Separation of Ethyl Acetate Fraction of Mengkudu Fruit (Morinda citrifolia L.) and its Hypoglycemic Activity by Glucose Tolerance Method

By Shintia Lintang Charisma

WORD COUNT 3425
TIME SUBMITTED 11-MAY-2020 11:25AM
PAPER ID 58536113

Research Journal of Chemistry and Environment_____Vol. 23(9) September (2019)
Res. J. Chem. Environ.

Separation of Ethyl Acetate Fraction of Mengkudu Fruit (*Morinda citrifolia* L.) and its Hypoglycemic Activity by Glucose Tolerance Method

Shintia Lintang Charisma[1,3*], Yasmiwar Susilawati[1], Ahmad Muhtadi[2] and Asman Sadino[2]
1. Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, INDONESIA
2. Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, INDONESIA
3. Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, INDONESIA
*shintia16001@gmail.com

Abstract

Mengkudu (Morinda citrifolia L.) is one of the Rubiaceae medicinal plant that has been used traditionally for lowering blood glucose level. Previous research showed that ethanolic extract and ethyl acetate fraction gave significant hypoglycemic activity. The ethanolic extract of mengkudu fruit at a dose of 300 mg/kg b.w can increase plasma insulin levels in the group of diabetic rats induced streptozotocin 12.52 µU/mL, while the group was given glyclazide (dose 5 mg/kg bw) of 13.27 µU/mL. The ethyl acetate fraction of mengkudu fruit also gave the best hypoglycemic activity (54.29%) followed by n-hexane fraction (34.18%) and water fraction (47.42%). at dose of 1200 mg/kg body weight on male Wistar rats. The aim of this study was to determine the hypoglycemic effect of ethyl acetate subfraction which was separated from ethyl acetate fraction by liquid vacuum chromatography. Subfraction which shows the strongest hypoglycemic effect, indicates that the subfraction contained hypoglycemic active compounds.

Based on TLC profile, the subfractions were grouped into five subfractions (Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E). The hypoglycemic effect of subfractions was evaluated on male mice by glucose tolerance method at a dose of 150 mg/kg body weight. Blood glucose level was measured at 30, 50, 90, 120 and 150 minutes after administration of subfractions. The subfractions Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E showed hypoglycemic effect at 150 minutes with reduction in relative blood glucose levels as 39.11%, 52.85%, 35.31% 43.55% and 33.78% respectively. The study showed that Mc-II-B subfraction indicates the highest hypoglycemic activity by glucose tolerance method.

Keywords: Separation, *Morinda citrifolia* L., hypoglycemic, glucose tolerance method.

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by elevating blood sugar levels.[5] 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.[8]

Diabetes mellitus is important for concern on health issue because the prevalence of diabetes has been steadily increasing over the past few decades.[3] Basic health research showed the incidence rate of diabetes in Indonesia nearly 6.9 % during 2013.[4] Mengkudu (*Morinda citrifolia* L.) has a long history as medicinal plant in many countries, especially in the continent of Polynesia, South Asia, South East Asia, parts of Australia and the Caribbean continents.[10] Based on the literature, mengkudu has been used as medicinal plant for diabetes treatment.[9]

Chemical compounds reported on mengkudu plants were polysaccharides, fatty acids, glycosides, iridoid, triterpenes, anthraquinones, coumarins, flavonoids, phytosterols, carotenoids and volatile compounds.[1] Mengkudu fruit contains ursolic acid, 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 and americanin A.[2]

Rao and Subramanian[12] showed that the ethanolic extract of mengkudu fruit at a dose of 300 mg/kg b.w can increase plasma insulin levels in the group of diabetic rats induced streptozotocin 12.52 µU/mL while the group was given glyclazide (dose 5 mg/kg bw) of 13.27 µU/mL. This research showed the ability of mengkudu fruit extract in increasing the production of insulin comparable with glyclazide which is one of the oral antidiabetic sulfonylurea group.[12]

Hypoglycemic activity of ethanolic extract of mengkudu fruit had been examined by glucose tolerance method on rats. In the glucose tolerance test on rats, 30, 60, 90 and 120 minutes after administration of the extract at dose of 1200 mg/kg b.w, serum glucose concentration decreased by 13.99%, 31.85%, 44.46% and 56.19 %.[7]

