Separation of Ethyl Acetate Fraction of Mengkudu Fruit (*Morinda citrifolia* Linn.) and Its Antidiabetic Activity by Glucose Tolerance Method on Mice

Shintia Lintang Charisma[1,3], Yasmiwar Susilawati[1], and Ahmad Muhtadi[2]
[1] *Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran*
[2] *Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia*
[3]*Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Jl. Raya Dukuh Waluh, Purwokerto, Indonesia*

Keywords: Separation, *Morinda citrifolia* Linn., Antidiabetic, Glucose tolerance method

Abstract: Mengkudu (*Morinda citrifolia* Linn.) is one of the medicinal plant that contains alkaloids, carbohydrates, flavonoids, glycosides, phenols, proteins, amino acids, saponins, steroids, tannins, antraquinone and terpenoids. Traditionally, mengkudu (*M. citrifolia* Linn.) fruit has been used for antidiabetic effect. Previous research showed that ethyl acetate fraction gave significant activity (54.29 %) at dose 1200 mg/kg body weight on male wistar rats. The aims of this study were to separate ethyl acetate fraction from mengkudu fruit by Vacuum Liquid Chromatography method and to study antidiabetic activities on male mice by glucose tolerance method. This research used separation guided by activity method which ethyl acetate fraction separated by Vacuum Liquid Chromatography and gave five subfractions (Ds II-A, Ds II-B, Ds II-C, Ds II-D and Ds II-E). The five subfractions then tested the antidiabetic activity at a dose of 150 mg/kg body weight on male mice by glucose tolerance method. Blood glucose level measured at 30, 50, 90, 120 and 150 minutes after administration of from mengkudu fruit (Ds II-A, Ds II-B, Ds II-C, Ds II-D and Ds II-E) showed antidiabetic activity with percentage of reduction relative blood glucose levels 150 minutes after sample administration were 39.11%, 52.85%, 35.31% 43.55%, and 33.78% respectively. These research showed that Ds-II-B subfraction indicate the highest antidiabetic activity by glucose tolerance method. Rutin, quercetin and scopoletin compounds indicate antidiabetic activity from ethyl acetate subfractions of mengkudu fruit.

1. INTRODUCTION

Diabetes mellitus is a type of chronic disease that characterized by elevating blood sugar levels (Dipiro, 2015). World Health Organization (WHO) has established several criteria that indicate diabetes mellitus, including fasting plasma glucose (no caloric intake of at least 8 hours) ≥126 mg/dL or 2 hours plasma glucose ≥200 mg/dL or random plasma glucose levels at ≥200 mg/dL (IDF, 2012).
Diabetes mellitus become an important for concern on health issue because the prevalence of diabetes has been steadily increasing over the past few decades (WHO,2016). The incidence rate of diabetes in Indonesia nearly 6.9 % during 2013 (Depkes RI, 2013).

Mengkudu (*Morinda citrifolia* Linn.) has a long been used as medicinal plants in many countries, especially people in the continent of Polynesia, South Asia, Southeast Asia, parts of Australia and the Caribbean continents (Pawlus and Douglas, 2007). Based on the literature, mengkudu has been used as medicinal plants for diabetes treatment (Nerurkar, 2015).

Chemical compounds reported on mengkudu plants were polysaccharides, fatty acids, glycosides, iridoid, triterpenes, anthraquinones, coumarins, flavonoids, phytosterols, carotenoids and volatile compounds (Ahmad, *et.al*., 2016). Mengkudu fruit contains caprylic acid, hexanoic acid, caproic acid, vitamin C, vitamin E, niacin, asperulosidic acid, quercetin, 2,6-di-O- (b-D-glucopyranosyl 1-O-octanoyl-b-D-glucopyranose, damnacanthal and americanin A (Assi, *et.al*., 2015).

Rao and Subramanian (2009) showed that the ethanolic extract of mengkudu fruit at a dose of 300 mg/kgBW can increase plasma insulin levels in the group of diabetic rats induced streptozotocin 12.52 µU/ml, while the group given glyclazide (dose 5 mg/kgBW) of 13.27 µU/ml. these research showed

Charisma, S., Susilawati, Y. and Muhtadi, A.
Separation of Ethyl Acetate Fraction of Mengkudu Fruit (Morinda citrifolia Linn.) and Its Antidiabetic Activity by Glucose Tolerance Method on Mice.
DOI: 10.5220/0009844900002406
In *Proceedings of BROMO Conference (BROMO 2018) - Symposium on Natural Product and Biodiversity*, page 1
ISBN: 978-989-758-347-6

that the ability of mengkudu fruit extract in increasing the production of insulin comparable with glyclazide which is one of the oral antidiabetic sulfonylurea group.

