Vol. 9 No. 1 April 2022

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

E-ISSN: 2580-8303
P-ISSN: 2406-9388

PUBLISHED BY:
FACULTY OF PHARMACY UNIVERSITAS AIRLANGGA in collaboration with
INDONESIAN PHARMACISTS ASSOCIATION (IAI) OF EAST JAVA

Accredited SINTA 2
No: B/1796/E5.2/KI.02.00/2020

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

Faculty of Pharmacy, Universitas Airlangga
Jl. Dr. Ir. H. Soekarno, Mulyorejo,
Surabaya, East Java – 60115, Indonesia
Phone. (6231) 5933150, Fax. (6231) 5932594
Website:
http://e-journal.unair.ac.id/index.php/JFIKI
Email: jfiki@ff.unair.ac.id

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia (Pharmacy and Pharmaceutical Sciences Journal) P-ISSN: 2406-9388; E-ISSN: 2580-8303 is an official journal published by the Faculty of Pharmacy, Universitas Airlangga in collaboration with Indonesian Pharmacists Association (IAI) of East Java which the articles can be accessed and downloaded online by the public (open-access journal).

This journal is a peer-reviewed journal published three times a year on topics of excellence of research outcomes in the fields of pharmacy service and practise, community medicine, pharmaceutical technology, and health science disciplines that are closely related. This journal only accepts English-language submission. The following are the research areas that this journal focuses on

1. Clinical Pharmacy
2. Community Pharmacy
3. Pharmaceutics
4. Pharmaceutical Chemistry
5. Pharmacognosy
6. Phytochemistry

This journal receives a manuscript from the research results, systematic reviews and meta analyses that are closely related to the health sector, particularly the pharmaceutical field. Selected manuscripts for publication in this journal will be sent to two reviewers, experts in their field, who are not affiliated with the same institution as the author(s). Reviewers are chosen based on the consideration of the editorial team. Manuscripts accepted for publication are edited copies checked for the grammar, punctuation, print style, and format. The entire process of submitting manuscripts to make a final decision for publishing isconducted online.

Table of Content

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 1-7
DOI: 10.20473/jfiki.v9i12022.1-7
Available online at https://e-journal.unair.ac.id/JFIKI/

Cost of Illness Type 2 Diabetes Mellitus Outpatient BPJS on Malang City Health Center

Ika Ratna Hidayati[1], Aniza Oktaviana[1]*, Liza Pristianty[2]
[1]Departement of Pharmacy, Faculty Health of Science, University of Muhammadiyah Malang
[2]Department of Phamacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: anizaoktaviana@gmail.com

Submitted: 27 October 2020
Accepted: 2 February 2022
Published: 26 April 2022

Abstract

Background*: Type 2 diabetes mellitus is a progressive illness that impacts the costs borne by patients. The Cost of Illness method can be used further to analyze the medical expenses for type 2 diabetes mellitus.* ***Objective****: Determine the annual total cost of type 2 diabetes mellitus outpatients in Indonesia Health Insurance (BPJS) participants and treated with metformin-glibenclamide.* ***Methods****: This study applied the non-probability sampling technique and the purposive sampling method to the cross-sectional approach. The research was conducted in the Mulyorejo Health Care of Malang City, using 58 patients as samples. The research instrument involves a systematic interview that has been tested for its validity. The data were analyzed using Microsoft Excel.* ***Results****: The direct medical cost per patient is IDR 173,560.00 – IDR 1,266,240.00. Non-medical direct cost is IDR 0.00 – IDR 240,000,00. The indirect cost is IDR 0.00 – IDR 1,920.000,00.* ***Conclusion****: The estimated annual total medical expenses of diabetes mellitus type 2 Indonesia Health Insurance (BPJS) outpatients employing metformin-glibenclamide therapy at Mulyorejo Health Center in Malang is IDR 173,560.00 – IDR 3,426,240.00.*

Keywords*: cost of illness, direct medical cost, direct non-medical cost, indirect cost, type 2 diabetes mellitus*

How to cite this article:
Hidayati, I. R., Oktaviana, A., & Pristianty, L. (2022). Cost of Illness Type 2 Diabetes Mellitus Outpatient BPJS on Malang City Health Center. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 1–7. http://doi.org/10.20473/jfiki.v9i12022.1-7

INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous metabolic disorder characterized by hyperglycemia caused by impaired insulin secretion, impaired insulin action, or both (Punthakee *et al*., 2018). DM is a chronic disease that requires long-term medical care. The DM classification consists of type 1 diabetes, type 2 diabetes, gestational diabetes mellitus (GDM), and other types of diabetes (American Diabetes Association, 2017). The therapy used in type 2 DM is pharmacological and non-pharmacological. The pharmacological treatment uses oral antidiabetic drugs and insulin, while non-pharmacological treatment involves healthy lifestyles changes (PERKENI, 2019). Based on the results of Riskesdas in 2018, the prevalence of DM in Indonesia was 10.9% in which 2.6%, in East Java and 1.9% in Malang, (Kementrian Kesehatan Republik Indonesia, 2018).

According to the International Diabetes Federation (IDF), in 2019, the total health expenditure related to diabetes mellitus in the world in 2017 was USD 727 billion, and in 2019 IDF estimated that the total price was USD 760 billion an increase of 45%. Treatment costs in developed countries range from 1500 - 9000 USD/DM patient/year, treatment costs in developing countries are between 50 – 2000 USD/DM patient/year, and in Indonesia, 80.22 USD/DM patient/year (International Diabetes Federation, 2019). The average annual cost (both direct and indirect) of type 2 DM in low and middle-income countries ranges from 29.91 USD/patient to 237.38 USD/patient (Afroz *et al*., 2018)

Cost is an essential factor in health services. According to Rascati (2013) in Essentials of Pharmacoeconomics, the cost of illness includes direct and indirect costs. The direct costs are divided into direct medical and direct non-medical costs. Direct medical costs include drug costs, patient counselling and consultation, inpatient, outpatient, emergency, ambulance services, and nursing services. Meanwhile, direct non-medical costs include transportation, food, and lodging costs during treatment. Indirect costs are costs due to the loss of productivity caused by a disease.

Mursalin & Soewondo (2016) stated that the average direct medical cost of type 2 DM outpatients at RSUD Dr Abdul Aziz Singkawang in one year amounted to Rp. 2,406,325.00. The largest cost type is medicine at 75.65%. At the same time, costs other than drugs are 24.35%, which include laboratory costs and additional costs (Mursalin & Soewondo, 2017). Research by Baroroh *et al*. (2016) showed that the average total cost of outpatient type 2 DM at PKU Muhammadiyah Bantul Hospital Yogyakarta without complications ranged from Rp. 247,309.00 - Rp. 686,753.00 per month. The average cost of outpatient type 2 DM with complications ranges from Rp 128,143.00 – Rp 1,174,342.00 per month due to the type of therapy and the cost of antidiabetic drugs well as the price of drug complications (Baroroh *et al*., 2016).

The high cost of health care and the prevalence of diabetes mellitus impairs a country's economy and productivity. Through the Ministry of Health of the Republic of Indonesia, the Government of Indonesia has made several programs to control diabetes mellitus, one of which is the National Health Insurance (JKN) program by the Health Social Security Administering Body (BPJS). Based on the Regulation of the Minister of Health of the Republic of Indonesia No. 52 of 2016 concerning Health Service Tariffs in the Implementation of the Health Insurance Program, BPJS fees are divided into capitation costs and non-capitation costs. The capitation fee is paid in advance by BPJS Health every month to the First-Tier Facility, which considers the number of registered participants without considering the type and amount of health services provided. In contrast, the non-capitation fee is a fee that is claimed by BPJS Health to the First Level Health Facility every month, considering the type and amount of health services provided. When the claims submitted for DM surpass the claim ceiling, it is frequently found that there is a disparity between the actual expenditures spent for therapy and the capitation rate.

Cost of Illness (COI) estimation plays an essential role in decision-making in a chronic disease such as diabetes mellitus because it can estimate the cost of illness (Darmawan *et al*., 2019). Therefore, research on the total cost of treatment for type 2 DM is necessary for making a policy in this JKN era.

This study aims to determine the total cost of treating type 2 DM patients with outpatient metformin-glibenclamide therapy for BPJS participants at the Mulyorejo Health Center in Malang City.

MATERIALS AND METHODS

This research is an observational study that uses a cross-sectional approach with data collection in a specific period (Masturoh & Anggita, 2018).

The sampling technique used is non-probability sampling in the form of consecutive selection. The

P-ISSN: 2406-9388
E-ISSN: 2580-8303

population in this study was all type 2 DM patients with outpatient metformin-glibenclamide therapy for BPJS participants at the Mulyorejo Health Center in Malang City. The research sample is a population that meets the following inclusion criteria:

1. Type 2 DM patients with metformin-glibenclamide therapy at the Mulyorejo Health Center Malang City
2. Patients aged 18 years and over
3. BPJS participant patients
4. Patients who are willing to be research respondents

The location of this research is in Public Health Center Mulyorejo Malang City. The study was carried out from July 29 to August 22, 2020. This research was ethically compliant with the ethics number No.E.5.a/188/KEPK-UMM/VII/2020, published on July 27, 2020, by the Health Research Ethics Commission University of Muhammadiyah Malang.

The variables in this study are direct medical costs, including registration fees, laboratory fees, costs for doctor examinations, costs for drugs obtained from health centers, and costs for purchasing drugs themselves. Transportation costs are an example of direct non-medical costs. In addition, there are indirect costs such as lost patient income and lost patient companion income.

Data collection

A structured interview was used to collect data, including a list of approved questions. Prior to collect data, the researcher obtained informed consent from the respondent by filling out a consent form, and then proceeded with the interview.

Validity test

The validity test used is in the form of content validity, which is to see the suitability of the contents of the interview guide with the variables you want to know. In this study, interview questions were derived from a literature review conducted by researchers, who then had experts test the validity of the interview questions.

Data analysis

After processing the data, they will be analyzed using Microsoft Excel. Based on the following calculations:

COI = (Direct medical costs × frequency of treatment in one year) + (Direct non-medical costs × frequency of treatment in one year) + (Indirect costs × frequency of treatment in one year).

The estimated total cost of treating type 2 DM patients BPJS outpatients with metformin-glibenclamide therapy in one year is obtained.

RESULTS AND DISCUSSION

Patient demographic data

A total of 58 patients met the inclusion criteria and participated in the interview. More than 70% (Table 1) of the subjects are female, and, based on previous research, this gender has a greater risk (71.2%) of suffering from DM than men because they have a less active lifestyle. On the other hand, women are more at risk of suffering from type 2 diabetes mellitus because women have a larger body mass (Rantung *et al*., 2015).

Most types of work are housewives 22 (38%), this is because the activities of homemakers are more often at home, and lack of exercise can cause obesity. The effects are significant changes in metabolic function and endocrine function, which can stimulate obesity (Wijaya *et al*., 2015). These effects can be a triggering factor for DM.

The highest education level was an elementary school with 25 patients (43%). Low education level affects the incidence of DM because a lower level of education can affect thinking patterns related to health awareness (Wijaya *et al*., 2015).

Financial limitations can limit patients from seeking information about their illness and affect their motivation to carry out treatment (Musdalifah & Nugroho, 2020). In this study, the highest significant patient income was < Rp 500,000.00, with 24 patients (41%). This is because socioeconomic status and knowledge about diabetes can affect self-care management with DM.

Demographic data based on BPJS participation obtained the highest BPJS class, namely BPJS class 3, with 24 patients (41%). Following previous research, BPJS class 3 is one of the most frequently used insurances (Nur *et al.*, 2018). Other researchers also stated that the BPJS class chosen by the most respondents was class 3, including PBI, which is generally included in class 3 payments (Lesmana & Sugiman, 2020).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Demographic data of type 2 diabetes mellitus patients with metformin-glibenclamide therapy outpatient BPJS participants

Demographic Data	Number of Respondents (%)
Sex	
Male	17(29)
Female	41(71)
Age	
18 - 25 years	0(0)
26 - 35 years	0(0)
36 - 45 years	4(7)
46 - 55 years	16(28)
56 - 65 years	21(36)
> 65 years	17(29)
Education	
No education	7(12)
Elementary school	25(43)
Junior high school	8(14)
High school	14(24)
College	4(7)
Occupation	
Student	0(0)
Civil servant	0(0)
Employee	6(10)
Entrepreneur	19(33)
Housemaker	22(38)
Unemployed	11(19)
Income	
< Rp 500,000.00	24(41)
Rp.500,000.00 – Rp 1000,000.00	14(24)
Rp 1000,000.00 – Rp 2000,000.00	7(12)
> Rp 2000,000.00	14(22)
BPJS Participant	
Contribution Assistance Recipients (PBI)*	18(31)
Independent Class 1	7(12)
Independent Class 2	9(16)
Independent Class 3	24(41)

*PBI: Penerima Bantuan Iuran

Table 2. Medical direct cost

Cost component	Cost Range Within 1 Year (IDR)	Average Range Per Patient (IDR)
Registration	120,000.00 – 5,760,000.00	40,000.00 – 120,000.00
Laboratory	120,000.00 – 5,760,000.00	40,000.00 – 120,000.00
Doctor's Examination	42,000.00 – 2,016,000.00	14,000.00 – 42,000,00
Drugs Obtained from the Health Center	79,560.00 – 7,399,080.00	79,560.00 – 318,240.00
Self-bought medicine	0.00 – 666,000.00	0.00 – 666,000.00
Medical Direct Cost Total Range	361,560.00 – 21,601,080.00	173,560.00 – 1,266,240.00

Medical direct cost

In this study, direct medical costs include registration fees, laboratory fees, charges for examinations by doctors, prices for drugs obtained from the public health center and the cost of self-bought medicine.

In this study, the average range of direct medical costs per patient in one year based on patient visits in Table 2 was 173,560.00 IDR - 1,266,240.00 IDR.

The components of registration fees and laboratory fees based on the health service retribution at the Mulyorejo Health Center are 10,000.00 IDR, so the range of registration fees per patient in one year based on the frequency of patient visits is 40,000.00 IDR – 120,000.00 IDR. The cost range is due to the different frequency of patient visits to the public health centre, namely four times, six times, and 12 times in one year.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Examination fees by doctors were referring to BPJS Health Regulation Number 2 of 2015 concerning Norms for Determining Capitation Amounts and Capitation Payments Based on Fulfillment of Service Commitments at First Level Health Facilities, for doctor services fees with the number of patients registered with BPJS 15,000.00 IDR – 20,000.00 IDR with 24-hour service time which is 3,500.00 IDR per patient. As a result, the cost of a doctor's examination per patient in a year ranges from 14,000.00 IDR to 42,000.00 IDR. There is a range in these costs due to the different frequency of patient visits to the public health center.

In the component of drug costs obtained from the public health center, namely metformin and glibenclamide, the cost per patient in one year is 79,560.00 IDR – 318,240.00 IDR. In one year, the range of costs per patient is 0.00 IDR – 666,000.00 IDR. This cost component is the most significant because the cost of herbal medicines is quite expensive, and the price of treatment when the patient buys it himself is higher than the medicine obtained from the public health center. Patients buy drugs independently for metformin and glibenclamide drugs because of the pandemic, so there are restrictions on going to the public health center (Puskesmas).

Non-medical direct costs

Non-medical direct costs include transportation costs. In this study, the average range of non-medical direct costs per patient in one year based on the frequency of patient visits in Table 3 is 0.00 IDR – 240,000.00 IDR. There are variations in transportation costs because the transportation used by patients varies, ranging from motorbikes, motorcycle taxis, and public transportation. However, some patients walk to the public health center because the distance from their homes to the public health center is not too far. Furthermore, the variation in transportation costs is due to the distance to the public health center and the frequency of visits.

Indirect cost

Indirect costs include lost patient income and lost patient companion income. In this study, the average range of indirect costs per patient in one year based on the frequency of patient visits in Table 4 is 0.00 IDR – 3,600,000.00 IDR.

In the cost component of income for patients lost due to illness, the cost range per patient in one year is 0.00 IDR – 2,400,000.00 IDR, and in the cost component of the income of the companion of patients lost due to illness, the cost range per patient in one year is 0.00 IDR – 1,200,000.00 IDR. There is a range of costs in these cost components because the amount of income and patient visits to the public health center vary.

The total *cost of illness*

From the data on direct medical costs, direct non-medical costs, and indirect costs that have been calculated and processed previously, the total cost of illness for type 2 diabetes mellitus patients can be seen in one year.

In this study, the average cost of illness per patient in one year based on the frequency of patient visits in Table 5 obtained 173,560.00 IDR – 5,106,240.00 IDR.

Table 3. Indirect cost

Cost Component	Cost Range Within 1 Year (IDR)	Average Range Per Patient (IDR)
Lost Patient Income	0.00 – 1,600,000.00	0.00 – 2,400,000.00
Lost Patient Companion Income	0.00 – 1,200,000.00	0.00 – 1,200,000.00
Total Indirect Cost Range	0.00 – 4,800,000.00	0.00 – 3,600,000.00

Table 4. Total *cost of illness*

Cost Component	Cost Range Within 1 Year (IDR)	Average Range Per Patient (IDR)
Medical Direct Cost	361,560.00 – 21,601,080.00	173,560.00 – 1,266,240.00
Non-Medical Direct Costs	0.00 – 720,000.00	0.00 – 240,000.00
Indirect Cost	0.00 – 3,600,000.00	0.00 – 3,600,000.00
Total Range Cost of Illness	361,650.00 – 25,921,080.00	173,560.00 – 5,106,240.00

Table 5. Non-medical direct costs

Total Non-Medical Direct Cost Range	Cost Range Within 1 Year (IDR)	Average Range Per Patient (IDR)
Transportation	0.00 – 720,000.00	0.00 – 240,000.00

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The results of this study indicate that indirect costs are more significant than direct costs because the indirect costs consist of loss of patient income and loss of income for patient companions, which, based on the study results, show that in a day, the lost income can reach 100,000.00 IDR so that when multiplied by the frequency of visits in one year yields a value greater than the total direct costs. In addition, patients do not incur charges for immediate medical expenses because they are registered as BPJS participants. These results are following research by Lebbe & Rinosha (2018) in Sri Lanka that indirect costs are more significant at $68.94 (1,021,346.00 IDR) per month than direct costs, which are $19 (281,485.00 IDR) per month.

Because BPJS bears several variable costs, it may help alleviate the burden on the economy of patients undergoing treatment. This study had some limitations, including the fact that it was conducted on a single public health center, the conditions of which could differ from those of other public health centers, so the results could not be generalized directly. Interviews were also conducted during the COVID-19 pandemic, impacting the difference in visits between one patient and another.

CONCLUSION

The research shows that the total cost of treating type 2 DM patients with outpatient metformin-glibenclamide therapy for BPJS participants at the Mulyorejo Health Center Malang City for one year is 173,560.00 IDR – 5,106,240.00 IDR. Direct medical costs per patient are 173,560.00 IDR – 1,266,240.00 IDR. Direct non-medical costs per patient are 0.00 IDR – 240,000.00 IDR. And the indirect costs per patient are 0.00 IDR – 3,600,000.00 IDR. Indirect costs are more significant than direct costs.

AUTHOR CONTRIBUTIONS

Conceptualization, L.P.; Methodology, I.R.H., L.P., A.O.; Software, A.O.; Validation, A.O., L.P., I.R.H.; Formal Analysis, A.O.; Investigation, A.O.; Resources, A.O.; Data Curation, A.O., L.P., I.R.H.; Writing - Original Draft, I.R.H., A.O., L.P.; Writing - Review & Editing, A.O., I.R.H.; Visualization, I.R.H., A.O.; Supervision, I.R.H.; Project Administration, I.R.H., A.O., L.P.; Funding Acquisition, I.R.H.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Afroz, A., Alramadan, M. J., Hossain, M. N., Romero, L., Alam, K., Magliano, D. J. & Billah B. (2018). Cost-of-Illness of Type 2 Diabetes Mellitus in Low and Lower-Middle Income Countries: a systematic review. *BMC Health Services Research*; *18*; 1–10. doi: 10.1136/bmjgh-2018-001258.

American Diabetes Association. (2017). Classification and Diagnosis of Diabetes. *Diabetes Care*; *40*; S11–S24.

Baroroh, F., Solikah, W. Y. & Urfiyya, Q. A. (2016). Analisis Biaya Terapi Diabetes Melitus Tipe 2 di Rumah Sakit PKU Muhammadiyah Bantul Yogyakarta. *Jurnal Farmasi Sains dan Praktis*; *I*; 11–22. doi: 10.31603/pharmacy.v1i2.230.

Darmawan, E., Putri, R. E. K. & Perwitasari, D. A. (2019). Cost of Illness Diabetes Melitus Tipe 2 dan Komplikasinya pada Peserta Jaminan Kesehatan Nasional (JKN) di Rawat Jalan Rumah Sakit Condong Catur Yogyakarta. *Jurnal Farmasi Indonesia*; *16*; 89–101. doi: 10.23917/pharmacon.v16i2.8915.

International Diabetes Federation. (2019). IDF Diabetes Atlas Ninth Edition. https://diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html. Accessed: 23 October 2020.

Kementrian Kesehatan Republik Indonesia. (2018). Hasil Utama Riset Kesehata Dasar (RISKESDAS) 2018. https://kesmas.kemkes.go.id/assets/upload/dir_519d41d8cd98f00/files/Hasil-riskesdas-2018_1274.pdf. Accessed: 23 October 2020.

Lebbe, S. M. A. & Rinosha, K. F. (2018). Economic Cost of Diabetes in Ampara District in Sri Lanka. *Journal of Politics and Law*; *11*; 146. doi: 10.5539/jpl.v11n4p146.

Lesmana, T. C. & Sugiman. (2020). Determinan Pemilihan Kelas BPJS Peserta Jaminan Kesehatan Nasional Mandiri. *Jurnal Endurance: Kajian Ilmiah Problema Kesehatan*; *5*; 216–226. doi: 10.22216/jen.v5i2.4979.

Masturoh, I. & Anggita, T. N. (2018). Metodologi Penelitian Kesehatan. http://bppsdmk.kemkes.go.id/pusdiksdmk/wp-content/uploads/2018/09/Metodologi-Penelitian-Kesehatan_SC.pdf. Accessed: 23 October 2020.

Mursalin & Soewondo, P. (2017). Analisis Estimasi Biaya Langsung Medis Penderita Rawat Jalan

Diabetes Mellitus Tipe 2 di RSUD Dr. Abdul Aziz Singkawang Tahun 2013. *Jurnal Ekonomi Kesehatan Indonesia*; *1*; 5–15. doi: 10.7454/eki.v1i2.1770.

Musdalifah & Nugroho, P. S. (2020). Hubungan Jenis Kelamin dan Tingkat Ekonomi dengan Kejadian Diabetes Melitus di Wilayah Kerja Puskesmas Palaran Kota Samarinda Tahun 2019. *Borneo Student Research*; *1*; 1238–1242.

Nur, R. A., Ab, I. & Setyowati, D. L. (2018). Analisis Fator yang Mempengaruhi Pemilihan Kelas Kepesertaan Jaminan Kesehatan Nasional. *Faletehan Health Journal*; *5*; 135-141. doi: 10.33746/fhj.v5i3.32.

PERKENI. (2019). Pedoman Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 Dewasa di Indonesia 2019. https://pbperkeni.or.id/wp-content/uploads/2020/07/Pedoman-Pengelolaan-DM-Tipe-2-Dewasa-di-Indonesia-eBook-PDF-1.pdf. Accessed: 23 October 2020.

Punthakee, Z., Goldenberg, R. & Katz, P. (2018). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Canadian Journal of Diabetes*; *42*; S10–S15. doi: 10.1016/j.jcjd.2017.10.003.

Rantung, J., Yetti, K. & Herawati, T. (2015). Hubungan Self-Care dengan Kualitas Hidup Pasien Diabetes Mellitus (DM) di Persatuan Diabetes Indonesia (PERSADIA) Cabang Cimahi. *Jurnal Skolastik Keperawatan*; *1*; 38–51. doi: 10.35974/jsk.v1i01.17.

Rascati, K. L. (2013). Essentials of Pharmacoeconomics: Second Edition (2nd ed). Philadelphia: Wolters Kluwer Health.

Wijaya, I. N., Faturrohmah, A., Agustin, W. W., Soesanto, T. G., Kartika, D. & Prasasti, H. (2015). Profil Kepatuhan Pasien Diabetes Melitus Puskesmas Wilayah Surabaya Timur Dalam Menggunakan Obat Dengan Metode Pill Count. *Jurnal Farmasi Komunitas*; *2*; 18–22.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 8-16
DOI: 10.20473/jfiki.v9i12022.8-16
Available online at https://e-journal.unair.ac.id/JFIKI/

Physicochemical Characteristics, Stability, and Irritability of Nanostructured Lipid Carrier System Stabilized with Different Surfactant Ratios

Dyah Rahmasari[1], Noorma Rosita[2]*, Widji Soeratri[2]
[1]Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Malang, Malang, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: noorma-r@ff.unair.ac.id

Submitted: 17 May 2021
Accepted: 3 November 2021
Published: 26 April 2022

Abstract

Background*: One of the vital variables affecting the stability and the characteristics of the Nanostructured Lipid Carrier (NLC) is the surfactant concentration. Using the two combinations of surfactants can cause higher stability and a better characteristic of NLC. Tween 80 and Span 20 are anionic surfactants whose combination has not been studied for use in NLC systems.* ***Objective****: Determine the effect of different surfactant ratios of Tween 80 and Span 20 on the physicochemical characteristics, stability, and irritability of NLC using the High Shear Homogenization (HSH) method.* ***Methods****: Four different surfactant ratios were used in the NLC formulation, in which the ratio of Tween 80:Span 20 were 5:5, 6:6, 7:7, and 8:8, respectively. In this NLC system, cetyl palmitate served as solid lipid, medium-chain triglyceride (Crodamol™) as liquid lipid, Tween 80, and Span 20 as surfactant components. NLC was characterized for organoleptic, viscosity, pH, zeta potential, particle morphology, particle size, and polydispersity index (PDI), then evaluated for stability using the real-time and freeze-thaw method, and irritability effect.* ***Results****: The different ratios of Tween 80 and Span 20 had no significant effect on the particle size, PI, and irritation score of the NLC system. On the other hand, it influenced all formulas' pH value, viscosity, zeta potential, and stability.* ***Conclusions****: The different ratios of surfactant combination affect the characteristics and stability of the NLC system.*

Keywords*: irritability, NLC, physicochemical characterization, stability, surfactant ratios*

How to cite this article:
Rahmasari, D., Rosita, N., & Soeratri, W. (2022). Physicochemical Characteristics, Stability, and Irritability of Nanostructured Lipid Carrier System Stabilized with Different Surfactant Ratios. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 8–16. http://doi.org/10.20473/jfiki.v9i12022.8-16

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

Over the past decade, many formulation studies about lipid carriers have increased. The rise in lipid carriers exploration as a nanotechnology delivery system is essentially due to the drawbacks of conventional drug delivery systems, like first-pass metabolism, which leads to a decrease of bioavailability, interaction due to food and drug, poor solubility drug, and high fluctuation of the drug concentration level in plasma (Brito *et al.*, 2019). Lipid nanoparticles are utilized as an alternative drug delivery system for the existing conventional particulate systems, like polymeric nanoparticles, or known as a liposome. This system enhances drug stability, increases the safety and the efficiency of drugs, provides targeted drug delivery, improves bioavailability for instance, and extends the drug's effect in the target tissue (Zahin *et al*., 2020).

Nanostructured Lipid Carriers (NLC) is one of the nanoparticle lipid-based systems developed from the Solid Lipid Nanoparticles (SLN). The NLC structure is the most significant advantage of this system compared to SLN. NLC is composed of a blend of spatially incompatible liquid lipids along with solid lipids providing spaces to host the active compound. Those lipids can be utilized in a high concentration (up to 95%) when compared to SLN. Due to the lipid protection, NLC can avoid the degradation of drugs and promotes drug-controlled release (Natarajan et al., 2017; Durán et al., 2019). The utilization of liquid lipid also gives a better drug loading and it can avoid drug expulsion for a long period. NLC presents many other advantages such as the improved penetration of drugs due to the increased permeation on the skin and the occlusive effect while decreasing the transepidermal water loss and increasing skin hydration (Pivetta *et al*., 2018). Thus, NLC formulations have been proposed to be suitable for cosmeceuticals, especially for poorly water-soluble (Ortiz *et al*., 2021) and weak-acid drugs (Rahmasari, 2018).

The surfactant concentration is one of the important factors that affect the stability, particle size, particle size, particle size distribution, degree of crystallization, and polymorphism of the Nanostructured Lipid Carrier (NLC). The surfactant acts as a stabilizer (Ortiz *et al*., 2021), and plays an important role in lipid nanoparticles formation. Surfactant reduces the surface tension and facilitates the particle partition during the homogenization process (de Souza *et al*., 2021). To produce a great NLC with good characteristics and stability, it is necessary to select and use the proper surfactant concentration (Witayaudom & Klinkesorn, 2017). It has been reported that the nanoparticle surface can be covered efficiently using Poloxamer with an optimum concentration of 3%. The nanoparticles' surface will be well-covered, and the aggregation among particles reduces with an adequate concentration of surfactant (Zirak & Pezeshki, 2015). Another study reported that the surfactant type influenced the quality of lycopene-loaded NLC. Surfactant type moreover appeared to have a vital role in the zeta potential of the NLC (Riangjanapatee & Okonogi, 2012). Karn-Orachai *et al*. (2014) reported that smaller NLC particles, lower crystallinity, and also a more homogenous mixture of solid lipid and oil are obtained by the two surfactants system. It indicates that the stability of NLC of mixed two surfactant systems showed to be held over more extended periods than the one or the three-surfactant systems.

This study aimed to explore the physicochemical characterization, stability, and irritability of NLC prepared at different ratios of Tween 80 and Span 20. In expansion, we assessed which surfactant ratio could deliver great NLC with good characterization, and stability and had no irritability effect.

MATERIALS AND METHODS

Materials

Cetyl palmitate was bought from BASF (Germany). Medium-chain triglyceride (Crodamol™) was a gifted sample from Croda (Singapore). Tween 80 was obtained from Kao Corporation (Japan). Span 20 was purchased from Brataco (Indonesia). All of these chemicals were in pharmaceutical grade.

Tools

High Shear Homogenizer (T25 Ultra-Turrax IKA®), Zeta Potential and Submicron Particle Size Analyzer (Delsa ™Nano), Zetasizer Nano (Malvern Panalytical), Transmission Electron Microscope (Jeol JEM-1400), pH Meter (Schott Glass Mainz, GC 824 type), Viscometer (Brookfield), and Light Microscope (Nikon H600L).

Methods

Preparation of NLC

This NLC preparation was made by the high shear homogenization method. Firstly, the cetyl palmitate, Crodamol™, and Span 20 were put in a glass and dissolved using a hot plate at 70°C. This blend was then stirred by using a high-speed homogenizer at a speed of 3400rpm for 5min. On the other hand, a beaker glass containing acetate buffer solution and Tween 80 was blended by heating at 70°C. The lipid phase was then added with this hot aqueous phase gradually and

P-ISSN: 2406-9388
E-ISSN: 2580-8303

homogenized at a speed of 20,000 rpm for 3 mins in five cycles. This preparation then cooled while stirring at 500 rpm for 30 mins until the best NLC system was obtained (Table 1).

Organoleptic test

Organoleptic tests were carried out by visually determining the odor, color, and consistency of the NLC systems.

pH evaluation

About 1 g of NLC system was dissolved in 20 mL distilled water, then immersed the electrode into the sample. A digital pH meter was used to measure the pH value of the NLC preparations, which was already calibrated. The pH values were observed until the screen showed a stable result.

Viscosity test

A viscometer was used to determine the viscosity of the samples. The 150 g samples were poured into the container, and the spindle had to be sunk into it. At that moment, the viscometer was turned on and maintained in place until the steady measurement result was achieved.

Zeta potential evaluation

Zeta potential value was measured by using a zetasizer. About 0.5 g of NLC system was dissolved in 20 mL of distilled water. Approximately 3 mL sample was diluted in 10 mL distilled water, then shaken with a vortex to prevent aggregation. After that, the sample was placed in the sample holder until the measurement result was stable.

Particle size and polydispersity index (PDI) evaluation

The particle size and PDI analysis were performed using a dynamic light scattering instrument (Particle Size Analyzer). The variance of the average intensity of light scattering from this instrument will be calculated as the particle size. The PDI shows the particle size homogeneity within the sample population (Hendradi *et al*., 2017).

Particle morphology evaluation

Transmission Electron Microscopy (TEM) was utilized to observe the morphology of the NLC systems. A sample drop was colored with 2% (w/v) of phosphotungstic acid solution and placed on a copper grid for observation with TEM (Gokce *et al*., 2012).

Stability test

Stability testing was held using the real-time and freeze-thaw method. In the real-time method, the NLC samples were stored at 30 ± 2°C for one month (30 days) (Dantas *et al*., 2016). In the freeze-thaw method, the NLC samples were stored at 4 ± 2°C for 24 hours and moved at 40 ± 2°C for 24 hours (counted as one cycle), then repeated up to six cycles (12 days) (Kumar & Dua, 2018). The organoleptic, pH values, particle size, and PDI were evaluated on the last day of storage.