Ramdhini[11] reported that all fractions of mengkudu fruit ethanolic extract can decrease plasma glucose level of male rats at a dose of 1200 mg/kg b.w. by glucose tolerance method. It was known that ethyl acetate fraction had the best hypoglycemic activity 54.29%) followed by *n*-hexane fraction (34.18%) and water fraction (47.42%). Separation and identification of active compounds containing the ethyl

acetate fraction have not been performed.[11] The aim of this study was to determine the hypoglycemic effect of ethyl acetate subfraction which was separated from ethyl acetate fraction by liquid vacuum chromatography. Subfraction which shows the strongest hypoglycemic effect, indicates that the subfraction contained hypoglycemic active compounds.

Material and Methods

Plant materials: The fresh ripe fruits of *M. citrifolia* L. were collected from Lembang, West Java, Indonesia, in October 2017. This plant was identified at Plants Taxonomy Laboratorium, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran. The specimen were collected by number of 444/HB/09/2017.

Plant extraction and phytochemical analysis: Dried mengkudu fruit (2 kg) was extracted in ethanol 70% for 24 hours with 3 times repetitions. The macerate was filtered, collected and concentrated with a rotary evaporator until the solvent has evaporated. The concentrated extract was then reheated at 55°C by water bath until a fixed weight was obtained. The extract was subjected to qualitative chemical tests for identifying of secondary metabolites.[6]

Fractionation of Ethanol Extract by Liquid-Liquid Extraction (LLE): The concentrate extract was dissolved in water, then filled into a separating funnel and add *n*-hexane as the same volume. The *n*-hexane layer separated from water layer. The water portion was fractionated using ethyl acetate in the same way as fractionation with *n*-hexane. The *n*-hexane (Mc-I), ethyl acetate (Mc-II) and water (Mc-III) fractions were evaporated and weighed to get the fractions yield.

Analysis of ethyl acetate fraction (Mc-II) by TLC: Ethyl acetate fraction of mengkudu fruit was spotted on TLC plate 60 F_{254} (Merck, Germany) and developed in mobile phase chloroform : ethyl acetate : methanol (7:2:1). TLC plate was visualized under UV light at wavelength 254 nm and 366 nm and sprayed by using 10% H_2SO_4 reagent.

Separation of ethyl acetate (Mc-II) fraction by VLC: The chromatographic column was packed by dry state in a vacuum condition to obtain the maximum packing density. The ethyl acetate fraction (Mc-II) was dissolved in a suitable solvent, inserted directly at the top of the column, sucked slowly into the package. The columns were eluted by *n*-hexane, ethyl acetate and ethanol with 10% eluent gradient. Result of these VLC separation obtained 19 fractions.

The fractions of VLC separation were analyzed by TLC and the same pattern spots were combined as one subfraction.

Hypoglicemic activity of subfractions

Animals preparation: Experiments were reviewed and approved by Research Ethics Committee Universitas Padjadjaran (Approval no. 132/ UN6.KEP/EC/ 2018). Swiss Webster male mice weighing 20-30g were 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 with adequate nutrient content.

Experimental Design: Before the experiment, normal mice fasted 16 hours, but water still available *ad libitum*. Mice were divided into seven groups, each consisting of four mice, Group I was administered 2% (w/v) PGA, Group II was positive control which was administered Glibenclamide 0.65 mg/kg b.w, Group III-VII were subfractions group which were administered (Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E) 150 mg/kgBW respectively. Glucose (2 g/kg b.w) was fed 30 min after the administration of subfractions. Blood sample was collected at 0, 30, 60, 90, 120 and 150 minutes after glucose administration and blood glucose level was estimated using electronic glucometer (Accu-Chek Active Glucometer) and glucostrips (Accu-Chek Active Diabetic Test Strips).

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

Results and Discussion

Extraction and phytochemical analysis: The percentage yield of ethanol extract was found to be 22.46% w/w. The qualitati phytochemical analysis of the ethanolic extract showed the presence of saponins, triterpenoids, flavonoids, alkaloids and phenolic compounds (Table 1).

