

The Potency of Flavonoid Compounds in water Extract Gyrinops Versteegii Leaves as Natural Antioxidants Sources

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Flavonoids can provide antioxidant effects by preventing the formation of ROS, directly capture ROS, protect lipophilic antioxidants and stimulate the increase of enzymatic antioxidants. Flavonoids are phenolic compounds that are widely found in medicinal plants, one of which is *Gyrinops versteegii* leaves. In this research to determine the potential of flavonoids in water extract *Gyrinops versteegii* as one source of natural antioxidants was investigated. This research begins with maceration of *Gyrinops versteegii* leaves with some solvents such as ethyl acetate, ethanol, methanol and water. Each of the extracts obtained measured the total content of Phenol. The extract, which had the highest total phenol content, measured the total flavonoid content and antioxidant capacity. The active extract as antioxidant was further isolated and identified its flavonoid content. Flavonoids obtained measured antioxidant capacity in vitro. Total phenol (mg GAE/100 g) of ethyl acetate extract = 443, ethanol extract = 1.510, methanol extract = 6.069 and water extract = 14.979, total flavonoid contents = 2298, 977 mg QE/100 gram, containing phenol, flavonoid, tannin, alkaloid and steroid compounds. Antioxidant capacity with IC_{50} = 3,45 ppm (5 min.) and 3,05 (60 min.). Identification of isolates with UV-Vis spectroscopy showed 2 absorption bands namely band I at 352 nm and band II at 256 nm. Addition of $AlCl_3$ / HCl shear reagent showed band I undergoing a 2 nm bathochromic shift. These results indicate that the resulting flavonoid is suspected to be a flavonoid group of flavonol substituted -OH group at C-5 or 5-hydroxy-flavonol. and its antioxidant capacity or IC_{50} = 17,14 ppm. These results indicate that the isolated flavonoid has very strong antioxidant activity and is potentially developed as a natural antioxidant.

Keyword : *Gyrinops versteegii* , capacity antioxidant, total phenol and flavonoid.

The utilization of medicinal plants or herbs (back to nature) in tackling health problems is increasing. This movement is motivated by environmental changes, lifestyle, and the development of disease patterns. The lack of negative effects arising in the use of medicinal plants and economically attract people to go back to use drugs derived from natural materials (back to nature). It is supported by thousands of herbs

used in traditional Chinese Medicine (TCM) which is currently developed in Hong Kong, Canada, The United States, Malaysia, Thailand, India, Singapore and Australia. Ayurvedic medicine is based on the system of Indonesia and India to used hundreds or more herbs for traditional medicine. Movement back to nature is followed by the research and development of medicinal plants in terms of bioactivity and effectiveness

as drugs with experimental animals and patients. This proved more and more stocks are ready fitofarmaka developed into modern medicine. One developed study are a natural antioxidant and immunomodulatory. Antioxidant compounds contained in medicinal plants are one of the active compounds that can prevent a reaction - free radical oxidation reactions later. The compounds are antioxidants include phenolic compounds and flavonoids. These compounds can capture or provide the hydrogen atoms in free radicals that lipid peroxidation reactions and reactions of DNA damage can be prevented. Basically, the body is already producing or endogenous antioxidant such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) but has not been able to deal with the excess of free radicals in the body resulting in an imbalance of antioxidant production and the amount of free radicals (Akhlaghi, *et al.*, 2009). This imbalance if not addressed can lead to oxidative stress oksidatif. Stres unresolved is what can lead to degenerative diseases such as diabetes mellitus and kanker. this imbalance can be overcome by exogenous antioxidant consumption (Adi Parwata, 2016)