Irritation test

The irritation test was held using in vivo histopathological scoring method. Male mice were sedated with ketamine (50 mg/Kg) intraperitoneally one hour before use. Then the back hair was shaved, and the samples applied. After 48 hours, the mice were sacrificed with the dislocation method. The back skin was cut using a microtome, then stained with hematoxylin-eosin (HE) and observed with a light microscope. Observation of skin irritation was carried out with histopathological scoring on several indicators of irritation, which are liquefaction, edema, collagen fibre swelling, inflammatory cell infiltration, and skin appendages degeneration (Shoviantari *et al*., 2020).

Ethics consideration

The Research Ethics Committee approved this research of Veterinary Medicine Faculty of Airlangga University (Animal Care and Use Committee, Approval Code: No. 738-KE).

Statistical analysis

The characterization, which includes pH, viscosity, zeta potential, particle size and PDI, stability, and irritability of NLC system, was analyzed using a one-way Analysis of Variance (ANOVA) method for parametric data and Kruskal-Wallis method for non-parametric data, at a 95% confidence interval, statistically. The data results were further analyzed using the Honestly Significant Difference (HSD) test (parametric data) and the Mann-Whitney test (non-parametric data).

Table 1. Composition of the NLC preparations (% w/w)

Composition	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)	Formula 4 (F4)
Cetyl palmitate	1	1	1	1
Crodamol™	4	4	4	4
Tween 80	5	6	7	8
Span 20	5	6	7	8
Acetate buffer (4.5 ± 0.5)	until 100	until 100	until 100	until 100

P-ISSN: 2406-9388
E-ISSN: 2580-8303

RESULTS AND DISCUSSION

Organoleptic test

The organoleptic observation showed that the NLC was white, odorless, had a liquid consistency and soft texture, as shown in Figure 1.

Figure 1. NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4

pH evaluation

The pH of all formulas was found about 4.36 to 4.57 (Table 2). It can be demonstrated that NLC systems can be utilized for topical preparation, in line with normal skin pH, which is 4 - 6 (Prakash *et al*., 2017). Based on the measurable examination of ANOVA followed by the post-hoc Tukey HSD test, the results revealed a significant difference (sig. value 0.010 < 0.05) in pH values, especially between Formula 4 with the other formulas. These results may be due to the acetate buffer solution (pH = 4.5) used in NLC, and in Formula 4 there was a substantial change in micellar molecular weight, which caused a change in pH (Bloor *et al*., 1970).

Viscosity test

The proper viscosity is required to enable NLC to adhere to the skin surface, thus increasing the drug penetration across the skin and the residence time (Hendradi *et al*., 2017). As can be seen in Table 2, the results showed a significant difference (sig. value 0.000 < 0.05) in viscosities among all formulas. It represents that different ratio of surfactant (Tween 80 and Span 20) affect the NLC systems' viscosity. This result was in line with the theory that viscosity increases with the addition of more surfactants because surfactants can change the morphology of the micelle from spherical form to cylindrical form. It causes electroviscous surfactant effects, leading to a bigger molecular weight (El Aferni *et al*., 2020).

Zeta potential evaluation

The zeta potential of all formula was found < (-)25mV (Table 2). Based on the statistical analysis of Kruskal-Wallis followed by the Mann-Whitney test, these results represented that there was a significant difference (sig. value 0.021 < 0.05) in zeta potential values among all formulas. It could indicate that NLC systems had been thought to be stable colloid dispersion. The NLC systems were considered to have sufficient repulsive force to attain a high degree of physical colloidal stability (Shnoudeh et al., 2019).

Particle size and polydispersity index (PDI) evaluation

The size of particles is a critical factor in producing nano-sized particles. It depicts the stability of the formulation. One of the factors which affects the particle size is the added surfactant (Suhaimi *et al*., 2015). This study found that the increment in the surfactant ratio contributed to bigger particle size (Table 2). Statistically, it showed no significant difference (sig. value 0.168 > 0.05) among all formulas. Polydispersity Index (PDI) exhibited the width of particle size distribution. The PDI range extended from 0 to 1. As the PDI value got to be closer to zero, the particles got to be more homogenous. From Table 2, it was indicated that all formulae has a homogenous particle and were considered to be acceptable (PDI < 0.3) (Danaei *et al*., 2018). It statistically showed no significant difference (sig. value 0.243 > 0.05) among all formulae. As can be seen, the high amount of surfactant produced smaller PDI. This may occur due to increasing surfactant, whichstrengthens the steric resistance effect by forming an adsorption layer on the particle surface (Wang *et al*., 2019), thereby preventing drug particles from aggregating (Pan *et al*., 2015) and making the size among particles homogenous.

Table 2. Physicochemical characterization of NLC systems

Formula	pH Value	Viscosity (cps)	Zeta Potential (mV)	Particle Size (nm)	PDI
1	4.57 ± 0.03	0.38 ± 0.02	-38.1 ± 0.29	161.60 ± 51.35	0.205 ± 0.04
2	4.54 ± 0.02	0.43 ± 0.02	-35.6 ± 0.44	115.93 ± 50.32	0.265 ± 0.22
3	4.52 ± 0.07	0.48 ± 0.02	-35.0 ± 0.15	174.90 ± 3.16	0.125 ± 0.03
4	4.36 ± 0.08	0.51 ± 0.02	-37.9 ± 0.12	186.90 ± 11.12	0.160 ± 0.02

P-ISSN: 2406-9388
E-ISSN: 2580-8303

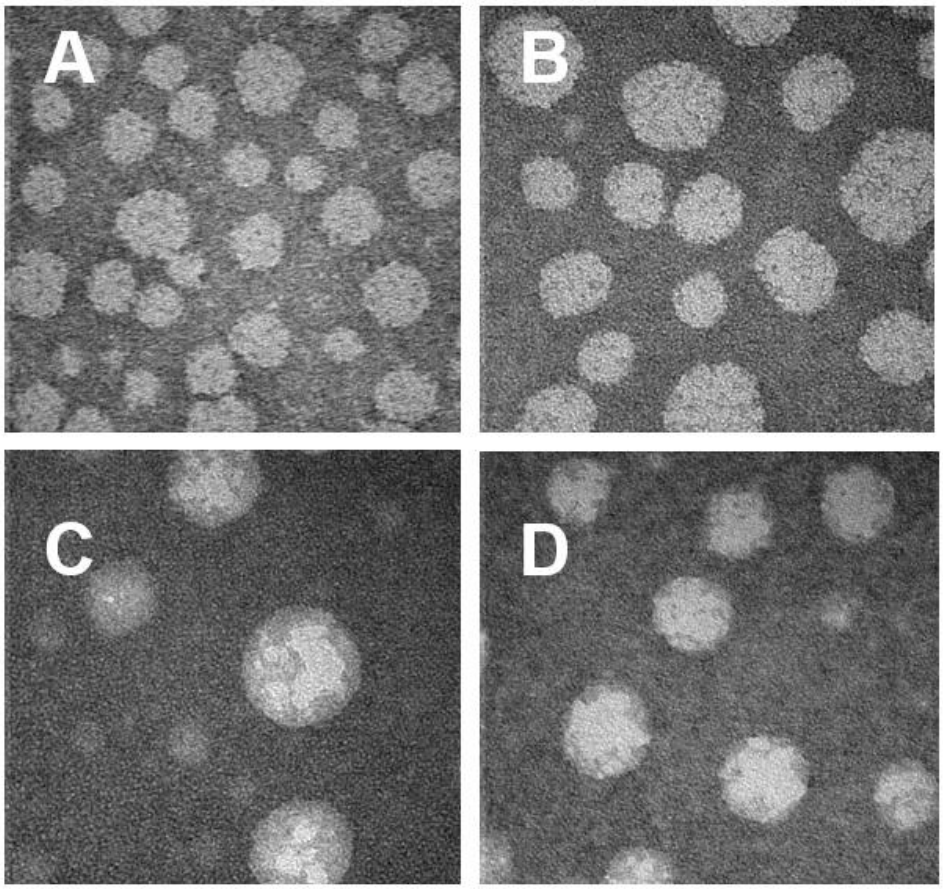


Figure 2. Particle morphology of NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4 using Transmission Electrone Microscope (TEM) on 50.000x magnification

Particle morphology evaluation

Figure 2 shows TEM images of the NLC systems. As indicated in these figures, particles depicted a mono-dispersed spheroid-like appearance with a clear boundary among each particle. The particles showed no visible aggregation, a uniform and spherical shape. These spherical particles have an uneven surface. Probably, this matter is formed from a liquid lipid that coats the inner particle of the systems and includes a flip-flop structure.

Stability test

In the real-time method (Table 3), a stability test was conducted to physically determine the system resilience of NLC when stored at room temperature. The NLC systems showed no significant changes in pH and PDI values, but a significant change in particle size, statistically with paired t-test method. There was an increment in the size of the particles, which indicates the incorporation of small particles or coalescence. After 30 days of storage with the real-time method, it can be concluded that the NLC system formed represents the stability of the NLC system in the absence of pH and PDI change. It can also be concluded that the surfactant concentration does not affect in 'real-time' method stability.

Table 3. Stability testing results of NLC systems in real-time method

For mula	Organoleptic	Parameters					
		pH		Particle Size (nm)		PDI	
		Before	After	Before	After	Before	After
1	No color change, odor change, precipitation and phase separation	4.57 ± 0.03	4.62 ± 0.02	161.60 ± 51.35	384.73 ± 3.71	0.205 ± 0.04	0.227 ± 0.09
2		4.54 ± 0.02	4.66 ± 0.06	115.93 ± 50.32	175.93 ± 39.12	0.265 ± 0.22	0.145 ± 0.06
3		4.52 ± 0.07	4.66 ± 0.02	174.90 ± 3.16	380.37 ± 73.27	0.125 ± 0.03	0.235 ± 0.04
4		4.36 ± 0.08	4.70 ± 0.02	186.90 ± 11.12	276.47 ± 62.73	0.160 ± 0.02	0.256 ± 0.02

P-ISSN: 2406-9388
E-ISSN: 2580-8303

In the freeze-thaw method (Table 4), a stability test was conducted to physically determine the system resilience of NLC when stored in extreme conditions. The NLC systems statistically showed significant changes in pH values, particle size, and PDI values with the paired t-test method. There was a decrease in PDI values and an increment in the pH values and size of the particles. An increase in temperature s crystal growth, indicating the aggregation of nanoparticles when the temperature increases, which tends to increase particle size (Catauro *et al*., 2018). Besides that, preparations containing Tween 80 and stored at 40°C undergo autoxidation to form more peroxides (Kishore *et al*., 2011). This autoxidation leads to the destabilizing effect of Tween 80 and increases the aggregation of particles (Agarkhed *et al*., 2013). After 12 days of storage with a freeze-thaw method, it can be concluded that the NLC system formed represents the instability of the NLC system in the presence of pH, particle size, dan PDI change. It can also be concluded that the surfactant concentration does not affect in 'freeze-thaw' method stability.

Irritation test

Safety of use is an important factor in developing such topical preparations. One of the safety parameters can be illustrated by the absence of skin irritation and can be done with histopathological observation (Figure 3). The histopathological scores of the back skin of mice after 48 h indicates that NLC systems had an average score that did not cause structural changes while Crodamol™ had a slight irritation (Table 5). Crodamol™ is a medium-chain triglyceride oil that acted as a skin sensitizer or caused dermal irritation in several studies (Traul *et al*., 2000). This result represents that the NLC system has less irritation risk than the liquid lipid. This phenomenon was probably caused by the addition of cetyl palmitate, which has the ability to moisturize to minimize the occurrence of irritation (Shoviantari *et al*., 2020).

Table 4. Stability testing results of NLC systems in freeze-thaw method

Formula	Organoleptic	Parameters					
		pH		Particle Size (nm)		PDI	
		Before	After	Before	After	Before	After
1	No color change, odor change, precipitation and phase separation	4.57 ± 0.03	5.46 ± 0.03	16.60 ± 51.35	289.4 ± 24.93	0.205 ± 0.04	0.138 ± 0.00
2		4.54 ± 0.02	5.43 ± 0.02	115.93 ± 50.32	275.47 ± 19.13	0.265 ± 0.22	0.123 ± 0.00
3		4.52 ± 0.07	5.20 ± 0.04	174.90 ± 3.16	264.93 ± 17.14	0.125 ± 0.03	0.139 ± 0.00
4		4.36 ± 0.08	4.70 ± 0.05	186.90 ± 11.12	244.33 ± 44.92	0.160 ± 0.02	0.159 ± 0.00

Table 5. Histopathological score NLC systems irritation test on male mice's back after 24 hours

Formula	Irritation Score	Classification
1	0.2 ± 0.28	Almost no change
2	0.4 ± 0.28	Almost no change
3	0.73 ± 0.19	Almost no change
4	0.87 ± 0.19	Almost no change
Crodamol™	1.6 ± 0	Slight irritation

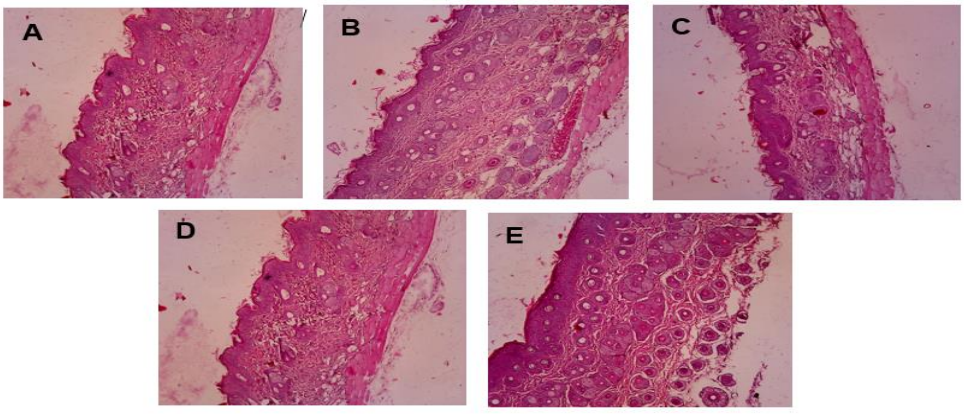


Figure 3. Microscopic images of mice skin backs after 48 h of NLC systems (A) formula 1; (B) formula 2; (C) formula 3; (D) formula 4 and (E) crodamol™; using the nikon H600L light microscope at 100x magnification

P-ISSN: 2406-9388
E-ISSN: 2580-8303

CONCLUSION

In this research, it can be concluded that the different surfactant ratios of Tween 80 and Span 20 affect the characteristics and stability of the NLC system and did not affect the irritability. There was no significant difference in particle size, PDI, and irritation score. There was a significant difference in the pH value, viscosity, zeta potential, and stability of samples for all formulas. The authors recommend Formula 3 with the surfactant ratio of Tween 80:Span 20 (7:7), as the best formula, due to the good physicochemical properties in the NLC system. These results also suggest the potential formula of NLC as a drug delivery system for weak-acid and poorly water-soluble drugs.

ACKNOWLEDGMENT

The authors are thankful to Croda, Singapore, for providing gift sample of liquid lipid, medium-chain triglyceride (Crodamol™).

AUTHOR CONTRIBUTIONS

Conceptualization, N.R., W.S.; Methodology, N.R.; Software, D.R.; Validation, W.S.; Formal Analysis, D.R.; Investigation, W.S.; Resources, D.R.; Data Curation, N.R.; Writing - Original Draft, D.R.; Writing - Review & Editing, D.R..; Visualization, N.R.; Supervision, W.S.; Project Administration, N.R.; Funding Acquisition, D.R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Agarkhed, M., O'Dell, C., Hsieh, M.-C., Zhang, J., Goldstein, J. & Srivastava, A. (2013). Effect of Polysorbate 80 Concentration on Thermal and Photostability of a Monoclonal Antibody. *AAPS PharmSciTech*; *14*: 1–9. doi: 10.1208/s12249-012-9878-0.

Bloor, J., Morrison, J. & Rhodes, C. (1970). Effect of pH on the Micellar Properties of a Nonionic Surfactant. *Journal of Pharmaceutical Sciences*; *59*: 387–391. doi: 10.1002/jps.2600590325.

Brito, R. S., Chandrasekhar, K. B. & Reddy, K. B. (2019). Formulation, In-Vitro and In-Vivo Pharmacokinetic Evaluation of Simvastatin Nanostructured Lipid Carrier Loaded Transdermal Drug Delivery System. *Future Journal of Pharmaceutical Sciences*; *5*: 1-14. doi: 10.1186/s43094-019-0008-7.

Catauro, M., Tranquillo, E., Dal Pogetto, G., Pasquali, M., Dell'Era, A. & Ciprioti, S. V. (2018). Influence of the Heat Treatment on the Particles Size and on the Crystalline Phase of TiO2 Synthesized by the Sol-Gel Method. *Materials*; *11*: 1-11. doi: 10.3390/ma11122364.

Danaei, M., Dehghankhold, M., Ataei, S., Davarani, F. H., Javanmard, R., Dokhani, A., Khorasani, S. & Mozafari, M. R. (2018). Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics*; *10*: 1–17. doi: 10.3390/pharmaceutics10020057.

Dantas, M. G. B., Reis, S. A. G. B., Damasceno, C., M., S., Rolim, L. A., Rolim-Neto, P. J., Carvalho, F. O., Quintans-Junioe, L. J. & Almeida, J. R. G. S. (2016). Development and Evaluation of Stability of a Gel Formulation Containing the Monoterpene Borneol. *Scientific World Journal*; *2016*: 1–4. doi: 10.1155/2016/7394685.

de Souza, G. L., Martinez, R. M, Bou-Chacra, N. A., Velasco, M. V. R., Rosado, C. & Baby, A. R. (2021). An Overview on Topical Administration of Carotenoids and Coenzyme q10 Loaded in lipid Nanoparticles. *Antioxidants*; *10*: 1–25. doi: 10.3390/antiox10071034.

Durán, N., Costa, A. F., Stanisic, D., Bernardes, J. S. & Tasic, L. (2019). Nanotoxicity and Dermal Application of Nanostructured Lipid Carrier Loaded with Hesperidin from Orange Residue. *Journal of Physics: Conference Series*; *1323*: 1-5. doi: 10.1088/1742-6596/1323/1/012021.

El Aferni, A., Guettari, M., Kamli, M., Tajour, T. & Ponton, A. (2020). A Structural Study of a Polymer-Surfactant System in Dilute and Entangled Regime: Effect of High Concentrations of Surfactant and Polymer Molecular Weight. *Journal of Molecular Structure; 1199*: 1-10. doi: 10.1016/j.molstruc.2019.127052.

Gokce, E. H., Korkmaz, E., Dellera, E., Sandri, G., Bonferoni, M. C. & Ozer, O. (2012). Resveratrol-Loaded Solid Lipid Nanoparticles Versus Nanostructured Lipid Carriers: Evaluation of Antioxidant Potential for Dermal Applications. *International Journal of Nanomedicine*; *7*: 1841–1850. doi: 10.2147/IJN.S29710.

Hendradi, E., Rosita, N. & Rahmadhanniar, E. (2017). Effect of Lipid Ratio of Stearic Acid and Oleic Acid on Characteristics of Nanostructure Lipid Carrier (NLC) System of Diethylammonium

Diclofenac. *Indonesian Journal of Pharmacy*; *28*: 198–204. doi: 10.14499/indonesianjpharm28iss4pp198.

Karn-Orachai, K., Smith, S. M., Phunpee, S., Treethong, A., Puttipipatkhachorn, S., Pratontep, S. & Ruktanonchai, U. R. (2014). The Effect of surfactant Composition on the Chemical and structural Properties of Nanostructured Lipid Carriers. *Journal of Microencapsulation*; *31*: 609–618. doi: 10.3109/02652048.2014.911374.

Kishore, R. S. K., Pappenberger, A., Dauphin, I. B., Ross, A., Buergi, B., Staempfli, A. & Mahler, H. -C. (2011). Degradation of Polysorbates 20 and 80: Studies on Thermal Autoxidation and Hydrolysis. *Journal of Pharmaceutical Sciences*; *100*: 721–731. doi: 10.1002/jps.22290.

Kumar, A. & Dua, J. (2018). Formulation and Evaluation of Itraconazole Niosomal Gel. *Asian Journal of Pharmaceutical Research and Development*; *6*: 76–80. doi: 10.22270/jddt.v9i4-s.3680.

Natarajan, J., Karri, V. V. S. R. & De, A. (2017). Nanostructured Lipid Carrier (NLC): A Promising Drug Delivery System. *Global Journal of Nanomedicine*; *1*: 120–125.

Ortiz, A. C., Yañez, O., Salas-Huenuleo, E. & Morales, J. O. (2021). Development of a Nanostructured Lipid Carrier (NLC) by a low-Energy Method, Comparison of Release Kinetics and Molecular Dynamics Simulation. *Pharmaceutics*; 13: 1-21. doi: 10.3390/pharmaceutics13040531.

Pan, Z., Cui, B., Zeng, Z., Feng, L., Liu, G., Cui, H. & Pan, H. (2015). Lambda-Cyhalothrin Nanosuspension Prepared by the Melt Emulsification-High Pressure Homogenization Method. *Journal of Nanomaterials*; *2015*: 1-9. doi: 10.1155/2015/123496.

Pivetta, T. P., Simões, S., Araújo, M. M., Carvalho, T., Arruda, C. & Marcato, P. D. (2018). Development of Nanoparticles from Natural Lipids for Topical Delivery of Thymol: Investigation of Its Anti-Inflammatory Properties. *Colloids and Surfaces B: Biointerfaces; 164*: 281–290. doi: 10.1016/j.colsurfb.2018.01.053.

Prakash, C., Bhargava, P., Tiwari, S., Majumdar, B. & Bhargava, R. K. (2017). Skin Surface pH in Acne Vulgaris: Insights from an Observational Study and Review of the Literature. *Journal of Clinical and Aesthetic Dermatology*; *10*: 33–39.

Rahmasari, D. (2018). Pengaruh Kadar Surfaktan Kombinasi Tween 80 dan Span 20 terhadap Stabilitas Fisik, Iritabilitas, dan Penetrasi Resveratrol dalam Nanostructured Lipid Carrier (NLC). Surabaya: Airlangga University.

Riangjanapatee, P. & Okonogi, S. (2012). Effect of Surfactant on Lycopene-Loaded Nanostructured Lipid Carriers. *Drug Discoveries & Therapeutics; 6*: 163–168.

Shnoudeh, A. J., Hamad, I., Abdo, R. W., Qadumii, L., Jaber, A. Y., Surchi, H. S. & Alkelany, S. Z. (2019). Synthesis, Characterization, and Applications of Metal Nanoparticles, Biomaterials and Bionanotechnology. *Advances in Pharmaceutical Product Development and Research*; *2019*: 527-612. doi: 10.1016/B978-0-12-814427-5.00015-9.

Shoviantari, F., Erawati, T. & Soeratri, W. (2020). Coenzyme Q10 Nanostructured Lipid Carriers as an Inducer of the Skin Fibroblast Cell and Its Irritability Test in a Mice Model. *Journal of Basic and Clinical Physiology and Pharmacology*; *30*: 1–7. doi: 10.1515/jbcpp-2019-0320.

Suhaimi, S. H., Hasham, R. & Rosli, N. A. (2015). Effects of Formulation Parameters on Particle Size and Polydispersity Index of Orthosiphon Stamineus Loaded Nanostructured Lipid Carrier. *Journal of Advanced Research in Applied Sciences and Engineering Technology*; *1*: 36–39.

Traul, K. A., Driedger, A., Ingle, D. L. & Nakhasi, D. (2000). Review of the Toxicologic Properties of Medium-Chain Triglycerides. *Food and Chemical Toxicology*; 38: 79–98. doi: 10.1016/s0278-6915(99)00106-4.

Wang, C., Cui, B., Guo, L., Wang, A., Zhao, X., Wang, Y., Sun, C., Zeng, Z., Zhi, H., Chen, H., Liu, G. & Cui, H. (2019). Fabrication and Evaluation of Lambda-Cyhalothrin Nanosuspension by One-Step Melt Emulsification Technique. *Nanomaterials*; *9*: 1–13. doi: 10.3390/nano9020145.

Witayaudom, P. & Klinkesorn, U. (2017). Effect of Surfactant Concentration and Solidification Temperature on the Characteristics and Stability of Nanostructured Lipid Carrier (NLC) Prepared from Rambutan (*Nephelium lappaceum* L.) Kernel Fat. *Journal of Colloid and Interface Science*; *505*: 1082–1092. doi: 10.1016/j.jcis.2017.07.008

Zahin, N., Anwar, R., Tewari, D., Kabir, M. T., Sajid, A., Mathew, B., Uddin, M. S., Aleya, L. & Abdel-Daim, M. M. (2020). Nanoparticles and Its Biomedical Applications in Health and Diseases: Special Focus on Drug Delivery. *Environmental Science and Pollution Research*; *27*: 19151–19168. doi: 10.1007/s11356-019-05211-0.

Zirak, M. B. & Pezeshki, A. (2015). Effect of Surfactant Concentration on the Particle Size, Stability and Potential Zeta of Beta Carotene Nano Lipid Carrier. *International Journal of Current Microbiology and Applied Sciences*; *4*: 924–932.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 17-23
DOI: 10.20473/jfiki.v9i12022.17-23
Available online at https://e-journal.unair.ac.id/JFIKI/

Synthesis and Activity Test of 1-Allyl-3-(4-tertiary-Butylbenzoyl) Thioureaas a Candidate of an Analgesic Drug

Rais Razak[1]*, Siswandono[2], Juni Ekowati[2]
[1]Faculty of Pharmacy, Muslim University of Indonesia, Makassar, Indonesia
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: raiz.razak@umi.ac.id

Submitted: 17 May 2021
Accepted: 21 Januari 2022
Published: 26 April 2022

Abstract

Background*: Urea derivatives showed good analgesic activity compared to diclofenac sodium. The addition of the allyl group to the thiourea and 4-tertiary-butylbenzoyl chlorideis expected to provide a better analgesic effect.* ***Objective****: The research aimed to synthesize 1-allyl-3-(4-tertiary-Butylbenzoyl) Thiourea and determine its analgesic activity in mice (Mus musculus).* ***Methods****: The synthesis was carried out by a modified Schotten-Baumann reaction, via nucleophilic substitution reaction of allylthiourea on 4-tertiary-butylbenzoyl chloride. A writhing test was performed to observe analgesic activity in the test compound. Confirmation of the structure of pure 1-allyl-3-(4-tertiary-Butylbenzoyl) Thiourea was obtained through UV, IR, ^{1}H-NMR, and ^{13}C-NMR data.* ***Results****: The compound showed better pain inhibition activity compared to diclofenac sodium, with ED_{50}19,018 mg/kg BW.* ***Conclusion****: The compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea showed better analgesic activity than diclofenac sodium.*

Keywords*: 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea, drug candidate, potential analgesic, urea derivatives*

How to cite this article:
Razak, R., Siswandono, & Ekowati, J. (2022). Synthesis and Activity Test of 1-Allyl-3-(4-tertiary-Butylbenzoyl) Thioureaas a Candidate of an Analgesic Drug. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 17-23. http://doi.org/10.20473/jfiki.v9i12022.17-23

INTRODUCTION

Pain is the most individualized common symptom of a disease characterized by a feeling of sensory and emotional discomfort as a signal of or potentially causing tissue damage (HCANJ, 2017). Pain management aims to increase the effectiveness of treatment, either reducing pain intensity or duration, improving quality of life, and preventing the risk of side effects (Cregg *et al*., 2013).

Non-steroid anti-inflammatory drugs (NSAIDs) are widely used to treat mild to moderate inflammation and pains (Sun *et al*., 2018). Although relatively safe, the use of this drug is also often associated with the overuse of medication (Auriel *et al*., 2014). NSAIDs work by inhibiting the cyclooxygenase enzyme, which converts arachidonic acid into prostaglandin in the inflammatory process (Somakala & Amir, 2017). NSAIDs can offer pain inhibiting effects without the severe side effects of sedation and respiratory depression common with opioid use (Bader *et al*., 2011). According to the National Health Service (NHS), although NSAIDs are often prescribed, not everyone is suitable for this drug, especially at severe pain sensation, which causes side effects (National Health Service, 2019).

One of the NSAIDs that is often used is diclofenac sodium. However, this drug has a short half-life and has some side effects, such as gastric ulcers, gastric irritation, and bleeding, thus limiting its use as a pain agent (Aielo *et al*., 2014). Therefore, the discovery of new drugs for pain treatment is an opportunity to develop a drug.

Urea derivatives have biological activity as antiviral, antibacterial, anti-Human Immunodeficiency Virus (HIV), analgesic, and anti-inflammation (Alagarsamy *et al*., 2013). The chlor and nitro substituents at the para position added to a benzoyl thiourea derivative were able to increase analgesic and anti-inflammatory activity, where the influence of the chlorsubstituent was superior to that of the nitrosubstituent. Compounds 4-nitrobenzoylthiourea and 4-chlorobenzoylthiourea showed strong analgesic activity with lower anti-inflammatory activity compared to diclofenac sodium (Budiati *et al*., 2010).

In-silico identification has become a popular approach in computer-aided drug discovery. In new drug development, in-silico screening is carried out to find the ranking or screening value of a compound based on the data structure using one or more computational procedures (Budiati et al., 2010). This approach can narrow down the search of a potential lead compound from a massive number of compound databases to select potential hits by using high-throughput molecular docking; or elucidate the mechanistic interaction of potential hits, which helps rationalise or optimise bioactivity (Yap *et al*., 2019). It has many advantages; among other things, it can reduce excessive use of tools and materials and save on trial costs (Dona *et al*., 2019). The addition of the allyl group to the thiourea is expected to provide a better analgesic effect.

MATERIALS AND METHODS

Materials

*Allylthiourea*p.a 98% (Aldrich), 4-tertiary-butylbenzoyl chloridep.s (Aldrich), and diclofenac sodium (Dexa Medica),Tetrahydrofuran p.a (Merck), Ethyl acetate p.a (Merck), Methanol p.a (Merck), n-hexane p.a (Merck), Triethylamine p.a (Merck), Chloroform p.a (Merck).

Tools

Computer Intel core i7 Memory 8 GB, Chemdraw Ultra 8.0, Chem3D Ultra 8.0, and Molegro Virtual Docker 5.0 programs are used as devices for *in-silico* testing. HEWLET PACKARD 8452A Diode Array Spectrophotometer, PERKIN ELMER Spectrum One FTIR Spectrophotometer, and BRUKER BioSpinAvance III NMR Spectrometer are used as structural identification tools.

Method

In-silico procedures

In-silico test of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea against the cyclooxygenase (COX-2) receptor (pdb: 1PXX) was carried out using Chembio Draw and Molegro Virtual Docker (MVD) computer programs. The 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea is docked with the receptor in the cavity position. The results obtained are the energy of the interaction between the test compound and the COX-2 receptor, in the form of a Rank Score (RS). *In-silico* testing is carried out as a basis for synthesizing the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Synthesis procedure

In a 250 mL round bottom flask, dissolve 0.0172 mol N-allylthiourea with 50 mL of tetrahydrofuran (THF) and 0.028 mol of triethylamine. Add a solution of 0.0143 mol benzoyl chloride derivative in 15 mL of THF gradually in the ice bath while stirring the mixture using a magnetic stirrer for 0.5 hours. Then the mixture is refluxed over a water bath and tested using thin-layer chromatography (TLC) every hour until it produces one spot on the TLC plate, indicating that the target compound has been formed. If there are still two stains, the reaction is considered

P-ISSN: 2406-9388
E-ISSN: 2580-8303

incomplete. When the reaction is deemed to be complete, the mixture is washed three times with saturated sodium carbonate solution, then the mixture is filtered over a Buechner funnel and recrystallized with methanol.

Animals

Male white mice aged 6-8 weeks were kept in cages at room temperature, maintained under a 12-hour light-dark cycle. All the animals were given an adaptation period of at two weeks and allowed free access to food and water *ad libitum*. The research was implemented after getting ethical clearance no. 512-KE Ethics Committee, Faculty of Veterinary Medicine Universitas Airlangga

Analgesic activity test

The experimental animals were divided into three test groups consisting of five experimental animals and given intraperitoneal injection (i.p). The division of the group included a negative control group that was given 1% CMC-Na suspension with a dose of 10 mL/kg BW, a positive control group was given a suspension of diclofenac sodium in 1% CMC-Na suspension at a dose of 12.5 mg/kg BW, and the test group given the test compound suspension in 1% CMC-Na suspension, at a dose of 6.25 mg/Kg BW; 12.5 mg/Kg BW; and 25 mg/Kg BW.

The experimental animals were observed in each group for 30 minutes to calculate the percentage of pain. The formula of pain resistance is calculated by comparing the number of writhes as the effect of pain resistance between the control and the test animals (Jakaria *et al*., 2015). The reduction in the amount of writhing in the test animals compared to the control group is evidence of an analgesic effect (Abdulmalik *et al*., 2011).

Researchers also count the median effective dose (ED) of 50%, the dose that produces 50% of the maximum response (Koyagura *et al*., 2015).

$$\%\ Inhibition = \frac{Mean\ number\ of\ writhes\ (control) - Mean\ number\ of\ writhes\ (test)}{Mean\ number\ of\ writhes\ (control)}\ x\ 100$$

After all the writhes data of the mice were obtained, then the ED50 value of each test compound was determined using probit analysis on SPSS 22.

RESULTS AND DISCUSSION

This study will compare the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea with benzoylthiourea. Benzoylthiourea was chosen as a comparison because it is a urea derivative tested for its activity as an analgesic and anti-inflammatory (Budiati *et al*., 2010).

The RS value is a value that reflects the bond energy required to form a bond between a ligand and its receptor. This value predicts the activity of the test compound when it binds to the receptor. The smaller the RS value, the more stable the resulting bond, and the greater the predicted activity (Suhud, 2015). The amino acids involved in the interaction of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoyltiourea at the COX-2 receptor can be seen in Table 1.