Table 1
Phytochemical Analysis of Mengkudu Fruit Ethanolic Extract

S.	Compound Groups	Results
1	Alkaloid	+
2	Flavonoid	+
3	Tannin	-
4	Saponin	+
5	Polyphenol	+
6	Quinone	+
7	Monoterpenoid	+
8	Sesquiterpenoid	+
9	Steroid	-
10	triterpenoid	-

Information:
(+): Detected
(-): Undetected

Fractionation of ethanolic extract by Liquid-Liquid Extraction (LLE): The yields of fractions obtained by LLE methode were *n*-hexane (5.15%); ethyl acetate (2.85%) and water fraction (16.74 %).

Analysis of ethyl acetate fraction (Mc-II) by Thin Layer Chromatography: Ethyl acetate fraction of mengkudu fruit was spotted on TLC plate 60 F_{254} (Merck, Germany) and developed in mobile phase chloroform: ethyl acetate: methanol (7:2:1). TLC plate was visualized under UV light 254 nm and 366 nm, then detected by using 10% H_2SO_4 spray reagent (Figure 1). Based on TLC analysis (Table 2), 6 spots were detected with *Rf* values $Rf_1 = 0.125$; $Rf_2 = 0.225$; $Rf_3 = 0.425$; $Rf_4 = 0.600$; $Rf_5 = 0.700$ and $Rf_6 = 0.825$.

Separation of ethyl acetat fraction (Mc-II) by VLC: A total of 50 g fraction (Mc-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 we obtained 19 fractions, which were then monitored by the pattern of spot through TLC. This monitoring was done to see the similarity of patterns to be combined into subfractions.
Based on the similarity of spot pattern, five subfractions were obtained namely Mc-II-A (fr 1-3), Mc-II-B (fr 4-6), Mc-II-C (fr 7-9), Mc-II-D (fr 10-12) and Mc-II-E (f 13-16). The weights of Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E were obtained as 2.65 g, 10.89 g, 13.65 g, 11.94 g and 2.58 g respectively so that the yields obtained of each subfraction were 0.118%, 0.488%, 0.612%, 0.535% and 0.116% respectively. The TLC profile of subfractions is shown in table 3.

Hypoglycemic activity 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. Based on figure 1, the relative blood glucose levels of mice reached a peak at 30 minutes after oral administration of glucose (2 g/kg b.w) and gradually decrease in 150 minutes.

Subfractions of mengkudu fruit at dose 150 mg/kg b.w showed hypoglycemic effect start at 30' until 150' after administration. The best hypoglicemic effect of subfractions (Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E) at a dose 150 mg/kgBW was demonstrated by Mc-II-B subfraction with reduction in relative blood glucose levels at 30', 60', 90', 120' and 150' were 40.92%, 56.87%, 35.72%, 45.99% and 52.85% respectively. Percentage decrease relative blood glucose levels of each group was shown in table 2 and figure 3.

Table 2
TLC Profile of Ethyl Acetate Fraction (Mc-II)

Spot	Rf	Color		
		254 nm	366 nm	H_2SO_4 10%
1	0.125	Dark blue	Dark blue	Pale red
2	0.225	Dark blue	Dark blue	Pale red
3	0.425	Dark blue	Dark blue	Pale red
4	0.6	Dark blue	Dark blue	White
5	0.7	Blue fluorescence	Blue fluorescence	White
6	0.825	Dark blue	Dark blue	Red violet

Table 3
TLC Profile of Subfractions

Group of Subfractions	Rf	Color		
		254 nm	366 nm	H_2SO_4 10%
Mc-II-A	0.894	Dark blue	Blue fluorescence	Red violet
Mc-II-B	0.237	Dark blue	Light blue	Pale red
	0.342	Dark blue	Blue fluorescence	Pale red
	0.474	Blue fluorescence	Blue fluorescence	Red violet
	0.658	Dark blue	Blue fluorescence	Red violet
Mc-II-C	0.158	Dark blue	Light blue	Brown red
	0.289	Dark blue	Light blue	Brown red
	0.474	Blue fluorescence	Blue fluorescence	Pale red
	0.684	Dark blue	Dark blue	White
Mc-II-D	0.105	Dark Blue	Dark blue	Pale red
Mc-II-E	-	-	-	-