Hartati (2003) had been studied antidiabetic activity of ethanolic extract of mengkudu fruit by glucose tolerance method on male rats. These study showed that glucose tolerance test on rats, 30, 60, 90 and 120 minutes after administration of the extract at dose of 1200 mg/kgBW, serum glucose concentration decreased by 13.99%, 31.85%, 44.46% and 56.19 %.

Based on Ramdhini (2005) reseach, fractions of mengkudu fruit extract can decrease plasma glucose level of male rats at a dose of 1200 mg/kgBW by glucose tolerance method. It is known that ethyl acetate fraction was the best antidiabetic activity (54.29%), followed by *n*-hexane fraction (34.18%) and water fraction (47.42%). Separation and identification of active compounds that contain in the ethyl acetate fraction have not been performed.

The aims of this study were : (a). To separate ethyl acetate fraction from mengkudu (*M. citrifolia* Linn.) fruit by Vacuum Liquid Chromatography method. (b). To study antidiabetic activities from mengkudu fruit subfractions on male white mice by glucose tolerance method.

2. METHODS

The fresh ripe fruit of *M. citrifolia* Linn. were collected from Lembang, West Java, Indonesia. This plant was identified at Plants Taxonomy Laboratorium, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran.

2.1 Extraction and Phytochemical Analysis

10 kg mengkudu fruit, mashed and then macerated in ethanol 70% for 24 hours with 3 times repetitions. The macerate were filtered, collected, then concentrated with a rotary evaporator until the ethanol vaporised. The concentrate extract than reheated at 55°C by waterbath until obtained a fixed weight. The extract was subjected to qualitative chemical tests for the identification of various phytoconstituents.

2.2 Fractionation of Ethanolic Extract by Liquid-Liquid Extraction (LLE)

The concentrate extract was dissolved in water, then filled into a separating funnel and added *n*-hexane as the same volume of water added. The two formed layers are separated, then the water layer was put back into the separating funnel and added *n*-hexane with the same volume. The water portion then introduced into the separating funnel again, and fractionated using ethyl acetate in the same way as fractionation with *n*-hexane. The three fractions *n*-hexane (Ds-I), ethyl acetate (Ds-II) and water (Ds-III) were evaporated with a rotary evaporator and continue the evaporation with with water bath until the weight is obtained constantly.

2.3 Analysis of Ethyl Acetate Fraction (Ds-II) by Thin Layer Chromatography (TLC)

Ethyl acetate fraction of mengkudu fruit were spotted on TLC plate 60 F254 (Merck, Germany) and developed in mobile phase obtained chloroform : ethyl acetate : methanol (7:2:1). TLC plate were visualized under UV light at wavelength 254 nm and 366 nm and spray by using 10% H_2SO_4 reagent.

2.4 Separation of Ethyl Acetate (Ds-II) Fraction by Vacuum Liquid Chromatography (VLC)

The chromatographic column was packed by dry state in a vacuum conditions to obtain the maximum packing density. The ethyl acetate fraction (Ds-II) was dissolved in a suitable solvent, inserted directly at the top of the column or on the absorbent layer, sucked slowly into the package by placing it. The columns were eluted by *n*-hexane, ethyl acetate and ethanol with a gradient system. The columns sucked to dry and every fraction was collected in the bottle. Result of these separation with VLC obtained 19 fractions.

The fractions of VLC separation were analyzed by TLC. Eluate which have the same pattern spots appearance on TLC are combined as one fraction then concentrated using rotary evaporator. Based on their similarity spot pattern, five subfractions were obtained (Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E).

2.5 Antidiabetic Activity of Subfractions

2.5.1 Experimental Animals Preparation

Animal experiments were reviewed and approved by Research Ethics Comittee Universitas Padjadjaran (approval no. 132/ UN6.KEP/EC/ 2018). Swiss Webster male mice weighing 20-30 g procured from Center for Life Sciences Institut Teknologi Bandung. The mice were adapted in the cage for approximately 7 days. During the adaptation, mice were given drink and food in the form of special feed of livestock with adequate nutrient content.

