

ANTIHYPERCHOLESTEROLEMIC EFFECT OF *Arcangelisia flava* STEM EXTRACT IN HYPERLIPIDEMIC RATS

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INTRODUCTION

Cardiovascular diseases especially coronary heart disease (CHD) is the main disease cause death in the world. The mortality rate of CHD (26.4%) is higher than the mortality rate of cancer (6%). Indonesian Health Department in 2002 reported that one of four people were died due to CHD. Hyperlipidemia is a major risk factor for CHD. Hyperlipidemia is lipid metabolism disorder characterized by an elevation of total cholesterol (TC), triglyceride and low density lipoprotein (LDL) cholesterol¹.

Arcangelisia flava Merr is a plant belongs to Menispermaceae family, commonly known as yellow root. *A. flava* is Indonesian medicinal plant that have been used traditionally for malaria, diabetes mellitus (DM), urinary stones, jaundice, diarrhea and skin abscess^{2,3,4}. The water extract of *A. flava* showed antimicrobial activity⁵. Methanolic extract of *A. flava* has antioxidant and cytotoxic effect against brine shrimps and breast cancer cells (MCF-7)⁴. The Ethyl acetate fraction of *A. flava* have been proven to reduce blood sugar level of diabetic rats⁶. The major chemical compounds of stem of *A. flava* is alkaloid including berberine, palmatine, jathorrhizine and columbamine⁷. Flavonoid, saponin, tanin and furanoditerpene are other compounds of *A. flava*⁸. Berberine isolated from *Coptis sinensis* can decrease total cholesterol and triglycerides by increasing expression of the hepatic low density lipoprotein receptor (LDLR)⁹.

Our previous study showed that methanol extract of leaf of *A. flava* reduced TC and LDL cholesterol. These extract was capable to decrease the atherogenic index value and the number of foam cells^{10,11}. Antihyperlipidemic effect of *A. flava* was caused by berberine and flavonoid in the extract. The berberine content of stem was higher than leaf of *A. flava*¹². In order to know the antihyperlipidemic effect of stem extract of *A. flava*, the influence of methanolic extract of *A. flava* stems (EMBAf) on total cholesterol, triglyceride, LDL cholesterol and High Density Lipoprotein (HDL) cholesterol levels has been determined in hyperlipidemic induced rats.

MATERIALS AND METHODS

This study was conducted at Laboratory of Biomedic in Faculty of Pharmacy, University of Jember. *A. flava* was collected from National Park of Meru Betiri in March 2015 and identified by Biology Department of

Faculty of Mathematics and Natural Sciences, University of Jember.

Apparatus : Rat balance, analytical balance, rotary vacuum evaporator, TLC densitometer, oral gavage for rat, Biolyzer 100.

Materials : methanol extract of *A. flava* stems, simvastatin, CMC-Na, n hexane, methanol, fructose 27,5%, aquadest, animal food high fat content, reagent for TC, TG and LDL-C.

Animals : All experiments were performed on Wistar male rat weighing 150-200 g. The animals were acclimatized for 1-2 weeks before being used for the experiments.

Standard pelleted diet and water were given ad libitum.

Preparation of Methanol Extract of *A. flava* Stems (EMBAf)

Dried *A. flava* stems were ultrasonicated with hexane (1:6) for 1 h. The residues were ultrasonicated for 1 h with methanol (1:6). Methanol filtrate were collected and evaporated using rotary vacuum evaporator until concentrated extract achieved. The extract was suspended in CMC Na 1% before use.

Preparation of Hyperlipidemia Induced Rats

Hyperlipidemic rats was artificially induced by orally administration of cholesterol and fructose for 45 days. Normal rats received water alone for 45 days. Treatment was started after induction and continued for 7 days.

Treatment Protocols and Statistical Analysis

Twenty hyperlipidemic rats were randomly divided into 4 groups. Group I received CMC Na 1% (control diabetic group), group II and III treated with 250 mg/Kg BW and 500 mg/Kg BW of EMBAf respectively (treatment group) and group IV received simvastatin 1.8 g/Kg BW (positive control). Normal rats were used as normal control. TC, TG, and LDL-C were determined with Biolyzer 100 on 0 (H₀) and 7 days after treatment (H_a). The HDL-C was calculated using formula HDL-C = TC-LDL-TG/5. The atherogenic index was calculated by using formula AI = TC/(HDL-TC).

Berberine content and Total Flavonoid content

The berberine of EMBAf was determined by TLC-densitometric method⁴. The stationary phase used silica gel (GF₂₅₄) plates while mobile phase used toluene : etanol: NH₃ 25% = 3:4:1 (v/v/v). Detection

and quantitation of berberine was performed by densitometry at the wavelength of 254 nm. Total flavonoid content was determined by using the aluminium chloride colorimetric method¹³. As much as 0.1-1 gram extract was diluted with ethanol to 25 mL. Then 0.5 mL extract was added in 1.5 mL ethanol, 0.1 mL AlCl₃ 10%, 0.1 mL natrium asetat and 2.8 mL water. The mixture was allowed to stand for 15 min, and absorbance was measured at 425 nm. Quercetin was used as standard. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quersetin (MgQE).

