International Journal of Applied Pharmaceutics

ISSN - 0975 - 7058 Vol 11, Special Issue 3, 2019

Research Article

ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF *ABELMOSCHUS MANIHOT* L. MEDIK LEAF FRACTION AGAINST CCL_4-INDUCED LIVER DAMAGE IN RATS

YOS BANNE[1]*, TATY SETYAWATI PONIDJAN[2], JOVIE MIEN DUMANAUW[1]

[1]Department of Pharmacy, Manado Health Polytechnic, Indonesia. [2]Department of Nursery, Manado Health Polytechnic, Indonesia.
Email: yosbanne_2518@yahoo.com

Received: 22 November 2018, Revised and Accepted: 18 February 2019

ABSTRACT

Objective: The objective of this research was to determine the antioxidant and hepatoprotective activity of *Abelmoschus manihot* L. Medik (AMLM) leaves in carbon tetrachloride-induced liver damage in rats (*Rattus norvegicus*).

Methods: Samples of dried leaf were macerated using 96% ethanol solvent to obtain a viscous extract. Fractionation was then performed using ethyl acetate and n-butanol solvent. The antioxidant activity test of each fraction was done by 1,1-diphenyl-2-picrylhydrazyl assay. For the hepatoprotective activity test, the fractions were suspended in Tween 20 0.4% solution with concentrations of 10 mg/mL and 20 mg/mL. Rats were divided into 6 treatment groups consisting of five rats/group. Group I (positive control) was given an oral suspension of Curcuma® tablet, Group II (negative control) was given Tween 20 0.4% solution, and Groups III and IV (ethyl acetate fraction) and V and VI (n-butanol fraction) were given fraction suspension. On the 1st–7th days, all groups were given the solution treatment, and on the 8th day, all groups were injected with CCl_4 intraperitoneally and the treatment was continued until the 11th day. On the 12th day, the blood of rats was taken and then measured the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT).

Results: The results of the antioxidant test showed that ethyl acetate and n-butanol fraction, respectively, had IC_{50} of 89.99 and 114.56 ppm. Measurement of SGOT showed the result for Groups I–VI, respectively, of 160±63.62, 260.53±18.98, 154.16±52.78, 177.43±13.70, 120.07±34.80, and 105.23±40.49 IU/L. Measurement of SGPT showed the result for Groups I–VI, respectively, of 101.87±29.24, 108.1±9.04, 57.73±49.05, 106.07±26.45, 66.9±20.05, and 146.63±84.89 UI/L.

Conclusion: The results of this research indicated that the ethyl acetate and n-butanol fraction of AMLM leaves have the antioxidant and hepatoprotective activity.

Keywords: *Abelmoschus manihot* L. leaf fraction, Hepatoprotective, Antioxidant.

 DOI: http://dx.doi.org/10.22159/ijap.2019.v11s3.M1009

INTRODUCTION

Abelmoschus manihot L. Medik (AMLM) is a tropical annual plant of Malvaceae that is common in North Sulawesi. In addition to food, it is also used as a traditional medicine. Empirically, people consume the boiled water of leaves to lower cholesterol. In addition, it is also used as an analgesic, to treat kidney and ulcer pain [1,2]. The leaf has been tested to prevent ovariectomy-induced femoral osteopenia (bone mineral density conditions lower than normal limits on joints due to surgical removal of the uterus/ovary) [3,4]. These plants can also improve glomerular filtration function ann reduce proteinuria and mesangium hyperplasia that can reduce kidney tissue damage [5].

AMLM plant contains mucilage consisting of polysaccharides and protein. This plant contains flavonoids that are quercetin-3-o-robinobioside, hyperine, isoquercetin, gossypetin-8-o-glucuronide, and myricetin [6-8]. The flower contains quercetin-3-o-ribinobioside, quercetin-3'-glycoside, hyperine, myricetin, anthocyanin, and hyperoside. Hyperoside has antiviral, antinociceptive, anti-inflammatory, cardioprotective, hepatoprotective, and protective effects against gastric mucosal (mucous membrane layer of the stomach) [3,4,9].

Flavonoids are one of the largest natural phenol groups found in all vascular plants. Phenols being an important antioxidant that readily reduce the free radicals [10]. Flavonoids are secondary metabolites, it have various important functions for health, among others in reducing the risk of cardiovascular disease, blood pressure, atherosclerosis, and as an antioxidant [11]. Antioxidants can protect biomolecules against oxidative stress so as to reduce the risk of cardiovascular disease as well as certain types of cancer [12,13]. In addition to being an antioxidant, flavonoids can also modulate cell signal pathways and their effects can be marked on cell function by altering protein and fat phosphorylation and modulation of gene expression [14].