Exogenous Antioxidants can be either synthetic and natural. Synthetic antioxidants that have been circulating in the community is Vitamin C, tocopherols, β -carotene, propyl gallate, butyl hidroksi anisole (BHA) and butyl hidroksi toluene which is used as an additive in some food and beverage packaging circulating in the community. Based on several studies of synthetic antioxidants consuming excess can cause toxicity effects and lower health (Wong S.P., *et al.*, 2006). Based on this reason many researchers are looking for alternative antioxidants derived from nature either of vegetables, fruits and traditional medicinal plants. In addition to vitamins contained in vegetables and fruits some phenolic compounds such as flavonoids that of the medicinal plants can be used as a natural antioxidant. One of the medicinal plants that could potentially are the leaves of plants *Gyrinops versteegii* and *Aquilaria* sp. (Tri (Noviyanti, 2011; Mulyaningsih, *et al.*, 2014)

Gyrinops versteegii and *Aquilaria* sp is a genus of the plant family Thymelaeaceae. This plant is a producer of agarwood or resin which has a high-quality essential oils and very fragrant.

Agarwood or resin can be formed at the plant when naturally infected by the fungus *fusarium* sp or injected directly with the inoculant of the fungus *fusarium* sp. (Tri (Noviyanti, 2011; Mulyaningsih, *et al.*, 2014)

Crude extract of *Aquilaria crassna* can reduce ischemia induces cardiac cells die or injured by initiating a p38 mitogen and further activating phosphorylation of protein kinase (MAPK). This extract can cure diarrhea and skin infections caused by *Staphylococcus aureus* (Jermsri, *et al.*, 2012 and Kamonwannasit, *et al.*, 2013).

Japanese society has traditionally use agarwood as herbal medicines to treat of stress or tranquilizers, protect the operation of the organs vital such as heart, lungs and liver. maintaining the health of the stomach, help sore throats with its anaesthetic, and detoktifikasi body (Noviyanti, 2011; Tri Mulyaningsih, *et al.*, 2014).

Traditionally in India, China, Tibet, Eropa, Timur Tengah, Malaysia dan Indonesia, agarwood used for sedative, antiseptic, expectorant, deodorants, anti-asthma, anesthesia, anti-fungal, antibacterial, anorexia, headache, gout, inflammatory, *Mycobacterium tuberculosis* drugs, especially humans, cirrhosis of the liver, abdominal pain, aphrodisiac, thyroid cancer, lung tumor, analgesic, cardiovascular disease, kidney disease, inhibit *Salmonella typhi* and *Salmonella flexneri* bacilli (Gunasekera, *et al.*, 1981; Taylor, *et al.*, 2001; Tri Mulyaningsih, *et al.*, 2014).

Gyrinops versteegii leaf water extract can decrease oxidative stress through decreased MDA levels and increased SOD enzyme activity and catalase in Wistar Rats given maximum physical activity (Adi Parwata, 2016). This extract can also inhibit DNA damage through decreased levels of 8-OHdG in Wistar rats given maximum physical activity.

Chemical constituents of plants *Gyrinops versteegii* including noroxo-agarofuran, agarospirol, 3,4-dihidroxy-dihydrofuran, vaterolactone, indol dan isolonifolen, coniferyl alkohol, guaiacol, catecol dan pyrogalol, veratrol, ambrox, p-methoxy-benzylacetone, aquilochin, Jinkohol, jinkohol ermol, and kusunol (Noviyanti, 2011).

Gyrinops versteegii is active as an antioxidant allegedly caused by the content of phenol and flavonoid compounds. Flavonoids

can provide antioxidant effects by preventing the formation of ROS, directly capture ROS, protect lipophilic antioxidants and stimulate the increase of enzymatic antioxidants. Flavonoids can directly capture superoxide free radicals so that the production of SOD can be improved. This condition can keep the balance of free radicals and enzymatic antioxidants so that the sustainability of oxidative stress can be inhibited (Akhlaghi, *et al.*, 2009). Flavonoid 5-hydroxy-7-methoxyflavanone has activity as an antioxidant and inhibits the growth of fibrosarcoma. This flavonoids also have activity as antiangiogenesis by decreasing VEGF and COX-2. These flavonoids can increase apoptosis by increasing p53 (Adi Parwata, 2016).