RESULTS AND DISCUSSION

The TC, TG, LDL-C and HDL-C before and after treatment are shown in Table 1. While the decrease of TC, TG, LDL-C and HD-C after treatment are shown in Table 2. EMBAf oral administration on

hyperlipidemic induced rats could reduce TC, TG and LDL cholesterol level.

EMBAf at a dose 250 mg/KgBW and 500 mg KgBW was able to decrease cholesterol level significantly on day-7 after treatment by 23.97±22.7 and 25.49±16.7 respectively. Increasing EMBAf dosis would decrease cholesterol level. EMBAf at dose 500 mg/Kg BW gave the highest percentage of cholesterol decrease level by 28.54±13,52 (Table 1, 2 and Figure 1) but statistically not significant compared to EMBAf at dose 250 mg/Kg BW. The percentage of cholesterol decrease level of EMBAf at all dose was higher than methanol extract of *A.flava* leaves (Maryani et al., 2016). The hypocholesterolemic effect of EMBAf might be caused by berberine and flavonoid. According to Jeong et al, 2009, berberine isolated from *Coptis sinensis* could lower TC level.

Table 1. Comparison of lipid profiles on hyperlipidemic induced rats

Group	TC (mg/dl)		TG (mg/dl)		LDL-C (mg/dl)		HDL-C (mg/dl)	
	H ₀	H ₈	H ₀	H ₈	H ₀	H ₈	H ₀	H ₈
Negative Control	92.12±13.1	99.59±12.9	48.04±10.2	48.31±25.1	27.23±3.4	27.69±12.5	55.28±19.5	62.24±19.3
Simvastatin 1.8 g /Kg BW	109.55±30.5	98.87±25.2	46.71±19.7	45.36±12.9	38.28±11.3	35.97±17.2	61.93±27.7	53.83±13.6
EMBAf 250 mg/Kg BW	107.54±10.5	83.56±24.2	59.82±25.9	52.03±18.9	43.57±12.0	32.60±12.1	52.00±12.0	40.56±26.7
EMBAf 500 mg/Kg BW	88.90±23.7	63.41±20.5	33.56±8.8	28.07±9.2	36.69±6.3	27.55±18.7	45,50±26.2	30,25±27.2

Values are the mean ± SD . H₀: lipid profiles on day 0 before treatment. H₈ : lipid profiles on day 7 after treatment. TC : Total cholesterol, TG : Triglyceride, LDL-C : Low Density Lipoprotein-Cholesterol, HDL-C : High Density Lipoprotein-Cholesterol. EMBAf : Methanol extract of *A. flava* stems

Table 2 Decrease of Lipid Profiles after EMBAf and Simvastatin supplementation

Group	Δ TC (mg/dL)	Δ TG (mg/dL)	Δ LDL-C (mg/dL)	Δ HDL-C (mg/dL)
Negative Control	-7.46±15.7	-0.27±15.2	-7.46±15.7	6.95±28.8
Simvastatin 1.8 g /Kg BW	10.68±24,1	1.35±24.2	2.31±24.1	-8.10±30.4
EMBAf 250 mg/Kg BW	23.97±22.7*	7,79±19.8	10.97±22.7	-9.97±25.3
EMBAf 500 mg/Kg BW	25.49±16.7*	5.50±10.2	9.14±16.4	-14.88±32.8

Values are the mean ± SD. *Significantly different to negative control (p,0.05). Δ TC, TG, LDL-C, HDL-C : Difference of TC, TG, LDL-C and HDL-C.(-) : show increasing of TC, TG, LDL-C, HDL-C.

As we can see in Table 2 and Figure 1, EMBAf was able to reduce the TG and LDL but statistically not significant compared to negative control groups. These results were not inline with our previous study using methanol extract of *A. flava* leafs. This condition was possibly caused by a large standard deviation due to individual variation of rats and the duration of treatment that was not enough. The HDL level was not significantly influenced in all groups. The EMBAf at dose 250 mg/Kg BW and 500 mg/Kg BW could increase HDL level by 9.97 ± 259 and 14.88 ± 32 respectively.

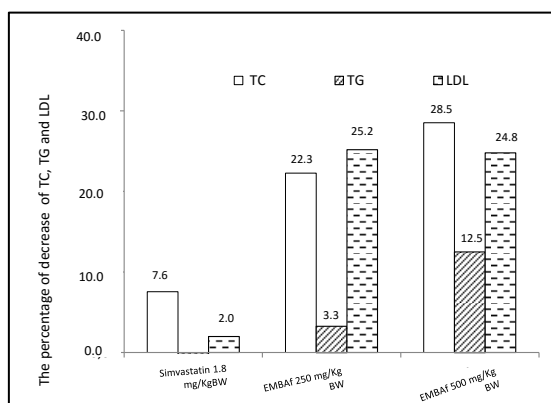


Figure 1. The percentage of decrease of TC, TG and LDL after treatment

The effect of EMBAf and simvastatin oral administration on atherogenic index is shown in Table 3. The atherogenic index in groups treated with EMBAf and simvastatin was decreased compared with negative control.

