al, 2001, Song et.al, 2002 ; Grasi et.al, 2010 and Ryzky 2015)

CONCLUSION

High contents of total phenol and flavonoids with very strong antioxidant activity, the water extract of *gyrinops versteegii* leaves with a dose of 100, 200, 400 mg / kgBW was able to reduce blood glucose levels in hyperglycemic Wistar rats with a consecutive value of 53.6%, 58.9% and 75.8%.

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