Based on the use as a medicinal plant and its chemical content, Gyrinop versteegii plant potential to be developed as an alternative natural antioxidant. In this research, the researchers want to prove that the content of flavonoids as antioxidants so that the results of the extract can be developed into standardized herbal (OHT) remedies based on the active ingredient of flavonoids

MATERIALS AND METHODS

Material

Fresh Gyrinops versteegii leaves, obtained from the village of Marga, Subdistrict Marga, Tabanan, Bali, Indonesia, male rats Wistar from BBVet, standard food of, ethanol GR (E Merck), ethyl acetate GR (E Merck), HCl GR (E Merck), NHCO_3 GR (E Merck), NH_4Cl GR (E Merck), methanol GR (E Merck), TCA, TBA, TEP, BHT (Sigma), PBS, xanthin oxidase, Na-EDTA, H_2O_2 , BSA 0,5%, 2.5 mM NBT, MDA Assay Kit from NWK Northwest, CMC-Na and Whatmann Filter Paper No.4 (E Merck)

Equipment

UV-Vis spectrophotometer (Varian), analytic Digital Balance Scale (Ohaus), Brand Memmert oven, polypropilin tube (Colom 18), rotary vacuum evaporator Brand Buchii, Vortex, water bath.

METHODS

Extraction of Gyrinops versteegii leaves

Extraction of Gyrinops versteegii leaves

Table 1. Water content of Gyrinops versteegii leaves powder

Sample	Start weight (gram)	End weight (gram)	Water contents (% w/w)
1	1,16993	1,06943	8,5905
2	1,46305	1,33747	8,5834
3	1,19854	1,09582	8,5870
% Average \pm SD			8,5870 \pm 0,004

Table 2. The Total phenol content of each extract

No.	Extract	Total Phenol Contents (mg GAE /100 gr)
1	Water	14.979
2	Methanol	6.069
3	Ethanol	1.510
4	Ethyl acetate	443

Table 3. IC_{50} of extract in 5 and 60 minutes treatment

Solvent	Conc. (ppm)	Abs. 5 min.	% Inhibit	IC_{50} (ppm) 5 min.	% Inhibit	Abs. 60 min.	IC_{50} ppm 60min
Water	0,00	0,966	0,00	3,45	0,00	0,987	3,05
	0,82	0,812	15,94		20,22	0,791	
	1,64	0,653	32,35		40,34	0,592	
	3,27	0,432	55,28		65,41	0,343	
	4,91	0,267	72,36		79,37	0,205	
	6,54	0,188	80,59		81,34	0,185	

Note : Abs. = Absorbance ; min = minutes

followed procedure Harborn, 1996, Biswas R. *et al.* 2005 and Ashafa, 2010. The fresh of *Gyrinops versteegii* leaves that have been dried to a powder blended with a size of 40 mesh. The powder of *Gyrinops versteegii* leaves, macerated with ethyl acetate, ethanol, methanol and water for 24 hours, strain extract and liquid extract obtained is evaporated until thick. Condensed extract ethyl acetate, ethanol, methanol and water collected is weighed and stored at a temperature of -20°C. This extract is used for test or further analysis.

Antioxidant Capacity Analysis

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 $\lambda_{max} = 517$ nm. Antioxidant capacity was calculated using linear regression equation $Y = ax + b$. Antioxidant capacity can be seen from the results % inhibite 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. Levels 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.

The most active extracts as antioxidants analyzed its chemical content by some of the color reagent and the other analysis.

Total Phenol Analysis

Total Phenol Analysis followed the procedure (Wolfe *et al.*, 2003 and Almey, 2010). Extract of ethyl acetate, ethanol, methanol and water dissolved in 5 mL volumetric flask. Pipette 0.4 mL, put in a test tube, add 0.4 mL reagent Folin - Clocalteu, divortex until homogeneous, allow 5-10 minutes, add 4.2 mL of Na_2CO_3 , then let stand for 90 minutes at room temperature. further absorbance is measured at maximum wavelength at 760 nm. Create a standard curve of gallic acid in 85% methanol with a concentration of 10-100 mg/L. Levels of Total Phenol is calculated by linear regression formula $Y = ax + b$. Data calculation results are expressed in units Gallic acid equivalent (GAE /100 gram samples. Extracts of the most active and has the highest antioxidant capacity is used as a treatment in Wistar rats to measure the levels of MDA.

