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PHARMACOLOGICAL POTENTIAL OF FLAVONOID COMPOUNDS IN ETHANOL EXTRACT ARTOCARPUS HETEROPHYLLUS LAM. LEAVES AS NATURAL ANTIOXIDANTS SOURCES

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ABSTRACT
Artocarpus heterophyllus Lam. is a one of the traditional medicinal plants in Asia. In traditional Chinese medicine, India and Indonesia leaves of Artocarpus heterophyllus Lam are used as antidiabet, antitumor, prevent cancer, fever, prevent osteoporosis, blood circulation, prevent hypertension, strengthen bone and flu medicine. The leaves of Artocarpus heterophyllus Lam. contains high levels of flavonoids (Marianne, 2011). Flavonoids as antioxidants can prevent degenerative diseases such as diabetes, heart disease and cancer. This research aimed to explore the antioxidant of flavonoids in Ethanol Extract Artocarpus heterophyllus Lam. Leaves. Dry powder of Artocarpus heterophyllus Lam. leaves was macerated with ethanol. Liquid extract ethanol was concentrated by rotary evapourator. Consentrated extract was analyzed total flavonoid and antioxidant capacity in vitro than the isolation, identification and antioxidant activity test of its flavonoid. The results obtained are total flavonoid (mg /100 g QE) of ethanol extract = 422 and antioxidant capacity (IC_{50} = mg/L) = 12,65. These results indicate that ethanol extract has the highest total flavonoids and the strongest antioxidant activity. Based on this result then continued with the isolation, identification and test of antioxidant activity of flavonoid compound on ethanol extract. Identification of isolates by spectroscopy of UV-Vis showed 2 absorption bands. Band I at 378.90 nm and band II at 279,60 nm. This result is thought to be a type flavonoid flavonol. Addition of $AlCl_3$ / HCl reagent showed band I undergoing a batochromic shift. This shows the presence of hydroxy groups in C3', C4' and C5 atoms. This isolation and identification results are suspected to be 5,3',4'-trihydroxy-flavonol. The antioxidant activity test of 5,3',4'-trihydroxy-flavonol yielded IC_{50} = 18.67 ppm. These results suggest that 5,3',4'-trihydroxy-flavonol has very strong antioxidant activity, and potentially developed as a natural antioxidant agent in preventing degenerative diseases.

KEYWORDS: Artocarpus heterophyllus Lam, antioxidant, total Flavonoid and Flavonol.

INTRODUCTION
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 develoved 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 enzymatic antioxidants 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. 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 (Akhlaghi, *et al.*, 2009).

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 hydroksi anisole (BHA) and butyl hidoksi 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 be used as a source of natural antioxidants are the leaves of plants Artocarpus heterophyllus Lam.

Artocarpus heterophyllus Lam. is a genus of the plant family Moraceae. Phytochemical screening of leaf Artocarpus heterophyllus Lam. contains flavonoids, alkaloids, saponins, steroids and tannins (Marianne, 2011). Leaf of Artocarpus heterophyllus Lam. In traditional Chinese medicine, India and Indonesia are used as antidiabet, antitumor, prevent cancer, fever, prevent osteoporosis, blood circulation, prevent hypertension, strengthen bone and flu medicine. The n-butanol extract contains isoquercetic flavonoid and has activity as antidiabetes (Omar, 2011). The ethanol extract of Artocarpus heterophyllus contains flavonoids of 7.55 mg/g (Wang, et.al., 2011).

Flavonoids in this plant significantly contribute to increase the activity of antioxidant enzymes and are able to regenerate the damaged beta cells of the kreas so that insulin deficiency can be overcome (Akhlaghi, *et al.*, 2009).. 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 peroxinitrit that destroys the endothelium vacorelaxation and disrupts the endothelium, resulting in ultimately leading to better blood circulation in the coronary arteries (Akhlaghi, *et al.*, 2009). Pinostrobin or 5-hydroxy-7-ethoxy-flavanones from Kaempferia pandurata Roxb has a significant effect in inhibiting the growth of fibrosarcoma (Parwata, et.al., 2016). Flavonoid flavonol from ethyl acetate extract of Euchresta horsfieldii Lesch Benn leaf has been able to increase the activity of superoxide dismutase (SOD) (Gunawan, et.al., 2017).