Table 3. Atherogenic Index after treatment

Group	AI (mg/dL)
Negative Control	1.19±0.6
Simvastatin 1.8 g /Kg BW	0.73±0.2
EMBAf 250 mg/Kg BW	0.85±0.5
EMBAf 500 mg/Kg BW	0.89±0.5

Values are the mean ± SD.

Phytochemical screening using TLC showed that EMBAf contains berberine and flavonoid. Determination of berberine and flavonoid showed that EMBAf contains $0.31 \pm 0.04\%$ w/w and 7.05 ± 0.26 mgQE respectively. The active substance from EMBAf is berberine^{4,9}. Berberine has antidiabetic, anticancer, antihyperlipidemic and antioxidant activities^{4,9,14}. The flavonoid in EMBAf acts as antioxidant that may be helpful to prevent LDL oxidation. Based on the results, EMBAf is prospective to be developed as herbal medicine for hypercholesterolemia.

CONCLUSION

Methanol extract of *A. flava* stems at dose 250 mg/Kg BW decreases total cholesterol level significantly compared to negative control. Methanol extract of *A. flava* stems at dose 250 mg/Kg BW decreases atherogenic indexes compared to negative control.

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REFERENCES

- Dipiro, J.T., Talbert, R.L., Yee, G.C., Matzke, G.R., Wells, B.G., and Posey, L.M. *Pharmacotherapy A Pathophysiologic Approach Seventh Edition*. New York: Mc Graw Hill Medical. 2112 (2008).
- Wiyanto. *Petunjuk Mengenai Tanaman di Indonesia dan Khasiatnya sebagai Obat Tradisional*, terj., Jilid 1, Yayasan Dana Sejahtera, Yogyakarta. (1993)
- Nagle Hinter and Barbara Nagle. *Pharmacology: An Introduction*, McGraw Hill, Boston, 256 (2005)
- Keawpradub, N., Dej-adisai, A., and Yuenyongsawad, S. Antioxidant and Cytotoxic Activities of Thai Medicinal Plants Named Khaminkhruea: *Arcangelisia flava*, *Coscinium blumeinum* and *Fibraurea tinctoria*. *The Songklanakarin Journal of Science and Technology*. 27(2). 455–427(2005).
- Larisu, MA, Sudarsono, Iravati, S, Nurrochmad A. Kajian ilmiah air rebusan katola (*Arcangelisia flava* L. Merr) obat diare berdarah masyarakat kabupaten muna sulawesi tenggara. *Majalah Farmasi Indonesia*, 21(4),283-289, (2010).
- Zhou J, Zhou S, Tang J, Zhang K, Guang L, Huang Y, Xu Y, Ying Y, Zhang L, Li D . Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/ high-fat diet-induced diabetic rats. *Eur J Pharmacol* 606:262-8(2009).
- Siwon, J.. *A Pharmacognostical Study Of Some Indonesian Medicinal Plants Of The Family Menispermaceae*, Leiden, 73-79 (1982).
- Kuni, T, Kangei K, Kawakami Y, Nagai Y, Nizu Y, Sato T. Indonesian medicinal plant I. New Furanoditerpenes from *Arcangelisia flava* Merr (1), *Chem Pharm Bull* 33(2), 479-487 (1995).
- H. W. Jeong, K. C. Hsu, M. Ham et al., "Berberine suppresses pro-inflammatory responses through AMPK activation in macrophages," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 296, no. 4, E955–E964. (2009).

10. Maryani, Ulfa and Rachmawati, Pengaruh Ekstrak Metanol Daun Kayu Kuning (*Arcangelisia flava* (L.)Merr.) terhadap Kadar Kolesterol Total dan Trigliserida Tikus Hiperlipidemia, *e-Jurnal Pustaka Kesehatan*,4 (1), Januari, 20-26, (2016).
11. Rahmawati, Y., Ulfa, E.U, and Rachmawati, E, Pengaruh Ekstrak Metanol Daun Kayu Kuning (*Arcangelisia flava* (L.) Merr) terhadap Histopatologi Aorta Tikus Wistar Hiperlipidemia *e-Jurnal Pustaka Kesehatan*,4(2), Mei, 241-248. (2016)
12. Ulfa, EU and Rachmawati, E, Standarisasi Ekstrak Batang Kayu Kuning (*Arcangelisia flava* (L.) Merr) , *Prosiding Seminar Nasional Farmasi Jember*,20-25,(2015).
13. Departemen Kesehatan Republik Indonesia, *Farmakope Herbal Indonesia*, Departemen Kesehatan Republik Indonesia, Jakarta, (2008)
14. Vuddanda, PR., Chakraborty, S., Singh, S., Berberine: a potential phytochemical with multispectrum therapeutic activities, *Expert Opin. Investig. Drugs*, 19(10), 1297-1308, (2010).