Total Flavonoid Contens Analysis

Total Flavonoid contens Analysis followed the procedure Chang and Wen (2002), Ashafa (2010) and Ordon-ez *et al.* (2006). Extract of water dissolved in 5 mL eyhanol in 10 mL volumetric flask, vortexed until homogeneous. Pipette 2,0 mL, put in a test tube, add 2,0 mL $AlCl_3$ 2%, vortexed until homogeneous then incubation

Tabel 4. Absorbance of quercetine Standard

No.	Concentration of standard quercetine (ppm)	Absorbance
1.	0	0
2.	2	0,065
3.	5	0,175
4.	8	0,265
5.	10	0,333
6.	15	0,565

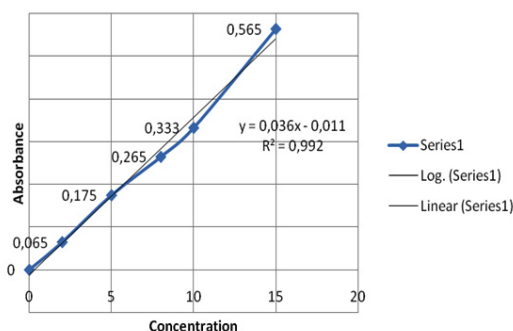


Fig. 1. Quercetin standard curve

in room temperature for 25 minutes. Furthermore absorbance at $\lambda_{\text{max}} = 415 \text{ nm}$. Total Flavonoid contents are integrated in the quersetin standard calibration curve (mg QE / gram).

Isolation and identification of flavonoid from water extract of *Gyrinops verstegii* leaves

Isolation and identifikasi of flavonoid from water extract of *Gyrinops verstegii* leaves followed procedure Biswas R. *et al.* (2005). The standardized Gaharu leaf powder is macerated with hot water. The obtained liquid extract is concentrated with an evaporator rotary. The concentrated extract obtained further in the press dryer until obtained by viscous extract. Subsequently the viscous extract obtained is separated by column chromatography with eluent which is a mixture of n-hexane: ethyl acetate: ethanol = 8: 2: 1. The fraction of the

separation results was then tested for its flavonoid content. A positive fraction containing flavonoids is purified by thin layer chromatography with some eluent of different polarity.

Top of Form

Bottom of Form

The obtained purification result is recrystallized with methanol to obtain needle-shaped crystals. The obtained crystals were further identified with 10% NaOH to determine the flavonoid species and identified by UV-Vis spectroscopy and shear reagents such as NaOH, AlCl_3 and $\text{AlCl}_3 / \text{HCl}$. These crystals are also identified by IR spectroscopy to know the functional groups. and analyzed again antioxidant activity with DPPH method.

RESULT AND DISCUSSION

Analysis water content of *Gyrinops verstegii* leaves powder

Measurement of water content of *Gyrinops verstegii* leaves powder was used oven method. The measurement of the water content was performed three replications as revealed in Table 1,

The result of water content of simplicia powder of *Gyrinops verstegii* leaves = 8, 98 % w/w. This result is in accordance with the standard of simplicia ingredients of Indonesian herbal

Table 5. Total Flavonoids contents

Sample	Weight	Total Flavonoid Contents
1	1,246	2297,149
2	1,248	2300,804
3	1,247	2298,977
Average \pm SD		2298,977 \pm 1,8275

Table 6. The Chemical Ingredients of extract

No.	Reagent	Compounds
1.	Willstater	(+) Flavonoid
2.	NaOH 10%	(+) Flavonoid
3.	Meyer	(-) Alkaloid
4.	Leiberman-Burchard	(+) Steroid
5.	Water	(-) Saponin
6.	FeCl_3	(+) Phenolic

Note : (+) = containing compounds to be analyzed,
(-) = not contains compounds being analyzed