Based on the use as a medicinal plant and its chemical content, Artocarpus heterophyllus Lam. plant potential to be developed as a source of natural antioxidants . This needs to be demonstrated in vitro by measuring the antioxidant capacity and Isolation of flavonoids in the most active extracts as antioxidants. Antioxidant capacity can be seen from the results % inhibit and IC_{50} . This rule is used to determine the concentration limit of ethanol extract of Artocarpus heterophyllus Lam.leaves endangering health. The isolation results were identified by UV-Vis spectroscopy and Infra Red spectroscopy.

MATERIALS AND METHODS

Material: Fresh Artocarpus heterophyllus Lam. 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).

Instrument: UV-Vis double beam (Varian), analytic Digital Balance Scale (Ohaus), Brand Memmert oven, polypropilin tube (Colom 18), centrifuge (MSE Micro Centaur), rotary vacuum evaporator Brand Buchii, Vortex, water bath, instrument sonde, pyrex measuring cup, pyrex test tubes, micro pipette, a set of mouse cage.

Methods

Extraction of artocarpus heterophyllus Lam. leaves

Extraction of Artocarpus heterophyllus Lam. leaves followed prosedure Harborn and Biswas R. The fresh of Artocarpus heterophyllus Lam. leaves that have been dried to a powder blended with a size of 40 mesh. The extraction process is preceded by the determination of the moisture content of the dry powder Artocarpus heterophyllus Lam. leaves with an oven method. Furthermore, the leaf powder extract was made with n-hexane solvent, ethyl acetate and ethanol. This study used a method of maceration. Which is the simplest extraction method that is by simply soaking the leaves of Artocarpus heterophyllus Lam. with n-hexana, ethyl acetate and ethanol in a simple container with a time of 24 hours , then filtered. The filtrate were then evaporated with a rotary evapourator to obtain a thick extract . Condensed extract ethyl acetate, ethanol, methanol and water collected is weighed and stored at a temperature of -20^0C. This extract is used for test or further analysis. Each of these extracts measured their antioxidant capacity. The most active extract as an antioxidant was determined its flavonoid content, isolation and identification. The type of flavonoids obtained measured its antioxidant capacity by the DPPH method.

Antioxidant capacity analysis

Antioxidant Capacity Analysis followed procedure Almey. 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 25^0C for 30 minutes so DPPH reacts with the sample. Each solution was measured absorbance at λ 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 . Activity of antioxidan 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.

Analysis of total phenol contens

Analysis of Total Phenol Contens followed the procedure Almey**.** 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 1.5 hours at room temperature , Furthermore absorbance at λ max = 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 (mg GAE /100 gram samples.

Analisis of total flavonoid contens

Total Flavonoid levels Analysis followed the procedure Chang and Wen. 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 in room temperature for 25 minutes. Furthermore absorbance at λ max = 415 nm.Total Flavonoid levels are integrated in the quersetin standard calibration curve (**mg QE/ 100 gram).**

Isolation and Identification of flavonoid from Artocarpus heterophyllus Lam. Leaves

Isolation and identifikasi of flavonoid from Artocarpus heterophyllus Lam. leaves followed prosedure Biswas R. and Adi Parwata. The extract which had the highest total flavonoid content and antioxidant activity was continued by isolating and identifying its flavonoids. The extract was further separated by Column Chromatography using eluent n-hexane, ethyl acetate and ethanol with a ratio of 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. 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 $AlCl_3$ / 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

Result

Extract Artocarpus heterophyllus Lam. Leaves

Water content of dried leaves powder of Artocarpus heterophyllus Lam. Leaves obtained 5.7 % w /v. The result of maceration with n-hexane solvent, ethyl acetate and ethanol obtained by viscous extract with weight and color shown in the following table:

Table 1: Maseration results from Artocarpus heterophyllus Lam. Leaves.