Table 1. Amino acids involved in the interaction between 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoyltiourea at the COX-2 receptor

Compound	Amino Acid	Type of Interaction
a	*Val 3523*	Steric
	Gln 3192	Steric
	Ala 3516	Steric
	Leu 3352	Steric
	Phe 3518	Steric
b	*Gly 3256*	Hydrogen
	Ser 3530	Steric

Based on docking data using MVD in Figure 1, the RS value of both 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and benzoylthiourea were -95,9587 and -67.5824 respectively. It was predicted that 1-allyl-3-(4-tertiary-butylbenzoyl) thioureahas better analgesic activity than benzoylthiourea. Theoretically, when calculated using the Chemdraw Ultra 8.0 and Chem3D Ultra 8.0 computer program, the1-allyl-3-(4-tertiary-butylbenzoyl) thioureahas a lipophilic parameter value (Clog P) 3.3871 and a steric parameter value (CMR) of 7.2461 and (Etot) -16.0039 kcal/mol. While benzoylthiourea has a lipophilic parameter value (Clog P) of 0.566 and a steric parameter value (CMR) of 5.2812 (Etot) of -46.7383 kcal/mol.

The synthesis design in this study was to carry out the nucleophilic acyl substitution reaction in one of the amine groups in allythiourea with the benzoyl chloride substituted for the tertiarybutyl group in a THF solvent in the alkaline atmosphere with triethylamine as a catalyst. The primary amine group on the number three N atom of the allylthiourea compound acts as a nucleophile that attacks the C carbonyl atom on the benzoyl chloride substituted for the tertiarybutyl group because the C atom lacks electrons and is more positive, as shown in Figure 2. This reaction is expected to increase the interaction of pain receptors.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

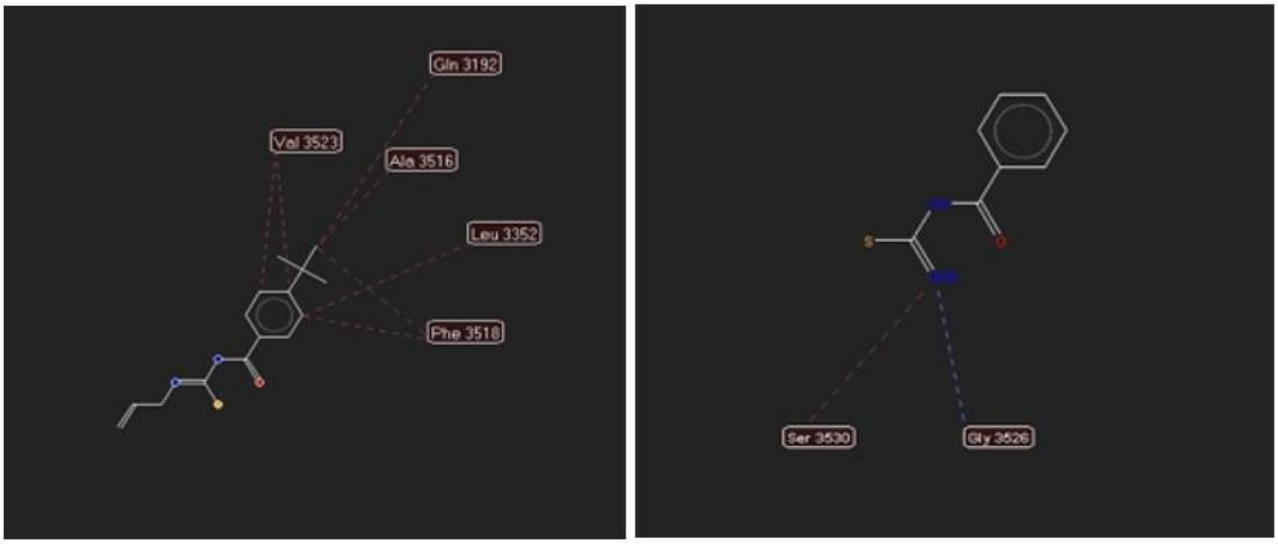


Figure 1. Docking result of (a) 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and (b) Benzoyltiourea on 1PXX receptor

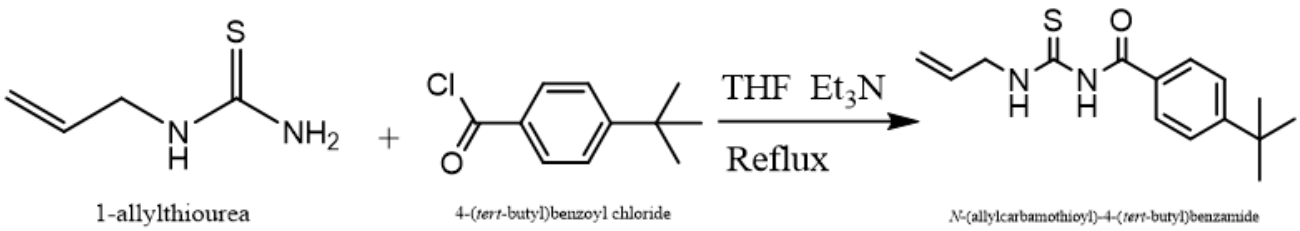


Figure 2. Synthesis of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

The compounds synthesized on the TLC chromatogram showed a single stain, and the melting point showed a range of less than 1 – 2ºC. The results of the synthesis obtained colorless crystals as much as 25.3%. mp 150-151 0C. TLC Rf: 0.78. Determination of the synthesized structure was carried out using ultra violet, infrared, and magnetic resonance; this was done to confirm the compound obtained with the desired target compound.

Table 2 shows IR data ν cm-1: 849 (Para substituted benzene); 1169 (-C=S); 1268 (-C-N); 1547 (-C=C aromatic); 1669 (-C=O amide); 3429 (-OH); 3244 (-NH).The interpretation of the IR spectrum for the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea in Figure 3 shows an absorption band at 1669 cm^{-1} which indicates the presence of the –C=O amide group; absorption of 1169 cm-1 indicates the presence of a –C=S group; absorption of 1268 cm^{-1} indicates the presence of –C-N group; 1428-1547 cm^{-1} indicates the presence of –C=C- aromatic group; and the absorption of 3429 cm^{-1} indicates the presence of –O-H groups. In addition, the absorption at 846 cm^{-1} indicates a para substitution pattern in the aromatic ring.

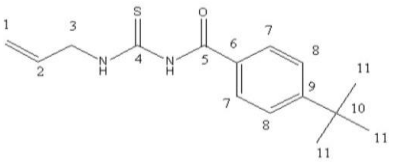


Figure 3. Structure of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Table 2. IR data of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Wave number (cm^{-1})	Type of vibration
849	Para disubtituted benzene
1169	-C=S
1268	-C-N
1547	Aromatic -C=C
1669	Amide -C=O
3429	-OH
3244	-NH

In the ^{1}HNMR spectrum (Table 3), the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea is a compound that has the same symmetrical plane as the last peak at 1.37 ppm and contained a singlet with an integration indicating 9 H atoms of methyl at the tertierbutyl position of the substitution on the aromatic ring.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. ^{13}C NMR and ^{1}H-NMR data of 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea

Position	δ_H ppm	δ_C ppm
(1)	5.22 (*dt, ^2J = 1.2 ,^3J = 10.4)*; 5.30 (*dt ,^2J* = 1.6, *^3J = 17.2*)	117.8
(2)	5.90 (*m*)	131.9
(3)	4.32 (*t)*	48.1
(4)	-	180.1
(5)	-	166.8
(6)	-	128.8
(7)	7.74 (*d,^3J* = 8.8)	127.8
(8)	7.48 (*d, ^3J* = 8.4)	127.4
(9)	-	157.6
(10)		35.2
(11)	1.37 (*s,^3J* = 6.8)	31.1
NH-C(5)	10.83 (*s*)	
NH-C(3)	9.4 *(s*)	

*$CDCl_3$ peak was reference at 7.24 ppm for ^{1}H NMR and 77 ppm for ^{13}C NMR; coupling constant (*J*) are reported in Hz

Table 4. The results of the analgesic activity of the compound1-allyl-3-(4-tertiary-butylbenzoyl) thiourea and diclofenac sodium

Compound	Doses	Mean percentage of pain inhibition	SD
Diclofenac sodium	12.5 mg/Kg BW	33.22%	8.26
1-allyl-3-(4-tertiary-butylbenzoyl) thiourea	6.25 mg/Kg BW	38.41%	9.20
	12.5 mg/Kg BW	48.10%	9.15
	25 mg/Kg BW	53.29%	6.36

In the ^{13}CNMR spectrum (Table 3) of the compound 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea, two peaks in the distant chemical shift can be seen at 166.8 ppm representing the carbon atom –C=O and at 180.1 ppm representing the carbon atom – C = S; this is because the carbon atom is bonded to another atom with a large electronegativity, namely -O and -S atoms. In the aromatic ring shift area, there are four peaks, namely at 127.8 ppm, at 127.4 ppm indicating two pairs of equivalent C atoms, at 128.8 ppm a C atom attached to a carbonyl group; and 157.6 ppm are -C-C atoms bonded to tertierbutyl on para substituents while the 35.2 ppm peak represents one -C-C atom and the last at 31.1 ppm there are three C atoms of three methyl groups.

Furthermore, the analgesic activity test was carried out using a writhing test on mice (*Mus musculus*) (Table 4). This method tests the sensation of pain felt by rats by observing the amount of writhing. Philosophically, pain cannot be directly monitored in animals but can only be estimated by examining their response to nociceptive stimuli (electrical, thermal, mechanical, or chemical), but chemical stimulation is the closest approach to clinical pain (Lee Bars *et al*., 2001). Therefore, the writhing test was chosen in this analgesic activity test by giving 1% acetic acid suspension in 1% CMC-Na at a dose of 10 mL/kg BW as pain induction. The diclofenac sodium was used as a comparison because it is one of the most widely used NSAIDs in pain management and anti-inflammatory. The analgesic activity of the test compound was inferred from a decrease in the writhings frequency (Gawade, 2012).

Better analgesic activity is shown by the compound1-allyl-3-(4-tertiary-butylbenzoyl) thiourea. Structural modification by adding Allyl groups and tertiarybutyl substitution to benzoyl ring can better affect analgesic activity (Shalas *et al*., 2016).

CONCLUSION

Based on this study, 1-allyl-3-(4-tertiary-butylbenzoyl) thiourea was successfully synthesized by the modified Schotten-Baumann reaction and it showed better analgesic activity than 12.5 mg diclofenac sodium. This compound needs further investigation as a potential analgesic drug candidate.

ACKNOWLEDGMENT

The researchers would like to thank the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Airlangga University, Indonesia. Special thanks to Prof. Dr. Siswandono, MS., Apt., who allowed the use of the license from Molegro Virtual Docking (MVD).

AUTHOR CONTRIBUTIONS

Conceptualization, S.S.; Methodology, S.S.; Software, R.R.; Validation, J.E.; Formal Analysis, R.R.; Investigation, R.R.; Resources, R.R.; Data Curation, J.E.; Writing - Original Draft, R.R.; Writing - Review & Editing, J.E..; Visualization, R.R.; Supervision, S.S.; Project Administration, S.S.; Funding Acquisition, R.R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abdulmalik, I. A., Sule, M. I., Yaro, A. H., Abdullahi, M. I., Abdulkadir, M. E., & Yusuf, H. (2011). Evaluation of Analgesic and Anti-Inflammatory Effects of Ethanol Extract of *Ficus Iteophylla* Leaves in Rodents. *African Journal of Traditional, Complementary and Alternative Medicines*; *8*; 462-466. doi: 10.4314/ajtcam.v8i4.19.

Aielo, P. B., Borges, F. A., Romeira, K. M., Miranda, M. C. R., Arruda, L. B. D., L Filho, P. N., Drago, B. de-C. & Herculano, R. D. (2014). Evaluation of Sodium Diclofenac Release Using Natural Rubber Latex as Carrier. *Materials Research*; *17*; 146-152. doi: 10.1590/S1516-14392014005000010.

Alagarsamy, V., Murugananthan, G. & Venkateshperumal, R. (2003). Synthesis, Analgesic, Anti-Inflammatory and Antibacterial Activities of Some Novel 2-Methyl-3-Substituted Quinazolin-4-(3H)-Ones. *Biological and Pharmaceutical Bulletin*; *26*; 1711-1714. doi: 10.1248/bpb.26.1711.

Auriel, E., Regev, K. & Korczyn, A. D. (2014). Nonsteroidal anti-inflammatory drugs exposure and the central nervous system. *Handbook of clinical neurology*; *119*; 577-584. doi: 10.1016/B978-0-7020-4086-3.00038-2.

Bader, P., Echtle, D., Fonteyne, V., De Meerleer, G., Papaioannou, E. G. & Vranken, J. H. (2011). Guidelines on Pain Management. Netherland: European Association of Urology.

Budiati, T., Suzana & Surdijati, S. (2010). Synthesis, Analgesic and Anti-Inflammatory Activities of Substituted Benzoylthioureas. *Majalah Farmasi Indonesia*; *21*; 68-76. doi: 10.14499/indonesianjpharm0iss0pp68-76.

Cregg, R., Russo, G., Gubbay, A., Brandfort, R. & Sato, H. (2013). Pharmacogenetics of Analgesic Drugs. *British Journal of Pain*; *7*; 189–208. doi: 10.1177/2049463713507439.

Dona, R., Frimayanti, N., Ikhtiarudin, I., Iskandar, B., Maulana, F. & Silalahi, N. T. (2019). Studi In Silico, Sintesis, dan Uji Sitotoksik Senyawa P-Metoksi Kalkon terhadap Sel Kanker Payudara MCF-7. *Jurnal Sains Farmasi & Klinis*; *6*; 243-249. doi: 10.25077/jsfk.6.3.243-249.2019.

Gawade, S. P. (2012). Acetic Acid Induced Painful Endogeneous Infliction in Writhing Tes on Mice. *Journal of Pharmacology & Pharmacotherapeutics*; *3*; 348. doi: 10.4103/0976-500X.103699.

HCANJ. (2017). Pain-Management-Guidelines. https://www.hcanj.org/files/2013/09/Pain-Management-Guidelines-_HCANJ-May-12-final.pdf. Accessed: 11 April 2019.

Jakaria, M., Parvez, M., Zaman, R., Arifujjaman., Hasan, M. I., Sayeed, M. A. & Ali, M. H. (2015). Investigations of Analgesic Activity of the Methanol Extract of *Haldina cordifolia* (Roxb.) Bark by using in vivo Animal Model Studies. *Research Journal of Botany*; *2015*; 98-103. doi: 10.3923/rjb.2015.98.103.

Koyagura, N., Nayak, N., Jamadar, M. G., Patil, A. M. & Kumar, H. (2015). Dose Response Relationship of Dexamethasone on Insulin Levels in Wistar Rats. *Journal of Chemical and Pharmaceutical Research*; *7*; 492-496.

Le Bars, D., Gozariu, M. & Cadden, S. W. (2001). Animal Models of Nociception. *Pharmacological reviews*; *53*; 597-652.

National Health Service. (2019). Pain Management Guidelines. New Jersey: Authors.

Shalas, A., Siswandono. & Rudyanto, M. (2016). Synthesis and Structure-Activity Relationship of 1-allyl-3-(2-chlorobenzoyl) Thiourea as Analgesic. *International Journal of Pharmacy and Pharmaceutical Science*; *8*; 297-298.

Somakala, K. & Amir, M. (2017). Synthesis, Characterization and Pharmacological Evaluation of Pyrazolyl Urea Derivatives as Potential Anti-Inflammatory Agents. *Acta Pharmaceuticasinica B*; *7*; 230-240. doi: 10.1016/j.apsb.2016.08.006.

Suhud, F. (2015). Uji Aktivitas In-Silico Senyawa Baru 1-benzil-3-benzoilurea Induk dan Tersubstitusi sebagai Agen Anti Proliferatif. *Jurnal Farmasi Indonesia*; *7*; 245-251.

Sun, K., Song, X., Jia, R., Yin, Z., Zou, Y., Li, L., Yin, L., He, C., Liang, X., Yue, G., Cui, Q. & Yang, Y.

(2018). Evaluation of Analgesic and anti-Inflammatory Activities of Water Extract of *Galla chinensis* in Vivo Models. *Evidence-based Complementary and Alaiternative Medicine*; *2018*; 1-7. doi: 10.1155/2018/6784032.

Yap, B. K., Lee, C. Y., Choi, S. B., Kamarulzaman, E. E., Hariono, M. & Wahab, H. A. (2019). In Silico Identification of Novel Inhibitors. *Encyclopedia of Bioinformatics and Computational Biology*; *2019*; 761-779. doi: 10.1016/j.jmgm.2012.11.010.0.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 24-31
DOI: 10.20473/jfiki.v9i12022.24-31
Available online at https://e-journal.unair.ac.id/JFIKI/

Calcium Decay Ability of Various Kirinyuh Leaf Extracts (*Chromolaena odorata* L.) on Kidney Stones

Dimas Danang Indriatmoko[1], Maulani[1], Tarso Rudiana[2*]
[1]Department of Pharmacy, Faculty of Science Pharmacy and Health, Universitas Mathla'ul Anwar, Pandeglang, Indonesia
[2]Department of Chemistry, Faculty of Science Pharmacy and Health, Universitas Mathla'ul Anwar, Pandeglang, Indonesia

*Corresponding author: tarso.rudiana@gmail.com

Submitted: 23 May 2021
Accepted: 18 December 2021
Published: 26 April 2022

Abstract

Background*: Kidney stones are one of the causes of chronic and acute kidney failure symptoms. The flavonoid compounds in Chromolaena odorata leave extract are thought to dissolve calcium in kidney stones.* ***Objective****: This study aims to determine the activity of C. odorata leaves extract as a dissolution agent for calcium kidney stones and to characterize the active extract with a liquid chromatograph mass spectrometry.* ***Methods****: The leaves of C. odorata were extracted by ultrasonication method using 3 solvents in stages, namely n-hexane, ethyl acetate, and methanol. The powder for kidney stones was immersed in an extract solution of n-hexane, ethyl acetate, and methanol for 5 hours at 37°C. The reaction results were analyzed for their absorbance using a UV-Vis spectrophotometer. The fraction with the best activity was analyzed for phytochemical content with various typical reagents and LCMS/MS.* ***Results****: Methanol extract of C. odorata with a concentration of 10,000 µg/mL can reduce calcium in kidney stones by 19,2 µg/mL. Based on phytochemical tests and LCMS/MS analysis, the methanol extract of C. odorata leaves contains compounds of the tannins, alkaloids, flavonoids, and steroids. Chromatogram at a retention time of 7.76; 8.96; and 10.01 in methanol extract of C. odorata identified 3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone; 5,7,4'-trihydroxy-3',5'-dimethoxyflavone, and 5,6,7,8,4'-pentamethoxyflavone compounds.* ***Conclusion****: Methanol extract with a concentration of 10,000 µg/mL had the best calcium decay activity in kidney stones of 19,2 µg/mL. 3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone;5,7,4'-trihydroxy-3',5'-dimethoxyflavone, and 5,6,7,8,4'-pentamethoxyflavone compounds are contained in methanol extract which is thought to play a role in shedding calcium in kidney stones.*

Keywords*: Chromolaena odorata, extraction, flavonoids, kidney stones, UV-Vis spectrophotometer*

How to cite this article:
Indriatmoko, D. D., Maulani, & Rudiana, T. (2022). Calcium Decay Ability of Various Kirinyuh Leaf Extracts (*Chromolaena odorata* L.) on Kidney Stones. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 24-31. http://doi.org/10.20473/jfiki.v9i12022.24-31

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

Acute and chronic decline in kidney function is a sign of kidney failure (Rostanti *et al*., 2016). Kidney failure is characterized by the failure of the kidneys to function optimally. People with kidney disease must undergo hemodialysis throughout their life or get a kidney donor through kidney transplant surgery (Savitri & Parmitasari, 2015). One of the causes of kidney failure is the presence of stones in the urinary tract, and kidney stones, and it is ranked fourth in the occurrence of kidney failure after hypertension, diabetes, and cholesterol (Pranandari & Woro, 2015). According to Purnomo (2003), Kidney stones with a calcium composition are the most common types of kidney stones. Traditional medicine is one of the therapies for kidney stones in addition to surgery, radiation and modern medicine. Treatment of kidney stones with traditional medicine is an alternative option because, besides being cheap, the side effects are also more negligible (Sasmito *et al*., 2001).

The use of traditional medicine has become the culture of Indonesian society. The use of plants is vital in the development of traditional medicine. Medicinal plants that can be used to treat back pain that leads to urinary tract stones include those from the families of Zingiberaceae, Acanthaceae, Poaceae, Lamiaceae, and Asteraceae (Nisa & Astana, 2018). The content of compounds such as flavonoids and phenolics in plants is used as bioactive compounds. Novalia *et al*. (2016) reported that the flavonoid content in the ethyl acetate fraction of *Clerodendron thomsonae* Balf (Lamiaceae) leaves could dissolve calcium in kidney stones. Flavonoids can dissolve calcium in kidney stones because the hydroxy groups on flavonoids can form complexes with calcium (Ratri, 2008), such as luteolin-7-*O*-glycoside compounds which are capable of preventing and dissolving kidney stones (Dhianawaty *et al*., 2003).

Kirinyuh (*Chromolaena odorata* L.) (Asteraceae) is a shrub that contains secondary metabolites such as flavonoids, phenols, tannins, saponins, and steroids. *C. odorata* extract contains isoflavone, flavone, flavonol, and chalcone group compounds (Saputra *et al*., 2017). Fitrah *et al*. (2017) succeeded in isolating a flavonoid derivative, namely methyl ether naringenin. Three chromome derivatives have been isolated from *C. odorata*, namely 5,7-dihydroxy-2-(4-methoxyphenyl) c hromen-4-on, 3,5-dihydroxy-2-(3-hydroxy-4-methoxyp henyl) compounds 4-on and 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-chromen-4-on (Jasnie, 2009). The potential content of flavonoid compounds in *C. odorata* can be used as a dissolution agent of calcium found in kidney stones. In this study, secondary metabolites were extracted from the leaves of *C. odorata* with *n*-hexane, ethyl acetate, and methanol as solvents. Each extract was tested for calcium decay in kidney stones. Extracts with the best activity were analyzed for phytoconstituent content by LCMS/MS.

MATERIALS AND METHODS

Sample collection

C. odorata leaves were collected from Serang Regency - Banten (6°05'22.0"S 106°09'25.4"E). Plants were identified in LIPI Research Center for Biology (Identification letter number 2499/IPH.1.01/If.07/XI/2018).

Materials

Technical grade organic solvent such as *n*-hexane, ethyl acetate, methanol, aquadest, ethanol, anhydrous acetic acid (Merck), 1% iron (III) chloride (Merck), chloroform (Merck), 2 N hydrochloric acid (Merck), metal Mg, Meyer reagent, Wagner reagent, calcium kidney stones, standard calcium chloride (Merck), murexid solution 0.5 N (Merck), silver nitrate (Merck), sulfuric acid (Merck), barium chloride (Merck), 0.1 N sodium hydroxide (Merck), dimethyl sulfoxide (Merck), Harnal D tablets.

Instruments

Various glass and non-glass equipment, analytical scales, waterbath, ultrasonicator (BAKU BK-3A), oven, vaccum rotary evaporator (IKA RB 10 basic), a set of UV-Vis spectrophotometers (Optima SP - 300 Spectrophotometer), a set of LC spectrophotometers MS (Acquaty UPLC®H-Class System, BEH C18, Xevo G2-S QTof).

Methods

Preparation sample and extraction

The leaves of *C. odorata* were collected, cleaned, and dried. *C. odorata* leaves are air-dried at room temperature. *C. odorata* leaves that have been dried are mashed using a blender. *C. odorata* leaf powder was extracted using ultrasonication with *n*-hexane, ethyl acetate, and methanol, alternating from nonpolar, semipolar, and polar. Dried *C. odorata* leaves powder (282 g) was put into an erlenmeyer flask and added with solvent *n*-hexane 1:5 (w/v) then extracted using an ultrasonicator for 30 minutes, and the macerate was separated from the residue (Januarti *et al*., 2017). The residue was extracted again using alternatingly different solvents, namely ethyl acetate and methanol. Each macerate was filtered and concentrated with a vacuum rotary evaporator, resulting in extracts of *n*-hexane,

P-ISSN: 2406-9388
E-ISSN: 2580-8303

ethyl acetate, and methanol from the leaves of *C. odorata*.

Calcium decay test in kidney stones

The calcium decay test in kidney stones consists of several stages, including preparation of kidney stones, identification of calcium in kidney stones, and analysis of calcium decay in kidney stones. Kidney stones (3 g) obtained from a patient with kidney stones were cleaned with aquadest and baked in an oven for 10 minutes at 100°C, after being cooled, they were crushed using a mortar.

Kidney stones identified the content of calcium, oxalate, and phosphate. Kidney stone powder is added with a sulfuric acid solution to detect the presence of calcium. Kidney stone powder was added with nitrate treatment to detect oxalate content, and to identify phosphate, kidney stone powder was added with barium chloride.

The standard solution of calcium is made following the research procedure Hayati *et al*. (2016) includes the making of $CaCl_2.2H_2O$ solutions with concentrations of 4, 6, 8, 10, 20, and 25 µg/mL. Each sample concentration was pipetted 1 mL and put into a 25 mL volumetric flask, added 1 mL of murexide solution, 2 mL of sodium hydroxide and made up to 25 mL with 96% ethanol. The solution was homogenized and the absorbance was measured using UV-Vis spectrophotometer at a wavelength of 500 nm, and the standard curve was determined.

Each extract of *n*-hexane, ethyl acetate, and methanol was made with a concentration series of 100, 500, 1000, 5000, and 10000 µg/mL with 96% ethanol as the solvent. Kidney stone powder was immersed in each extract solution at 37°C for 5 hours and stirred every 15 minutes. The reaction results were filtered using Whatman filter paper, then 1 mL of the filtrate was taken and put into a 25 mL volumetric flask, 1 mL of murexide solution, and 2 mL of 0.1 N sodium hydroxide and 96% ethanol were added to the mark. The solution was homogenized and the absorbance was read at 500 nm (Novalia *et al*., 2016). The negative control in this study was kidney stone powder at 96% ethanol. The positive control used was Harnal D tablet.

Phytochemical analysis

Phytochemical analysis of the extract included qualitative tests for saponins, tannins, alkaloids, flavonoids, triterpenoids and steroids. The extract was dissolved in methanol and put in several tubes, the first tube was added with aqua dest for the detection of saponins, the second tube was added with aqua dest and $FeCl_3$ for the detection of tannins, the third and fourth tubes were added Mayer and Wager reagents for the identification of alkaloids respectively, the fifth tube was added with aqua dest, Mg and HCl for flavonoid identification. The sixth tube was added with Liebermann Burchard reagent for triterpenoid/steroid (Fathoni *et al.,* 2019).

Analysis of the most active extract using LCMS/MS

Methanol extract has the best activity as a dissolution agent in kidney stones. Chemical content contained in the methanol extract of *C. odorata* leaves was analyzed using LCMS/MS. A total of 0.5 mg of methanol extract of *C. odorata* was dissolved in methanol then pipette 10 µL of the sample and then injected into LCMS/MS with a stationary phase column C-18 (2 x 150 mm), methanol: water (9:1) as the mobile phase with flow rate. 0.3 mL/minute (Rudiana *et al.*, 2019).

RESULTS AND DISCUSSION

Extraction and calcium decay test in kidney stones

The leaves of *C. odorata* (282 g) were extracted using an ultrasonicator using *n*-hexane, ethyl acetate, and methanol as solvents. The ultrasonication method has advantages, including faster and more efficient extraction times in the use of solvents (Febriyanti *et al*., 2016). The ultrasonic filtrate is filtered using filter paper. The macerate was concentrated using a vacuum rotary evaporator at 45°C to obtain concentrated extracts of *n*-hexane, ethyl acetate, and methanol.

Table 1. Yield value data of *C. odorata* extract

Initial powder weight (g)	Extract	Mass (g)	% Yield
282	*n*-Hexane	12.44	4.41
	Ethyl acetate	23.48	8.32
	Methanol	37.97	13.46

Methanol extract had the highest % yield, amounting to 13.46% (Table 1). The high% yield in methanol extract indicated that *C. odorata* leaves contained many polar compounds such as flavonoids, phenolics, and alkaloids. Methanol can dissolve compounds with high polarity due to OH groups such as flavonoids, phenolics, and alkaloids (Rudiana *et al*., 2018). The terpenoid and steroid compounds are soluble in non-polar to semi-polar solvents (Saidi *et al*., 2018).

The yield of ethyl acetate solvent is smaller than that of methanol but larger than that of *n*-hexane (Table 1); this is presumably due to the presence of a methoxy group in the chemical structure of ethyl acetate. The presence of the methoxy group causes ethyl acetate to form hydrogen bonds with compounds contained in the

P-ISSN: 2406-9388
E-ISSN: 2580-8303

sample. The hydrogen bond formed in the ethyl acetate solvent is weaker than the hydrogen bond formed in the methanol solvent so that it affects the yield of the ethyl acetate solvent, which is less (Romandanu *et al*., 2014).

Analysis of calcium dissolution in kidney stones was carried out using photometry using a UV-Vis spectrophotometer. Each test solution was incubated at 37°C for 5 hours. The incubation temperature of 37°C corresponds to average human body temperature (Kukus *et al*., 2009). Based on research Hayati *et al*. (2016) The optimum incubation time is 5 hours. Kidney stones in the body can move due to urine flow, water flow or movement due to the activity of the human body (Efendi *et al*., 2012), so every 15 minutes during the incubation of the test solution, stirring was carried out (Oktari *et al*., 2014). The purpose of stirring is to obtain conditions such as those that occur in the body, especially in the kidney organ, which then moves to the urinary tract where kidney stones are usually found (Dewi *et al*., 2016).

The calcium solubility test of kidney stones was carried out *in vitro* where the measurement of dissolved calcium levels was carried out using a UV-Visible spectrophotometer at a maximum of 500 nm. The dissolved calcium content was calculated based on the standard curve equation y = 0.3627 + 0.004x (R^2 = 0.9984). The measurement of dissolved calcium levels can be seen in Figure 1.

The bar chart in Figure 1, shows that the methanol extract of *C. odorata* has a greater effect in dissolving calcium kidney stones than the *n*-hexane and ethyl acetate solutions.

This study showed that *in vitro* studies of methanol extract with a 10,000 µg/mL concentration can dissolve calcium kidney stones by 19.2 µg/mL (Figure 1). A positive control using the drug Harnal D was able to dissolve calcium kidney stones by 3.33 µg/mL (Figure 1). The increasing concentration of the extract was followed by the increasing ability to dissolve calcium kidney stones. The high concentration of the extract is directly proportional to the number of flavonoid levels, causing the ability to dissolve kidney stones will also increase. The highest ability to dissolve calcium kidney stones was in 10,000 µg/mL methanol extract. Ethyl acetate is a semipolar solvent. Compounds such as aglycone flavonoids, methylated flavonoids, tannins, and some alkaloid compounds can be extracted by ethyl acetate solvent (Rudiana *et al*., 2018). The solvent of *n*-hexane can attract nonpolar compounds such as steroids and terpenoids. Low polarity compounds such as methylated flavonoids, steroids, and triterpenoids have a low ability to dissolve calcium in kidney stones.

Data for the solubility of calcium kidney stones were statistically tested with the normality test (Shapiro-Wilk) and the homogeneity test (Brown-Forsythe and Welch) and followed by a parametric test, namely ANOVA (Analysis of Variance). Based on the statistical analysis results, the percent solubility of calcium for kidney stones is normally distributed with a significance value > 0.05, so the variable data for the percent solubility of calcium for kidney stones is normally distributed. In the homogeneity test with Brown-Forsythe and Welch, it is known that the significance value is 0.000, and the homogeneity test value at the significance level is $\leqslant$ 0.05 so that the data on the variance value is homogeneous. The One Way Anova test results showed that the percent solubility of kidney stone calcium had different variant values (α = 0.05).

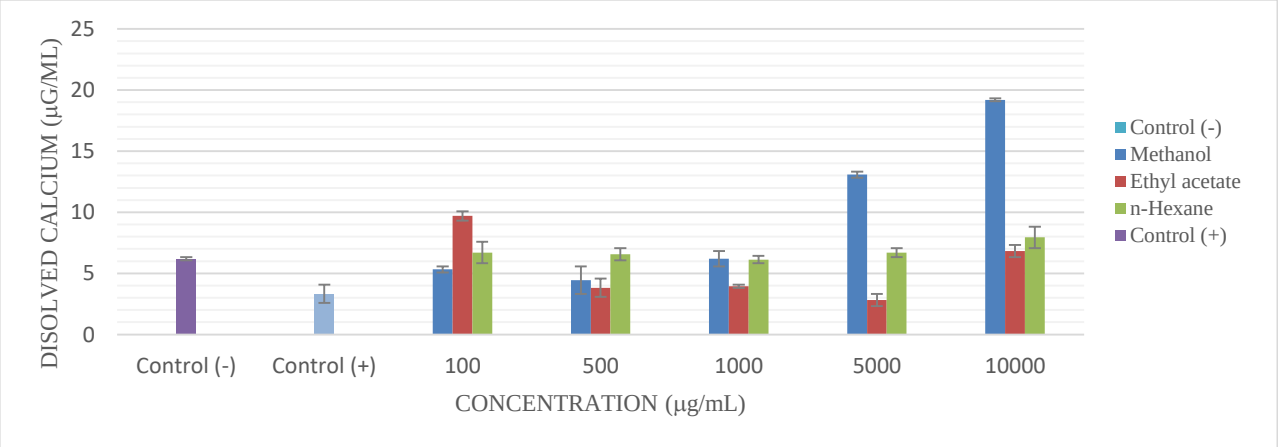


Figure 1. Comparison of the average dissolved kidney stone calcium levels in each extract

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Characterization of kidney stone decreasing active extract

Methanol extract of *C. odorata* leaves had the best activity compared to *n*-hexane and ethyl acetate extracts. The methanol extract *C. odorata* leaves was characterized and determined its chemical content. The results of phytochemical testing of the methanol extract of the leaves of *C. odorata* are presented in Table 2.