Table 7. Purity Test of flavonoid isolate by TLC

No.	Mobile phase	Ratio	Result
1	n-BuOH: CH_3COOH : H_2O	4:1:5	1 stain
2	CHCl_3 : EtOH	3:1	1 stain
3	n-BuOH: CH_3COOH : H_2O	3:1	1 stain
4	n- C_6H_6 : $\text{CH}_3\text{COOC}_2\text{H}_5$	1:1	1 stain
5.	CH_3COOH : H_2O : HCl	30:10:1	1 stain
6.	n- C_6H_6 : $\text{CH}_3\text{COOC}_2\text{H}_5$	3:1	1 stain

Table 8. Chemical Ingredients of isolates

No.	Reagent	Discoloration	Indicated
1.	Willstater	Colorless to pink	Flavonoid
2.	Bate Smith Metcalfe	Colorless to red	Flavonoid
2.	NaOH 10%	Colorless to brown	Flavonoid flavonol
3.	FeCl_3	Colorless to pink	Flavonoid

Note : All the Reagent result flavonoid

medicine. These results indicate that the water content is <10% w/w. This result is in accordance with the standard of simplicia ingredients of Indonesian herbal medicine which states that the water content of herbal medicine simplicia powder Indonesia must be <10% w / w

Analysis Total Phenol Contents

Extract of water, methanol, ethanol and ethyl acetate from the leaves of *Gyrinops versteegii* obtained through the extraction process. Extraction is a process of withdrawal of the desired component of an ingredient. This study used a method of maceration. Which is the simplest extraction method that is by simply soaking the leaves of *Gyrinops versteegii* with solvent water , methanol , ethanol and ethyl acetate in a simple container with a time of 24 hours , then filtered . The filtrate were then evaporated with a rotary evaporator to obtain a thick extract. Extraction results obtained brownish viscous extract . The total phenol content

of each extract is extract water , methanol, ethanol and ethyl acetate as revealed in table 2,

The table above shows that the gaharu leaf water extract has the highest total phenol content. The water extract then measured the total flavonoid content and its antioxidant activity.

Analysis Antioxidant Capacity

Antioxidant capacity of measurement results of *Gyrinops versteegii* leaves water extracts are revealed in the table 3,

Based on the above results, it can be proved water extract of *Gyrinops versteegii* leaves had the highest total phenol contents and antioxidant capacity with $IC_{50} < 50$ ppm. This means that the water extract of *Gyrinops versteegii* leaves potential to be developed into a source of natural antioxidants , the next need to be tested its total flavonoid contents and Chemical Ingredients of water extract of *Gyrinops versteegii* leaves.

Determination of Total Flavonoids Contents of *Gyrinops versteegii*

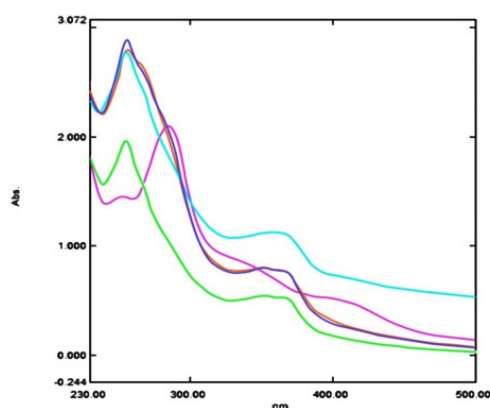
The measurement of Total flavonoid contents from *Gyrinops versteegii* leaves water extract preceded by making standard curve of quercetine with some concentration as revealed in table 4,

This result is used to make the calibration curve like the following :

Measurement of total flavonoid content performed three replications so that the total

Table 9. Identification isolates by IR spectroscopy

No.	Wavenumber (cm ⁻¹)	Intensity	Indicated
1	3453,58	Sharp	-OH
2	2925,09	Sharp	-CH aliphatic
3	1729,22	Sharp	>C=O
4	1642,42	Sharp	>C=C< aromatic
5	1249,88	Sharp	-C-O