No.	Extract	Color	Weight (gram)
1.	n-hexane	Green	19,60
2.	Ethyl acetate	green brown	21,04
3.	Ethanol	Brown	24,76

Each extract measured the total contents of flavonoids and flavonoid types. The results obtained are shown in the following table :

Table 2: Total flavonoid Contents and Flavonoid types of Artocarpus heterophyllus Lam.
Leaves

No.	Extract	Total flavonoid contents (mg QE/100 gr)	Color			Types Flavonoid
			Wilstatter	Bate-Smith	NaOH 10%	
1.	N-hexane	18,07	Green	Green	Yellow	Flavon (+)
2.	Ethyl acetate	249,94	Green	Dark red	Light yellow	Dihidroksi flavonol (++)
3.	Ethanol	429,90	Red	Orange	Orange	Flavonol (+++)

Note : (+++): bright color intensity with color reagents and the highest total flavonoid contents.

Each extract also measured its antioxidant capacity. The measurement results can be seen in the following table 3:

Table 3: Results of antioxidant capacity measurement of each extract.

Extract	Concentration (mg/L)	Absorbance		Persamaan linier R^2	% inhibition	IC_{50} (mg/L)
		Blanco	Test sample			
N-heksana	4	0,204	0,192	y = 1,2496x + 5,552 R^2 = 0,7739	5,88	35,57
	6		0,177		13,23	
	8		0,171		16,18	
	10		0,154		24,51	
	20		0,147		27,94	
Ethyl acetate	4	0,220	0,211	y = 0,9285x + 4,9948 R^2 = 0,7739	4,09	48,48
	6		0,194		11,82	
	8		0,187		15,00	
	10		0,183		16,82	
	20		0,172		21,81	
Ethanol	4	0,222	0,160	y = 2,7201x + 15,597 R^2 = 0.9813	27,93	12.65
	6		0,150		32,43	
	8		0,148		33,33	
	10		0,123		44,59	
	20		0,066		70,27	

The linear graphs to calculate IC_{50} from each extract can be seen in the following graph :

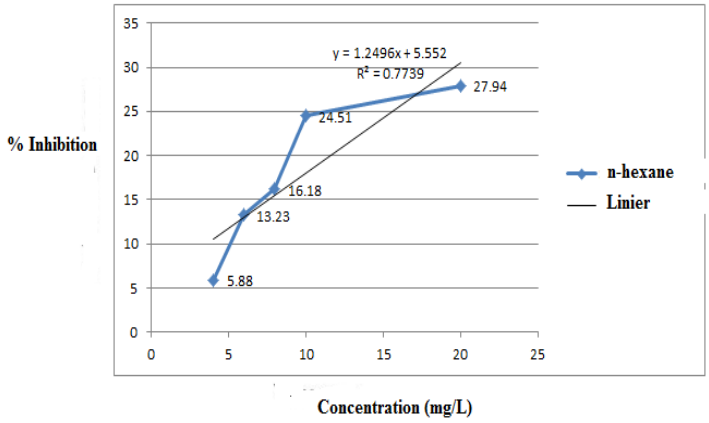


Figure 1: Linear curve of n-hexane extract.

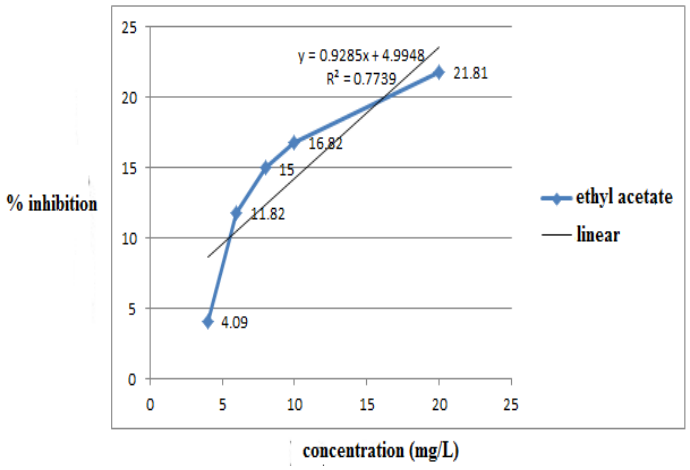


Figure 2: Linear curve of ethyl acetate extract.