Table 2. Phytochemical screening of the methanol extract of *C. odorata*

No.	Secondary metabolites	Result
1.	Saponin	+
2.	Tannin	+
3.	Alkaloid	+
4.	Flavonoid	+
5.	Steroid	+
6.	Triterpenoid	-

+ : detected; - : not detected

C. odorata leaf methanol extract contains saponins, tannins, alkaloids, flavonoids and steroids (Table 2). In this test, saponins were identified in the methanol extract of *C. odorata* which was characterized by the formation of a stable foam. Tannin compounds were identified in the methanol extract of the leaves of *C. odorata*, the formation of a green-black color in the analysis of tannins was thought to come from a complex compound between Fe metal and tannins contained in the methanol extract of *C. odorata* (Sukarno, 2017). The methanol extract of *C. odorata* contains alkaloids that are characterized by the formation of a white precipitate when reacted with Meyer reagent and an orange residue in Wagner reagent (Sukarno, 2017).

The methanol extract of *C. odorata* contains flavonoids characterized by the occurrence of a color change to orange color when the methanol extract of *C. odorata* is added with Mg metals and HCl. The flavonoids contained in the methanol extract of *C. odorata* are reduced, causing an orange color (Simaremare, 2014). The steroid test was based on the color change of Liebermann Burchard reagent with methanol extract of *C. odorata*. The green ring was identified in this test so that the methanol extract of the leaves of *C. odorata* contains steroid class compounds (Ayoola *et al.*, 2008).

The methanol extract of *C. odorata* was analyzed using LCMS/MS which aims to determine the content of its chemical compounds. The chromatogram of the methanol extract of *C. odorata* is presented in Figure 2. The chromatogram shows 13 peaks at a retention time of 1.23; 5.20; 6.23; 7.32; 7.76; 8.13; 8.96; 9.31; 10.01; 10.68; 11.04; 11.70; and 12.16 minutes.

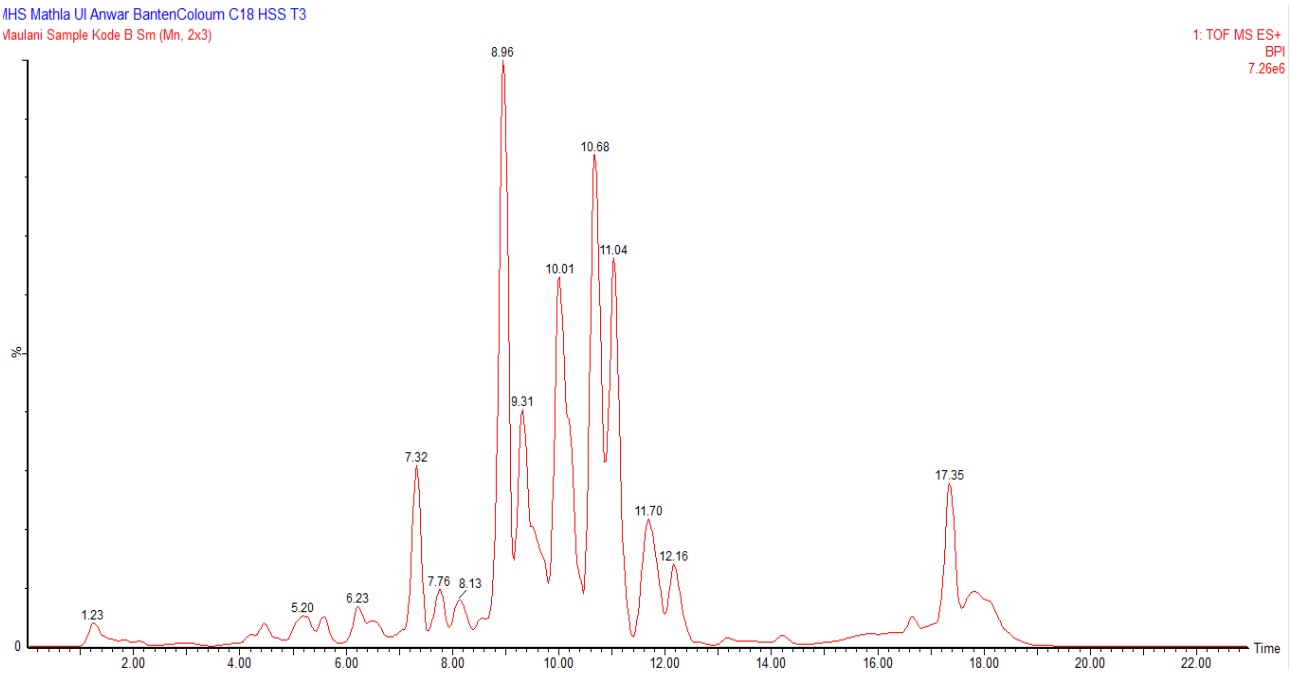
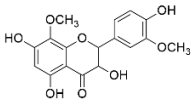
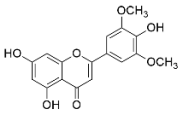
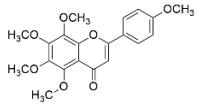


Figure 2. Total Ion Chromatogram of the methanol extract of *C. odorata* leaves

Table 3. Tabulation of interpretation results of LCMS/MS data from methanol extract of *C. odorata* leaves

No.	Retention time (minute)	Molecular weight (g/mol)	Molecular Formula	Compound Name	Molecular Structure
1.	7.76	346.0689	$C_{17}H_{14}O_8$	3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone (**1**)	
2.	8.96	331.0812	$C_{17}H_{14}O_7$	5,7,4'-trihydroxy-3',5'-dimethoxyflavone (**2**)	
3.	10.01	372.1209	$C_{20}H_{20}O_7$	5,6,7,8,4'-pentamethoxyflavone (**3**)	

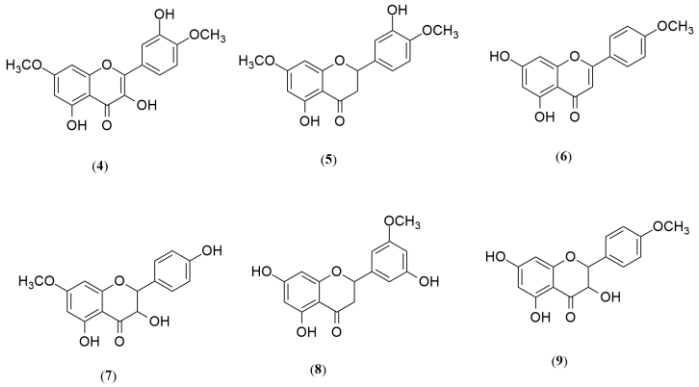


Figure 3. Structure chemical compounds from *C. odorata* (Pisutthanan *et al*., 2005)

Several flavonoid compounds with a flavonoid basic framework have been isolated from *C. odorata*. Pisutthanan *et al*. (2005) reported 6 flavonoid compounds with a flavone framework, namely 3,5,3'-trihydroxy-7,4'-dimethoxyflavone (4), 5,3'-dihydroxy-7,4'-dimethoxyflavanone (5), 5,7-dihydroxy-4'-methoxyflavone (6), 3,5,4'-trihydroxy-7-methoxyflavanone (7), 5,7,3'-trihydroxy-5'-methoxyflavanon (8), and 3,5,7-trihydroxy-4'-methoxyflavanone (9), chemical structure can be seen Figure 3. Compounds 1, 2, and 3 (Tabel 3) identified in this study have a basic framework similar to Pisutthanan et al. (2005) and the number of substituents in rings A and B. The difference in substituents is thought to be due to the difference in the location of different plants so that the enzymatic reactions that occur in plants will produce other chemical structures.

Compounds 1 and 2 are flavonoid derivative compounds with a hydroxyl group (OH). The –OH group in compounds 1 and 2 can react with calcium to form a Ca-flavonoid chelate complex. These complex compounds are more soluble in water, so the water contained in the urine will help remove kidney stones (Nisma, 2011).

CONCLUSION

Extract methanol from the leaves of *C. odorata* with a concentration 10,000 µg/mL can dissolve calcium kidney stones by 19.2 µg/mL. The methanolic extract of *C. odorata* leaves contains 3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone; 5,7,4'-trihydroxy-3',5'-dimethoxyflavone, and 5,6,7,8,4'-pentamethoxyflavone compounds.

ACKNOWLEDGMENT

The author would like to thank Mr Nasrullah (Ciwandan - Cilegon), who was willing to voluntarily provide kidney stones after kidney stone removal surgery at Kurnia Hospital, Cilegon. Mr. Azhar at the POLRI Center for Laboratory Affairs and Farhan Riza Afandi at the Chemistry Study Program at UIN Syarif Hidayatullah Jakarta assisted in analysing LCMS/MS. Adawiah at the Center for Integrated Laboratory of FST UIN Syarif Hidayatullah Jakarta and a laboratory

assistant at the Chemical Laboratory of STAK Cilegon who has assisted in the analysis of kidney stone decay.

AUTHOR CONTRIBUTIONS

Conceptualization, D.D.I., M.M., T.R.; Methodology, D.D.I.; Software, T.R.; Validation, T.R.; Formal Analysis, D.D.I.; Investigation, T.R.; Resources, M.M.; Data Curation, T.R.; Writing - Original Draft, M.M., T.R.; Writing - Review & Editing, D.D.I., T.R.; Visualization, D.D.I.; Supervision, T.R.; Project Administration, M.M.; Funding Acquisition, M.M.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C. & Atangbayila, T. O. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*; *7*; 1020-1024. doi: 10.4314/tjpr.v7i3.14686.

Dewi, E. K. M., Walanda, D. K. & Sabang, S. M. (2016). Pengaruh Ekstrak Seledri (*Apium graveolens*) Terhadap Kelarutan Kalsium dalam Batu Ginjal. *Jurnal Akademika Kimia; 5*; 127-132.

Dhianawaty, D., Padmawinata, K., Soediro, I. & Soemardji, A. A. (2003). Isolasi, Karakterisasi dan Uji Aktivitas Pencegahan Antikalkuli Luteolin-7-*O*-Glukosida dari Daun *Sonchus arvenis* L. pada Tikus dengan Metode Matriks Asam Glikolat. *Jurnal Bionatura*; *5*; 196-202.

Efendi, F., Desnauli, E. & Nursalam. (2012). Indikator Kualitas Hidup Pasien Gagal Ginjal Kronis yang Menjalani Hemodialisa Berdasarkan Strategi Koping. *Jurnal Ners*; *6*; 187–191. doi: 10.20473/jn.v6i2.3990.

Fathoni, A., Rudiana, T. & Adawiah, A. (2019) Characterization and Antioxidant Assay of Yellow Frangipani Flower (*Plumeria alba*) Extract. *Jurnal Pendidikan Kimia*; *11*; 1–7. doi: 10.24114/jpkim.v11i1.13034.

Febriyanti, A. P., Iswarin, S. J. & Digjayanti, T. (2016). Perbandingan Kadar Asiatikosida dalam Ekstrak Etanol 70% Pegagan (*Centella asiatica* (L) Urben) dengan Metode Ekstraksi Maserasi dan Sonikasi Secara LC-MS/MS. *Jurnal Farmasi*; *4*; 50–57. doi: 10.24252/jurfar.v4i2.2246.

Fitrah, M., Winarno, H. & Simanjuntak, P. (2017) Isolasi dan Identifikasi Senyawa Kimia Zat Anti Kanker dari Daun Kopasanda (*Chromolaena odorata* (L.). *Jurnal Ilmu Kefarmasian Indonesia*; *15*; 77–81.

Hayati, M., Ramadhan, A. M. & Ibrahim, A. (2016). Efek Peluruhan Kalsium Batu Ginjal Ekstrak Metanol Daun Bungur (*Lagerstroemia speciosa* Pers.) secara *In Vitro*. *Proceeding*; Mulawarman Pharmaceuticals Conferences, Samarinda. doi: 10.25026/mpc.v3i2.122.

Januarti, B. I., Santoso, A. & Razak, A. S. (2017) Flavonoid Extraction of Teak Leaf (*Tectona grandis* L.) with Ultrasonic Method (Study of Material: Solvent Ratio and Extraction Time). *Media Farmasi Indonesia*; *12*; 1263–1270.

Jasnie, F. H. (2009). Biological Activities and Chemical Constituents of *Chromolaena odorata* (L.) King & Robinson. *PhD Thesis*; Faculty of Science University of Malaya, Kuala Lumpur.

Kukus, Y., Wenny, S. & Fransiska, L. (2009). Suhu Tubuh: Homeostatis dan Efek Terhadap Kinerja Tubuh Manusia. *Jurnal Biomedik*; *1*; 107–118. doi: 10.35790/jbm.1.2.2009.824.

Nisa, U. & Astana, P. R. W. (2018). Studi Etnofarmakologi Tumbuhan Obat untuk Mengobati Gangguan Batu Saluran Kemih di Sumatera Indonesia. *Buletin Penelitian Kesehatan*; *46*; 275-286. doi: 10.22435/bpk.v46i4.436.

Nisma, F. (2011). Pengaruh Penambahan Ekstrak Etanol 70% Buah Anggur Biru (*Vitis vinifera* L.) Terhadap Kelarutan Kalsium Batu Ginjal. *Skripsi*; Fakultas MIPA Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta.

Novalia, N., Ramadhan, A. M. & Ibrahim, A. (2016). Efek Peluruhan Kalsium Batu Ginjal Fraksi Etil Asetat Daun Nona Makan Sirih (*Clerodendron thomsonae* Balf.f.) secara In Vitro. *Proceeding*; Mulawarman Pharmaceuticals Conferences, Samarinda.

Oktari, T., Fitmawati, & Sofiyanti, N. (2014) Identifikasi dan Uji Fitokimia Ekstrak Alami Tanaman Antiurolithiasis. *Jurnal Online Mahasiswa Fakultas Matematika dan Ilmu Pengetahuan Alam*; *1*; 1-9.

Pisutthanan, N., Liawruangrath, S., Bremer, J. B. & Liawruangrath, B. (2005). Chemical Constituents Biological Activities of *Chromoleana odorata*. *Chiang Mai Journal of Science*; *32*; 139-148.

Pranandari, R. & Woro, S. (2018) Faktor Resiko Gagal Ginjal Kronik di Unit Hemodialisis RSUD Wates Kulon Progo. *Majalah Farmaseutik*; *11*; 316–320.

Purnomo, B. B. (2003). Dasar-Dasar Urologi, Edisi 2. Jakarta: Penerbit CV Sagung Seto.

Ratri, W. N. (2008). Uji Kelarutan Batu Ginjal Kalsium Dalam Fraksi Air dan Fraksi Etil Asetat Daun Jagung (*Zea mays* l) secara In Vitro dengan Metode Spektrofotometri Serapan Atom. *Skripsi*; Fakultas Farmasi Universitas Muhammadiyah, Surakarta.

Romandanu, Rahcmawati, S. H. & Lestari, S. D. (2014) Pengujian Aktivitas Antioksidan Ekstrak Bunga Lotus (*Nelumbo nucifera*). *Fishtech*; *3*; 1-7. doi: 10.36706/fishtech.v3i1.3523.

Rostanti, A., Bawotong, J. & Onibala, F. (2016). Faktor-Faktor yang Berhubungan Dengan Kepatuhan Menjalani Terapi Hemodialisa pada Penyakit Ginjal Kronik Di Ruangan Dahlia Dan Melati RSUP Prof. DR. R. D. Kandou Manado. *Jurnal Keperawatan UNSRAT*; *4*; 1-6. doi: 10.35790/jkp.v4i2.12873.

Rudiana, T., Fitriyanti, F. & Adawiah, A. (2018). Aktivitas Antioksidan dari Batang Gandaria (*Bouea macrophylla* Griff). *EduChemia (Jurnal Kimia dan Pendidikan)*; *3*; 195-205. doi: 10.30870/educhemia.v3i2.3328.

Rudiana, T., Suryani, N., Indriatmoko, D. D., Yusransyah, Amelia, A., Noviany & Hadi, S. (2019). Characterization of Antioxidative Fraction of Plant Stem *Bouea macrophylla* Griff. *Journal of Physics: Conference Series*; *1341*; 1-8.

Saidi, N., Ginting, B., Murniana & Mustanir. (2018) Analisis Metabolit Sekunder. Banda Aceh: Syiah Kuala Press.

Saputra, A., Gani, A. & Erlidawati, E. (2017). Antioxidant Activity of Siam Gulma (*Chromoleana odorata* L.) Using 1,1-Diphenyl-2-Picrylhydrazil Method. *Jurnal IPA & Pembelajaran IPA*; *1*; 131–142. doi: 10.24815/jipi.v1i2.9687.

Sasmito, D., Kamal, Z. & Kristanto, J. (2001) Kemampuan Ekstrak Air dan Fraksi Etil Asetat Daun Benalu Mindi (*Dendrophthoe falcata* LF Ettingsh) Melarutkan Batu Ginjal Kalsium *in vitro* yang Diuji Dengan Metode Aktivasi Neutron Cepat. *Majalah Farmasi Indonesia*; *12*; 120–127.

Savitri, Y. A. & Parmitasari, D. L. N. (2015). Kepatuhan Pasien Gagal Ginjal Kronis dalam Melakukan Diet Ditinjau dari Dukungan Sosial Keluarga. *Psikodimensia*; *14*; 1–10. doi: 10.24167/psiko.v14i1.369.

Simaremare, E. S. (2014). Skrining Fitokimia Ekstrak Etanol Daun Gatal (*Laportea decumana* (Roxb.) Wedd). *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*; *11*; 98-107. doi: 10.30595/pji.v11i1.855.

Sukarno. (2017). Uji Aktivitas Antibakteri Ekstrak Etanol, Etil Asetat dan *n*-Heksana Daun Laruna (*Chromolaena odorata* L.) terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Skripsi*; Fakultas Sains dan Teknologi Universitas Islam Negeri Alauddin, Makassar.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 32-38
DOI: 10.20473/jfiki.v9i12022.32-38
Available online at https://e-journal.unair.ac.id/JFIKI/

Synthesis and Antiplatelet Activity of 4-Hidroxy-3-Methoxycinnamic Acid

Erika Christy Marentek[1], Tutuk Budiati[1], Juni Ekowati[2*]
[1]Faculty of Pharmacy, Universitas Katolik Widya Mandala, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: juni-e@ff.unair.ac.id

Submitted: 11 July 2021
Accepted: 15 December 2021
Published: 26 April 2022

Abstract

Background*: Cinnamic acid and its derivatives have been widely studied for their efficacy because of the pharmacological effect on good health and well being. Microwave irradiation is more time effective to synthesize than conventional heating method because it conducts heat faster and shortens the reaction time.* ***Objective****: This study aimed to synthesize 4-hydroxy-3-methoxycinnamic acid using microwave irradiation and its antiplatelet activity by blood clotting time method.* ***Methods****: Synthesis of 4-hydroxy-3-methoxycinamic acid with malonic acid and 4-hydroxy-3-methoxybenzalehyde as a starting material using ammonium acetate catalyst with microwave irradiation (960 Watt, 4 minutes). The synthesis results were tested for purity by thin-layer chromatography, a melting point determination and structure identification (UV-Vis, infrared, and proton NMR spectrometry). The antiplatelet activity test consisted of a negative control group CMC-Na, a positive control acetosal, cinnamic acid, and 4-hydroxy-3-methoxycinnamic acid, each group consisted of 3 different doses, namely 0.0037 mmol/Kg (I), 0.0069 mmol/Kg (II) and 0.0139 mmol/Kg (III).* ***Results****: Synthesis of 4-hydroxy-3-methoxycinamic acid had a yield percentage of 30.55%. The test results showed that the 4-hydroxy-3-methoxycinnamic acid compound has antiplatelet activity with an ED30 value of 1.3080 mg/Kg BW and antiplatelet activity comparable to acetosal.* ***Conclusion****: 4-hydroxy-3-methoxycinamic acid can be synthesized by microwave irradiation and had antiplatelet activity 1.7 fold greater than cinnamic acid.*

Keywords*: 4-hydroxy-3-methoxycinamic acid, antiplatelet, good health and well being, microwave irradiation*

How to cite this article:
Marentek, E. C., Budiati, T., & Ekowati, E. (2022). Synthesis and Antiplatelet Activity of 4-Hidroxy-3-Methoxycinnamic Acid. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 32-38. http://doi.org/10.20473/jfiki.v9i12022.32-38

INTRODUCTION

Thromboembolism is a pathological process that leads to the formation of intravascular clots that can reduce blood flow to vital organs. Therapy for diseases associated with thromboembolism is antithrombotic, including antiplatelet (Acosta *et al*., 2016). Antiplatelets work by reducing platelet aggregation to prevent thrombus formation. Platelet inhibitory drugs have been identified, such as cyclooxygenase-1 inhibitors, adenosine diphosphate (ADP) antagonists and blockade of glycoprotein IIb/IIIa receptors on platelets (Hoffman *et al*., 2018). However, it is necessary to synthesize compounds with better therapeutic effects and minimal side effects.

Cinnamic acid derivatives have various pharmacological effects such as anti-inflammatory, antiplatelet, antimicrobial and antidiabetic (Sharma, 2011). The compound 4-hydroxy-3-methoxy cinnamic acid or ferulic acid (Figure 1) is one of the cinnamic acid derivatives that can be synthesized by Knoevenagel condensation. Knoevenagel condensation is a reaction involving an active methylene compound (a CH_2 flanked by two electron-withdrawing groups) and an aldehyde or ketone to yield an α,β-unsaturated product (McMurry, 2016). Ferulic acid is a cinnamic acid derivative, estimated to have the exact mechanism in inhibiting platelet aggregation by inhibiting the Ca^{2+} channels on the cell membrane surface and interfering with phospholipase C in releasing Ca^{2+} to reduce the interaction between ADP and $P2Y_{12}$ receptors and inhibiting the synthesis of thromboxane A_2 (TXA_2), which can activate platelet aggregation (Yang et al., 2013).

This study aims to synthesize 4-hydroxy-3-methoxycinnamic acid compound by reacting malonic acid with 4-hydroxy-3-methoxybenzaldehyde and ammonium acetate as catalyst using microwave irradiation. Because it is more cost effective, we prefer to produce 4-hydroxy-3-methoxycinnamic acid. The synthesized product was then compared to cinnamic acid and acetosal for antiplatelet activity utilizing a blood clotting time method with mice blood. Acetosal was chosen for this study because it is commonly used as an antiplatelet medication.

MATERIALS AND METHODS

Materials

Malonic acid (Sigma, Aldrich), 4-hydroxy-3-methoxybenzaldehyde (Merck, Germany), ammonium acetate, chloroform p.a. (Mallinckrodt, USA), ethanol p.a. (Mallinckrodt, USA), methanol p.a. (Mallinckrodt, USA), and silica gel 60 F_{254} (MACHEREY-NAGEL GmbH & Co. KG, Jerman).

Tools

Analytical balance (Sartorius, Germany), Sakura MW 9600 microwave oven with an output power of 1600 W with a frequency of 2,450 MHz, hot plate-magnetic stirrer, melting point apparatus (Stuart Scientific SMP1, UK), UV spectrophotometer (Hitachi UV-Vis U2910, Japan), infrared spectrophotometer (UATR Two Perkin Elmer, USA), nuclear magnetic resonance spectrometer (FT-NMR JEOL ECS-400, USA).

Method

Synthesis of 4-hydroxy-3-methoxycinnamic acid (ferulic acid)

Malonic acid was being crushed as much as 1.56 g (15 mmol) with ammonium acetate 770.8 mg (10 mmol) in an Erlenmeyer flask. 4-hydroxy-3-methoxybenzaldehyde 760.75 mg (5 mmol) was added to the mixture and stirred for several seconds. Erlenmeyer was removed from the microwave oven (960 Watt, 4 minutes) after the reaction, cooled, and added 2 N HCl little by little while stirring until a precipitate formed, filtered and washed with water to remove residual acid. The precipitated of ferulic acid was recrystallized from 70% ethanol.

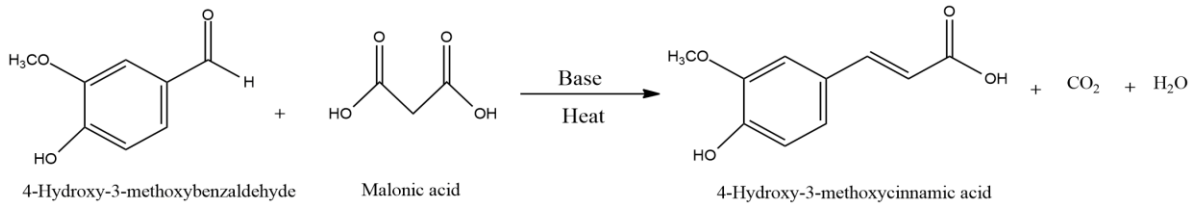


Figure 1. Scheme of 4-hydroxy-3-methoxycinnamic acid reaction

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Purity test and identification of synthesized compounds

The purity test was conducted by determining the melting point and thin-layer chromatography (TLC) test using 3 mobile phases with different polarity indexes and using 4-hydroxy-3-methoxybenzaldehyde as a standard.

Structure identification of the synthesized compounds was carried out by ultraviolet (uv) spectrophotometric test with ethanol solvent, infrared (IR) spectrophotometric test at a wavelength of 4000 - 600 cm^{-1} and proton NMR (^{1}H-NMR) spectrometry test with acetone-d6 solvent.

Antiplatelet activity test

Healthy mice aged 8-12 weeks and weighing 20-22 grams were used to test the antiplatelet activity of 4-hydroxy-3-methoxy cinnamic acid compounds. Mice were divided into ten treatment groups, namely the negative control group (CMC-Na 0.5%), positive control (acetosal) doses of 40 mg, 75 mg and 150 mg, groups of cinnamic acid and ferulic acid compounds equivalent to doses of acetosal 40 mg, 75 mg and 150 mg, specifically 0.0037 mmol/Kg (I), 0.0069 mmol/Kg (II) and 0.0139 mmol/Kg (III).

Test compounds such as acetosal, cinnamic acid and ferulic acid were prepared in suspension form using 0.5% CMC-Na solution because the test compounds are not soluble in water. Acetosal is less stable and easily hydrolyzed in water, so the test solution is made every day. The maximum administration volume was 0.3 mL because the test compound was administered after eating, so it did not irritate the stomach because it was acidic.

Each mouse was conditioned for one week, and the length of time for fibrin formation was measured in the mice's blood samples on day 0. The mice's tale was cleaned with 70% alcohol, then pierced with a scalpel as far as 2 cm from the edge of the tail with a wound depth of 2 mm. The blood sample was then dripped into an object-glass and observed every 15 seconds to determine the onset of fibrin formation. Afterwards, the wounds in mice were given an antiseptic solution. Each test solution was administered orally for seven days. Observation of the onset of fibrin formation was analyzed on day 8 to determine the effect of the test solution. The results were then statistically tested by the one-way ANOVA method. The Ethical Commission approved the antiplatelet activity test in this experiment of Universitas Airlangga No.: 2.KE.103.11.2020.

RESULTS AND DISCUSSION

4-Hydroxy-3-methoxycinnamic acid (ferulic acid)

The 4-hydroxy-3-methoxycinnamic acid compound was light yellow crystals as shown in Figure 2. The synthesis of 4-hydroxy-3-methoxycinnamic acid was replicated three times. The percentage yield of the synthesis results were 31.92%, 28.83% and 30.89%, respectively, with an average of 30.55% ± 1.57. The results of determining the melting point obtained an average result of 170 - 171.5ºC. In a study conducted by Ekowati (2016), the melting point test of 4-hydroxy-3-methoxycinnamic acid compounds obtained an average of 170 - 171°C. That melting point range is the same as the one produced in this study. Each replication of ferulic acid was tested by TLC with some mobile phases such as ethyl acetate: n-hexane (1:1, v/v; Rf 0.27), acetone: chloroform (1:4, v/v); Rf 0.38), and methanol: ethyl acetate (7:3, v/v; Rf 0.61).

Figure 2. 4-Hydroxy-3-methoxycinnamic acid crystal

Structure identification of ferulic acid was carried out by analyzing the test results of the ultraviolet, infrared and ^{1}H-NMR spectra. The UV spectra of ferulic acid gave a maximum absorption peak at 313 nm and the starting material, 4-hydroxy-3-methoxybenzaldehyde, gave an absorption peak at 309 nm (Figure 3). The presence of conjugated double bonds cause the wavelength of the light absorbed becomes longer (Pavia, 2015). The IR spectrum (Figure 4) of ferulic acid shows the absorption at wave numbers 3433 cm^{-1} (phenolic OH), 1688 cm^{-1} (cojugated C=O), 1590 and 1431 cm^{-1} (aromatic C=C), 1265 and 1033 cm^{-1} (C-O ether). ^{1}H NMR (400 MHz, acetone-D_6, δ ppm) showed signals at 8.08 (s, 1H), 7.57 (d, J = 15.9 Hz, 1H), 7.30 (d, J = 1.9 Hz, 1H), 7.11 (dd, J = 8.2, 2.0 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.34 (d, J = 15.9 Hz, 1H), 3.89 (s, 3H).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

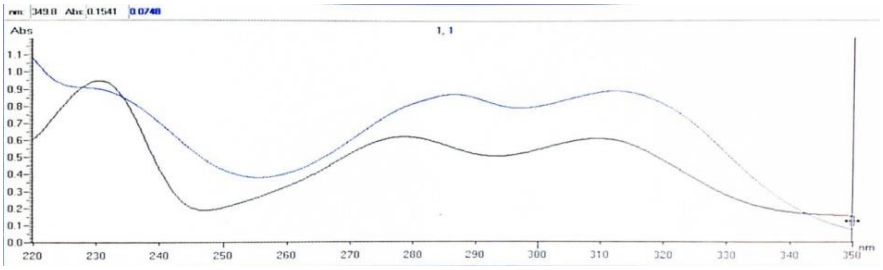


Figure 3. UV spectra overlays of 4-hydroxy-3-methoxybenzaldehyde and 4-hydroxy-3-methoxycinnamic acid

Information :

——— = Ultra violet spectrum pattern of 4-hydroxy-3-methoxybenzaldehyde

——— = Ultra violet spectrum pattern of 4-hydroxy-3-methoxycinnamic acid

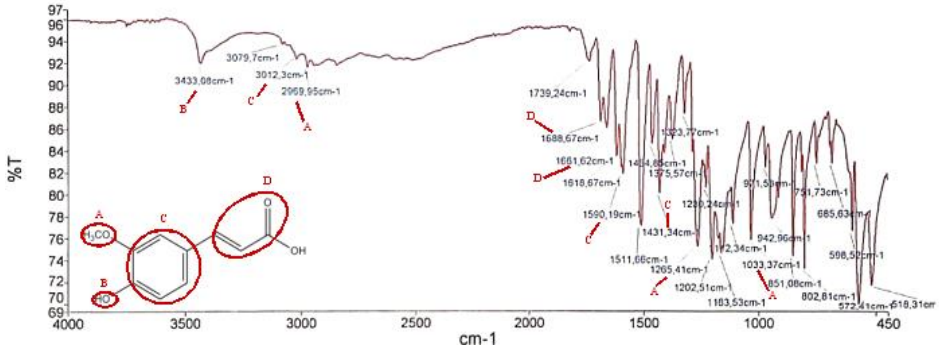


Figure 4. IR spectrum of 4-hydroxy-3-methoxycinnamic acid

The benzene ring in the synthesized compound can be proven based on IR spectrum data at wave numbers 1590 dan 1431 cm^{-1} which shows the presence of aromatic C=C bonds and aromatic C-H sp^2 which is indicated at an absorption of 3012 cm^{-1}. This is also evidenced by data on the ^{1}H-NMR spectrum (Figure 5) with a chemical shift of 7.30 ppm and a multiplicity of doublets, 7.11 ppm and multiplicity of double doublets, as well as 6.84 ppm and multiplicity of doublets. These three kinds of chemical shifts indicate the presence of 3 types of protons attached to the benzene ring because of the presence of absorption at a chemical shift of 6.5 - 8 ppm (Smith, 2011). The alkene group conjugated with carboxylate in the ^{1}H-NMR spectrum data is indicated by doublet absorption with each absorption showing 1 proton at a chemical shift of 7.57 ppm with J = 15.9 Hz and at a chemical shift of 6.34 ppm J = 15.9 Hz. Based on these data, it was concluded that the protons in Cα dan Cβ are in the trans conformation indicated by a coupling constant in the 11 - 18 Hz range representing protons in an alkene with the trans conformation (Smith, 2011). The company of a hydroxyl group (-OH) bound to the benzene ring is indicated by IR absorption at a wavenumber of 3433 cm^{-1} and ^{1}H-NMR spectrum data with a chemical shift at 8.08 ppm with singlet multiplicity and 1 proton. The H atom bound to the phenolic O atom has a chemical change in 4.0 - 7.0 ppm, but this chemical shift can vary depending on the concentration, temperature and solvent (Pavia *et al.*, 2015). The presence of a methoxy group (-OCH_3) bound to the benzene ring is indicated by IR absorptions at wavenumbers 1265 dan 1033 cm^{-1} by C-O eter dan C-H sp^3 bonds at wavenumbers 2969 cm^{-1} and ^{1}H-NMR spectrum data with a chemical shift of 3.89 ppm (*s*, 3H) which according to the literature, namely C-H sp^3 bound to the O atom undergoes a chemical shift in the range of 2.5 - 4 ppm (Smith, 2011).