Table 4
Relative Blood Glucose Levels (%) of Mengkudu Fruit Subfractions

Groups	Relative Blood Glucose Levels (%)					
	0'	30'	60'	90'	120'	150'
Normal Control	100±0	105.58±0.44*	100.41±0.34*	97.25±1.24*	88.98±0.29*	76.08±1.05*
Positive Control	100±0	238.07±10.7*	125.33±10.23*	76.75±8.87*	69.18±0.83*	58.82±3.84*
Negative Control	100±0	392.02±8.6	303.94±13.96	225.93±7.36	147.92±0.75	143.14±3.72
Mc-II-A	100±0	276.77±54.57*	156.61±16.63*	140.96±15.28	127.44±20.93	87.15±8.44*
Mc-II-B	100±0	232.18±11.86*	131.09±11.34*	99.64±6.21*	79.89±5.11*	67.48±4.05*
Mc-II-C	100±0	251.2±21.18*	143.7±0.9*	119.35±1.6*	113.05±6.74*	92.59±0.32*
Mc-II-D	100±0	319.85±12.41	238.89±7.3*	108.37±7.33*	77.41±2.44*	80.8±2.35*
Mc-II-E	100±0	337.24±27.38	142.05±14.95*	98.84±5.88*	91.88±6.01*	94.86±5.55*

The data displayed is Average ± SD n = 3. *Statistically significant (p <0.05)
Statistically significant was compared to negative controls using Anova test followed by Tukey test

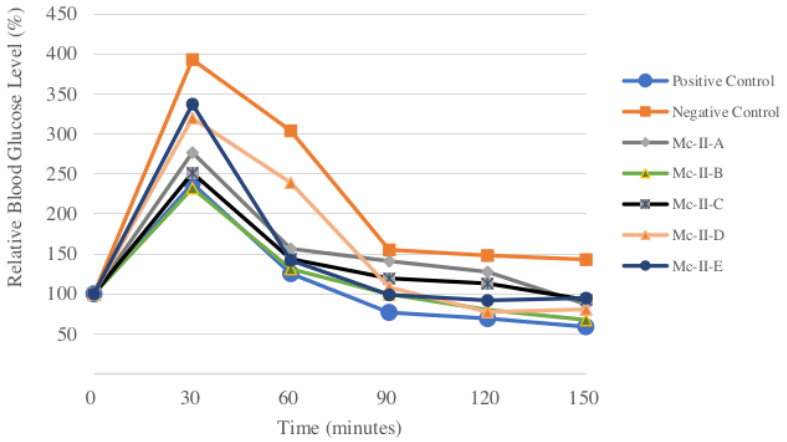

Figure 1: Hypoglicemic effect of mengkudu fruit subfractions

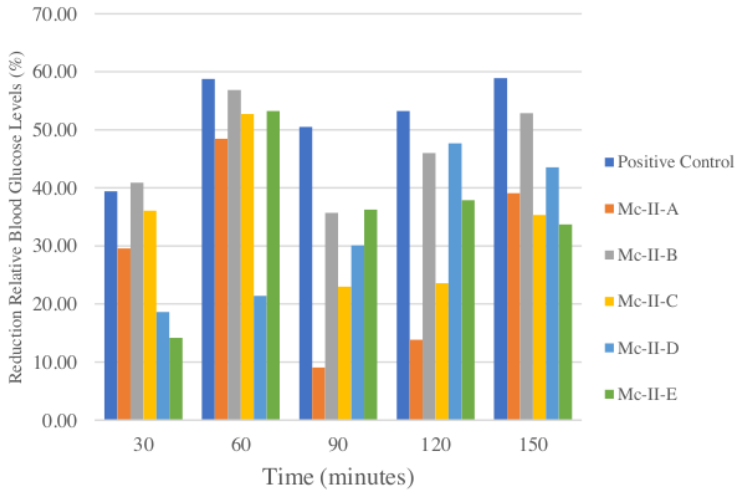

Figure 2: Decrease Level of Relative Blood Glucose of Mengkudu Fruit Subfractions

Based on the analysis of ANOVA with 95% confidence level, it can be concluded that there was a significant difference between the treatment given to decrease blood glucose levels of mice. Then a further test with Tukey was conducted to determine the significant differences between the positive control group, Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-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 was significantly different compared with the negative control group ($p < 0.05$).

Hypoglycemic effect of ethyl acetate subfractions is caused in 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.[2,14] These compounds are possible to have hypoglicemic effect. The structure of the phytochemical compounds found in mengkudu fruit is shown in figure 3.