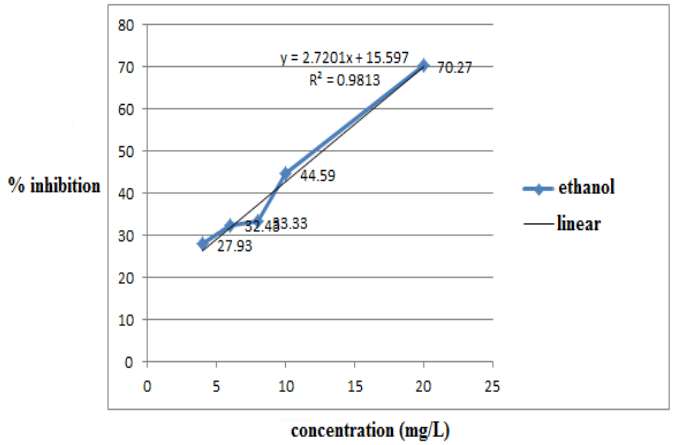


Figure 3: Linear curve of ethanol extract.

The above results show that ethanol extract is the most active extract and has the most flavonoid content then the ethanol extract was isolated and identified the flavonoid type with the following stages :

A. Separation of flavonoids by column chromatography

Separation by column chromatography is preceded by the selection of the best eluent with thin layer chromatography. The best eluent selection uses several eluents with different polarities. The best eluent selected is the eluent that produces the most stains and the Rf range between the stains is quite large. The eluent used and the result of separation can be seen in the following table:

Table 4: The selection of the best eluent with thin layer chromatography.

No.	Eluent	number of stains	Rf value	Information
1	ethanol : ethyl acetate : water = (8:1,5:0,5)	-	-	Not separate
2	ethanol : ethyl acetate = (3:7)	-	-	Not separate
3	ethanol : ethyl acetate :: n-hexane = (8:1:1)		-	Not separate
4	n-hexane: ethanol = (6:4)	-	-	Not separate
5	ethanol : ethyl acetate: n-hexane = (5:4:1)	-	-	Not separate
6	n-hexane : ethyl acetate : ethanol = (8:2:1)	9	0,05; 0,35; 0,44; 0,49; 0,54; 0,59; 0,91; 0,96; 0,99	Separate

Based on the above table then the best eluent used as eluent in column chromatography is n-hexane : ethyl acetate : ethanol = (8:2:1). The result of column chromatography separation was obtained 170 fraction and through KLT process of grafting obtained 15 fraction with same separation pattern. Fifteenth of this fraction tested the flavonoid content was only 6 fractions that contain positive flavonoids. The six fractions that contain positive flavonoids, one fraction shows the intensity of color so bright that it can be said the most or at most flavonoid content, as shown in the following table.

Table 5: Result of separation by column chromatography.

Fraction	Number of stain	The initial color	Color reagent			Information
			NaOH 10%	Bath-Smith	Wilstatter	
A	1	Colorless	Colorless	Light yellow	Colorless	+
B	2	Light yellow	Light yellow	Light yellow	Colorless	+
C	2	Yellow	Light yellow	Light yellow	Colorless	+
D	2	Light green	Yellow	Reddish yellow	Greenish yellow	++
E	1	Light green	Orange	Yellow	Yellow	+++
F	2	Light yellow	Light yellow	Yellow	Yellow	++

Note : + = less color intensity (low flavonoid content)
++ = bright color intensity (medium flavonoid content)
+++ = very bright color intensity (high flavonoid content)

Based on the data in the above table it turns out that the fraction of E is suspected to contain the highest flavonoid and then purified by thin layer chromatography with several eluents of different polarity. Some of the eluents used in the purification as well as the results obtained can be seen in the following table :

Table 6: Eluent used in purification and purification results.