2.5.2 Experimental Design

The glucose tolerance method was performed in overnight fasted (16 hours) normal mice, ad libitum. Mice were divided into seven groups, each consisting of four mice were administered 2% (w/v) PGA, glibenclamide 0.65 mg/kgBW, and subfractions (Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E) 150 mg/kgBW, respectively. Glucose (2 g/kgBW) was fed 30 min after the administration of subfractions. Blood sample was collected at 0, 30, 60, 90, 120 and 150 minutes of glucose administration and blood glucose level was estimated using electronic glucometer (Accu-Chek Active Glucometer) and glucostrips (Accu-Chek Active Diabetic Test Strips).

2.6 Data Analysis

Data from blood glucose measurement, further analyzed by statistics using variant analysis (ANAVA) design with fixed model at level of 0.05 and 0.01 and further analyzed by Tukey test method at level 0.05.

3. RESULTS

This plant was taxonomically determined at Plants Taxonomy Laboratorium, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran as *Morinda citrifolia* Linn. of the family Rubiaceae, collection number 444/HB/09/2017.

3.1 Extraction and Preliminary Phytochemical Analysis

The percentage yield of ethanol extract was found to be 22.46% w/w. The qualitative phytochemical analysis of the ethanolic extract showed the presence of saponins, triterpenoids, flavonoids, alkaloids and phenolic compounds.

3.2 Fractionation of Ethanolic Extract by Liquid-Liquid Extraction (LLE)

The yield of fractions obtained by LLE method were *n*-hexane fraction (Ds-I) 103.18 g (5.15%); ethyl acetate fraction (Ds-II) 54.18 g (2.85%) and water fraction (Ds-III) 334.86 g (16.74 %).

3.3 Analysis of Ethyl Acetate Fraction (Ds-II) by Thin Layer Chromatography (TLC)

Ethyl acetate fraction of mengkudu fruit were spotted on TLC plate 60 F254 (Merck, Germany) and developed in mobile phase obtained chloroform : ethyl acetate : methanol (7:2:1). TLC plate were visualized under UV light at wavelength (a) 254 nm and (b) 366 nm then detected by using 10% H_2SO_4 spray reagent (Figure 1). Based on TLC analysis, there were 6 spots have detected with Rf values Rf_1 = 0.125; Rf_2 = 0.225; Rf_3 = 0.425; Rf_4 = 0.6; Rf_5 = 0.7 and Rf_6 = 0.825 respectively.

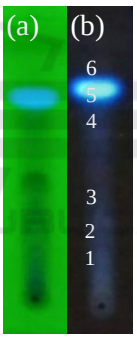

Figure 1. TLC profile of ethyl acetat fraction (Ds-II) after being developed with chloroform : ethyl acetate : methanol (7:2:1) as mobile phase. TLC plate were visualized under UV light at wavelength (a). 254 nm and (b) 366 nm.

3.4 Fractionation of Hs-II fraction by Vacuum Liquid Chromatography (VLC)

A total of 50 g fraction (Ds-II) was separated by Vacuum Liquid Chromatography (VLC). The principle of separation in VLC is based on adsorption chromatography. Higher polar compounds will be more attached to silica gel and more retained in the VLC column. Polar silica gel will bind compounds that are relatively more polar. Compounds with lower levels of polarity will come

out first carried by eluen so that the separation of the compound occurs based on differences in polarity.

The mobile phase used in VLC is *n*-hexane: EtOAc: EtOH with gradient system. From VLC obtained 19 fractions, which then monitored the pattern of spot through TLC. This monitoring was done to see the similarity of pattern to be combined into subfractions.

Based on the similarity of spot pattern, five subfractions were obtained, namely Ds-II-A (fractions 1-3), Ds-II-B (fractions 4-6), Ds-II-C (fraction 7-9), Ds-II-D (fractions 10-12) and Ds-II-E (fractions 13-16). The weight of Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E were obtained 2.65 g, 10.89 g, 13.65 g, 11.94 g and 2.58 g respectively. So that the yield obtained of each subfraction were 0.118%, 0.488%, 0.612%, 0.535% and 0.116% respectively.