MATERIALS AND METHODS

Materials

Distilled water, olive oil, 96% ethanol, absolute ethanol (pa), ethyl acetate (pa), chloroform (pa), n-butanol (pa), 1,1-diphenyl-2-picrylhydrazyl (DPPH), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) determinants were used. Animal Test: Male Wistar rats, 3 months of age with an average weight of 150-200 g.

Sample preparation and extraction

AMLM leaves were taken in the morning, washed, and dried in an aerated way. The dried leaves were then powdered. About 1.5 kg of AMLM leaf powder macerated with 96% ethanol solvent. The filtrate was collected and concentrated with the rotary evaporator to obtain a viscous extract [15]. The extract obtained then weighed.

Fractionation of AMLM leaf extract

About 4 g of the viscous extract was dispersed in 100 mL mixture of 96% ethanol and distilled water (ratio 1:4), inserted into separating funnel and then added 100 ml of ethyl acetate, shaken vigorously, and allowed to stand until the two liquids separated. The ethyl acetate fraction was collected and concentrated with the rotary evaporator, and then, the

result weighed. The same work was carried out using n-butanol solvent.

Antioxidant activity test with DPPH method

This test was conducted to determine the presence or absence of antioxidant activity as a free radical scavenger [16]. A total of 1 mL of AMLM fraction with a concentration of 150 µg/mL was added with 2 mL of DPPH solution in 0.08 mM methanol. The mixture is then diverted and left for 30 min at room temperature in the dark. The absorbance was measured at a wavelength of 517 nm, and methanol was used as a blank. The percentage of DPPH free radical scavenging activity is calculated according to the following equation [17]:

$$AA\%=100-\left[\frac{(Abs\,sample-Abs\,blank)\times 100}{Abs\,control}\right]\times 100$$

The IC_{50} calculated by the intercept function.

Hepatoprotector activity testing

A total of 30 rats divided into six groups consisting of five each, adapted to the environment for 7 days. Group I (positive control) was given an oral suspension of Curcuma® tablet, Group II (negative control) was given 0.4% Tween 20 solution, and Groups III and IV (ethyl acetate fraction) and V and VI (n-butanol fraction) were given fraction suspension. The fractions were suspended in 0.4% Tween 20 solution with concentrations of 10 mg/mL and 20 mg/mL. On the 1st–7th days, all groups were given the solution treatment, and on the 8th day, all groups were injected with CCl_4 at a dose of 0.2 mL/200 g BW of rat intraperitoneally and the treatment was continued until the 11th day. On the 12th day, the blood serum of rats was taken and then measured the SGOT and SGPT.

RESULTS AND DISCUSSION

Extraction and fractionation

1.5 kg of dried AMLM leaves extracted with maceration method using 96% ethanol solvent, obtained 65.6 g of viscous extract (rendemen 4,37%). The extract was fractionated and obtained 11.6 g of ethyl acetate fraction and 10.3 g of n-butanol fraction. The use of ethanol as a solvent because it has a wide extraction ability that is able to attract both nonpolar, semi-polar, and polar substances, in addition from the results of the previous research, showed that the ethanol solvent produced the most extracts compared to other solvents [15].

The content of active ingredients in the leaf that is suspected to have antioxidant and hepatoprotective activity is flavonoids. The flavonoid content in this sample is of different kinds and differs in their degree of polarity. In general, flavonoids are polar, but there is also a semi-polar depending on the type of flavone compound that binds to glucoside. Previous research had shown that some types of flavonoid compounds contained in the leaves are soluble in semi-polar solvents (such as chloroform and ethyl acetate) and also in polar solvents (such as n-butanol). It is the underlying choice of solvent used in fractionation.

Antioxidant activity

The results are presented in Table 1. Assay of antioxidant activity was performed by DPPH method which is a stable free radical and can be used to determine the properties of free radical damping activity of the fractions [18].