No	Eluent	Comparison	Rf value
1	n-butanol : acetic acid : water	3:1:1	0,60
2	n-hexane : n-butanol	6:4	0,13
3	n-butanol :water : ethyl acetate	6:1:2	0,51
4	Ethanol : ethyl acetate	2:8	0,58
5	n-hexane : ethyl acetate : n-butanol	8:2:1	0,24
6	n-butanol : ethyl acetate : acetic acid	2:7:1	0,74

Note : Rf = retention factor

The data in the table shows that fraction E by using some eluent turns out to produce one stain. These results show that the fraction of E is purely chromatographed. Furthermore, recycling E fraction is made to make the crystals more pure and ready for identification with UV-Vis spectroscopy with sliding reagent and FTIR spectrocopy.

Before being analyzed by UV-Vis spectroscopy and FTIR, it is necessary to determine the flavonoid group in isolate with 10% NaOH, reagent. The results of the analysis showed a change of color from yellow to orange. this indicates that the isolates obtained flavonoid flavanon flavanon, flavonol or chalcon.

The result of the analysis with UV-Visible spectrophotometers showed two absorption bands at wavelength 379 nm for band I and absorption at wavelength 280 nm for band II. These results indicate that the isolates belong to the flavonol group with the hydroxy group on C-3. Theoretically, flavonol compounds with hydroxy groups on C-3 show absorption band I at wavelength 378.90 nm and absorption band II at wavelength 279,60 nm.

Hydroxylation of A or B or C rings may affect the shift of the absorption bands I and II. Hydroxylation in the ring can be proved by shear reagents such as $AlCl_3$ / HCl, NaOH, ethanol, ethanol + NaOH, ethanol + $AlCl_3$, H_3BO_3 and ethanol + $AlCl_3$ + HCl. The hydroxylation of A ring will affect the absorption band II while the hydroxylation in the B and C rings will affect the absorption band I.

The results of identification of isolates with UV-Visible spectrophotometers and shear reagents were obtained as shown in the following table :

Table 7: Results of UV-Visible Spectroscopy analysis and shear reagents.

Isolate + reagent	λ max (nm)		Maximum wavelength shift (nm)		Indicate
	Band I	Band II	Band I	Band II	
EtOH	378,90	279,60			Flavonol (OH free on carbon number 3 ring C)
EtOH+NaOH	405,20	289,80	+26,30	+10,20	3',4'-OH, o-di-OH on ring B and 3- OH adjacent to ring A
EtOH+NaOH (after 5 minutes of silence)	404,00	285,60	+25,10	+6,00	
EtOH +$AlCl_3$	408,20	279,60	+ 29,30	279,60	o-diOH on ring B
EtOH +$AlCl_3$ + $AlCl_3$	415,80	266,60	+36,90	-13,00	5-OH

Identification by IR spectroscopy showed some peaks as shown by the data in the following Table 8 :

Table 8: Identification isolates by IR spectroscopy.

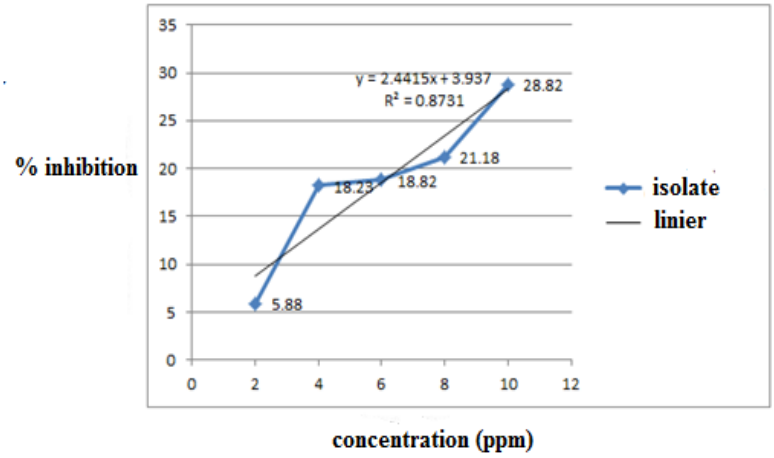
No.	Wavenumber (cm^{-1})	Intensity	Indicated
1	3452,58	sharp	-OH
2	2924,09	sharp	-CH aliphatic
3	1728,22	sharp	>C=O
4	1641,42	sharp	>C=C< aromatic
5	1249,87	sharp	-C-O alcohol