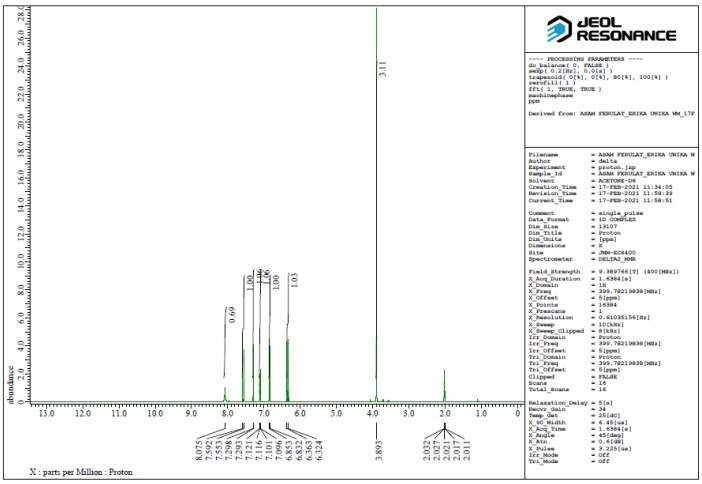
Antiplatelet activity test

The test compounds consisted of positive control (acetosal) at doses of 40 mg, 75 mg and 150 mg, groups of cinnamic acid and ferulic acid compounds which were equivalent to the molar doses of acetosal 40 mg, 75 mg and 150 mg, namely 3.7 x 10^{-3} mmol/Kg (I), 6.9 x 10^{-3} mmol/Kg (II) dan 13.9 x 10^{-3} mmol/Kg (III) which can be seen in Table 1. The selection of these three doses refers to the dose of acetosal as an antiplatelet, which is 75 - 325 mg (Katzung, 2018), a dose of 40 mg to see whether the potential test compound can provide antiplatelet activity in low doses. The dose of 75 mg was chosen because it is the minimum dose as an antiplatelet, and the dose of 150 mg was chosen to see the potential of the test compound if it was increased twice from the minimum dose.

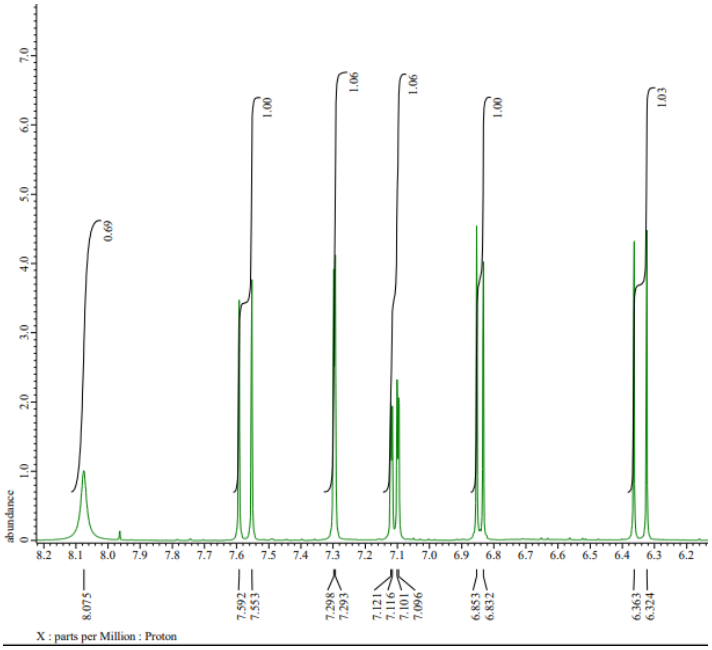
P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. %Antiplatelet activity and ED_{30} of the test compounds

Compounds	Dosage (mg/Kg)	% Activity	ED_{30}	
			mg/kg BW	mmol/kg
Acetosal (MW : 180.16 mg/mmol)	8.2	24.69	1.004	5.81×10^{-3}
	15.375	35.11		
	30.75	44.67		
Cinnamic Acid (MW : 148.16 mg/mmol)	8.2	8.68	1.695	11.45×10^{-3}
	15.375	20.84		
	30.75	35.48		
4-Hydroxy-3-methoxycinnamic acid (MW : 194.18 mg/mmol)	8.2	22.27	1.308	6.74×10^{-3}
	15.375	32.69		
	30.75	43.67		



a)



b)

Figure 5. a) ^{1}H-NMR spectrum of 4-hydroxy-3-methoxycinnamic acid using acetone-d*6* solvent; b) magnification of chemical shift : 6.324 - 8.075 ppm

P-ISSN: 2406-9388
E-ISSN: 2580-8303

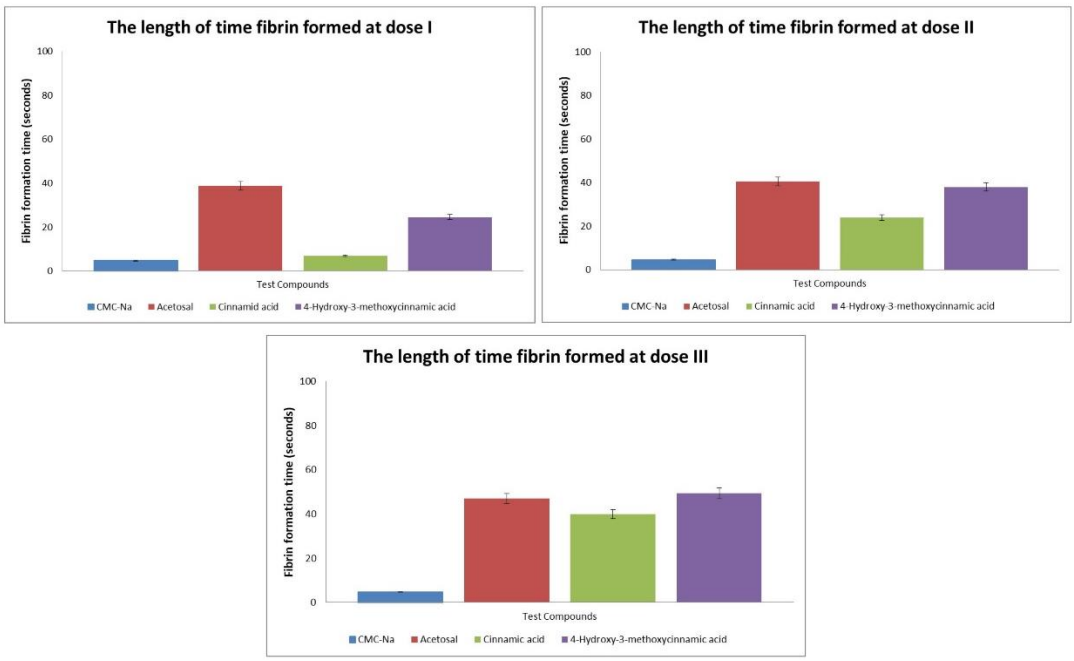


Figure 6. Comparison of blood clotting time at doses I, II and III of the test compounds

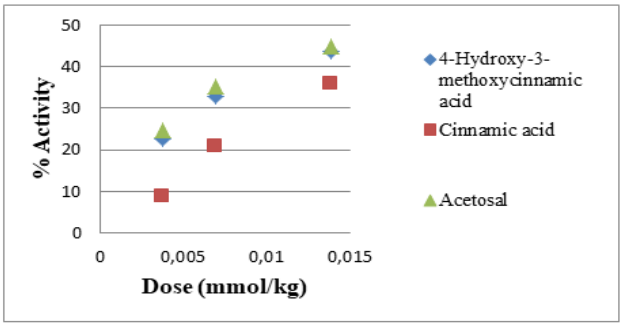


Figure 7. Graph of dose vs % activity of the test coumpounds

The difference between the time of fibrin formation after and before being administered the test solution, as shown in Figure 6, was used to calculate the blood clotting time in each test compound dose. This indicates that ferulic acid has an antiplatelet effect with a longer onset of fibrin formed and has activity comparable to acetosal at a dose of 3.7 x 10^{-3} mmol/Kg (I).

The blood clotting time in each test compound in dose II it was stated that all test groups had an effect on mice and produced antiplatelet activity with a longer time for fibrin formation Figure 6. The results in the ferulic acid group gave significantly different results from the cinnamic acid group ($P < 0.05$), where blood clotting time in ferulic acid was longer, indicating that its activity is higher than in cinnamic acid. However, the antiplatelet activity of ferulic acid compared to acetosal did not give significantly different results, so that it can be said to have similar activity ($P > 0.05$).

The blood clotting time in each test compound at dose III, ferulic acid and cinnamic acid groups compared to acetosal did not give significantly different results, so it can be said that all test groups provided antiplatelet activity Figure 6. Comparison of blood clotting time at doses I, II and III of each test compound can be seen in Figure 6.

The ED_{30} value is the amount of dose that can produce 30% activity. The ED_{30} value in determining antiplatelet activity aims to compare the antiplatelet activity of ferulic acid with acetosal and cinnamic acid to assess the effect of adding hydroxy and methoxy groups. The correlation coefficient (r) of the dose linearity equation to the percent activity of the test group consisting of ferulic acid, cinnamic acid and acetosal is 0.98. The graph of the linearity equation of the molar dose of each test compound to the percent antiplatelet activity at each dose is shown in Figure 7.

The ED_{30} value Table 1 from the results of the regression equation for each compound namely ferulic acid, cinnamic acid and acetosal was 67.4 x 10^{-3} mmol/Kg or 1.080 mg/Kg; 11.45 x 10^{-3} mmol/Kg

P-ISSN: 2406-9388
E-ISSN: 2580-8303

or 1.6958 mg/Kg; and 58.1 x 10^{-3} mmol/Kg or 1.0466 mg/Kg. The test compound ferulic acid had better activity than cinnamic acid which could be due to the presence of hydroxy and methoxy groups substituted in the cinnamic acid compound. The ED30 value of ferulic acid proves that this compound has a better activity of 1.7 times compared to cinnamic acid because it has a smaller ED30 value by comparing the ED30 value in mmol/Kg.

Based on research conducted by Ekowati *et al.* (2019), the addition of hydroxy and methoxy groups can increase antiplatelet activity. Referring to the study by Ekowati *et al*. (2019) regarding testing the antiplatelet activity of p-coumaric acid derivative compounds, including p-methoxycinnamic acid and p-hydroxycinnamic acid in a dose of 80 mg with the same method as this study, which produced the same antiplatelet activity compared to acetosal for p-methoxycinnamic compounds and antiplatelet activity of p-hydroxycinnamic acid compound which is lower than acetosal but still has an antiplatelet effect.

CONCLUSION

4-Hydroxy-3-methoxy cinnamic acid can be synthesized with the help of microwave irradiation using malonic acid and 4-hydroxy-3-methoxy benzaldehyde as the starting material and ammonium acetate as a catalyst at 960 Watt (P60) for 4 minutes which produces a yield percentage of 30.55%. Ferulic acid has antiplatelet activity which was tested by the method of measuring the length of time fibrin formed in the blood of mice with an ED_{30} value of 1.308 mg/KgBW.

The test for blood clotting time in the ferulic acid group gave significantly different results from the cinnamic acid group ($P < 0.05$), where the length of time fibrin formed in ferulic acid was longer, indicating that its activity is higher than that of cinnamic acid. However, the antiplatelet activity of dose II of ferulic acid compared to acetosal did not give significantly different results, so it can be said to have comparable activity ($P > 0.05$).

AUTHOR CONTRIBUTIONS

Conceptualization, J.E.; Methodology, J.E.; Software, E.C.M.; Validation, T.B.; Formal Analysis, T.B.; Investigation, E.C.M.; Resources, E.C.M.; Data Curation, J.E.; Writing - Original Draft, T.B.; Writing - Review & Editing, J.E.; Visualization, E.C.M.; Supervision, T.B.; Project Administration, T.B.; Funding Acquisition, J.E.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Acosta, R. D., Abraham, N. S., Chandrasekhara, V., Chathadi, K. V., Early, D. S., Eloubeidi, M. A. & Hwang, J. H. (2016). The Management of Antithrombotic Agents for Patients Undergoing GI Endoscopy. *Gastrointestinal endoscopy*; *83*; 3-16. doi: 10.1016/j.gie.2015.09.035.

Ekowati, J., Diyah, N. W. & Syahrani, A. (2019). Synthesis and Antiplatelet Activites of Some Derivatives of p-Coumaric Acid. *Chemistry & Chemical Technology*; *13*; 296-302. doi: 10.23939/chcht13.03.296.

Ekowati, J., Rahman, S. D., Isadiartuti, D., Widyowati, R. & Budiati, T. (2016). Synthesis of Ferulic Acid and Its Non Covalent Inclusion with Hydroxypropyl-β-Cyclodextrin. *International Journal of Pharmaceutical and Clinical Research*; *8*; 198-205.

Hoffman, R., Benz Jr, E. J., Silberstein, L. E., Heslop, H., Anastasi, J., Weitz, J., Anastasi, J., Salama, M. E. & Abutalib, S. A. (2018). Hemostasis and Thrombosis (*Hematology: Basic Principles and Practice*). Philadelphia: Elsevier Health Sciences.

Katzung, B. G. (2018). Drug Used to Treat Diseases of the Blood, Inflammation, & Gout. *Basic and Clinical Pharmacology; 14*; 591-642.

McMurry, J. (2016). Organic Chemistry 9th. California: Cengage Learning.

Pavia, D. L., Lampman, G. M., Kriz, G. S. & Vyvyan, J. R. (2015). Introduction to Spectroscopy 5th. California: Cengage Learning.

Sharma, P. (2011). Cinnamic Acid Derivatives: a New Chapter of Various Pharmacological Activities. *Journal of Chemical and Pharmaceutical Research*; *3*; 403-423.

Smith, J. G. (2011). Organic Chemistry 3th. New York: McGraw Hill.

Yang, X. Z., Diao, X. J., Yang, W. H., Li, F., He, G. W., Gong, G. Q. & Xu, Y. G. (2013). Design, Synthesis and Antithrombotic Evaluation of Novel Dabigatran Prodrugs Containing Methyl Ferulate. *Bioorganic & Medicinal Chemistry Letters*; *23*; 2089-2092. doi: 10.1016/j.bmcl.2013.01.126.

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 39-47
DOI: 10.20473/jfiki.v9i12022.39-47
Available online at https://e-journal.unair.ac.id/JFIKI/

Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Gendarusa Leaves *In Vivo*

Luthfiatu Kanina[1], Mabluhatus Solehah[1], Hani Plumeriastuti[2], Retno Widyowati[3], Bambang Prajogo Eko Wardoyo[3]*
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Veterinary Pathology, Faculty of Veteninary, Universitas Airlangga, Surabaya, Indonesia
[3]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: prajogo_ew@ff.unair.ac.id

Submitted: 11 July 2021
Accepted: 15 December 2021
Published: 26 April 2022

Abstract

Background*: Justicia gendarussa Burm. f., has been used traditionally in Indonesia for antifertility. Nowadays, a capsule containing 70% ethanol extract of J. gendarussa leaves has been studied for safety.* ***Objective****: This study aimed to determine the acute and sub-chronic toxicity of 70% ethanol extract of J. gendarussa leaves in vivo.* ***Methods****: In the acute toxicity study, a single dose of 2,000 mg/Kg BW was orally administered to mice (n = 10), which were monitored for 24 days. For the subchronic toxicity study, rats were randomly divided into four groups (n = 10). The control group received distilled water, while the treatment groups received a repeated dose of 40, 200, and 1,000 mg/Kg BW orally for 90 days. Blood samples were collected for hematological and biochemical evaluations. Gross pathology and histopathology of liver and kidneys were assessed.* ***Results****: No mortality and non-observed adverse effect level (NOAEL) were observed in the acute toxicity study. The hematological analysis did not show significant differences in the subchronic toxicity study. The SPGT, SGOT, and creatinine values showed no change in groups 2 and 4, but the level of SGPT increased in groups 3. The increasing level of BUN was observed in all treated groups. Abnormalities or histopathological changes were observed in the liver and kidney in groups 3 and 4.* ***Conclusions****: Using 70% ethanol extract of J. gendarussa leaves at a therapeutic dose is safer, but it needs attention at a higher dose.*

Keywords*: acute toxicity, Justicia gendarussa Burm. f., sub-chronic toxicity*

How to cite this article:
Kanina, L., Solehah, M., Plumeriastuti, H., Widyowati, R., & Wardoyo, B. P. E. (2022). Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Gendarusa Leaves *In Vivo*. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 39-47. http://doi.org/10.20473/jfiki.v9i12022.39-47

INTRODUCTION

Justicia gendarussa Burm.f. (JG), commonly-known as Gendarusa, can be found in Indonesia, Malaysia, Sri Lanka, and India (Heyne, 1987). In Papua, Indonesia, JG is said to be a traditional male antifertility medicine (Moeso & Agus,1985). *In vitro* and *in vivo* antifertility studies of JG n-butanol fractions reveal a competitive and reversible suppression of the spermatozoa hyaluronidase enzyme (Prajogo *et al*., 2009). The presence of flavonoid molecules, particularly C-glycosyl flavone groups with an apigenin base structure, is responsible for the hyaluronidase enzyme's inhibitory activity. Apigenin and vitexin, a glycoside of apigenin, have anti-inflammatory and anticancer properties in JG (Prajogo *et al*., 2008). As nonhormonal male contraception, JG natural medicine can be turned into a phytopharmaceutical product (Prajogo *et al*., 2008).

According to an FDA (Food and Drug Administration) report in the Poisonous Plant Database (Plant List), the JG plant is one of the potentially poisonous plants (Solehah, 2018). Based on previous research, the acute toxicity test using the water phase and ethanol phase of 60% JG leaves in rats shows relatively harmless results (Ekaputri, 2015). In addition, the subacute toxicity test in rabbits does not affect the liver and kidney function parameters. The water and ethanol phases of 60% JG leave also do not have a teratogenic effect on rats. Even the water phase does not have a carcinogenic effect, but it does not provide histopathological changes in rats' testes, liver, kidneys, intestines, and lungs. However, giving the water phase for 52 days can cause changes in liver histopathology and erosion of the small intestine mucosa but does not show differences in kidney histopathology. Therefore, before being used as a medicine, further research is needed regarding the subchronic toxicity of this plant using ethanolic solvent (Ekaputri, 2015).

MATERIALS AND METHODS

This study was designed through the Animal Care and Use Committee (ACUC), Faculty of Veterinary Universitas Airlangga, with the reference number of 715-KE.

Materials

The 70% ethanol extract of gendarusa leaves was obtained from a series of processes in all parts of the *Justicia gendarussa* Burm. F leaves from Gempol, Pasuruan that was identified by Indah Yulia Ningsih with number herbarium 01. Na citrate, 70% ethanol, CMC Na, aquadest, acetonitrile, and methanol were used as chemical materials. Male Balb/C mice were used as experimental animals weighing 15 - 30 grams and aged 6 - 8 weeks, and male Wistar rats weighing between 150 - 300 grams and 6 - 8 weeks old were used as experimental animals. Variation in body weight did not exceed 20% of the average body weight.

Preparation of dried leaves

JG leaves that were nine months old were harvested. Fresh leaves were sorted to separate them from impurities or foreign materials such as soil, gravel, grass, unnecessary plant parts, etc. After sorting, they were washed and then dried in the oven at a temperature of 50^{o}C. Subsequently, the leaves were made into powder by grinding.

Preparation of alkaloid free dried leaves

The JG dried leaves were acidified with a solution of citric acid with a pH of 3. Acidification was carried out for 3 x 24 hours (with stirring) and then washed with aquadest until the pH was neutral. Then, it was dried at 50^{o}C until the drying shrinkage reached 10%.

Preparation of 70% ethanolic extract

Alkaloid free dried leaves were extracted with 70% ethanol for 12 hours without heating by maceration technique. This process was repeated three times and the filtrate obtained from each process was collected. The filtrate was then concentrated until reaching a total solid of $\geq$ 90%.

Preparation of animal models

The animal models used in the acute and sub-chronic toxicity test evaluations were male mice and rats. Before treatment, both were adapted to the environment for one week. They were kept in the same way and had the same diet. Then, they were weighed to calculate the dosage setting. The animal models were randomized in such a way that the distribution of body weight was evenly distributed across groups with variations in body weight not exceeding 20% of the mean of body weight. Their body weights were measured before, during, and after treatment. Monitoring of weight gain was done twice a week.

Preparation of CMC Na compound

The administration of each dose was in the form of an extract suspended in 0.5% CMC Na. The negative control was given 0.5% CMC Na. Preparation of 0.5% CMC Na was by weighing 0.5 grams of CMC Na, sprinkled over 20 times of hot water, allowed to expand (± 15 minutes), and crushed until it formed mucilage. Then, it was transferred to a bottle calibrated and added water to 100 ml. Afterwards it was given to the control group orally.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Acute toxicity test

Dose of acute toxicity test

The dosing method used for the acute toxicity test was the fixed-dose method, namely the preliminary, main, and limit tests. In the preliminary test, the fixed doses selected were 5, 50, 300, and 2,000 mg/Kg BW as doses expected as toxic effects. The limit dose of this experiment was 2,000 mg/Kg BW. Previous research on LD_{50} oral administration of 60% ethanol fraction and water fraction of JC leaves in mice was 17.82630 g/Kg BW and 15.63389 g/Kg BW (Ekaputri, 2015) the primary test was carried out at a dose rate of 2000 mg/Kg BW.

Provision of test preparations and volume of administration

Two groups of mice were prepared, in which each group consisted of 10 mice. Each experimental animal was given JC extract for the treatment group according to its respective weight. Before the treatment, the mice were fasting for 3 - 4 hours; after that, drinking water could be given. The administration was administered orally once and then observed for 24 hours and continued for 14 days. Control group was given 0.5% CMC Na suspension. Treatment was given JC extract, where one mouse was for the preliminary test and the other four were for the main test. The volume of fluid given was 2 mL/100 g BW.

Observation of animal behavior

The test animals were observed individually for at least the first 30 minutes after administration of the test preparation, periodically every four hours for 24 hours, and once a day for 14 days. The development and remission of toxicity symptoms were thoroughly recorded in separate records for each animal (particularly if there was a propensity for delayed toxic indications). If the animal had poisoning symptoms on a regular basis, additional observations were made. Skin, hair, eyes, mucous membranes, the respiratory system, the autonomic nervous system, central nervous system, somatomotor activity, and behavior were all observed. Furthermore, the following circumstances were observed: shaking, convulsions, salivation, diarrhea, weakness, drowsiness, and coma. The following items were observed during the observation period: animal behaviors, such as walking backwards and walking on the stomach, and animal body weight would be tracked at the time of the test preparation and for at least a week afterward.

Subchronic toxicity test

Dose of sub-chronic test

three dose groups and one control group were used. The doses chosen for subchronic toxicity tests were based on acute toxicity data and consideration of doses that had pharmacological effects. The highest dose of the test substance caused toxic effects but did not cause death or severe toxicity symptoms, medium doses caused milder toxic symptoms, while the lowest dose did not cause toxic symptoms (NOAEL).

Provision of test preparations and volume of administration

Four groups of rats were prepared, in which each group consisted of 10 rats. Each experimental animal was given JC extract according to their respective dosages for all groups except the control group. The administration was carried out orally (with a maximum volume of 1 - 2 mL of test preparation/100 g animal body weight) every three days a week for 90 days. During treatment, the toxic and clinical symptoms were observed every day. Meanwhile, weight monitoring was carried out twice a week. Animals were weighed daily to determine the volume of test preparation to be administered. After 90 days of treatment, the blood of each rat was taken intracardially. Then, the SGOT, SGPT, BUN, and creatinine levels were measured and complete blood profile testing by kinetic enzymatic methods based on IFCC (International Federation of Clinical Chemistry) (Sardini, 2007). In addition, the liver, kidneys, small intestine, lungs, lymph, and heart were also taken from rats for microscopic observation. From the SGOT, SGPT, BUN, and creatinine levels and microscopic observation of the liver, kidney, small intestine, lung, lymph, and heart, could be analyzed for the presence of organ damage or not. The following was each group of treatments: [I] Control group (group 1): it was given 0.5% CMC Na suspension. [II] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [III] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [IV] Group 4: it was given JC extract at a dose of 1000 mg/Kg BW. From the acute toxicity, it was known that LD_{50} score was up to 2,000 mg/Kg BW (NOAEL) or based on OECD included in the fifth category.

Analysis of Enzyme SGOT, SGPT, BUN, and creatinin

All data obtained from the biochemical parameters were analyzed with ANOVA (One Way) at the 95% degree of confidence to determine whether there were significant differences between treatment groups.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Body weight of mice in acute toxicity test

	Body weight of mice (g)		
	Initial experiment	**7th day**	**24th day**
Control group	28 ± 1	29 ± 1	31 ± 2
Test group dose (2000 mg/Kg BW)	28 ± 1	31 ± 1	32 ± 1

Table 2. Results of biochemical examination

	SGOT	**SGPT**	**BUN**	**Creatinine**
Group 1	131.9 ± 40.9	58.5 ± 18.9	20.7 ± 3.7	0.6 ± 0.2
Group 2	144.9 ± 35.5	65.4 ± 11.1	26,0 ± 2.4	0.6 ± 0.1
Group 3	173.4 ± 69.2	84.0 ± 40.9	24.2 ± 2.7	0.5 ± 0.2
Group 4	159.4 ± 34.4	61.5 ± 19.5	29.5 ± 2.5	0.6 ± 0.6

Note: average ± standard deviation

Blood draw

Blood was taken using a sterile syringe and always kept from being exposed to water (to avoid hemolysis). After the animals were anesthetized with ketamine solution, blood was taken from the heart slowly using a 3 m syringe, one syringe for one animal, and then put into a blood tube for further testing.

Execution of experimental animals

The experimental animals were executed by "dislocating the neck bones" to remove the liver, kidneys, small intestine, lungs, lymph, and heart. Furthermore, surgery was carried out to remove these organs. Then, the organs obtained were fixed with a buffer solution of 10% formalin to make histopathological preparations.

Histopathological preparations

Procedures for making histopathological preparations were based on BPOM regulations (Solehah, 2018).

Examination of histopathological preparations

Light microscopy was used to observe the rat organ preparations microscopically. At first, 100 times magnification was used and then a 400 magnification was used. The method of assessment was in the form of scoring (Sukardja, 1998).

Analysis of histopathological preparation data

The data on changes in the histopathological image of the rat organs that had been given a score were processed by ranking then analyzed using the Kruskal Wallis test as non-parametric statistical tests.

RESULTS AND DISCUSSION

Acute toxicity

The administration of 70% ethanol extract of JG leaves at a dose of 2,000 mg/Kg BW of the mice was found not to cause death. There were no toxic symptoms, such as standing hair, yellow eyes, and abnormal behaviors (not staying in one place and not biting certain body parts). From the acute toxicity, it was known that the LD_{50} score was up to 2,000 mg/Kg BW (NOAEL). Table 1 describes the observation of the body weight of mice in the acute toxicity test.

Subchronic toxicity

In the subchronic toxicity test for 90 days of the experimental animals with 70% ethanol extract of JG leaves, it was found no mortality, behavioral observations, and signs of clinical toxicity, such as not causing changes in behavior (walking backward, biting certain body parts, yellowing eyes, and standing hair).

Biochemical data from the results of subchronic toxicity tests such as SGOT, SGPT, BUN, and creatinine be seen in Table 2. The p-value of the SGOT level (0.170) was greater than 0.05, indicating that there was no significant difference between treatment groups. On the other hand, the SGPT level p-value (0.039) was less than 0.05, showing a difference between treatment groups (group 2, 3, and 4). The 200 mg/Kg BW dosage group (group 3) was substantially different from the control group according to the post hoc test results. BUN level as kidney function measurement had a p-value of 0.000, which was less than 0.05, indicating a difference between treatment groups. The post hoc test revealed that the 200 mg/kg BW dosage group differed significantly from the control group (Table 3). Then hematology data from the results of subchronic toxicity test be seen in Table 4. There was no significant difference between treatment groups because the p-value for creatinine (0.176) was greater than 0.05. In general, the test groups' p-values of leukocytes and hematocrit were less than 0.05, which indicated the difference between treatment groups (Table 5). The difference in leukocytes was described in group 3 with p-value of 0.044, while the level of hematocrit was observed in group 4 (p = 0.039).

The histopathological changes were carried out using scoring referred to Arsad *et al.* (2014) and the scoring data of hepar and kidney are shown in Table 6. On the microscopic examination of the liver and kidneys, a dose of 40 mg/Kg BW (group 2) JC extract did not affect all histopathological parameters of the

P-ISSN: 2406-9388
E-ISSN: 2580-8303

liver and kidneys, while a dose of 200 mg/Kg BW (group 3) and 1,000 mg/Kg BW (group 4), microscopic changes in the liver and kidneys of all histopathological parameters were shown at Figure 1 and 2.

Table 3. Results of biochemical statistical data processing of rat blood with SPSS

Parameter	Difference between treatment groups (p)	Difference between treatment groups(p)		
		1 & 2	1 & 3	1 & 4
SGOT	0.170	-	-	-
SGPT	0.039	0.330	0.012	0.232
BUN	0.000	0.002	0.083	0.000
Creatinine	0.176	-	-	-

Note : Control group (group 1): it was given 0.5% CMC Na suspension. Treatment group [I] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [II] Group 3: it was given JC extract at a dose of 200 mg/kg BW. [III] Group 4: it was given JC extract at a dose of o1000 mg/Kg BW

Table 4. Results of hematology examination

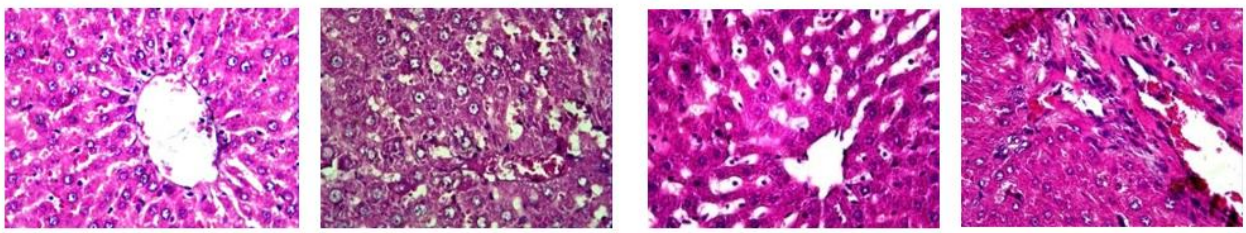
Parameter	Group 1	Group 2	Group 3	Group 4
HB	14.0 ± 1.1	14.1 ± 0.8	13.9 ± 0,8	13.5 ± 0.8
Leukocyte	17.8 ± 4.3	16.9 ± 2.1	17.4 ± 6.0	16.2 ± 2.9
Thrombocyte	1139 ± 162.5	1082.6 ± 151.7	948.8 ± 99.8	942.4 ± 212.2
Eosinophil	4.7 ± 1. 2	6.8 ± 2.0	4.6 ± 1.2	5.3 ± 1.5
Basophil	0.7 ± 0.5	0.2 ± 0.4	0.2 ± 0.4	0.8 ± 0.6
Neutrophil	17.1 ± 7.3	14.2 ± 3.3	10.7 ± 4.3	16.0 ± 4.9
Lymphocyte	70.3 ± 6.6	70.4 ± 3.7	77.3 ± 5.1	71.5 ± 5.0
Monocyte	7.2 ± 3.5	8.4 ± 3.5	7.2 ± 1.4	6.4 ± 1.9
LED	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Erythrocyte	8.6 ± 0.7	8.5 ± 0.42	8.3 ± 0.6	8. 2 ± 0,4
Hematocyte	47.8 ± 3.8	45.7 ± 2.9	44.1 ± 3.0	43.4 ± 3.3
MCV	53.7 ± 2.5	52.5 ± 0,9	53.3 ± 2.1	53.2 ± 2,0
MCH	16.4 ± 0.8	16.7 ± 0.4	17.0 ± 0,6	16.4 ± 0.5
MCHC	30.8 ± 06	31.9 ± 0.6	31.7 ± 0.6	31.0 ± 0.6
G	54.5 ± 15.9	37.0 ± 17.8	65.5 ± 23.0	38.0 ± 19.9

Note : average ± standard deviation

Table 5. Results of hematology statistical data processing of rat hematology with SPSS

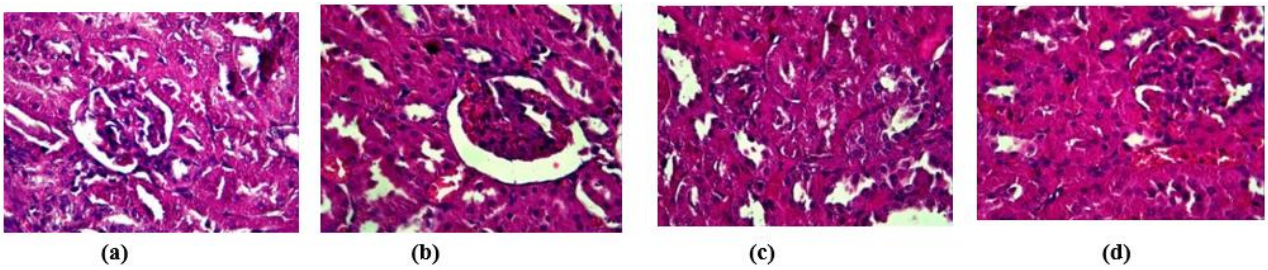
Parameter	Difference between treatment Group (p)	Difference between treatment groups(p)		
		1 & 2	1 & 3	1 & 4
Hemoglobin	0.444	-	-	-
Leukocyte	0.004	1.000	0.044	1.000
Thrombocyte	0.080	-	-	-
Erythrocyte	0.601	-	-	-
Hematocyte	0.033	0.295	0.121	0.039

Note: Control group (group 1): it was given 0.5% CMC Na suspension. Treatment group [I] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [II] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [III] Group 4: it was given JC extract at a dose of o1000 mg/Kg BW.