Note :
— isolates in methanol
— isolates in methanol + NaOH
— isolates in methanol + NaOH after 5 minutes
— isolates in methanol + AlCl₃ + HCl

Fig. 2. UV-Vis spectra of isolates in methanol and shear reagents

flavonoids = 2298, 977 mg QE/100 gram as revealed in table 5 :

Determination of Chemical Ingredients Water extract of *Gyrinops versteegii* leaves

The result of measurement of chemical content showed that the water extract of *Gyrinops versteegii* leaves contains terpenoid and flavonoid compounds, as revealed in Table 6,

Based on the above results and discussions, in particular the high content of flavonoid compounds and the very strong antioxidant activity of water extracts, the researchers wanted to isolate and identify the type of flavonoids contained. Furthermore, the isolated flavonoids were analyzed for their antioxidant activity.

Isolated and Identified of Flavonoid on Water extract of *Gyrinops versteegii* leaves

Prior to the identification of spectroscopic isolates in purity test and flavonoid type. The flavonoid isolate obtained is a light yellow needle crystal. The purity test with thin layer chromatography (TLC) with multiple eluents of different polarity is obtained by a single spot as revealed in table 7,

The results above show that the flavonoid suspected isolates are considered pure by thin layer chromatography. Furthermore, this isolate was analyzed flavonoid group and its flavonoid structure by spectroscopy

Analysis of chemical constituents showed that isolates indicated positive flavonoids of flavonol or dihydroflavonol, as revealed in Table 8 :

Identification with a UV-Vis spectrophotometer

Identification with UV-Vis spectrophotometer showed the λ_{max} = 256 nm (band II) and 352 nm (band I). Subsequent analysis when isolates in methanol was coupled with $AlCl_3 + HCl$, the absorption band II has a bathochromic shift of about 2 nm as shown by the following figure 2,

Identification with a Infra Red spectrophotometer

Identification by IR spectroscopy showed some peaks as shown by the data in the following table 9 :

The results of antioxidant activity measurements showed that the isolates had strong antioxidant activity with 17,14 ppm. These results are shown in the following Table 10 :

Table 10. Measurement of antioxidant capacity of isolates

Sample	Concentration (ppm)	Absorbance		% inhibition	Linear equations	IC ₅₀ (ppm)
		Blanco	Test sample			
Isolate	2	0.170	0.160	5,89	$y = 2,883x + 0,588$ $R^2 = 0,994$	17,14
	4		0.139	13,24		
	6		0.138	18,84		
	8		0.134	23,2		
	10		0.121	28,86		

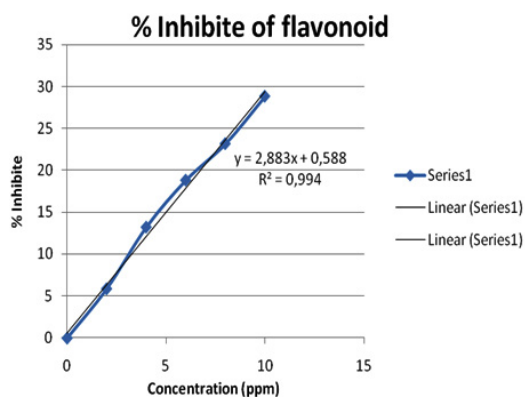


Fig. 3. Graph antioxidant capacity of isolates

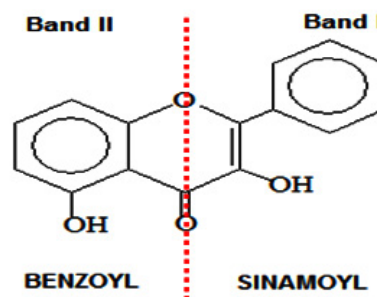


Fig. 4. Graph of sinamoyl dan benzoyl of Flavonoids

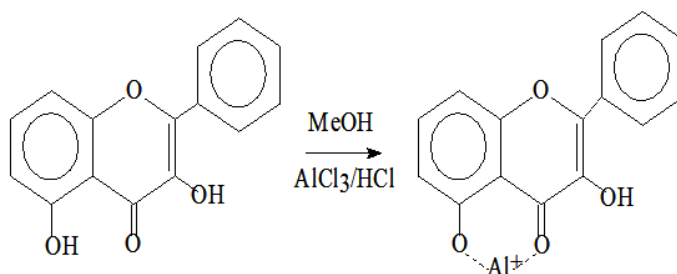


Fig. 5. Reaction of 5-hydroxy-flavonol with AlCl_3/HCl

IC_{50} results are obtained from the graph between concentration (mg / L) versus % oxidation resistance of free radicals by isolates as shown in the following figure 3,