An HPLC analysis from previous study showed that the ethyl acetate fraction of mengkudu fruit contained flavonoid rutin and quercetin and scopoletin compound from coumarin group.[15] These chemical compounds indicate hypoglycemic effect from ethyl acetate subfractions of mengkudu fruit.

Conclusion

Ethyl acetate subfractions from mengkudu fruit (Mc-II-A, Mc-II-B, Mc-II-C, Mc-II-D and Mc-II-E) showed hypoglicemic effect with reduction in relative glucose levels as 39.11%; 52.85%; 35.31%; 43.55% and 33.73% at 150 minutes after sample administration.

Table 4
Decrease Relative Blood Glucose Levels (%) of Mengkudu Fruit Subfractions

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

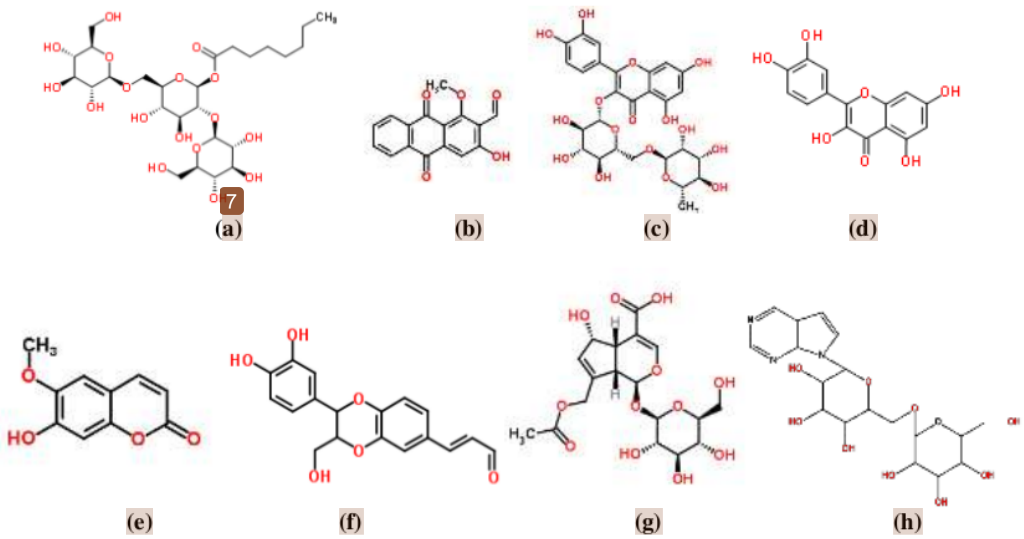

Figure 3: Phytochemical constituents of mengkudu fruit (a). 6-di-O- β-D-glucopyranosyl 1-O-octanoyl- β-D glucopyranose; (b). Damnacanthal; (c). Rutin; (d). Quercetin; (e). Scopoletin; (f). Americanin A; (g). Asperulosidic acid and (h). Xeronine.[2,14]

The research showed that Mc-II-B subfraction indicates the highest hypoglicemic effect by glucose tolerance method. Rutin, quercetin and scopoletin compounds indicate hypoglycemic effect from ethyl acetate subfractions of mengkudu fruit because based on polarity, they are semi-polar compounds which can be dissolved in the ethyl acetate subfraction. However, the research for the active hypoglycemic compound of Mc-II-B subfraction needs to be explored further.

Acknowledgement

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

References

1. Ahmad A.N., Mat Daud Z.A. and Amin Ismail, Review on potential therapeutic effect of *Morinda citrifolia* L., *Current Opinion in Food Science*, **8**, 62–67 **(2016)**

2. Assi Reem A., Darwis Yusrida, Abdulbaqi Ibrahim M., khan Arshad A., Vuanghao Lim and Laghari M.H., *Morinda citrifolia* (Noni): a comprehensive review on its industrial uses, pharmacological activities and clinical trials, *Arabian Journal of Chemistry*, **10(5)**, 691-707 **(2015)**