(a) (b) (c) (d)

Figure 1. Tissue section of hepar (a) group 1, (b) group 2, (c) group 3, (d) group 4



Note: This picture represented certain of hepar and kidney tissue

Figure 2. Tissue Section of kidney (a) group 1, (b) group 2, (c) group 3, (d) group 4

Table 6. Scoring degree of change in the organ of rats

Organ	Observed change of each group (average)	Group			
		K-(group 1)	P1 (group 2)	P2 (group 3)	P3 (group 4)
Hepar	Activated kupper cells	0.60	0.78	1.02	1.50
	Sinusinoiddillatation	0.40	0.52	0.87	1.76
	Cytoplasm Vacuole	1.20	1.00	2.54	2.84
	Karyolysis	1.10	1.02	2.20	2.24
	Karyopicnotis	1.00	0.96	1.74	1.86
	Average	**0.86**	**0.86**	**1.67**	**2.04**
Kidney	Granular Cast	0.10	0.08	1.06	1.16
	Cellular Cast	0.10	0.10	1.00	1.16
	Protein Cast	0.20	0.18	0.88	0.50
	Pyknotic cells	0.60	0.62	0.90	1.26
	Hydropic degeneration	0.40	0.40	1.60	1.72
	Average	**0.28**	**0.27**	**1.08**	**1.16**

Note: Control group [K] (group 1): it was given 0.5% CMC Na suspension. Treatment group [P1] Group 2: it was given JC extract at a dose of 40 mg/Kg BW. [P2] Group 3: it was given JC extract at a dose of 200 mg/Kg BW. [P4] Group 4: it was given JC extract at a dose of o1000 mg/Kg BW

Discussion

In the acute toxicity test, there were no hazardous symptoms, and no animals died due to it. According to the findings, the 70% ethanol extract of JC was not harmful, thus, it may be claimed that these changes are not toxicologically important. As a result, this research demonstrates that JC extract does not produce acute toxicity at the dosage examined and with LD50 values more than 2,000 mg/Kg. The limit check methodology is intended to serve as a reference for identifying crude extracts based on the estimated dose stage at which the animal would live (Roopashree *et al*., 2009). Based on the categorization of acute system toxicity established by the OECD (Kennedy *et al*., 1986), the crude extract of JC extract was once assigned category 5 popularity (LD_{50} > 2000 mg/Kg), which was once the lowest toxicity class.

The median lethal dose is the dose needed to kill one or more tested populations in a group after a certain amount of test duration. According to Kennedy *et al.* (1986), extract for treatment or drug with an oral LD_{50} greater than 2,000 mg/Kg is considered safe or nearly harmless. Some medicinal plants have been studied and reported to be toxic to both humans and animals (Kennedy *et al.*, 1986). Therefore, it has to be emphasized that the standard use of any plant for medicinal purposes by no means ensures the protection of such plants. The facts that the acute and subchronic toxicity research on medicinal vegetation or preparations derived from it have to be bought to extend the self-belief in its protection to humans, particularly for the use in the improvement of prescribed pharmaceuticals (Ukwuani *et al*., 2012). Choosing the best test and dosing regimens showing a large margin of safety is crucial in planning human security. Because no hazardous outcomes were discovered during the acute toxicity investigation, a comparison was conducted to look at the subchronic toxicity of JC extract in rats for up to 90 days to compile the complete toxicology information for this ancient medicinal plant.

The oral subchronic toxicity test is used to detect hazardous effects when repeated doses of the test preparation are given orally to test animals for a portion of their lives, but not more than 10% of their whole lives. Subchronic studies show the negative consequences of continuous or repetitive exposure to plant extracts or chemicals in experimental animals like rats during a portion of their lifespan. They are designed to identify

no observable adverse impact levels and provide information on target organ toxicity (NRC, 2006). Subchronic evaluation can also aid in selecting dosing regimens for longer-term investigations. As a result, the subchronic toxicity of JC extract was tested in rats for 90 days at dosages of 40, 200, and 1,000 mg/Kg/day. There was no satellite group in this study. For at least 14 days after treatment, an extra satellite group administered with the maximum dose should be considered to observe reversibility, persistence or delayed onset of systemic toxic effects, and recovery from toxic effects. Satellite groups are animal groups that are part of a toxicity study's conception and execution. Moreover, the satellite group will be treated and housed in the same conditions as the animals in the main experimental. Based on subchronic toxicity test findings of JC extract, the data of weight organ, especially kidney and liver were not recorded. The usefulness of weighing organs in toxicity research consists of their sensitivity to predict toxicity, enzyme induction, physiologic perturbations, and acute injury; this function of weighing is regularly a goal organ of toxicity; it correlates nicely with histopathological changes; there is little inter animal variability; historic managed varied statistics are available (Ukwuani *et al*., 2014). The serum hematology and scientific biochemistry analyses are done to evaluate the possible changes in hepatic and renal features influenced by the extracts. Liver and kidney feature evaluations are essential in the toxicity comparison of drug and plant extracts as they are each fundamental for the survival of an organism (Ukwuani *et al*., 2014).

From the study results based on biochemical parameters, it was known that at a dose of 40 mg/KgBW, the ethanol extract of 70% leaves of JC extract did not affect SGOT, SGPT, and Creatinine but did affect BUN. However, the 200 mg/Kg BW dose affected SGPT, and the BUN parameter was affected by both the 40 mg/Kg BW dose and the 1,000 mg/Kg BW dose, but it was not affected by the 200 mg/Kg BW dose. SGPT values were found to be normal in the control group with Doses of 40 mg/kg BW and 1,000 mg/Kg BW, but there was an increase at a dose of 200 mg/Kg BW, probably due to the appearance of SGPT first at a higher dose (dose of 1,000 mg/Kg BW). It can be assumed that there has been an improvement at this high dose at the 91st day of surgery. Meanwhile, at lower doses, the increase in SGPT occurred afterwards so that, on the day of surgery, there was no improvement. Renal dysfunction can be assessed using concurrent measurements of urea, creatinine, and uric acid, and their normal ranges replicate at the decreased probability of renal problem (Roopashree *et al*., 2009). In the current study, adjustments in creatinine level in JC extract dealt with corporation confirmed non-significant variations indicating a normal renal function. However, modifications in the BUN stage at 200 mg/Kg BW doses confirmed that there were enormous variations, indicating an abnormal renal function

The amount of the harmful effect of JC extract on an animal's blood can be determined using hematological measures. It can also be used to explain why blood is drawn to specific characteristics of a plant extract or its derivatives (Yakubu *et al*., 2006). Furthermore, such an assessment is useful to threat comparison since changes in the hematological system have a higher predictive value for human toxicity when statistics from animal studies are translated (Olson *et al*., 2000). The JC extract does not affect the erythropoiesis, shape, or osmotic fragility of red blood cells, as evidenced by its nonsignificant impact on complete red blood cells, suggestion of corpuscular volume, suggestion of corpuscular Hb, and platelets (Olorunnisola *et al*., 2012). Leukocytes are the first line of cell defence that respond to infectious agents, tissue injury, or inflammatory processes. Furthermore, in general, the p-values of leukocytes and hematocrit in the test group that are much less than 0.05 indicate the distinction between treatment groups. There is an exchange in hemotogram; however, no mortality facts are shown.

In comparison to the control group, macroscopic examinations of the organs of rats treated with several doses of JC extract show no changes in shade in Figure 1 and Figure 2. Organ hypertrophy is a first-hand indicator of chemical or organic substance toxicity. However, this study examined hypertrophy of organs at 200 mg/Kg BW and 1,000 mg/Kg BW of agencies. Any insult to the parenchymal liver cells causes blood transaminase levels to rise (Slichter, 2004). In addition, when compared to the control group and changes in the BUN stage at 200 mg/Kg BW dosages, there was no significant increase in creatinine in the subchronic administration of JC extract. This study used histological findings of kidney tissue to confirm this finding. These results suggest that JC extract at a 40 mg/kg BW concentration has no effect on liver or kidney characteristics and has no hazardous effects. However, it is necessary to study the changes and improvements in several test parameters at doses of 200 mg/Kg BW and 1,000 mg/Kg BW.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

CONCLUSION

Using 70% ethanol extract of *J. gendarussa* leaves at a therapeutic dose is safer, but it needs attention at a higher dose. These results suggest that further research is needed to ensure its safety for a clinical study.

ACKNOWLEDGMENT

The authors would like to deliver to our gratitude to the all crews that supported this experiment from Gandarussa team and Department of Veterinary, Universitas Airlangga.

AUTHOR CONTRIBUTIONS

Conceptualization, B.P.E.W.; Methodology, B.P.E.W.; Validation, B.P.E.W.; Formal Analysis, T.B.; Investigation, L.K.; Resources, M.S.; Data Curation, R.W.; Writing - Original Draft, H.P.; Writing - Review & Editing, R.W., B.P.E.W.; Visualization, L.K.; Supervision, R.W., B.P.E.W.; Funding Acquisition, L.K., B.P.E.W.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Arsad, S. S., Esa, N. M. & Hamzah, H. (2014). Histopathologic Changes in Liver and Kidney Tissue from Male Sparague Dawley Rats Treated with *Rhapidophora decursiva* (Roxb). *Journal of Cytology and Histology*; *2014*; 1-6. doi: 10.4172/2157-7099.S4-001.

Ekaputri, A. A. (2015). Studi Toksisitas secara In Silico Kandungan Senyawa Flavonoid dan Alkaloid pada Daun *Justicia gendarussa* Burm.f. *Skripsi*.; Fakultas Farmasi Universitas Airlangga, Surabaya.

Heyne. (1987). Tumbuhan berguna indonesia, Jilid 3. Jakarta: Yayasan Sarana Wana.

Kennedy, G. L., Ferenz R. L. & Burgess, B. A. (1986). Estimation of acute Oral Toxicity in Rats by Determination of the Approximatelethal Dose Rather than the LD50. *Journal of Applied Toxicology*; *6*; 145–148. doi: 10.1002/jat.2550060302.

Moeso, M. & Agus, P. (1985). Laporan perjalanan ke Jayapura Sentani (Irian Jaya). Yogyakarta: Fakultas Biologi UGM.

National Research Council (NRC). (2006). Toxicity Testing for Assessing Environmental Agents. (Interim Report). Washington, DC: National Academies Press**.**

Olorunnisola, O. S., Bradley, G. & Afolayan, A. J., (2012). Acute and Sub-Chronic Toxicity Studies of Methanolic Extract of *Tulbaghiaviolacea* Rhizomes in Wistar Rats. *African Journal of Biotechnology*; *11*; 14934–14940.

Olson, H., Betton, G. & Robinson, D. (2000). Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology*; *32*; 56–67. doi: 10.1006/rtph.2000.1399.

Prajogo, B. E. W, Juliaan, F., Hinting, A., Pramesti, M.P., Anggraeni, M., Radjaram & Musta'ina, A. (2008). Laporan Pelaksanaan Uji Klinik Fase I; Universitas Airlangga dan Badan Koordinasi Keluarga Berencana Nasional. Surabaya: Fakultas Farmasi Universitas Airlangga.

Prajogo, B. E. W., Juliaan, F., Hinting, A., Pramesti, M. P. Anggraeni, M., Radjaram & Musta'ina, A. (2009). Laporan Pelaksanaan Uji Klinik Fase II; Universitas Airlangga dan Badan Koordinasi Keluarga Berencana Nasional. Surabaya: Fakultas Farmasi Universitas Airlangga.

Ratih, G. A. M., Imawati, M. F., Nugroho, R. R., Prajogo, B. E. W. & Indrayanto, G. (2019). Phytochemicals of Gandarusa (*Justicia gendarussa*) and Its Preparations. *Natural Product Communications*; *2019*; 1-10. doi: 10.1177/1934578X19851406.

Roopashree, T. S., Raman, D., Rani, R. H. S., & Narendra, C. (2009). Acute Oral Toxicity Studies of Antipsoriatic Herbal Mixture Comprising of Aqueous Extracts of *Calendula officinalis, Momordica charantia, Cassia tora* and *Azadirachta indica* Seed Oil. *Thai Journal of Pharmaceutical Science*; *33*; 74–83.

Sardini, S. (2007). Penentuan Aktivitas Enzim GOT dan GPT dalam Serum dengan Metode Reaksi Kinetik Enzimatik sesuai IFCC. Jakarta: BATAN.

Slichter, S. J. (2004). Relationship between Platelet Count and Bleeding Risk in Thrombocytopenic Patients Transfusion. *Medicine Reviews*; *18*; 153–167. doi: 10.1016/j.tmrv.2004.03.003

Solehah, M. (2018). Profil Toksisitas Oral Akut dan Subkronis 90 Hari Ekstrak Etanol 70% Daun JC (*Justicia gendarussa Burm. f.). Tesis*; Fakultas Farmasi Universitas Airlangga, Surabaya.

Sukardja, I. D. G. (1998). Correlation of Clinical and Pathological Diagnosis of Neoplasm. *Modern Pathology for Service and Resesarch on Cancer*; *1*; 15-35. doi: 10.1016/j.jvoice.2020.10.003.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Ukwuani, A. N., Abubakar, M. G., Hassan, S. W. & Agaie, B. M. (2012). Toxicological Studies of Hydromethanolic Leaves Extract of *Grewia crenata. International Journal of Pharmaceutical Scienceand Drug Research*; *4*; 245–249.

Yakubu, M. T., Akanji, M. A. & Oladiji, A. T. (2007). Haematologicalevaluation in Male Albino Rats Following Chronic Administration of Aqueous Extract of *Fadogia agrestis* Stem. *Pharmacognosy Magazin*e; *3*; 34–38.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 48-54
DOI: 10.20473/jfiki.v9i12022.48-54
Available online at https://e-journal.unair.ac.id/JFIKI/

Anti-Hepatitis C Virus Activity of Various Indonesian Plants from Balikpapan Botanical Garden, East Borneo

Rina Puspitasari[1], Tutik Sri Wahyuni[2,3], Achmad Fuad Hafid[2,3], Adita Ayu Permanasari[3], Lidya Tumewu[3], Aty Widyawaruyanti[2,3]*

[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Center for Natural Product Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: aty-w@ff.unair.ac.id

Submitted: 7 September 2021
Accepted: 12 December 2021
Published: 26 April 2022

Abstract

Background*: Hepatitis C Virus infection is a serious health problem that leads to chronic liver disease, liver cirrhosis, hepatocellular carcinoma, which causes high morbidity. Direct-Acting Antiviral Agents have been used as anti-hepatitis C Virus therapy. However, it was covered only in limited patients due to the high cost. Moreover, serious side effects and resistance cases were also reported in some HCV genotypes.* ***Objective****: This research aimed to find new anti-HCV from some Indonesia plants collected from Balikpapan Botanical Garden, East Borneo.* ***Methods****: Twenty-one leaf and stem barks extracts were successively extracted in n-hexane, dichloromethane, and methanol. Extracts were screened for their anti-HCV activity under in vitro culture cells in the concentration of 30 µg/mL. Plant extracts were inoculated in the Human Hepatocellular 7it and infected with HCV Japanese Fulminant Hepatitis strain 1a. Determination of 50% Inhibitory Concentration (IC_{50}) value was further conducted at concentration of 100; 30; 10; 1; 0.1; 0.01 µg/ml of extracts.* ***Results****: In vitro anti-HCV activity revealed that among 21 plants extract, 11 extracts, namely, n-hexane extract from Luvunga scandens leaves, DCM extract from the leaf of L. scandens, Artocarpus sericicarpus, Artocarpus dadah, Eusideroxylon zwageri, Neolitsea cassiaefolia, methanol extract from A. sericicarpus and A. anisophyllus leaves, DCM extract from A. anisophyllus and A. elmeri stem bark, methanol extract from A. dadah stem bark, having potential inhibition with IC_{50} range 0.08 ± 0.05 to 12.01 ± 0.95 µg/mL.* ***Conclusions****: These results indicate that the eleven extracts could be good candidates as sources of anti-HCV agents.*

Keywords*: health, hepatitis C virus, herbal medicine, medicinal plant extracts*

How to cite this article:
Puspitasari, R., Wahyuni, T. S., Hafid, A. H., Permanasari, A. A., Tumewu, L., & Widyawaruyanti, A. (2022). Anti-Hepatitis C Virus Activity of Various Indonesian Plants from Balikpapan Botanical Garden, East Borneo. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 48-54. http://doi.org/10.20473/jfiki.v9i12022.48-54

INTRODUCTION

Hepatitis C Virus (HCV) infection is a global problem that is still endemic in several countries (Hajarizadeh *et al*., 2013). The global prevalence of HCV infection is estimated to be 2 - 3%, equivalent to 130 - 170 million people. As reported by the World Health Organization (WHO) in 2017, 71 million people out of the world's total population were infected with HCV. Each year there are about 3 – 4 million cases of new infections (Morozov & Lagaye, 2018). About 350,000 people die of HCV each year (World Health Organization (WHO), 2019). Hepatitis virus is a significant public health problem in Indonesia, with about 2.5 million infected with HCV (Jonathan *et al*., 2018). HCV can cause chronic liver disease, cirrhosis of the liver, hepatocellular carcinoma, often called a malignant liver tumor, and complications of other conditions resulting from lipid and glucose metabolic disorders (Morozov & Lagaye, 2018).

HCV treatment has been in place for almost 15 years, with *pegylated interferon-alpha* and ribavirin with an infection recovery rate reaching 45% in the 1.65% in genotype three and about 85% in genotype 2. Development of treatment continues to be undertaken; the WHO recommends *Direct Acting Antivirals* (DAAs) therapy using a combination of sofosbuvir, daclatasvir, and ledipasvir (Morozov & Lagaye, 2018). In 2011, telaprevir and boceprevir were approved for use in the treatment of HCV with a combination of *pegylated interferon-alpha* and ribavirin. This treatment had a *sustained virologic response* (SVR) rate between 75% to 90% while reducing the duration of treatment. Combining sofosbuvir, daclatasvir, and sofosbuvir/ledipasvir, which were standards used according to WHO guidelines, could achieve healing levels above 95% (World Health Organization, 2019). But this therapy was expensive, had side effects, and might give rise to resistance (Ashfaq *et al*., 2011; Morozov & Lagaye, 2018). Therefore, there was a need to develop a more safe, efficacious, and affordable new drug therapy.

Indonesia is a country that possesses large biodiversity, including plants that can be utilized for treatment. Of the world's approximately 40,000 species of flora, Indonesia owns 30,000 plants, and about 940 species of the plants are known to be effective as medications Plant species are estimated to include 90% of the number of medicinal plants circulating in Asia (Hafid *et al*., 2017). It is known that certain medicinal plants have antivirus activity, including anti-HCV (Wahyuni *et al*., 2016).

Our previous studies reported several ethanol extracts from some Indonesian medicinal plants which have been screened and possessed anti-HCV activity. Ethanol extract of *Toona sureni*, *Melicope latifolia*, *Ficus fistulosa* leaves, *Melanolepis multiglandulosa* stem; 13.9; 3.5; 15.0; and 17.1 µg/mL, respectively. Some plants from the family Rutaceae were also reported to be active anti-HCV are *Ruta angustifolia* and *Melicope latifolia*. In Indonesia, *R. angustifolia* has been known as a traditional medicine for liver disease and jaundice. This plant contains coumarin, alkaloid, and flavonoid compounds. The dichloromethane extract of *R. angustifolia* leaves indicates anti-HCV activity with IC_{50} value of 1,6 µg/mL. Two active compounds of anti-HCV, chalepin, and pseudane IX, are obtained from isolation and identification of dichloromethane fraction of *R. angustifolia* leaves using High Performance Liquid Chromatography (HPLC) (Wahyuni *et al*., 2013; 2014).

Infusion of *Persea americana* leaves, the family Lauraceae, strongly inhibits type 1 herpes simplex virus (HSV-1), *Aujeszky* virus (ADV), and type 3 adenovirus (AD3) in cell culture. Isolation and identification with the basis of anti-HSV one and ADV testing showed Afzelin and quercetin *3-O-α-D-arabinopyranoside* exhibiting anti-HSV-1 activity resistant to acyclovir (De Almeida *et al*., 1998). Methanol and water extracts from medicinal plants used in traditional Sudanese medicine, such as *Boswellia carterii and Acacia,* exhibit over 90% activity at 100 µg/mL (Hussein *et al*., 2000).

Several plants of the genus *Artocarpus* have been reported to be active for anti-HCV, such as *Artocarpus heterophyllus, Artocarpus altilis*, and *Artocarpus camansi*. The dichloromethane extract of *A. heterophyllus* leaves has an IC_{50} of as much as 1.5 ± 0.6 µg/mL against the JFH1a virus. The genus *Artocarpus* consists of about 50 species and is widespread in tropical and subtropical regions (Wetprasit *et al*., 2000).

This study conducted anti-HCV activity from 21 extracts of eight plants. All of the extracts were tested for their anti-HCV activity against the JFH1a virus.

MATERIALS AND METHODS

Materials

Chemicals and reagents

The materials for anti-HCV assay solutions are methanol, dichloromethane, n-hexane, Huh7it hepatocyte cells, JFH1a hepatitis C virus, *Dimethyl Sulfoxide* (DMSO, Merck, Darmstadt, Germany), *Dubelco's Modified Eagle Medium* (DMEM, GIBCO, USA), 10% *Fetal Bovine Serum* (FBS, biowest, USA),

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Non-Essential Amino Acid (NEAA, GIBCO, New York), *Dubelco's Phosphate Buffered Saline* (DPBS, SIGMA, USA), *Penicillin Streptomycin* (GIBCO, USA), *Trypsin*-EDTA (GIBCO, USA), *Bovine Serum Albumin* (BSA, Roche, Germany), formaldehyde (Merck, Germany), TritonX-100 (Promega, USA), *thermo staining* 3,3'-*diaminobenzidine* (DAB, Thermo scientific, USA), hepatitis patient antiserum C, HRP-*Goat-anti-human Ig* (MBL).

Plant materials

Plant materials were collected from Balikpapan Botanical Garden, East Borneo. The plant was verified by the Indonesian Institute of Sciences Botanical Garden, Purwodadi, Pasuruan Malang. Plant identification certificate number is 0074/IPH.06/HM/XII/ 2015.

Cells and virus

Huh 7it cells and *Japanese Fulminant Hepatitis strain 1a* (JFH1a) virus were used and developed at the Center for Natural Product Medicine Research and Development, Institute of Tropical Diseases, C Campus of Universitas Airlangga, Surabaya, East Java.

Method

Extraction

Extraction was conducted by ultrasonic method (UAE, *Ultrasonic assisted extraction*). A total of 800 grams of simplicia powder was extracted using the 3000 mL solvent n-hexane, dichloromethane, and methanol. Simplicia powder was extracted for six minutes and put in an ultrasonic machine. Every two minutes, the extract was stirred, then put in an ultrasonic machine again and filtered. The next step is evaporated extract using a rotary evaporator. The concentrated extract was drained inside the oven at a temperature of 40^{o}C until obtaining a constant weight.

***In vitro* anti-HCV activity test**

Anti-HCV activity assays were conducted in the following method. Ten mg of dried extracts were weighed and suspended in 100 µL of dimethyl sulfoxide (DMSO). The sample was diluted to obtain a concentration of 30 µg/mL for screening evaluation, while dose-dependent inhibition assays were also evaluated at 100, 30, 10, 1, 0.1, and 0.01 µg/mL. The mixture of sample and virus were inoculated to the Huh7it cells culture with *Multiplicity of Infection* (MOI) 0.1 at 48-well plates. After two hours of incubation, the cell was washed. The old medium was replaced with a new medium and further incubated in an extract-filled medium of the same concentration for 48 hours. After 48 hours of post-infection, culture supernatants were harvested for virus titration assay (Hafid *et al*., 2017). These procedures performed viral titration and immunostaining. First, a virus supernatant was made 30 times dilution in the medium and then inoculated into Huh7it cells. After four hours of incubation, the cells were cultured with a medium for 48 hours. After 48 hours of incubation, the cell was fixated with a formaldehyde solution and 0.5% X-100 triton. Cells were immunostained by adding the primary (human serum) and secondary antibodies (HRP-*Goat-anti-human* Ig (MBL)). Reagent of 3,3'-*diaminobenzidine* (DAB) Thermo staining was added, and infected cells were calculated under the microscope. The percent of infected cells was detected under the microscope. The percentage inhibitory was determined by SPSS probit analysis to obtain IC_{50} values.

RESULTS AND DISCUSSION

This study was conducted to determine the anti-HCV activity of some extracts from plants that originated from the Balikpapan Botanical Garden, East Borneo. The selection of plants is based on the chemotaxonomy approach of the plant. Several studies reported that plants from the family Rutaceae, Moraceae and Lauraceae showed anti-virus activity, including potential anti-virus Hepatitis C (De Almeida *et al*., 1998; Wahyuni *et al*., 2013; 2014). Plants from that family are reported to contain various metabolite compounds, such as flavonoids, terpenoids, lignin, sulfide, polyline, thiophene, protein, and peptides that were thought to be able as anti-HCV agents (Wahyuni *et al.*, 2013).

We evaluated 21 plants extract against HCV at 30 µg/mL (Table 1). The result showed that 11 extracts had higher percentage of inhibition (≥ 80%). Therefore, further analysis was conducted to n-hexane extract of *Luvunga scandens* leaves, dichloromethane extract of *L. scandens*, *Artocarpus sericicarpus*, *Artocarpus dadah*, *Eusideroxylon zwageri*, *Neolitsea cassiaefolia* leaves, methanol extract of *A. sericicarpus*, *Artocarpus anisophyllus* leaves, dichloromethane extract of *A. anisophyllus*, *Alseodaphane elmeri* stem bark and methanol extract of *A. dadah* stem bark for evaluating their 50% inhibitory effect. The IC_{50} values of the 11 plants extract were demonstrated to possess potential activities with the IC_{50} value of 0.08 to 12.01 µg/mL (Table 2).

Some flavonoid compounds reported having anti-HCV activity are *Epigalocatekin-3-gallate* (EGCG) (Ciesek *et al*., 2011), quercetin, luteolin, apigenin, and ladanein (Calland *et al*., 2012), naringenin, silymarin/silibin (Wagoner *et al*., 2011). A wide variety

P-ISSN: 2406-9388
E-ISSN: 2580-8303

of chemical components of medicinal plants, such as flavonoid, terpenoid, lignin, sulfide, polyphenolic, coumarin, saponin, furyl compound, alkaloid, and polyline, thiophene, protein, and peptide, was identified to inhibit various viruses (Wahyuni *et al*., 2016). In addition, some of them were known to be efficient in limiting the genome replication of RNA or DNA and its antioxidant activity (Ashfaq & Idrees, 2014).

L. scandens is a plant of the family Rutaceae and also mediated strong activity against HCV. The leaves extracts of this plant showed 100% and 96.15% inhibition against the HCV JFH1a virus both in n-hexane and DCM solvents. It was reported that isobutylglucosinolates were isolated from the roots of *L. scandens.* However, there is no information yet on its antiviral activities (Sirinut *et al*., 2017). Other plants from the *Rutaceae* genus, such as *Ruta angustifolia* and *Melicope latifolia*, have inhibited HCV. Meanwhile, less attention was given to its antiviral activity study.

Table 1. Percentage inhibition of extracts at a concentration of 30 µg/mL against JFH1a virus

Sample				Infection	Inhibition*
Plant	Familia	Part	Solvent	(%)	(%)
Luvunga scandens	Rutaceae	Leaves	n-Hexane	0.00	100.00 ± 0.00
Artocarpus sericicarpus	Moraceae	Leaves	n-Hexane	84.62	15.38 ± 21.76
Neolitsea cassiaefolia	Lauraceae	Leaves	n-Hexane	88.46	11.54 ± 1.81
Luvunga scandens	Rutaceae	Leaves	Dichloromethane	3.85	96.15 ± 5.44
Artocarpus sericicarpus	Moraceae	Leaves	Dichloromethane	17.95	82.05 ± 7.25
Artocarpus dadah	Moraceae	Leaves	Dichloromethane	1.28	98.72 ± 1.81
Eusideroxylon zwageri	Lauraceae	Leaves	Dichloromethane	2.56	97.44 ± 3.63
Neolitsea cassiaefolia	Lauraceae	Leaves	Dichloromethane	12.82	87.18 ± 7.25
Melicope glabra	Rutaceae	Leaves	Methanol	29.49	70.51 ± 9.07
Artocarpus sericicarpus	Moraceae	Leaves	Methanol	3.85	96.15 ± 5.44
Artocarpus anisophyllus	Moraceae	Leaves	Methanol	8.97	91.03 ± 9.07
Artocarpus dadah	Moraceae	Leaves	Methanol	30.77	69.23 ± 7.25
Neolitsea cassiaefolia	Lauraceae	Leaves	Methanol	55.13	44.87 ± 16.32
Melicope glabra	Rutaceae	Stem bark	Dichloromethane	58.97	41.03 ± 10.88
Artocarpus sericicarpus	Moraceae	Stem bark	Dichloromethane	21.79	78.21 ± 9.07
Artocarpus anisophyllus	Moraceae	Stem bark	Dichloromethane	2.56	97.44 ± 3.63
Alseodaphane elmeri	Lauraceae	Stem bark	Dichloromethane	0.00	100.00 ± 0.00
Luvunga scandens	Rutaceae	Stem bark	Methanol	100.00	0.00
Artocarpus sericicarpus	Moraceae	Stem bark	Methanol	55.13	44.87 ± 1.81
Artocarpus anisophyllus	Moraceae	Stem bark	Methanol	57.69	42.31 ± 9.07
Artocarpus dadah	Moraceae	Stem bark	Methanol	0.00	100.00 ± 0.00

*Data represent means ± SD of data from two independent experiments

Table 2. Anti HCV activity (IC_{50}) of extracts against HCV JFH1a

Sample				IC_{50}*
Plant	Familia	Part	Solvent	(µg/mL)
Luvunga scandens	Rutaceae	Leaves	n-Hexane	2.53 ± 0.55
Luvunga scandens	Rutaceae	Leaves	Dichloromethane	0.08 ± 0.02
Artocarpus sericicarpus	Moraceae	Leaves	Dichloromethane	0.08 ± 0.05
Artocarpus dadah	Moraceae	Leaves	Dichloromethane	0.58 ± 0.18
Eusideroxylon zwageri	Lauraceae	Leaves	Dichloromethane	6.23 ± 0.30
Neolitsea cassiaefolia	Lauraceae	Leaves	Dichloromethane	7.73 ± 0.92
Artocarpus sericicarpus	Moraceae	Leaves	Methanol	8.04 ± 2.90
Artocarpus anisophyllus	Moraceae	Leaves	Methanol	2.71 ± 0.14
Artocarpus anisophyllus	Moraceae	Stem bark	Dichloromethane	4.62 ± 0.99
Alseodaphane elmeri	Lauraceae	Stem bark	Dichloromethane	4.65 ± 0.72
Artocarpus dadah	Moraceae	Stem bark	Methanol	12.01 ± 0.95

*Data represent means ± SD of data from two independent experiments

P-ISSN: 2406-9388
E-ISSN: 2580-8303

E. zwageri is a plant of the family Lauraceae; DCM extracts of leaves of this plant have a percent inhibition of the HCV JFH1a virus of 97.44 ± 3.63. So in traditional medicine, it possesses potential as a natural antibacterial (Mariani *et al*., 2020). Methanol extract of *E. zwageri* leaves was thought to have chemical components such as alkaloids, flavonoids, steroids, phenolics, and saponins that were also owned by the extract of parts of its stem (Wila *et al*., 2018). According to Radulović *et al*. (2013), such compounds generally owned by plants influenced bacterial growth or might take a role as an antibacterial. Dichloromethane extracts of *N. cassiaefolia* leaves from family Lauraceae have a percent inhibition to the JFH1a HCV virus of 87.18 ± 7.25 (Radulović *et al.*, 2013).

In addition, three coumarins were isolated and characterized from *Viola yedoensis* using various chromatographic procedures. Among the isolated compounds dimer-coumarin 5.5'-bi (6.7-dihydroxycumarine) significantly inhibited NS3/4A proteases scoring IC_{50} 0.5 g/mL (Zhang *et al*., 2013).

Diosgenin (3-hydroxy-5-spirostene), a plant-derived sapogenin, effectively blocked the replication of HCV subgenomic replica systems at mRNA and protein levels. The value of the EC_{50} diosgenin was 3.8 μmol (Wang *et al.*, 2011). *Silybum marianum* (SM) seeds and their fractions inhibit the HCV core gene of 3a genotype, and a combination of SM and its fractions with interferon will be a better option to treat HCV infection (Ashfaq *et al*., 2011). Silibinin, a combination of two diastereoisomers, was the primary component of silymarin responsible for anti-HCV activity (Polyak *et al*., 2007).