The results of antioxidant activity measurements showed that the isolates had strong antioxidant activity. These results are shown in the following Table 9:

Table 9: Measurement of antioxidant capacity of isolates.

Sample	Concentration (mg/L)	Absorbance		% inhibition	Linear equations	IC_{50} (mg/L)
		Blanco	Test sample			
Isolate	2	0.170	0.160	5.88	**y = 2,4415x + 3,937 R^2 = 0,8731**	18,67
	4		0.139	18.23		
	6		0.138	18.82		
	8		0.134	21.18		
	10		0.121	28.82		

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 picture:



Picture 5: Graph measurement of antioxidant capacity of isolates.

DISCUSSION

Isolates obtained was in the form of needles white crystalline. Purity analysis with chromatographic by TLC showed one peak with a stain and a different polarity eluent. This shows that all this purity test results showed that the isolate was pure chromatographic. Based on these results further isolates identified spectroscopically.

Phytochemical screening reagents NaOH 10% indicated that the isolates do indeed flavanon flavanon, flavonol or chalcon.

Identification with UV-Vis spectrophotometer showed the λ max = 279,6 nm (band II) and 378,90 nm (band I). These results indicate that the isolates belong to the flavonol group with the hydroxy group on C-3. Theoretically, flavonol compounds with hydroxy groups on C-3. This was in agreement to the literature in which flavonoids flavonol show two absorption bands i.e. between 250-280 nm (band II) and 350-385 nm (band II) that caused by the cinnamoyl and benzoyl groups of flavonol as shown in the structure below[11]:

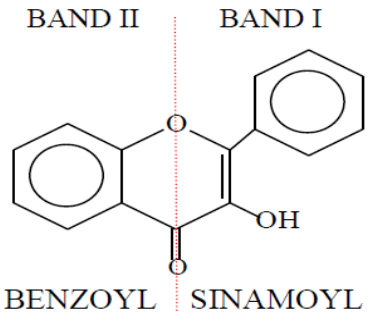


Figure 6: Structure of flavonol.

Batochromic shifts in band I after addition of NaOH reagent in ethanol isolate solution indicate the presence of hydroxy group at ring B at number 3' and 4', as shown by the following figure[1,2,3,11]

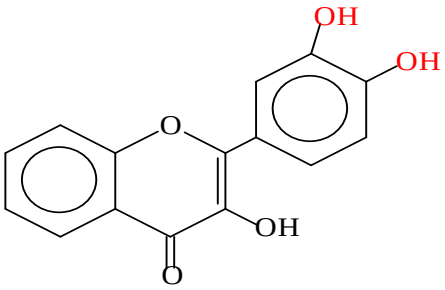


Figure 7: The substituted flavonol structure of the hydroxy group on C3 'and C4'.

Batochromic shifts in bands I and II after the addition of $AlCl_3$ and HCl reagents to an isolated ethanol solution indicate the presence of hydroxy groups in ring B at 3 'and 4' and hydroxy at number 5 in ring A, as shown by the following figure.[1,2,3,11]

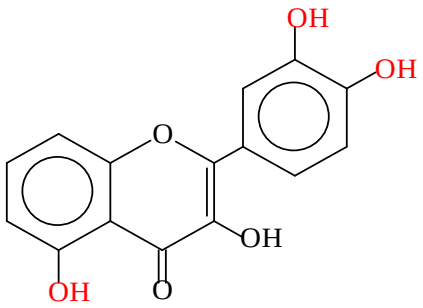


Figure 8: The structure of the substituted flavonol of the hydroxy group on C5 and C3'-C4'.