3.5 Antidiabetic Activity of Subfraction

Antidiabetic activity of mengkudu fruit subfractions were performed by glucose tolerance method. Mice were divided into seven groups, each consisting of four mice were administered 2% (w/v) PGA, glibenclamide 0.65 mg/kgBW, and subfractions (Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E) 150 mg/kgBW, respectively. Glucose (2 g/kgBW) was fed 30 min after the administration of subfractions. Blood sample was collected at 0, 30, 60, 90, 120 and 150 minutes of glucose administration and blood glucose level was estimated using electronic glucometer (Accu-Chek Active Glucometer) and glucostrips (Accu-Chek Active Diabetic Test Strips).Based on Figure 2, the relative blood glucose levels of mice reached a peak at 30 minutes after oral administration of glucose (2 g/kgBW) and gradually decrease in 150 minutes.

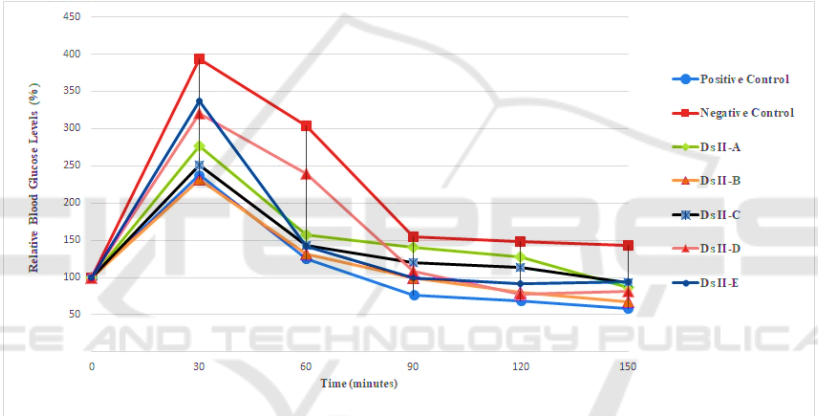

Figure 2. Antidiabetic Activity of Mengkudu Fruit Subfractions

Subfractions of mengkudu fruit at dose 150 mg/kgBW showed antidiabetic activity at minute 30 until minute 150 after administration.The best antidiabetic activity of subfractions (Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E) at a dose 150 mg/kgBW was demonstrated by Ds-II-B subfraction with percent reduction relative blood glucose levels at minute 30, 60, 90, 120 and 150 were 40.92%, 56.87%, 35.72%, 45.99% and 52.85% respectively. Percentage reduction relative blood glucose levels each group shown in Table 2 and Figure 3.

Table 2. Percent Reduction Relative Blood Glucose Levels of Mengkudu Fruit Subfractions

Groups	Reduction Relative Blood Glucose Levels (%)				
	30'	60'	90'	120'	150'
Positive Control	39.43	58.77	50.49	53.23	58.91
Ds II-A	29.58	48.47	9.07	13.84	39.11
Ds II-B	40.92	56.87	35.72	45.99	52.85
Ds II-C	36.09	52.72	23.01	23.58	35.31
Ds II-D	18.62	21.40	30.10	47.67	43.55
Ds II-E	14.19	53.26	36.24	37.88	33.73

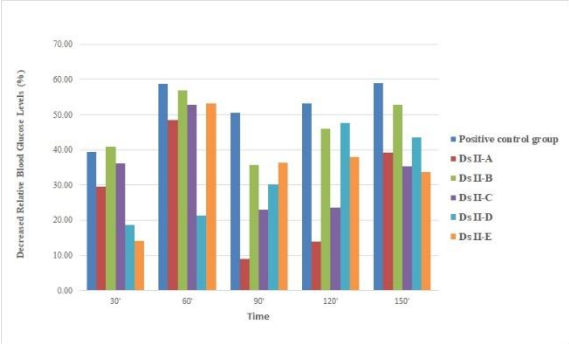

Figure 3. Percent Reduction Relative Blood Glucose Levels of Mengkudu Fruit Subfractions

Based on the analysis of Anava with 95% confidence level can be concluded that there was a significant difference between the treatment given to decreased blood glucose levels of mice. Then a follow-up test with Tukey was conducted to determine the significant differences between the positive control group, Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E compared with the negative control group. The results obtained that the decrease in relative blood glucose levels of the positive control group and all the test groups significantly different compared with the negative control group (sig> 0.05).