Naringenin was the primary flavanone found in grapefruit and tested on being able to suppress the activity of core proteins in Huh7 cells and also effectively block the formation of HCV particles (Nahmias *et al*., 2008). *Epigallocatechin-3-gallate* (EGCG) was found in green tea extracts and can inhibit HCV activity (Ciesek *et al*., 2011). Quercetin present in vegetables, fruits, grains, and leaves acted as an anti-HCV agent (Gonzalez *et al*., 2009). Ladanein was a flavon isolated from *Marrubium peregrinum L*. (Lamiaceae). Ladanein had an IC_{50} of 2.5 μmol (Haid *et al*., 2012).

This study identified 11 extracts from eight plant species that exhibited anti-HCV activity. The active substances from extracts were not identified yet. Therefore, those extracts were the potential to be studied, especially on isolation and identification of anti-HCV active compounds.

CONCLUSION

A total of 11 extracts can be candidates for anti-HCV drug material sources. They are n-hexane extract of *Luvunga scandens* leaves, dichloromethane extract of *Luvunga scandens*, *Artocarpus sericicarpus*, *Artocarpus dadah*, *Eusideroxylon zwageri*, *Neolitsea cassiaefolia* leaves, methanol extract of *Artocarpus sericicarpus*, *Artocarpus anisophyllus* leaves, dichloromethane extract of *Artocarpus anisophyllus*, *Alseodaphane elmeri* stem bark, and methanol extract of *Artocarpus dadah* stem bark.

ACKNOWLEDGMENT

Huge appreciation goes to the Center for Natural Product Medicine Research and Development, Institute of Tropical Disease for the research facility. The authors are grateful to Professor Hak Hotta and Dr Chie Aoki Utsubo (Kobe University) for providing HCV-JFH1a and hepatocyte line cells Huh7it. This study was supported by Universitas Airlangga through Riset Mandat grant.

AUTHOR CONTRIBUTIONS

Conceptualization, A.W., T.S.W.; Methodology, A.W., T.S.W.; Validation, A.W., T.S.W.; Formal Analysis, R.P., A.A.P.; Investigation, R.P., A.A.P., L.T.; Resources, A.W., T.S.W.; Data Curation, R.P., L.T.; Writing - Original Draft, R.P.; Writing - Review & Editing, R.P., T.S.W., A.A.P., L.T., A.W.; Visualization, R.P., T.S.W., A.A.P., L.T., A.W.; Supervision, A.A.P., L.T.; Project Administration, A.W., T.S.W.; Funding Acquisition, A.W., T.S.W.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Ashfaq, U. A. & Idrees, S. (2014). Medicinal Plants Against Hepatitis C Virus. *World Journal of Gastroenterology*; *20*; 2941–2947. doi: 10.3748/wjg.v20.i11.2941.

Ashfaq, U. A, Masoud, M. S., Nawaz, Z. & Riazuddin, S. (2011). Glycyrrhizin as Antiviral Agent Against Hepatitis C Virus. *Journal of Translational Medicine*; *9*; 1-7. doi: 10.1186/1479-5876-9-112.

Ashfaq, U. A., Javed, T., Rehman, S., Nawaz, Z. & Riazuddin, S. (2011). Inhibition of HCV 3a Core Gene Through Silymarin and Its Fractions. *Virology Journal*; *8*; 1-7.

Calland, N., Dubuisson, J., Rouillé, Y. & Séron, K. (2012). Hepatitis C Virus and Natural Compounds: a New Antiviral Approach?. *Viruses*; *4*; 2197–2217. doi: 10.3390/v4102197.

Ciesek, S., von Hahn, T., Colpitts, C. C., Schang, L. M., Friesland, M., Steinmann, J., Manns, M. P., Ott, M., Wedemeyer, H., Meuleman, P., Pietschmann, T. & Steinmann, E. (2011). The Green Tea Polyphenol, Epigallocatechin-3-Gallate, Inhibits Hepatitis C Virus Entry. *Hepatology (Baltimore, Md.)*; *54*; 1947–1955. doi: 10.1002/hep.24610.

De Almeida, A. P., Miranda, M. M. F. S., Simoni, I. C., Wigg, M. D., Lagrota, M. H. C. & Costa, S. S. (1998). Flavonol Monoglycosides Isolated from the Antiviral Fractions of *Persea americana* (Lauraceae) Leaf Infusion. *Phytotherapy Research*; *12*; 562–567. doi: 10.1002/(SICI)1099-1573(199812)12:8<562::AID-PTR356>3.0.CO;2-6.

Gonzalez, O., Fontanes, V., Raychaudhuri, S., Loo, R., Loo, J., Arumugaswami, V., Sun, R., Dasgupta, A. & French, S. W. (2009). The Heat Shock Protein Inhibitor Quercetin Attenuates Hepatitis C Virus Production. *Hepatology (Baltimore, Md.)*; *50*; 1756–1764. doi: 10.1002/hep.23232.

Hafid, A. F., Aoki-Utsubo, C., Permanasari, A. A., Adianti, M., Tumewu, L., Widyawaruyanti, A., Wahyuningsih, S. P. A., Wahyuni, T. S., Lusida, M. I., Soetjipto & Hotta, H. (2017). Antiviral Activity of the Dichloromethane Extracts from *Artocarpus heterophyllus* Leaves Against Hepatitis C Virus. *Asian Pacific Journal of Tropical Biomedicine*; *7*; 633–639. doi: 10.1016/j.apjtb.2017.06.003.

Hafid, A. F., Puliansari, N., Lestari, N. S., Tumewu, L., Rahman, A. & Widyawaruyanti, A. (2017). Skrining Aktivitas Antimalaria Beberapa Tanaman Indonesia Hasil Eksplorasi dari Hutan Raya Cangar, Batu-Malang, Jawa Timur. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*; *3*; 7-11. doi: 10.20473/jfiki.v3i12016.7-11.

Haid, S., Novodomská, A., Gentzsch, J., Grethe, C., Geuenich, S., Bankwitz, D., Chhatwal, P., Jannack, B., Hennebelle, T., Bailleul, F., Keppler, O. T., Poenisch, M., Bartenschlager, R., Hernandez, C., Lemasson, M., Rosenberg, A. R., Wong-Staal, F., Davioud-Charvet, E., & Pietschmann, T. (2012). A Plant-Derived Flavonoid Inhibits Entry of All HCV Genotypes Into Human Hepatocytes. *Gastroenterology*; *143*; 213-222e5. doi: 10.1053/j.gastro.2012.03.036.

Hajarizadeh, B., Grebely, J. & Dore, G. J. (2013). Epidemiology and Natural History of HCV Infection. *Nature Reviews. Gastroenterology & Hepatology*; *10*; 553–562. doi: 10.1038/nrgastro.2013.107

Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. & Shimotohno, K. (2000). Inhibitory Effects of Sudanese Medicinal Plant Extracts on Hepatitis C Virus (HCV) Protease. *Phytotherapy Research: PTR*; *14*; 510–516. doi: 10.1002/1099-1573(200011)14:7<510::aid-ptr646>3.0.co;2-b.

Jonathan, S., Jack, W. & Suzanne, W. (2018). Situation Analysis of Viral Hepatitis in Indonesia : A Policy Report. *Coalition Eradicate Viral Hepatitis in Asia Pacific (CEVHAP)*; *3*; 1-23.

Mariani, Y., Yusro, F. & Wardenaar, E. (2020). Aktivitas Ekstrak Metanol Daun Ulin (*Eusideroxylon zwageri* Teijsm & Binn) Terhadap Empat Jenis Bakteri Patogen. *Jurnal Biologi Tropis*; *20*; 94–101. doi: 10.29303/jbt.v20i1.1642.

Morozov, V. A. & Lagaye, S. (2018). Hepatitis C Virus: Morphogenesis, Infection and Therapy. *World Journal of Hepatology*; *10*; 186–212. doi: 10.4254/wjh.v10.i2.186.

Nahmias, Y., Goldwasser, J., Casali, M., van Poll, D., Wakita, T., Chung, R. T. & Yarmush, M. L. (2008). Apolipoprotein B-dependent Hepatitis C Virus Secretion is Inhibited by the Grapefruit Flavonoid Naringenin. *Hepatology (Baltimore, Md.)*; *47*; 1437–1445. doi: 10.1002/hep.22197.

Polyak, S. J., Morishima, C., Shuhart, M. C., Wang, C. C., Liu, Y. & Lee, D. Y.-W. (2007). Inhibition of T-cell Inflammatory Cytokines, Hepatocyte NF-kappaB Signaling, and HCV Infection by Standardized Silymarin. *Gastroenterology*; *132*; 1925–1936. doi: 10.1053/j.gastro.2007.02.038.

Radulović, N. S., Blagojević, P. D., Stojanović-Radić, Z. Z. & Stojanović, N. M. (2013). Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action. *Current Medicinal Chemistry*; *20*; 932–952.

Sirinut, P., Petchkongkeaw, A., Romsaiyud, J., Prateeptongkum, S. & Thongyoo, P. (2017). Phytochemical Constituents from the Root of Luvunga Scandens and Biological Activity Evaluation. *Natural Product Communications*; *12*; 1483-1484. doi: 10.1177/1934578X1701200926.

Wagoner, J., Morishima, C., Graf, T. N., Oberlies, N. H., Teissier, E., Pécheur, E.-I., Tavis, J. E. & Polyak, S. J. (2011). Differential In Vitro Effects

of Intravenous Versus Oral Formulations of Silibinin on the HCV Life Cycle and Inflammation. *PloS One*; *6*; 1-9. doi: 10.1371/journal.pone.0016464.

Wahyuni, T. S., Tumewu, L., Permanasari, A. A., Apriani, E., Adianti, M., Rahman, A., Widyawaruyanti, A., Lusida, M. I., Fuad, A., Soetjipto, Nasronudin, Fuchino, H., Kawahara, N., Shoji, I., Deng, L., Aoki, C. & Hotta, H. (2013). Antiviral Activities of Indonesian Medicinal Plants in the East Java region Against Hepatitis C Virus. *Virology Journal*; *10*; 1-9. doi: 10.1186/1743-422X-10-259.

Wahyuni, T. S., Utsubo, C. A. & Hotta, H. (2016). Promising Anti-Hepatitis C Virus Compounds from Natural Resources. *Natural Product Communications*; *11*; 1193-1200.

Wahyuni, T. S., Widyawaruyanti, A., Lusida, M. I., Fuad, A., Soetjipto, Fuchino, H., Kawahara, N., Hayashi, Y., Aoki, C. & Hotta, H. (2014). Inhibition of Hepatitis C Virus Replication by Chalepin and Pseudane IX Isolated from *Ruta angustifolia* Leaves. *Fitoterapia*; *99*; 276–283. doi: 10.1016/j.fitote.2014.10.011.

Wang, Y. -J., Pan, K. -L., Hsieh, T. -C., Chang, T. -Y., Lin, W. -H. & Hsu, J. T. -A. (2011). Diosgenin, a Plant-Derived Sapogenin, Exhibits Antiviral Activity In Vitro Against Hepatitis C Virus. *Journal of Natural Products*; *74*; 580–584. doi: 10.1021/np100578u.

Wetprasit, N., Threesangsri, W., Klamklai, N. & Chulavatnatol, M. (2000). Jackfruit Lectin: Properties of Mitogenicity and the Inhibition of Herpesvirus Infection. *Japanese Journal of Infectious Diseases*; *53*; 156–161.

Wila, H., Yusro, F. & Mariani, Y. (2018). Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Kulit Batang (*Eusideroxylon zwageri*) terhadap *Escherichia coli* dan *Salmonella typhi*. *Jurnal Tengkawang*; *8*; 38-49. doi: 10.26418/jt.v8i1.30147.

World Health Organization (WHO). (2019). Hepatitis C. https://www.who.int/news-room/fact-sheets/detail/hepatitis-c. Accessed: 21 April 2021.

Zhang, L., Li, M. -Y., Wang, L. -W., Gao, J. & Ma, C. -M. (2013). Isolation, Identification, Quantification and Inhibitory Activity on HCV Protease of Coumarins from *Viola yedoensis*. *Canadian Chemical Transactions*; *1*; 157–164. doi: 10.13179/canchemtrans.2013.01.03.0025.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 55-61
DOI: 10.20473/jfiki.v9i12022.55-61
Available online at https://e-journal.unair.ac.id/JFIKI/

Ethnomedicine Study on *Justicia gendarussa* for Male Contraception at the Nimboran Ethnic, Jayapura

Niluh Indrawati[1], Retno Widyowati[2,3], Pilipus Kopeuw[4], Bambang Prajogo Eko Wardoyo[2,3*]
[1]Master Program in Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Departemen of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Natural Product Drug Discovery and Development-Research Group, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[4]Sentanu Protestant Christian College, Jayapura, Indonesia

*Corresponding author: prajogo_ew@ff.unair.ac.id

Submitted: 23 September 2021
Accepted: 2 February 2022
Published: 26 April 2022

Abstract

Background*: Justicia gendarussa from Papua has traditionally been used for the treatment of several diseases, and phytochemical studies have been carried out since 1987.* ***Objective:*** *This study aimed to determine the use of this plant as a male contraceptive that the Nimboran Ethnic and their perspectives have long used.* ***Method:*** *A qualitative method with an ethnographic approach was used, while data were collected through interviews, observations, and documentation. The informants were selected using purposive and snowball sampling.* ***Result:*** *The results showed that 44% of people use it to delay pregnancy, 24% due to young marriage with poor economic conditions, 14% after moving to another place 12% because of tribal wars over fertile land to multiply offspring. The preparation and usage of this plant through the collection, mixing, and manufacturing method and in terms of dosage, time, and duration of use were explored more deeply by conducting interviews with 50 informants. The effectiveness and success as a method of contraception are presented in the way the community has known the plant over different generations, and this method is still used as an alternative option considering the very wide area profile from one place to another.* ***Conclusion:*** *Justicia gendarussa is used by the Nimboran Ethnic group as an ingredient in traditional medicines, especially for male contraception.*

Keywords*: Justicia gendarussa, ethnomedicine, male contraceptive, medicine, nimboran ethnic*

How to cite this article:
Indrawati, I., Widyowati, R., Kopeuw, P., & Wardoyo, B. E. P. (2022). Ethnomedicine Study on *Justicia gendarussa* for Male Contraception at the Nimboran Ethnic, Jayapura. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 55-61. http://doi.org/10.20473/jfiki.v9i12022.55-61

INTRODUCTION

Indonesia is a developing country with the fourth highest population globally; hence, to overcome the high rate of increase in the inhabitants, the government promotes a Family Planning program. This program is carried out to help individuals or married couples avoid undesired births, obtain the desired delivery, set pregnancy intervals, and determine the number of children in a family (BKKBN Provinsi Papua, 2015; Sulistyawati, 2013). Data from the Indonesian Demographic and Health Survey (IDHS) 2002 - 2003 showed that the females practising contraception were 98.7%, while the males that were only 1.3% later reached 1.81% in 2014 (Kemenkes, 2014). The use of modern methods among married women has the same percentage from 2002 to 2017 of IDHS (57%-58%). Meanwhile, traditional methods tend to increase from 2002 to 2017 (Kemenkes, 2018). The standard methods of male contraception are interrupted intercourse (coitus interruptus/withdrawal), condoms, and vasectomy (male sterilization). Furthermore, they have weaknesses, including less effectiveness (4 – 24%), psychological resistance, a high failure rate (3 – 15%), and possible causation of prostate cancer despite being reversible (Prajogo, 2017). Therefore, the development of male contraception and increasing participation are needed to support the achievement of Family Planning programs in Indonesia, improve maternal health, and combat HIV/AIDS and other sexually transmitted diseases.

Justicia gendarussa (Gandarusa) is usually used by Nimboran Distric for several diseases therapy, one of them as a male antifertility. Their phytochemical studies have been carried out since 1987. Gandarusa has 12 flavonoids with the main component of gendarusin A (6,8-di-α-L-arabinopyranosil-4',5,7-trihydroxyflavone) which is a compound that exerts hyaluronidase enzyme inhibitory activity on the acrosome head of spermatozoa during fertilization (Prajogo, 2002). This plant also contains potassium, flavonoids, justicin, steroids or triterpenoids, 0.4% tannins, alkaloids, aromatic amines, iridoids, and coumarins (Prajogo, 2002). It tends to be consumed by humans provided pharmaceutical quality, and clinical trials' requirements are met while being developed for the traditional medicine industry. The pharmaceutical quality guarantees that the desired efficacious substance (gendarusin A) has been sufficiently absorbed and the systemic circulation is reached to cause clinical effects (Prajogo *et al*., 2011).

The use of gandarusa by the Papuan people is local wisdom that has been practised for a long time and needs to be explored in-depth and preserved. Local wisdom from ethnic groups who inhabit a certain area utilizes natural materials in the form of plants, animals, and minerals around them to support their lives and survival. The utilization varies depending on the place of residence, ethnicity, indigenous beliefs, relationships to other community groups, and religion (Swerdlow, 2003; Atakpama *et al*., 2012; Atato *et al*., 2010; Avocevou-Ayisso *et al*., 2012; Ayantunde *et al*., 2008).

As described above, the early history of gandarusa usage in the Papuan community needs to be provided. This study was expected to provide new information and knowledge on the use of gandarusa for male contraception while preserving the cultural heritage of medicinal plants through ethnomedicine study. This study is a branch of medical anthropology that discusses the origin of disease, causes, and treatment methods according to certain groups of people. The ethnomedicine aspect is an aspect that appears along with the development of human culture. In medical anthropology, ethnomedicine gives rise to various terminology (Foster, 1986; Bhasin, 2007; Daval, 2009).

MATERIALS AND METHODS

Study design

In this study, a qualitative method with an ethnographic approach was used. Gandarusa from Nimboran District, Papua Province, was determined or identified at the Purwodadi Botanical Gardens, and plant specimens were stored in the Natural Product Drug Discovery and Development-Research Group (NPD3-RG). Then, data was collected through observations, interviews, and documentation (Nina & Lisa, 1996; Taek et al., 2019). The informants were selected using purposive and snowball sampling.

Location and Time

This study was performed for eight months (September 2020 – May 2021) in the Nimboran District and its surroundings with a radius of 54 km. These distrik is a large area and was divided into 12 villages that village are Gemebs, Kuwase, Menyu, Kaitemun, Benyom, Tabri, Singgriway, Kuipon, Pobaim, Kuwase, Oyengsi, dan Imsar (BKKBN Provinsi Papua, 2015; Kopeuw, 2017; Moeso & Agus, 1985).

Data analysis

The data were analyzed descriptively and presented in a tabular form.

RESULTS AND DISCUSSION

The community that has used gandarusa over different generations and knows information about this plant was approached as the informant. The inclusion

P-ISSN: 2406-9388
E-ISSN: 2580-8303

criteria were as follows: (1) the indigenous people of the Nimboran Ethnic, (2) males aged 35 to 55 years, (3) commonly used gandarusa, (4) have used gandarusa as male contraception, and (5) living within 12 villages in the Nimboran District/Sub-District. Meanwhile, the exclusion criteria were (1) immigrant communities living among the Nimboran Ethnic group, (2) lacking information on the usage of gandarusa, and (3) not familiar with the way to use gandarusa for male contraception. The informant selection started from official data obtained from the National Population and Family Planning Board, Statistic Indonesia, the local Health Service, the Regional Institute Research and Development, the district government regarding the results or publications of a similar study and the local community. Then, the following informants were recruited using the snowball sampling method (based on the information from previous informants). The number of informants was limited until the information reached the saturation point. So, the total informants in this study were 85 males. However, 35 males do not meet the requirements. The demographic profile of informants from the Nimboran District showed in Table 1.

Table 1. Demographic profile of the informants

Informant data	Demographic profile
Gender	Male
Age	35 - 55
Education level	Primary level
	Secondary level
	High education level
Language	Nimboran (local language)
Mastery	Nimboran and Indonesia
Main profession	Farmer and employee

The plant determination showed that *Justicia gendarussa* (gandarusa) from the Nimboran tribe has the following characteristics: usually grows wild in the forest, in the form of shrubs with a height of about 0.8 - 2 m, woody trunks, leaves have thorns & pinnate, purple flowers, and tubular, and two-lipped. They have the same characteristics as references (Dalimartha, 2001; Prajogo, 2014; Depkes RI, 1995).

Frans is one of the informants from Genyem village said gandarusa has a local name as lekik or nukhu. Gandarusa is used widely by Nimboran Ethnics. This plant is waist-high adults with many branches, has been known by the previous seven generations and is often found in forests or yards. Based on interview results with informants from 12 villages, it was informed that 64% of them used gandarusa leaves to delay pregnancy, 22% of them applied gandarusa roots for treating bone pain and bruises and 14% of them used gandarusa stems for particular purposes such as spiritual strength and walking ability for children under five months old was faster (Figure 1).

In this study, interviews with 50 informants were conducted. About 44% of informants said gandarusa was used to delay pregnancy, 24% said it was used for young marriage, 14% said it was used after moving to a new loaction because the area was too far from medical personnel, and 12% was used during the tribal war Nimboran Ethnic's previous generation, while the remaining (6%) said it was used for other purposes (Table 2). Actually, they don't mind having a large family, but there was a conflict that forced them to relocate to a new location with good and rich land as well as access to water. Hence, the community frequently sought advice from traditional elders or healers to to resolve the issue.

Table 2. Used of gandarusa

Time to use *Gandarusa*	Informants
To delay pregnancy	22
Due to young marriage	12
After moving to another place	7
During tribal war	6
Other	3

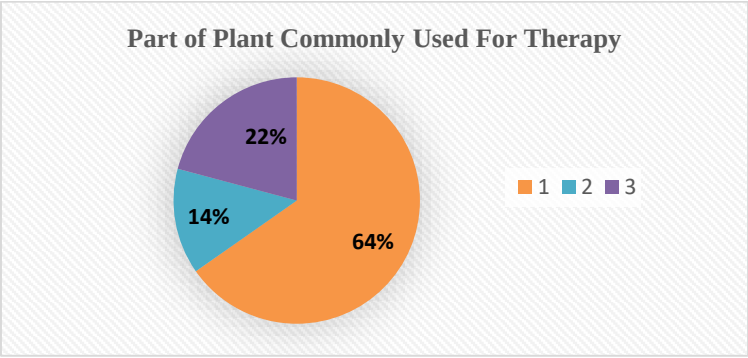


Figure 1. Part of plant commonly used for therapy; 1. leaves, 2. stem, 3. root

P-ISSN: 2406-9388
E-ISSN: 2580-8303

There was some information about gandarusa for male contraception, especially the number of leaves. Table 3 showed that the majority of informants used 10 pieces of leaves. Ingronang from Tabri Lekik Village used gandarusa leaf by picking ten pieces by themself, then passed and boiling them with water. The water extract was offered to the husband and wife for delayed pregnancy, and this activity was performed for one year. The leaves are better to pick in the morning before the sun starts shining fully. It is called self-preparation.

Table 3. Pieces of Gandarusa leaf for male contraception

The number of leaves	Informants
10 pieces	16
15 pieces	13
17 pieces	8
8 pieces	6
16 pieces	4
18 pieces	2
12 pieces	1

According to WHO, gandarusa is a dangerous plant because it contains risk alkaloids. Therefore, this study carried out the side effects of gandarusa used daily by the Nimboran ethnic. The results are shown in Table 4. Based on interview data, most informants who used gandarusa leaves did not feel any side effects. Only 2% experienced side effects such as red eyes, decreased appetite, heartburn, drowsiness, throat disorders, bloating, dizziness, decreased libido, and dry mouth.

Table 4. Adverse effect of Gandarusa

Side Effect	Informants
No side effects	49
Side effects (red eyes, decreased appetite, heartburn, drowsiness, throat disorders, bloating, dizziness, decreased libido, and dry mouth)	1

Yacob one of the informants from Tabri Village said that Papuans fell health and illness from nature and things beyond human ability. Real limitations of healthy and sick persons are due to interference from a supernatural power or spirits, while others are related to nature, climate, water, soil, and human.

According to the IDHS, married females aged 15 - 49 on one type of modern contraception is relatively high, namely 61.5% in Papua and 92.7% in West Papua. Furthermore, 65.7% of the married females in Papua know about traditional contraception, while in West Papua, 43.6% used this method. High-level knowledge does not automatically lead to high coverage of family planning in the two provinces. According to the 2007 IDHS, Contraceptive Prevalence Rate (CPR) in Papua was only 24.5 %, and 37.5 % in West Papua, a very low coverage compared to the national level, which can also be seen from the high Total Fertility Rate (TFR) in the two provinces, namely 2.9 and 3.4 respectively. Unmet needs for contraception are still high at 15.8 % for Papua and 16.5 %for West Papua (BKKBN Provinsi Papua, 2017).

Efforts to control population growth are carried out through the Family Planning Program, which is characterized by changes in the number, structure, composition, and distribution of a balanced population following the carrying capacity and capacity of the environment. In Papua Province, there are 5 (five) traditional territories: (a) Anim Ha, which consists of 4 districts, namely Merauke, Asmat, Mappi, and Boven Digul; (b) La Pago consists of 6 districts, namely Mimika, Nabire, Paniai, Dogiyai, Deiyai, and Intan Jaya; (c) Mamta consists of 5 districts, namely Jayapura City, Jayapura, Keerom, Sarmi, and Mamberamo Raya; (d) Saireri consists of 4 districts, namely Biak Numfor, Yapen Islands, Waropen, and Supiori; (e) Mee Pago consists of 10 districts, namely Bintang Mountains, Jayawijaya, Lanny Jaya, Yahukimo, Tolikara, Yalimo, Nduga, Puncak Jaya, Central Mamberamo, and Peak (BKKBN Provinsi Papua, 2017; Moeso & Agus, 1985).

Since becoming part of the district/city government, the BKKBN can be placed in various agencies according to the local government needs and vice versa. Provided the officer does not come from the BKKBN, there is a tendency that the program will not develop according to needs. This change in institutional status affects the number and competence of staff in managing KB programs.

Traditional treatment methods are potentially analysed based on an in-depth understanding of the Papua culture. Therefore, traditional medicine from this province can be classified into six treatment patterns as follows (Enos, 2015; Kopeuw, 2017):

a. Amulet Treatment Pattern

Amulets are anything with magical powers, often plants that smell strong and are dark in color. This pattern is known in the Kepala Burung (Bird's Head) area, especially Meibrat and Aifat. According to Elmberg, the principle is to use substantial objects or amulets to protect against disease.

b. Possession Treatment Pattern

According to Van Longhem, a healer is often possessed while treating the patient. Hence, the dominance of supernatural powers in this treatment is very pronounced, as in the amulet type (Loghem, 1951).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

This pattern is known in the bird's wing area, the Telik Arguni.

c. Blood Suction Treatment Pattern

This pattern is known in the ethnic living along the Tor River in the Sarmi, Marindanim, Kaimana, and Asmat areas. According to Oosterwal, the principle is that disease happens because of dirty blood, which when sucked, can lead to a curative effect (Oosterwal, 1962).

d. Trampling Treatment Pattern

According to Oosterwal, the principle is that disease occurs because the body is possessed by the spirit. Therefore, the evil spirit is released by trampling the patient's body, starting from two legs, then continuing until the head (Oosterwal, 1962). This pattern is known in the ethnic living along the Tor River in the Sarmi area.

e. Massage Treatment Pattern

This pattern is known in the ethnic living in the southern area of Merauke (Asmat) and south of Jayapura Regency (Towe). According to Van Amelsvoort, the principle is that disease occurs due to being possessed by the spirit and can be removed by massaging the whole body of patients (Amelsvoort, 2003).

f. Incense Treatment Pattern

This treatment is known in the ethnic living in the south of Jayapura bordering the Jayawijaya district, namely the Towe and Ubrub. The principle is that disease when the body is possessed by the spirit, causing the loss of a balance between the body and soul. The evil spirits and empirical causes of disease can be expelled with the steam from the concoction of heated leaves.

g. Elderly Prayer Treatment Pattern

People come to the traditional elders with several complaints and ask for prayers to cure diseases in the Namblong language. Then, elders informed medicinal plants to cure the disease and easily found in the yard or the surrounding forest. This treatment is known in the Nimboran Ethnic in Jayapura District.

Based on the concept of health and illness from the cultural perspective of the Papuan community, there are two categories based on their scope of life (Foster, 1986). The first category is health and sick as a supernatural event due to interference of supernatural power from humans. While the second category is health and sick as rationalistic due to interventions of nature, climate, water, soil, etc. and the community's behavior such as poor social relations, mental conditions, and others.

The Nimboran Ethnic group's method of processing medicinal plants into a potion is as follows:

a. Without Mixing (Direct Use).

This method directly uses plant parts without mixing or being processed in treating certain diseases. The leaves and stems are directly spread on the ground.

b. Mixture

1) Single form: mix the plant parts by pounding, then obtain the sap and put it on the sick area while the mixture is brewed and drunk immediately.

2) Compound form (mixture): this method involves adding certain mixtures into the initial preparation to provide a more potent/high-efficiency healing effect. The types and procedures for their use have been passed down down the generations and are recorded as traditional knowledge. The plant species such as vegetable pesticides are used to eradicate pests in their crops. The smell produced from the mixtures is powerful hence rats/pests do not dare to approach their crops, and the plant species for magical purposes is used as swanggi medicine. Several species can be used to cure diseases, resurrect the dead, and defend against enemy attacks.

Based on the field observation in 12 villages in the Nimboran District, they used gandarussa to delay pregnancy 44%, due to young marriage with poor economic conditions 24%, after moving to another place 14% and due to the tribal war as 12%. There is 64% of this ethnic group used the leaves of gendarussa as a drink for their husbands to delay pregnancy. Then, 22 % used the roots to treat bone pain and bruises, and 14% used the stems to provide the power of special prayers. Hence children under five months tend to walk promptly.

Gandarusa leaves used to delay pregnancy were between 8 to 18 pieces. Up to 32% of the community used ten pieces of leaves, 26% used 15 pieces, 16% used 17 pieces, 12% used eight pieces, 8% used 16 pieces, 4% used 18 pieces, and 2% used 12 pieces. It was stated that the leaves used were old, and the plants were waist-high for adults. The Nimboran Tribe, Jayapura in Papua Province uses gandarusa with 10. waist-high leaves sheets by boiling water and drinking by men at night.

Out of the 50 informants, around 98% stated that there were no side effects felt while using gandarusa, where the time of use varied from 6 months to one year. The 2% remaining had side effects when consuming the plant, such as red eyes, decreased appetite, heartburn, drowsiness, throat disorders, bloating, dizziness, decreased libido, and dry mouth.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

CONCLUSION

Gandarusa is used by the Nimboran Ethnic group in Jayapura, Papua Province, as an ingredient in traditional medicines, especially for male contraception. The preparation and usage of gandarusa through the collection, mixing, and manufacturing method and in terms of dosage, time, and duration of use were explored more deeply by conducting interviews with 50 informants spread over 12 villages. Gandarusa as a traditional male contraceptive in the Nimboran Tribe, Jayapura in Papua Province, is very effective and successful. The community's knowledge of the gendarussa plant and continued usage of it as an alternative remedy in a wide range of areas demonstrates its success and usefulness as a contraceptive strategy in this tribe.

AUTHOR CONTRIBUTIONS

Conceptualization, B.P.E.W.; Methodology, B.P.E.W.; Validation, B.P.E.W.; Investigation, N.I.; Resources, P.K.; Data Curation, R.W.; Writing - Original Draft, N.I.; Writing - Review & Editing, R.W., B.P.E.W.; Visualization, N.I.; Supervision, R.W., B.P.E.W.; Funding Acquisition, N.I., B.P.E.W.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Amelsvoort, V. (2003). An Epidemiological Approach to Study Fatigue in the Working Population: the Maastricht Cohort Study. *Occupational and Environmental Medicine*; *60*; i32–i39. doi: 10.1136/oem.60.suppl_1.i32.

Atakpama, W., Batawila, K., Wala, K., Douma, M., Péréki, H., Dimobe, K., Akpagana, K. & Gbeassor, M. (2012). Ethnobotanical Knowledge of *Sterculia setigera* Del. in the Sudanian Zone of Togo (West Africa). *ISRN Botany*; *7*; 231–257. doi: 10.5402/2012/723157.

Atato, A., Wala, K., Batawila, K., Woegan, A. Y. & Akpagana, A. (2010). Diversité des Fruitiers Ligneux Spontanés du Togo. *Fruit, Vegetable and Cereal Science and Biotechnology*; *4*; 1–9.

Avocevou-Ayisso, C., Avohou, T. H., Oumorou, M., Sinsin, B. (2012). Ethnobotany of *Pentadesma butyracea* in Benin: a Quantitative Approach. *Ethnobotany Research and Applications*; *10*; 151–166.

Ayantunde, A. A., Briejer, M., Hiernaux, P., Udo H. M. J. & Tabo, R. (2008). Botanical Knowledge and Its Differentiation by Age, Gender and Ethnicity in Southwestern Niger. *Human Ecology*; *36*; 881–889. doi: 10.1007/s10745-008-9200-7.

Bhasin. (2007). Medical Antropology: a Review. *Journal Ethnomedicine*; *1*; 1–20. doi: 10.1080/09735070.2007.11886296.

BKKBN Provinsi Papua. (2015). Pembangunan Kependudukan dan Keluarga Berencana. Jayapura: BKKBN Provinsi Papua.

BKKBN Provinsi Papua. (2017). Pembangunan Kependudukan dan Keluarga Berencana. Jayapura: BKKBN Provinsi Papua.