Based on the theory of addition of $AlCl_3$ to the ethanol solution of the isolates will form the Al complex with the OH group on C5 or C3'-C4 'as shown in the following figure[1,2,3,11]

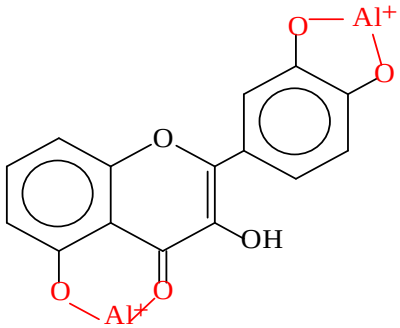


Figure 9: Formation of Al complex at the time of addition of $AlCl_3$.

This structure will return as originally at the addition of a few drops of concentrated HCl as shown in the following figure.[1,2,3,11]

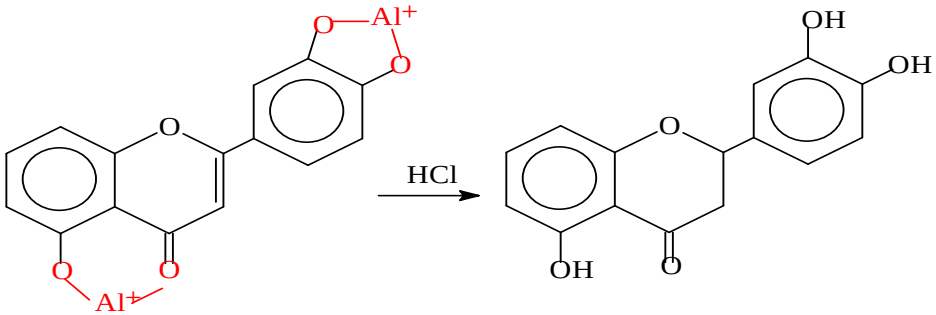


Figure 10: Structural changes in the addition of concentrated HCl.

The addition of shear reagents NaOH, $AlCl_3$ and $AlCl_3$/HCl, showed that the isolates produced were thought to be flavonoid compounds of the flavonol group ie, 5,3',4' trihydroxyflavonol as shown in the following figure[1,2,3,11]

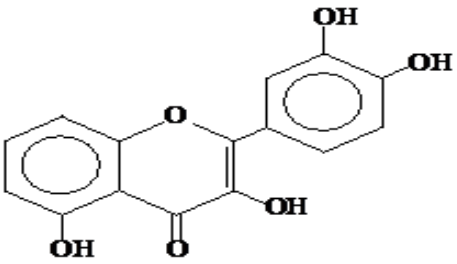


Figure 11: Structure of 5,3',4'-trihydroxyflavonol.

Identification with IR Spectoscopy showed that the isolates obtained contained carbonyl group (> C = O), > C = C < aromatic, -OH and > C-O. The results of antioxidant capacity test showed IC_{50} results of 18.67 ppm[2,3,4] These results indicate that isolates has **very strong antioxidant activity.** This means that the isolates contain flavonoid compounds is **5, 3',4'-trihydroxyflavonol** that have very strong antioxidant capacity.[11]

CONCLUSION

Based on the results of the research, it can be concluded as follows :

1. The flavonoid isolate produced from Artocarpus heterophyllus Lam. leaves is a class of flavonoid compounds **5, 3',4'-trihydroxyflavonol**
2. Compounds 5,3',4'-trihydroxyflavonol have very **strong antioxidant capacity with IC_{50}** = 18,67 ppm

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' declaration

The authors here by declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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REFERENCES