DISCUSSION

Acquisition of water content less than 10%, in accordance with the standards set by *Materia Medika Indonesia*. *Materia Medika Indonesia* is the standard of a medicinal plant powder or *simplicia* of medicinal material derived from plants. This suitability identifies the *simplicia* *Gyrinops versteegii* can be used for research in the development of Traditional Medicinal Plants. Research in the development of Traditional Medicines from Herbs to Standardized Herbal Medicine and *Phytopharmaca* can be done in the form of standardization of *simplicia*, standardization of extract, bioactivity test, isolation, identification and determination of its active chemical content, bioactivity test of isolates and formulations. In this research, standardization of extract, bioactivity test, isolation and identification of flavonoids content of extract and bioactivity test of flavonoid isolate were produced so that *Gyrinops versteegii* extract can be used as natural antioxidant based on flavonoid content (Koleva, 2002).

The Results of *in vitro* studies prove water extract of *Gyrinops versteegii* leaves have the highest antioxidant capacity compared with other solvents because it has the smallest IC_{50} , ie 3.45 mg/L(5 minutes) and 3.05 mg/L(60 min). The IC_{50} means that the concentrations of 3.45 and 3.05 mg/L, the water extract of *Gyrinops versteegii* leaves already can inhibit 50% of the oxidation

reaction of free radicals (Table 2). The potent antioxidant capacity is thought to be caused by a high content of total phenol and flavonoid contents. Phenolic compounds as flavonoids in the water extract of leaves *Gyrinops versteegii* able to inhibit and neutralize free radicals (DPPH). Based on these results that the water extract of *Gyrinops versteegii* leaves can be regarded as a natural antioxidant and developed further analyzing *in vivo* antioxidant activity (Yu *et al.*, 2002).

The results of antioxidant bioactivity measurements *in vivo* showed that *Gyrinops verstexi* leaves water extract at doses of 100 and 200 mg / kg BB could significantly ($p < 0.05$) decrease MDA levels in wistar rats that given maximum physical activity (Adi Parwata, 2016). Bioactivity in lowering levels of MDA caused by the quantity of phenolic compounds (as flavonoids) at doses is able to reduce or neutralize ROS reaction products of metabolism of the body with fatty acids having a double bond (PUFA). This would result in lipid peroxidation product called MDA (Middleton *et al.*, 2000 ; F.Hosseini-mehr *et al.* , 2006; Mathew *et al.*, 2006)

Oral intake of *Gyrinops versteegii* leaf extract at doses of 50, 100 and 200 mg / kg BW can significantly ($p < 0,05$) increase SOD enzyme activity and catalase in Wistar rats given maximum activity (Adi Parwata, 2016). This is due to the quantity of the content of this class of compounds phenol / flavonoid which is more and work synergistically, can directly capture or neutralize the free radical superoxide, peroxy-nitrite so flavonod stimulate the formation of enzymatic antioxidants SOD and endothel dysfunction can be reduced. Phenol compounds such as flavonoids

can also function as an inducer that activates Nrf2 in the cytoplasm. Nrf2 in the cytoplasm will dissociate and translocate to the nucleus. In the nucleus Nrf2 associates to the promoter of a gene called Antioxidant Response Element (ARE), thus triggering the expression of the antioxidant coding gene to produce SOD (Middleton Jr. *et al.*, 2000; Akhlaghi M. and Brian B., 2009).