The results showed that IC_{50} value of ethyl acetate fraction is smaller than n-butanol fraction. It is probably due to the flavonoid content in the ethyl acetate fraction much than in the n-butanol fraction. Flavonoids can act as an antioxidant by capturing free radicals and releasing hydrogen atoms from their hydroxyl groups, so it is important in maintaining a balance between oxidants and antioxidants in the body [19]. The ability of a substance as a hepatoprotector is related to the antioxidant activity. Antioxidants can protect biomolecules against oxidative stress so as to reduce the risk of cardiovascular disease as well as certain types of cancer [12]. The human body produces antioxidants naturally but is not strong enough to compete against free radicals, so there is still a need for intake from outside [20].

Hepatoprotective activity

The hepatoprotective activity test was performed in carbon tetrachloride-induced rats. This was intended to trigger liver damage in rats because it is a hepatotoxicant [21]. Treatment with AMLM leaf fraction which has hepatoprotective and antioxidant activity will help to protect the liver from damage and improve liver function damaged by carbon tetrachloride. Liver damage can be observed directly with organ histopathology or by measuring the levels of SGOT and SGPT enzymes in the blood [22]. The average SGOT and SGPT values of group treatment are presented in Table 2.

The results showed an increase in levels of SGOT and SGPT in rat blood after administration of carbon tetrachloride as seen in the negative control group. In normal rat, SGOT levels were 141±67.4 UI/L and SGPT 12.6±4.40 UI/L [23]. The ethyl acetate and n-butanol fraction lowers the SGOT and SGPT level. This might be due to the flavonoid content in the AMLM leaf extract as it has the antioxidant activity. At the SGPT data, it can be seen that increased doses lead to elevated serum levels, so testing should be performed to determine the appropriate dose. The increased liver enzyme indicates liver damage both in acute and chronic [24]. When liver cells are damaged, SGPT enzyme secretes from the liver cells into the blood circulation and will be measured through laboratory tests. The more damaged liver cells, the higher the measured SGOT/SGPT levels in the blood.

CONCLUSION

Based on research result of the antioxidant and hepatoprotective activity of AMLM leaf fractions, it can be concluded that ethyl acetate and n-butanol fraction have an antioxidant activity with IC_{50}, respectively, 89.99 and 114.56 ppm. Both fractions have the hepatoprotective activity. The hepatoprotective activity of AMLM leaves may be due to the presence of flavonoids.

SUGGESTION

It is advisable to test the non-polar fraction of AMLM leaf to compare the results obtained according to the theory that there are also non-polar flavonoids and also to do the histopathological assay to the liver to see the effect of the fraction on the process of repairing the liver. Further tests should be performed to determine the appropriate dose.

Table 1: Antioxidant activity of AMLM leaf fractions with DPPH method

Group of treatment	IC_{50} (ppm)
Ethyl acetate fraction	89.99
n-Butanol fraction	114.56

AMLM: *Abelmoschus manihot* L. Medik, DPPH: 1,1-Diphenyl-2-picrylhydrazyl

Table 2: Hepatoprotective activity of AMLM leaf fraction in CCl_4-induced rats

Group of treatment	SGOT (UI/L)+	SGPT (UI/L)+
Positive control	160±63.62*	101.87±29.24
Negative control	260.53±18.98	108.1±9.04
Ethyl acetate fraction 10 mg/mL	154.16±52.78*	57.73±49.05
Ethyl acetate fraction 20 mg/mL	177.43±13.70*	106.07±26.45
n-Butanol fraction 10 mg/mL	120.07±34.80*	66.9±20.05
n-Butanol fraction 20 mg/mL	105.23±40.49*	146.63±84.89

+Values are expressed as mean±SD, n=5. Data are compared against negative control group, one-way ANOVA LSD comparison test, *p<0.05. ANOVA: Analysis of variance, LSD: Least significant difference, AMLM: *Abelmoschus manihot* L. Medik, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

ACKNOWLEDGMENT

The authors would like to thank the Ministry of Health of the Republic of Indonesia for funding assistance and to the Director of Manado Health Polytechnic for the facilities used in this research.

CONFLICTS OF INTEREST

All authors have none to declare.