1. Adi Parwata, Sukardiman, Mulya HS., Alit Widhiartini. Inhibition of Fibrosarcoma Growth by 5-Hydroxy-7-Ethoxy- Flavanons from Kae mpferia Pandurata Roxb. *Biomedical & Pharmacology Journal*, 2016; 9(3): 941-948.
2. Adi Parwata, Putra Manuaba, Sutirta Yasa. The Potency of Flavonoid Compounds in water Extract Gyrinops versteegii Leaves as Natural Antioxidants Sources, *Biomedical and Pharmacology Journal*, 2018; 11(3): 1501-1511.
3. Adi Parwata, Laksmiwati, Sudiarta, Dina MN,Sutirta Yasa. The Contents of Phenol and Flavonoid Compounds in Water Extract of Gyrinops versteegii Leaves have Potentially as Natural Antioxidants and Hypoglicemic in Hyperglycemic Wistar Rats, 2018.
4. Akhlaghi M. and Brian B., Mechanisms of flavonoid protection against myocardial ischemia–reperfusion injury. *Journal of Molecular and Cellular Cardiology*, 2009; 46: 309–17.
5. Almey A., Khan AJ., Zahir S., Suleiman M., Aisyah Rahim K., Total phenolic content and primary antioxidan activity of methanolic and ethanolic extract of aromatic plants' leaves, *International Food Research Journal*, 2010; 17: 1077-88.
6. Biswas, R. Dasgupta, A. Mitra, A. Roy, S.K. Dutta, P.K. Achari, B. Dastidar, S.G. dan Chatterjee, T.K., Isolation, purification and characterization of four pure compounds from the root extract of *Pluchea indica Less* and the potentiality of the root extract and the pure compounds for antimicrobial activity. *European Bulletin of Drug Research*, 2005; 13: 63-70.
7. Chang S., Yang M. and Wen H.C.J., Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods, ***J. Food Drug Anal***, 2002; 10: 178-182.
8. Grassi D.,Giovambattista Desideri and Claudio Ferri. Flavonoids : Antioxidants Against Atherosclerosis. *Nutrients*, 2010; 2: 889-902.
9. Gunawan IWG., N M Puspawati, N W Rika Kumara Dewi and M. Oka Adi Parwata. Effect Of Euchresta Horsfieldii Lesch Benn Leaf Extract On Increases Enzyme Activity Of Superoxide Dismutase And Glutathione Peroxidase In Rats with Maximum Physical Activity. J. P*harm. Sci. & Res*, 2017; 9(5): 578-582
10. Harborne, J.B, Metode Fitokimia Penuntun Cara Modern Menganalisa Tumbuhan. (Kosasih Padmawinata). ITB. Bandung, 1996.
11. Jiang M.Z., Yan H., Wen Y., Li X.M. In vitro and in vivo studies of antioxidant activities of flavonoids from *Adiantum capillus-veneris* L. *African Journal of Pharmacology*, 2011; *5(18):* 2079-85.
12. Lecumberri E.,Goya,L.,Mateos, R.,Alfa, M., Ramos, R., Izquierdo-Pulido M.and L.Bravo. A Diet Rich in Dietary Fiber from Cocoa Improvees Lipid Profil and Reduces Malondialdehyde in Hypercholesterolemic Rats. *Nutrition*, 2007; 23: 332-41.
13. Markham,K.R., *Cara Mengidentifikasi Flavonoid*, diterjemahkan oleh Kosasih Padmawinata, Penerbit ITB Bandung, 1988.
14. Middleton Jr.E., Kandaswami C. and Theoharides T.C. The Effect of Plant Flavonoids on Mammalian Cells : Implication for Inflammation Heart Disease and Cancer**.** *Pharmacological Review*, 2000; 52: 673-751.
15. Omar, S.H., El-Beshbishy, H.A., Moussa, Z., Taha, K.F., and Singab, A.N.B., Antioxidant Activity of *Artocarpusheterophyllus* Lam. (Jack Fruit) Leaf Extracts: Remarkable Attenuations of Hyperglycemia and Hyperlipidemia in Streptozotocin-Diabetic Rats, *The Scientific Word Journal*, 2011; 788-800.
16. Wong, S.P., L.P. Leong and J.H.W. Koh, Antioxidant activities of Aqueous extracts of selected plant, Food Chem., 2006; 99: 775-783.