Phytochemical screening showed that the water extract of *Gyrinops versteegii* leaves contains phenolic compounds, steroid, terpenoids and flavonoids (Diouf, *et al.*, 2009). The compounds are believed to be active here as a natural antioxidant compounds. Natural phenol compounds (such as flavonoids, chlorophyll, terpenoids) usually contains many -OH groups, >C=O and >C=C< groups. These group is able to donate electrons or one atom of hydrogen for ROS to ROS becomes stable and form new free radicals that are less reactive. 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 (Akhlaghi M. and Brian B., 2009). The results of this study supported some research content of compounds such as flavonoids, tannins and anthocyanins contained in chocolate (cocoa fiber) can reduce the production of MDA compound when consumed for three weeks by the Wistar rats with hypercholesterolaemia conditions (Lecumberri, *et al.*, 2007). Flavonoid in *Adiantum capillus-veneris* L. has a high antioxidant capacity and may reduce levels of MDA (Jiang, *et al.*, 2011). Flavonoids in lotus leaf (*Nelumbo Gaertn nuficera*) can reduce oxidative stress by lowering levels of MDA (Xu, H.C. and Wang, M.Y. 2014).

Identification with UV-Vis spectrophotometer showed the λ_{max} = 256 nm (band II) and shoulder = 352 nm (band I). This was in agreement to the literature in which flavonoids flavonol which is substituted OH in C-5 show two absorption bands i.e. between 250-280 nm (band II) and 350-385 nm that caused by the cinnamoyl and benzoyl groups of flavanones as shown by the following figure 4, (Silverstein *et al.*, 1981; Adi Parwata, 2016)

The occurrence of the bathochromic shift in band I identifies the flavonoid flavonol group substituted by -OH groups in C-5 can be prevented by the reaction of flavonol in methanol with $AlCl_3$

as shown by the following figure 5 (Silverstein *et al.*, 1981; Markam, 1988)

The results of identification with Infrared Spectrophotometer (FTIR) show the functional groups -OH, >C=O, >C=C< (aromatic) and >C-O. The results of identification with Infrared Spectrophotometer (FTIR) show the functional groups -OH, >C=O, >C=C< (aromatic) and >C-O. This is according to the structure flavonoid flavonol substituted -OH group at C-5 (Silverstein *et al.*, 1981; Markam, 1988).

Result of antioxidant capacity analysis from flavonoid isolate obtained IC_{50} = 17,14 ppm. these results suggest that isolate flavonoids have very strong antioxidant bioactivity because IC_{50} < 50 ppm. This proves that the flavonoid contained in *Gyrinops versteegii* leaf water extract has the potential to be used as an alternative natural antioxidant. As antioxidant flavonoid isolated can prevent the occurrence of sustainable ROS reaction. Flavonoids can provide antioxidant effects by preventing the formation of ROS, directly capture ROS, protect lipophilic antioxidants and stimulate the increase of enzymatic antioxidants so as to prevent oxidative stress that can cause several degenerative diseases such as heart disease, diabetes mellitus and cancer (Zeng *et al.*, 2001; Yu *et al.*, 2002; Akhlaghi M. and Brian B., 2009)

Flavonoids can bind to hydroxy radicals ($\cdot OH$) so that DNA damage can be prevented. Prevention of DNA damage automatically prevents the occurrence of cancer due to DNA damage (Chabowska, *et al.*, 2009; Gupta, *et al.*, 2010). Flavonoids can directly capture superoxide and peroxynitrite. Through superoxide capture, flavonoids can increase the bioavailability of NO and inhibit the formation of peroxynitrite or flavonoids can directly capture peroxynitrite that damage the endothelium vasorelaxation and disrupt the endothelium, thus ultimately leading to better blood circulation in the coronary arteries and can automatically prevent heart disease (Middleton *et al.*, 2000; F.Hosseini-mehr *et al.*, 2006; Mathew *et al.*, 2006 and Maisuthisakul, 2007).

CONCLUSION

The flavonoid isolate from water extract of *Gyrinops versteegii* leaves is 5-hydroxy-flavonol and have bioactivity as natural antioxidant.

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