Antidiabetic activity of ethyl acetate subfractions caused by enrichment of phytochemical constituents in mengkudu fruit such as caprylic acid, hexanoic acid, caproic acid, vitamin C, vitamin E, niacin, asperulosidic acid, quercetin, 2,6-di-O- (β-D-glucopyranosyl 1-O-octanoyl- β-D glucopyranose, damnacanthal, americanin A, xeronin and scopoletin (Yashaswini *et al*., 2014; Assi, *et. al*., 2015). These compounds are possible to have antidiabetic activity.

An HPLC analysis from previous study showed that the ethyl acetate fraction of mengkudu fruit contained rutin and quercetin from flavonoid grup, and scopoletin compund from coumarin group (Pandy, *et.al*., 2017). These chemical compounds indicate antidiabetic activity from ethyl acetate subfractions of mengkudu fruit.

4. CONCLUSION

Ethyl acetate subfractions from Mengkudu (*M.* citrifolia Linn.) fruit (Ds-II-A, Ds-II-B, Ds-II-C, Ds-II-D and Ds-II-E) showed antidiabetic activity with percentage of reduction relative glucose levels were 39.11%; 52.85%; 35.31%; 43.55% and 33.73% at 150 minutes after sample administration.

These research showed that Ds-II-B subfraction indicate the highest antidiabetic activity by glucose tolerance method. Rutin, quercetin and scopoletin compounds indicate antidiabetic activity from ethyl acetate subfractions of mengkudu fruit. However, the research for the active antidiabetic compound of Ds-II-B subfraction needs to be explored further.

5. ACKNOWLEDGEMENT

The author would like to thank DRPMI Universitas Padjadjaran and Prof. Dr. Ahmad Muhtadi, MS.,Apt. for financial support by Academic Leadership Grant (ALG) scheme in the year of 2017.

6. REFERENCES

Ahmad, Mat Daud dan Ismail. 2016. Review on potential therapeutic effect of *Morinda citrifolia* L., *Current Opinion in Food Science.*

Assi, Reem A., Yusrida Darwis, Ibrahim M. Abdulbaqi, Arshad A. khan, Lim Vuanghao, M.H. Laghari. 2015. *Morinda citrifolia* (Noni): a comprehensive review on its industrial uses, pharmacological activities,and clinical trials. *Arabian Journal of Chemistry*.

Departemen Kesehatan Republik Indonesia. 2013. Laporan Hasil Riset Kesehatan Dasar Indonesia. Jakarta : Badan Penelitian dan Pengembangan Kesehatan, Kementrian Kesehatan RI.

Dipiro, J.T., B.G Wells., T. L. Schwinghammer dan C. V. Dipiro. 2015. *Pharmacotherapy Handbook*, Mc Graw Hill Education. New York, 9th edition.

Hartati, Sri. 2013. Aktivitas antidiabetes ekstrak etanol buah mengkudu (*Morinda citrifolia* L.) pada tikus putih. Skripsi. Universitas Padjadjaran.

IDF .2012. *The Global Guideline for Type 2 Diabetes*, International Diabetes Federation. Brussels.

Nerurkar, P.V., Phoebe W. Hang dan Erik Saksa. 2015. Anti-Diabetic potential of noni : the yin and the yang, *Molecules Journal*.

Pawlus,A.D. dan A.Douglas. 2007. Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni), *Journal of Pharmacy and Pharmacology*.

Ramdhini, N.S. 2005. Aktivitas antidiabetes fraksi air, etil asetat dan *n*-heksana buah mengkudu (*Morinda citrifolia* L.) pada tikus putih. Skripsi. Universitas Padjadjaran.

Rao UM and Subramanian S. 2009. Biochemical evaluation of antihyperglycemic and antioxidative effects of *Morinda citrifolia* fruit extract studied in streptozotocin-induceddiabetic rats, *Med Chem Res*,.

WHO (*World Health Organization*), 2016. Global Report On Diabetes, *WHO Library Cataloguing-in-*

Publication Data. 20 Avenue Appia, 1211 Geneva 27,
Switzerland.