REFERENCES

1. Mamahit LP, Sukamto NH. One organic acid compound isolated from Gedi (*Abelmoschus manihot* L. Medik) leaves from North Sulawesi. Chem Prog 2010;3:42-5.
2. Winda MR, Yamlean PV, Sudewi S. Formulation dan evaluation of green Gedi (*Abelmoschus manihot*) leaves extract tablets prepared with wet granulation method. Pharmacon 2016;5:243-50.
3. Wu LL, Yang XB, Huang ZM, Liu HZ, Wu GX. *In vivo* and *in vitro* antiviral activity of hyperoside extracted from *Abelmoschus manihot* (L) medik. Acta Pharmacol Sin 2007;28:404-9.
4. Jain PS, Bari SB, Surana SJ. Isolation of stigmasterol and γ-sitosterol from petroleum ether extract of woody stem of *Abelmoschus manihot*. Asian J Biol Sc 2009;2:112-7.
5. Shao-Yu Z, Nai-Ning S, Wen-Yuan G, Wei J, Hong-Quan D, Pei-Gen X. Progress in the treatment of chronic glomerolunephritis with traditional Chinese medicine. Asian J Pharm Pharm 2006;6:317-25.
6. Liu Y, Lai X, Ling X, Zhao Y, Cui J. Interactions between thrombin with flavonoids from *Abelmoschus manihot* (L.) Medicus by CZE. Chromatographia 2006;64:45-50.
7. Taroreh M. Raharjo S, Hastuti P, Agnes M. Antioxidative activities of various fractions of Gedi's leaf extracts (*Abelmoschus manihot* L. Medik). Agri Sci Procedia 2016;9:271-8.
8. Taroreh MI, Widiyantoro A, Murdiati A, Hastuti P, Raharjo S. Identification of flavonoid from leaves of Gedi (*Abelmoschus manihot* L. Medik) and its antioxidant activity. AIP Conf Proc 2016;1755:80010.
9. Qiu Y, Ai PF, Song JJ, Liu C, Li ZW. Total flavonoid extracted from *Abelmoschus manihot* L. medik flowers attenuates d-galactose-induced oxidative stress in mouse liver through the Nrf2 pathway. J Med Food 2017; 20:557-67.
10. Bhat MA, Ganie SA, Dar KB, Ali R, Hamid R. In vitro antioxidant Potential and hepatoprotective activity of *Taxus wallichiana*. Asian J Pharm Clin Res 2018;11:237-43.
11. Hodgson JM, Croft KD. Dietary flavonoids: Effect on endothelial function and blood pressure. J Sci Food Agri 2006;86:2492-8.
12. Abu Bakar MF, Mohamed M, Rahmat A, Fry J. Phytochemicals and antioxidant activity of different parts of Bambangan (*Mangifera pajang*) and Tarap (*Artocarpus odoratissimsu*). Food Chem 2009;113:479-83.
13. Sudewi S, Lolo WA, Wawongan M, Rifai Y, Rante H. The ability of *Abelmoschus manihot* L. leaves in scavenging of free radical DPPH and total flavonoid determination. IOP Conf Ser Mat Sci Eng 2017;l259:12020.
14. Ciz M, Denev P, Kratchanova M, Vasicek O, Ambrozova G, Lojek A, *et al.* Flavonoids inhibit the respiratory burst of neutrophils in mammals. Oxid Med Cell Longev 2012;2012:181295.
15. Pine AT, Alam G, Attamin F. Standardisasi Mutu Ekstrak Daun Gedi (*Abelmoschus manihot* L. Medik) Dan Uji Efek Antioksidan Dengan Metode DPPH 2011. Available from: http://www.pasca.unhas.ac.id/jurnal/files. [Last accessed on 2017 Dec 21].
16. Chandini SK, Ganesan P, Bhaskar N. *In vitro* antioxidant activities of there selected brown seaweeds. India Food Chem 2008;107:707-13.
17. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15:127-30.
18. Hanani E, Mun'in A, Sekarini R. Identification of antioxidant compound in *Callyspongia* sp. sponge from Seribu Islands. Majalah Ilmu Kefarmasian 2005;2:127-33.
19. Koncazak I, Okuno S, Yoshimoto M, Yamakawa O. Caffeoylquinic acids generated *in vitro* in high anthocyanin-accumulating sweet potato cell line. J Biomed Biotechnol 2004;5:28792.
20. Raharjo MH. Tanaman Berkhasiat Antioksidan. Jakarta: Penebar Swadaya; 2005.
22. Delgado-Montemayor C, Cordero-Perez P, Salazar-Aranda R, Waksman-Minsky N. Models of hepatoprotective activity assessment. Med Univ 2015;17:222-8.
23. Yogi B, Mishra A. Hepatoprotective effect of polyherbal formulation Against carbontetrachloride-induced hepatic injury in albino rats: A toxicity screening approach. Asian J Pharm Clin Res 2017;10:192-5.
24. Cahyono JB. Hepatitis. Jakarta: Kanisius; 2009.