3. Danaei G., Mariel M.F., Lu Yuan, Gitanjali M.S., Melanie J.C., Christopher J.P., John K.L., Farshad F., Young-Ho K., Gretchen A.S., Rao Mayuree, Mohammed K.A., Leanne M.R., Carolyn A.R. and Majid E., National, regional and global trends in fasting plasma glucose and diabetes prevalence since 1980, systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants, *Lancet*, **378(9785)**, 31-40 **(2011)**

4. Departemen Kesehatan Republik Indonesia, Laporan Hasil Riset Kesehatan Dasar Indonesia, Jakarta, Badan Penelitian dan Pengembangan Kesehatan, Kementrian Kesehatan RI **(2013)**

5. Dipiro J.T., Wells B.G., Schwinghammer T.L. and Dipiro C.V., Pharmacotherapy Handbook, Ninth Edition, New York, McGraw Hills Education **(2015)**

6. Farnsworth N.R., Biological and Phytochemical Screening of Plants, *Journal Pharm Sci*, **55**, 225-276 **(1966)**

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

8. International Diabetes Federation (IDF), IDF Diabetes Atlas, International Diabetes Federation, Brussels, 8th edition **(2017)**

9. Nerurkar P.V., Hang Phoebe W. and Saksa dan Erik, Anti-Diabetic potential of noni: the yin and the yang, *Molecules Journal*, **20**, 17684-17719 **(2015)**

10. Pawlus A.D. and Douglas dan A., Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni), *Journal of Pharmacy and Pharmacology*, **59(12)**, 1587-1609 **(2007)**

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

12. Rao U.M. and Subramanian S., Biochemical evaluation of antihyperglycemic and antioxidative effects of *Morinda citrifolia* fruit extract studied in streptozotocin-induceddiabetic rats, *Med Chem Res*, **18**, 433-446 **(2009)**

13. WHO (World Health Organization), Global Report on Diabetes, WHO Library Cataloguing-in-Publication Data, 20 Avenue Appia, 1211 Geneva 27, Switzerland **(2016)**

14. Yashahswini S., Venugopal C.K., Hegde R.V. and Mokashi A.N., Noni: A new medicinal plant for the tropics, *African Journal of Plant Science*, **8**, 243-247 **(2014)**

15. Pandey A. and Tripathi S., Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug, *Journal of Pharmacognosy Phytochemistry*, **2**, 115-119 **(2014)**.

(Received 19th September 2018, accepted 10th June 2019)

Separation of Ethyl Acetate Fraction of Mengkudu Fruit (Morinda citrifolia L.) and its Hypoglycemic Activity by Glucose Tolerance Method

ORIGINALITY REPORT

7%

SIMILARITY INDEX

PRIMARY SOURCES

1	media.neliti.com Internet	55 words — 2%
2	article.scholarena.com Internet	20 words — 1%
3	Abdelrahim, Maha, Babiker Elamin, Dalia Khalil, and Samia Badwi. "Antidiarrhoeal Activity of Ethanolic Extract of Adansonia digitata Fruit Pulp in Rats.", Journal of Physiology and Pharmacology Advances, 2013. Crossref	19 words — 1%
4	www.scitepress.org Internet	17 words — 1%
5	Erwin Ross. "Indicator Reagents", Ullmann s Encyclopedia of Industrial Chemistry, 06/15/2000 Crossref	16 words — 1%
6	cronfa.swan.ac.uk Internet	16 words — 1%
7	www.hindawi.com Internet	12 words — < 1%
8	ojs.iik.ac.id Internet	11 words — < 1%
9	link.springer.com Internet	10 words — < 1%

10	unsri.portalgaruda.org Internet	10 words — < 1%
11	Indri Hapsari, Marchaban Marchaban, Chairun Wiedyaningsih, Susi Ari Kristina. "The Extemporaneous Compounding at Primary Health Care Centers: Characteristic and Personnel", Global Journal of Health Science, 2018 Crossref	8 words — < 1%
12	S. M. Al-Salem, A. R. Khan. "Monitoring and Modelling the Trends of Primary and Secondary Air Pollution Precursors: The Case of the State of Kuwait", International Journal of Chemical Engineering, 2010 Crossref	7 words — < 1%
13	Amir H. Mahmoudkhani, Vratislav Langer. " Structure of [(CH) NH] CoCl at elevated temperatures ", Acta Crystallographica Section B Structural Science, 1999 Crossref	7 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		