Dalimartha, S. (2001). Atlas Tumbuhan Obat Indonesia Jilid 2. Jakarta: Trubus Agriwidya.

Daval. (2009). Conservation and Cultivation of Ethno Medicinal Plants in Jharkhand In: Trivedi PC (ed.). *Medical plants utilization and convervation*; 130-136. Jaipur: Aaviskar Publisher's Distributor.

Depkes RI. (1995). Materia Medika Indonesia Jilid VI. Jakarta: Departemen Kesehatan RI.

Enos, E. Y. (2015). Papeda: Masyarakat Papua (Studi pada Masyarakat Sentani di Kabupaten Jayapura, Provinsi Papua dan Suku Moi di Kota. Yogyakarta: Kepel Press.

Foster, A. (1986). Antropologi Kesehatan (Terjemahan). Jakarta: UI-Press.

Kemenkes. (2014). Profil Kesehatan Indonesia Tahun 2013. Jakarta: Kemenkes RI.

Kemenkes. (2018). Indonesia Demographic and Health Survey 2017. Jakarta: Kemenkes RI.

Kopeuw, P. M. (2017). Menggali Budaya Sentani di Papua Untuk Indonesia. Sentani: CV. Pealtwo Hiyakhe Press.

Loghem, V., Holländer, L. P., Dausset, J., Hässig, A. & Julliard, J. (1951). Formerly Bulletin of the Central Laboratory of the NRC Blood Transfusion Service. Guangzhou: Wiley.Moeso, S. & Agus, P. (1985). Laporan Perjalanan ke Jayapura Sentani (Irian Jaya). Yogyakarta: Universitas Gadjah Mada.

Nina L. E. & Lisa, X. G. (1996). Ethnomedicine in Maluku, Eastern Indonesia. *Cakalele*; *7*; 33–50.

Oosterwal, G. (1962). De Papoea, zijn Ook Mensen Als Wij. s'Gravenhag: Vanhoeve.

Prajogo, B. E. W. (2002). Aktivitas Antifertilitas Flavonoid Daun *Justicia gendarussa* Burm.f. *Disertasi*; Fakultas Farmasi Universitas Airlangga, Surabaya.

Prajogo, B. E. W., Juliaan, F., Hinting, A., Pramesti, M. P., Anggraeni, M., Radjaram, A. & Musta'ina. (2011). Laporan Pelaksanaan Uji Klinik Fase III

Universitas Airlangga dan Badan Koordinasi Keluarga Berencana Nasional. Surabaya: Universitas Airlangga.

Prajogo, B. E. W. (2014). Autentik Tanaman *Justicia gendarussa* Burm. f. sebagai Bahan Baku Obat Kontrasepsi Pria. Surabaya: Airlangga University Press dengan LP3 UNAIR.

Prajogo, B. E. W. (2017). Air Gandarusa (*Justicia gendarussa* Burm. f.) dan Gambaran Gen Hyaluronidase Lewat Analisis PCR, Indonesia Journal, Clinical Paythology and Medical Laboratory. Surabaya: Airlangga University Press.

Sulistyawati, A. (2013). Pelayanan Keluarga Berencana. Jakarta: Salemba Medika.

Swerdlow, J. L. (2003). Nature's Medicine: Plants that Heal. Washington DC: National Geographic.

Taek, M. M., Banilado, L., Neonbasu G., Vianey W. Y., Prajogo, B. & Agil, M. (2019). Ethnomedicine of Tetun Ethnic People in West Timor Indonesia; Philosophy and Practice in the Treatment of Malaria. *Integrative Medicine Research*; *8*; 139-144. doi: 10.1016/j.imr.2019.05.005.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 62-68
DOI: 10.20473/jfiki.v9i12022.62-68
Available online at https://e-journal.unair.ac.id/JFIKI/

HPLC Method Optimization for Simultaneous Determination of Quercetin, Luteolin, Sinensetin, and Stigmasterol in Herbal Medicines

Asih Imulda Hari Purwani[1,3], Riesta Primaharinastiti[2], Mochammad Yuwono[2]*
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[3]Bhakti Wiyata Institute of Health, Kediri, Indonesia

*Corresponding author: yuwono05@yahoo.com

Submitted: 31 October 2021
Accepted: 21 January 2022
Published: 26 April 2022

Abstract

Background*: Quercetin, luteolin, sinensetin and stigmasterol each is the main marker compound in extracts of Sonchus arvensis, Plantago major, Orthosiphon stamineus, and Strobilanthes crispus, respectively. These extracts show nephrolithiasis activity. For quality control of herbal medicines, a high performance liquid chromatography (HPLC) method has been developed in this study using quercetin, luteolin, sinensetin and stigmasterol as phytochemical markers.* ***Objective****: to show optimal conditions of analysis and evaluate the stability of quercetin, luteolin, sinensetin and stigmasterol.* ***Methods****: The optimal conditions for analysis were carried out by determining the composition of the mobile phase, the flow rate, and the detector's wavelength. Zorbax Eclipse Plus C18 150 x 4.6 mm, 5 µm was used as the column. The stability test was done by analyzing the standard and samples stored at 4ºC for 0, 3, 6 and 24 hours.* ***Results****: The best separation of the extract was achieved under isocratic conditions using a mixture of water: methanol: phosphoric acid: acetic acid : acetonitrile (50: 30: 0.05: 0.05: 20 v/v/v/ v/v) as mobile phase with detector wavelength of 352 nm, a mobile phase flow rate of 1 mL/min, and a sample injection volume of 10 µL.* ***Conclusion****: In this study, the optimal condition for analysis of quercetin, luteolin, sinensetin and stigmasterol. Quercetin, luteolin, sinensetin and stigmasterol were not stable during 6 hours storage, therefore, standard solutions and samples should be made fresh to maintain the stability.*

Keywords*: HPLC, luteolin, quercetin, sinensetin, stigmasterol*

How to cite this article:
Purwani, A. I. H., Primaharinastiti, P., & Yuwono, M. (2022). HPLC Method Optimization for Simultaneous Determination of Quercetin, Luteolin, Sinensetin, and Stigmasterol in Herbal Medicines. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 62-68. http://doi.org/10.20473/jfiki.v9i12022.62-68

INTRODUCTION

Herbal plants have been widely used as formulae for herbal medicines because they contain bioactive compounds that provide therapeutic effects for the body. Herbal plants are widely developed in several countries, such as *Plantago major*, *Orthosiphon stamineus*, *Sonchus arvensis* and *Strobilanthes crispus* which are empirically used to treat and prevent nephrolithiasis. Extracts of leaves, stems and roots of this plant have long been used as medicine in various countries to treat kidney stones, antifungals, bladder, antioxidants, gastrointestinal infections, diabetes and anticancer (Hossain & Ismail, 2012; Kartini *et al*., 2014).

Many studies have been carried out on *Plantago major*, *Orthosiphon stamineus*, *Sonchus arvensis* and *Strobilanthes crispus* are proving the effect of nephrolithiasis (Aziz *et al*., 2004; Arafat *et al*., 2008; Kartini & Azminah, 2012; Adnyana *et al*., 2013;). The effect of nephrolithiasis is due to luteolin, sinensetin, quercetin and stigmasterol compounds. Arafat *et al*. (2008) proved that these compounds play a role in controlling the crystallization process of kidney stones. Hossain & Ismail (2012) also confirmed that luteolin, sinensetin, quercetin and stigmasterol compounds could inhibit electrolyte reabsorption in the loop of Henle so that it has a diuretic effect. The structure of luteolin, sinensetin, quercetin and stigmasterol can be seen in Figure 1.

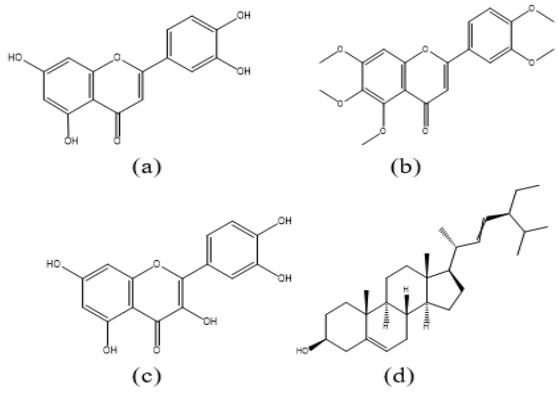


Figure 1. Structure of luteolin (a), sinensetin (b), quercetin (c) dan stigmasterol (d)

In the development of polypharmacy herbal medicine, the selection of marker compounds and methods of identification/quantification of marker compounds play an essential role in ensuring the quality of herbal medicines. The analytical method commonly used to identify marker compounds is High-Performance Liquid Chromatography (HPLC) with a DAD detector (Shah **et al**., 2010; Ang et al., 2014; Lee et al., 2015). Identification and quantitation of extracts in polypharmacy herbal medicines is a challenge. Therefore, developing and validating the HPLC method becomes essential to obtain, fast and straightforward procedures in quality control laboratories. The research carried out is limited to determining marker compounds in single herbal plants using the HPTLC method and has not been applied to a mixture of herbal medicinal preparations (Hossain and Ismail, 2012; Kartini et al., 2014). Several analytical methods for the determination of quercetin, luteolin, sinensetin and stigmasterol have been developed using the UHPLC-QToF-MS method and also reported (Ouyang et al., 2016; Yang *et al*., 2019; Velamuri *et al*., 2020); however, these instruments are expensive and require expertise. who are specialized in instrument operation.

The determination of sinensetin levels in *Orthosiphon stamineus* extract using the HPLC method has been reported by Yam *et al.* (2012) using the mobile phase of acetonitrile: isopropyl alcohol: phosphoric acid (30: 15: 55 v/v/v), but the peak resolution between sinensetin and eupatorin in the extract is low. As a result, the sinensetin complex has not entirely dissociated. This study aimed to obtain optimal conditions for analysis of luteolin, sinensetin, quercetin and stigmasterol using the HPLC and evaluating the stability of luteolin, sinensetin, quercetin and stigmasterol during storage in 4^{o}C for 0, 3, 6 and 24 hours.

MATERIALS AND METHODS

Material

Quercetin standard (Sigma Aldrich), Luteolin standard (Sigma Aldrich), sinensetin standard (Sigma Aldrich), stigmasterol standard (Sigma Aldrich), Herbal medicine products contain four kinds of plants. *Sonchus arvensis*, *Plantago major*, *Orthosiphon stamineus* and *Strobilanthes crispus* were obtained from pharmaceutical industry in Surabaya, methanol (Merck, US/Canada), phosphoric acid (Merck, US/Canada), acetic acid (Merck, US/Canada), methanol (Merck, US/Canada), HPLC grade water (Merck, US/Canada), acetonitrile (Merck, US/Canada).

Instrument

HPLC Shimadzu LC-6AD equipped with a DAD detector, a Zorbax Eclipse Plus C18 column 150 x 4.6 mm x 5 mm (E, Merck, Darmstadt, Germany).

Method

Preparation of standards

Weighed 10.0 mg standard, dissolved in 10 mL of methanol in a volumetric flask. This master standard solution was diluted to make a working standard solution of 20 - 100 ppm.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 1. Variation of mobile phase composition tested to obtain optimal conditions

No	Mobile phase composition	Volume ratio
1.	Water : methanol : acetic acid : acetonitrile	60 : 30 : 0.05 : 10
2.	Water : methanol : phosphoric acid : acetic acid : acetonitrile	40 : 40 : 0.05 : 0.05 : 20
3.	Water : methanol : phosphoric acid : acetic acid : acetonitrile	50 : 30 : 0.05 : 0.05 : 20
4.	Methanol : 0.2 % formic acid	With the following gradient elution program : 0-10 minutes, 15 - 30% A, 10 - 45 minutes, 30 - 50% A, 45 - 50 minutes, 50 - 80% A, 50 - 55 minutes, 80 - 95% A, 55 - 60 minutes, 100% A
5.	Acetonitrile: 0.2% formic acid	With the following gradient elution program : 0 - 10 minutes, 15 - 30% A, 10 - 45 minutes, 30 - 50% A, 45 - 50 minutes, 50 - 80% A, 50 - 55 minutes, 80 - 95% A, 55 - 60 minutes, 100% A

Sample preparation

Weighed 50 mg of herbal medicine and added 5 mL of methanol in a volumetric flask. The mixture was sonicated for 15 minutes and filtered through a 0.45 µm membrane filter before being injected into the HPLC system.

Preparation of mobile phase

Variation of mobile phase compositions is shown in Table 1.

Optimization of the analytical conditions

Optimization of the analytical conditions was carried out by changing the mobile phase's composition and the mobile phase's flow rate. The mobile phase was sonicated for 10 minutes before use. Optimization of the mobile phase flow rate at 0.6 - 2.0 mL/min range. Parameters for optimal conditions were retention time (Rt), peak shape and best resolution (Rs) >1.5.

Stability test of the test solution

A stability test was carried out on four standard solutions and four samples. Each tube was labelled 0 hours, 3 hours, 6 hours, and 24 hours. The test solution was analysed over the time period specified on the label. All tubes were stored at 4°C

RESULTS AND DISCUSSION

The result of mobile phase optimization is shown in Table 2. The mobile phase no. 1 showed a resolution of 0.98 - 1.07; thus, luteolin, sinensetin, quercetin and stigmasterol were not perfectly separated with the peaks of impurities. In addition, the peak was also *fronting*, so that mobile phase 1 was not selected. The composition of the mobile phase 2 showed good resolution between the peaks of luteolin, sinensetin, quercetin and stigmasterol, namely > 1.5 but the peak was *fronting*. This could affect the analysis results so that mobile phase 2 was not selected. The composition of mobile phases 4 and 5 did not show any peaks of luteolin, sinensetin, quercetin and stigmasterol so mobile phase 4 and 5 were not selected. The composition of mobile phase no. 3 showed the most optimal resolution of 1.5 - 7.7, which is > 1.5, and symmetrical peaks, so this mobile phase was selected and used for further analysis. The optimal conditions obtained in this study were used to test the stability of the pre-validation step of the method. This HPLC method can identify four marker compounds contained in herbal medicine. In the formulation development of herbal drugs, the selection of marker compounds and methods for identification/quantification of the marker compounds play an essential role in the quality control of herbal medicines to ensure that the products produced are consistent, safe and efficacious.

The selected wavelength was 352 nm. At a wavelength of 352 nm, the area produced by luteolin, sinensetin, quercetin and stigmasterol peaks and the resulting resolution was better so that the selected wavelength used was 352 nm (Figure 2).

Table 2. Experimental data on variations in the composition of the mobile phase

Mobile phase	Rt (minutes)	Rs	Tailing factor
1	1.31	0.84	*Fronting*
	2.06	0.98	
	2.89	-	
2	1.70	1.4	*Fronting*
	2.04	1.5	
	2.09	1.9	
	3.40	2.1	
3	3.08	1.5	Symmetric
	4.07	2.4	
	4.62	1.7	
	8.00	7.7	
4	NA	NA	NA
5	NA	NA	NA

*NA: no peak detected

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. Results of optimization of injection volume

Concentration (ng/µL)	Injection volume (µL)	Area (mAU)	Tailing factor
8.10	6	2391	Symmetric
		2475	
		2732	
		3668	
8.10	10	2483	Symmetric
		2547	
		2841	
		3724	
8.10	20	2671	*Fronting*
		2836	
		3085	
		3946	

Table 4. The result of the optimization of flow rate of mobile phase on analytes peaks

flow rate (mL/menit)	Rs (> 1,5)	N (> 1)	α (> 2000)
0.6	0.84	1098	1.24
	0.94	1056	1.26
	1.1	1176	1.29
	2.1	1210	2.31
1.0	1.5	2191	1.34
	2.4	2351	1.32
	1.7	2780	1.38
	7.7	3782	7.74
2.0	1.7	2182	1.32
	2.5	2462	1.34
	1.8	2878	1.36
	7.9	3985	7.72

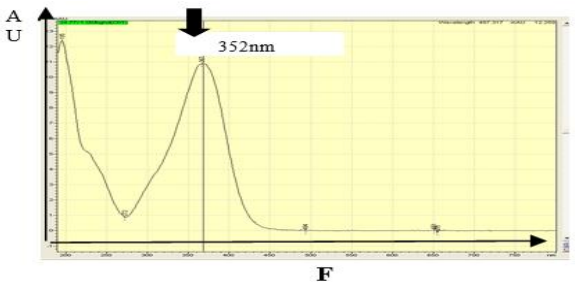


Figure 2. The spectrum of luteolin compounds and its maximum wavelength

The optimisation results of the injection volume are listed in Table 3. Based on the results obtained, the injection volumes that gave a symmetrical peak shape are 6 µL and 10 µL. At an injection volume of 20 µL, the peak *fronting* was too much due to the injection volume. Based on research by Shallajan *et al*. (2012), Yam *et al*. (2012), Lee *et al.* (2016), Rajagopal *et al*. (2017) and Khuluk *et al*. (2021), the injection volume of 10 µL was preferred in this study.

The mobile phase flow rate has been optimized in this study. The optimization results are listed in Table 4. The results obtained indicate that the mobile phase flow rate can affect the value of Rs. The best separation was obtained with the values of Rs > 1.5, N > 1 and the value of which met the criteria and had a good chromatogram shape. The best separation was obtained at a flow rate of 2.0 mL/min. However, the mobile phase flow rate of 1.0 mL/min also met the requirements of Rs, which is > 1.5. To shorten analysis time, a mobile phase flow rate of 1.0 mL/min was selected.

Stability of the test solution

The stability test was carried out to evaluate the stability of the test solution at a specific storage time. The analyte in the test solution was considered to be stable if the difference in area and retention time was not more than 2% to the analyte in the test solution that has just been made and immediately analyzed (Indrayanto, 2012). The stability test results are shown in Table 5 and Table 6.

Analysis was carried out using SPSS to determine whether there was a significant difference between the area and retention time at each observation time. The data obtained showed a normal and homogeneous distribution, so one-way ANOVA was used. The results of the analysis showed that there were significant differences in the area average and Rt at each observation time. This indicates that the longer the standard solution is stored, the lower the concentration is (Figure 3). Based on the data obtained, it is known that the test solution was unstable after six hours of storage.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 5. The results of stability tests of standard solutions in range 0 - 24 hours

Observation Time (hours)	Analytes Standard	Average Area (n = 3)	Area Difference (%)	Rt	Difference Rt (%)
0	Luteolin	7143.1 ± 0.5	-	3.09	-
	Stigmasterol	3163.3 ± 0.5	-	4.08	-
	Sinensetin	3127.1 ± 0.5	-	8.01	-
	Quercetin	5632.4 ± 0.4	-	4.62	-
3	Luteolin	7088.9 ± 0.4	0.7	3.08	0.3
	Stigmasterol	3073.3 ± 0.4	0.2	4.05	0.7
	Sinensetin	3067.1 ± 0.3	1.9	8.01	0
	Quercetin	5573.7 ± 0.3	1.0	4.60	0.4
6	Luteolin	6892.9 ± 0.3	3.5	3.01	2.5
	Stigmasterol	2987.1 ± 0,4	5.5	4.02	1.4
	Sinensetin	2984.2 ± 0.3	4.5	7.83	2.2
	Quercetin	5435.4 ± 0.4	3.4	4.51	2.3
24	Luteolin	6752.9 ± 0.3	5.4	2.98	3.5
	Stigmasterol	2895.5 ± 0.4	8.4	3.95	3.1
	Sinensetin	2878.7 ± 0.4	7.9	7.81	2.4
	Quercetin	5374.2 ± 0.3	4.5	4.46	3.4

Table 6. The results of the stability test of the sample solution in range 0-24 hours

Observation Time (hours)	Sample	Average Area (n = 3)	Area Difference (%)	Rt	Difference Rt (%)
0	Luteolin	7058.6 ± 0.5	-	3.09	-
	Stigmasterol	3093.2 ± 0.5	-	4.08	-
	Sinensetin	3018.0 ± 0.5	-	8.01	-
	Quercetin	5462.3 ± 0.4	-	4.62	-
3	Luteolin	7047.3 ± 0.4	0.1	3.05	1.2
	Stigmasterol	3076.2 ± 0.4	0.5	4.03	1.4
	Sinensetin	3012.4 ± 0.3	0.1	8.00	0.1
	Quercetin	5383.5 ± 0.3	1.4	4.58	0.8
6	Luteolin	6783.5 ± 0.3	3.8	3.00	2.9
	Stigmasterol	2893.2 ± 0.4	6.4	3.96	2.8
	Sinensetin	2876.9 ± 0.3	4.6	7.69	3.9
	Quercetin	5265.2 ± 0.4	3.6	4.48	3.0
24	Luteolin	6704.7 ± 0.3	5.0	2.94	4.8
	Stigmasterol	2837.1 ± 0.4	8.2	3.92	3.9
	Sinensetin	2840.3 ± 0.4	5.8	7.58	5.3
	Quercetin	5185.4 ± 0.3	5.0	4.36	5.6

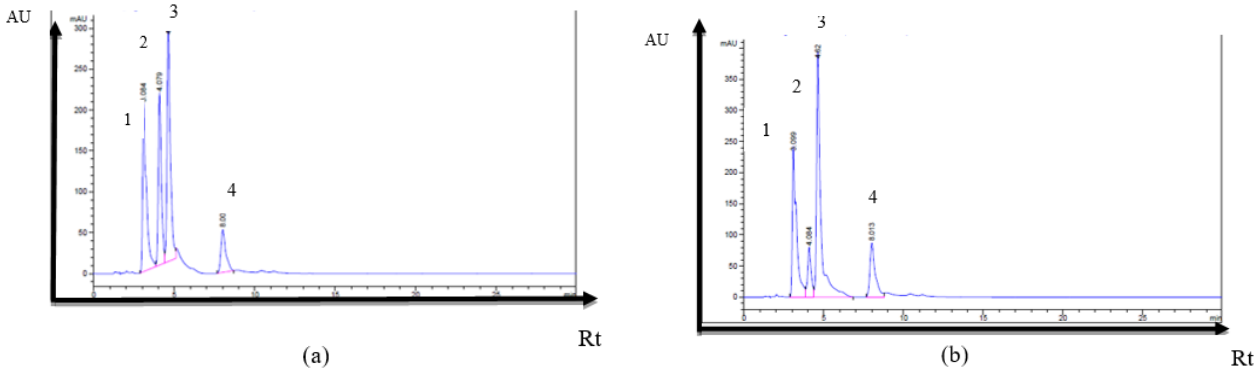


Figure 3. Chromatograms of standard (1) luteolin, (2) stigmasterol, (3) quercetin and (4) sinensetin (a) and herbal medicine samples (b) using the mobile phase water: methanol: phosphoric acid: acetic acid: acetonitrile (50:30:0.05:0.05:20 v/v/v/v/v)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

CONCLUSION

The optimal condition obtained for the analysis of luteolin, sinensetin, quercetin and stigmasterol in herbal medicine is a mixture of water: methanol: phosphoric acid: acetic acid: acetonitrile (50: 30: 0.05: 0.05: 20) as isocratic mobile phase using a Zorbax Eclipse Plus C18 column (150 x 4.6 mm, 5 µm), detector wavelength of 352 nm, injection volume of 10 µL and mobile phase flow rate of 1 mL/min. After six hours of storage, the test solution's stability deteriorated, as evidenced by a drop in area and a shift in retention time on the chromatogram. When doing the analysis, it is recommended that the test solution be made fresh. Validation of the method for the optimal conditions that have been established can be done in the future study.

AUTHOR CONTRIBUTIONS

Conceptualization, M.Y.; Methodology, M.Y.; Software, M.Y.; Validation, A.I.H.P.; Formal Analysis, A.I.H.P.; Investigation, A.I.H.P.; Resources, M.Y.; Data Curation, M.Y.; Writing - Original Draft, A.I.H.P.; Writing - Review & Editing, A.I.H.P.; Visualization, A.I.H.P.; Supervision, R.P.; Project Administration, R.P.; Funding Acquisition, A.I.H.P.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adnyana, I. K., Setiawan, F. & Insanu, M. (2013). From Ethnopharmacology to Clinical Study of *Orthosiphon stamineus* benth. *International Journal of Pharmacy and Pharmaceutical Sciences*; *5*; 66-73.

Ang, L. F., Yam, M. F., Fung, Y. T. T., Kiang, P. K & Darwin, Y. (2014). HPLC Method for Simultaneous Quantitative Detection of Quercetin and Curcuminoids in Traditional Chinese Medicine. *Journal of Pharmacopuncture*; *4*; 36-49. doi: 10.3831/KPI.2014.17.035.

Arafat, O. M., Tham, S. Y., Sadikun, A., Zhari, I., Haughton, P. J. & Asmawi, M. Z. (2008). Studies on Diuretic and Hypouricemic Effects of *Orthosiphon stamineus* Methanol Extracts in Rats. *Journal of Ethnopharmacology*; *118*; 354-360. doi: 10.1016/j.jep.2008.04.015.

Aziz, S. A., See, T. L., Khuay, L. Y., Osman, K. & Bakar, M. A. A. (2004). In Vitro Effects of *Plantago major* Extract on Urolithiasis. *Malaysian Journal of Medical Science*; *12*; 22-26.

Hossain, M. A. & Ismail, Z. (2012). Quantification and Enrichment of Sinensetin in the Leaves of *Orthosiphon stamineus*. *Arabian Journal of Chemistry*; *9*; 1338-1341. doi: 10.1016/j.arabjc.2012.02.016.

Indrayanto, G. (2012). Validation of Analytical Methods. *Profiles of Drug Subtances, Excipients, And Related Methodology*; *37*; 439–463. doi: 10.1016/B978-0-12-397220-0.00012-X.

Kartini & Azminah. (2012). Chromatographic Fingerprinting and Clustering of *Plantago major L.* from Different Areas in Indonesia. *Asian Journal of Pharmaceutical and Clinical Research*; *4*; 191-195.

Kartini., Piyaviriyakul, S., Siripong, P. & Vallisuta, O. (2014). HPTLC Simultaneous Quantification of Triterpene Acids for Quality Control of *Plantago major L.* and Evaluation of their Cytotoxic and Antioxidant Activities. *Industrial Crops and Products*; *60*; 239-246. doi: 10.1016/j.indcrop.2014.06.020.

Khuluk, R. H., Yunita, A., Rohaeti, E., Syafitri, U. D., Linda, R., Lim, L. W., Takeuchi, T. & Rafi, M. (2021). An HPLC – DAD Method to Quantify Flavonoids in *Sonchus arvensis* and Able to Classify the Plant Parts and Their Geographical Area through Principal Component Analysis. *Journal of Separations*; *8*; 2-10. doi: 10.3390/separations8020012.

Lee, D. G., Lee, J., Kim, K. T., Lee, S. W., Kim, Y. O., Cho, I. H., Kim, H. J., Park, C. G. & Lee, S. (2016). High Performance Liquid Chromatography Analysis of Phytosterol in *Panax gingseng* root Grown Under Different Conditions. *Journal of Gingseng Research*; *42*; 16-20. doi: 10.1016/j.jgr.2016.10.004.

Lee, J., Weon, J. B., Yun, M. R. & Ma, C. J. (2015). Simultaneous Determination Three Phytosterol Compounds, Campesterol, Stigmasterol and Daucosterol in *Artemisiaapiacea* by High Performance Liquid Chromatography Diode Array Ultraviolet/Visible Detector. *Pharmacognosy Magazine*; *11*; 297-303. doi: 10.4103/0973-1296.153082.

Ouyang, H., Li, T., He, M., Li, Z., Tan, T., Zhang, W., Li, Y., Feng, Y. & Yang, S. (2016). Identification and Quantification Analysis on the Chemical Constituents from Traditional Mongolian Medicine Flos Scabiosae Using UHPLC - DAD – Q - TOF - MS Combined with UHPLC – QqQ -

MS. *Journal of Chromatographic Sience*; *54*; 1028-1036. doi: 10.1093/chromsci/bmw041.

Rajagopal, S. S., Vazhayil, B. K., Varghese, L. & Nanjaian, M. (2017). Development and Validation of RP-HPLC Method for Simultaneous Determination of Apigenin and Luteolin in Ethanol Extract of *Clerodendrum serratum* (Linn) Leaves. *Asian Journal of Applied Secience*; *5*; 2321-0893.

Shah, U. M., Patel, S. M., Patel, P. H., Hingorani, L. & Jadhav, R. B. (2010). Development and Validation of a simple Isocratic HPLC Method for Simultaneous Estimation of Phytosterols in *Cissus quadrangularis. Indian Journal of Pharmaceutical Science*; *4*; 753-758. doi: 10.4103/0250-474X.84587.

Shallajan, S., Menon, S., Sayed, N. & Tiwari, B. (2012). Simultaneous Estimation of Three Triterpenoids from *Carissa carandas* Using Validated High Performance Liquid Chromstography. *International Journal of Green Pharmacy*; *3*; 242-247. doi: 10.22377/ijgp.v6i3.269.

Velamuri, R., Sharma, Y., Fagan, J. & Schaefer, J. (2020). Application og UHPLC – QTOF – MS in Phytochemical Profilling of Sage *(Salvia officinalis)* and Rosemary *(Rosmarinus officinalis)*. *Planta Medica*; *7*; 133-144. doi: 10.1055/a-1272-2903.

Yam, M. F., Mohamed, E. A. H., Ang, L. F., Pei, L., Darwis, Y., Mahmud, R., Asmawi, M. Z., Basir, R. & Ahmad, M. (2012). A Simple Isocratic HPLC Method for the Simultaneous Determination of Sinensetin, Eupatorin, And 3'-Hydroxy-5, 6, 7, 4'-Tetramethoxyflavone in *Orthosiphon stamineus* Extracts. *Journal of Acupuncture and Meridian Studies*; *5*; 176-182. doi: 10.1016/j.jams.2012.05.005.

Yang, W., Li, W., Zhang, L., He, S., Xiao, N., Qiao, Y., Sui, H., Liang, L. & Chen, J. (2019). Simultaneous Determination of 15 Flavonoids in Scutellaria barbata – Hedyotis diffusa Herb Pair by HPLC Q-TOF MS. *Journal of AOAC International*; *102*; 11-16. doi: 10.5740/jaoacint.17-0469.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 69-74
DOI: 10.20473/jfiki.v9i12022.69-74
Available online at https://e-journal.unair.ac.id/JFIKI/

Potential Drug–Drug Interactions in Ambulatory Patients with Hypertension: a Retrospective Study

Marcha Debby Saraswati[1,2], Shafira Muti Ardiana[3], Budi Suprapti[2,3]*, M. Yusuf Assegaf[2,4], Suharjono[3], Khusnul Fitri Hamidah[2]

[1]Magister of Clinical Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Universitas Airlangga Teaching Hospital, Surabaya, Indonesia
[3]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[4]Department of Cardiology and Vascular Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: budi-s@ff.unair.ac.id

Submitted: 4 November 2021
Accepted: 2 February 2022
Published: 26 April 2022

Abstract

Background*: Patients with cardiovascular diseases (CVD) are vulnerable to experiencing drug-drug interactions (DDIs). DDIs are a concern among patients receiving multiple drug regimens but they are also an avoidable cause of adverse drug reactions. The study of potential DDIs (pDDIs) would make it easier for the healthcare provider to deliver better patient care and mitigate pDDIs incidence.* ***Objective****: This study aimed to assess the frequency, severity level and risk factors associated with pDDIs among medications used to treat hypertensive ambulatory patients in Universitas Airlangga hospital.* ***Methods****: A retrospective observational study was carried out from electronic prescriptions received by hypertensive patients in March 2021. Data collection includes demographic data, the profile of antihypertensive drug use, and pDDIs. pDDIs were identified by severity using Lexicomp Drug Interaction Checker (Application). Univariate logistic regression analysis was used to find associated factors of major pDDIs. A p-value less than 0.05 (≤ 0.05) was considered statistically significant.* ***Results****: From 704 patients, 53.98% women and 46.02% men, 89.06% (n = 627) patients had minor to major pDDIs; 1354 pDDIs were identified, 89.4% (n = 1,210) were moderate and 9.8% (n = 133) were major class. Multiple antihypertensive drug regimens had significance associated with the major pDDIs occurrence.* ***Conclusion****: We found a high prevalence of pDDIs among hypertensive patients. The majority of pDDIs were of moderate severity. Multiple antihypertensive drug regimens were associated factors in the presence of major pDDIs.*

Keywords*: ambulatory patient, cardiovascular diseases, hypertension, potential drug-drug interaction*

How to cite this article:
Saraswati, M. D., Ardiana, S. M., Suprapti, B., Assegaf, M. Y., Suharjono, & Hamidah, K. F. (2022). Potential Drug–Drug Interactions in Ambulatory Patients with Hypertension: a Retrospective Study. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 69-74. http://doi.org/10.20473/jfiki.v9i12022.69-74

INTRODUCTION

Non-communicable diseases (NCDs) accounted for the most significant proportion of global deaths, contributing to 73.4% of causes of death of total deaths in 2017. It was estimated that the most significant number of deaths was from cardiovascular diseases (CVDs) (17.8 million). Hypertension is one of the CVDs. Deaths between 2007 and 2017 increased by 46.6% (Roth *et al.*, 2018). By causing an estimated 9.4 million deaths worldwide annually, hypertension has been shown to be one of the leading causes of death (WHO, 2019). In Indonesia, from 2013 to 2018, the prevalence of hypertension increased from 25.8% to 34.1% (Indonesia Ministry of Health, 2013; 2018).

Due to multiple comorbidities and complications, patients with hypertension need to be treated with a different treatment plan, including various drugs. A study on a combination of antihypertensive medications showed that most hypertensive patients use between two and five medications, which vary according to comorbidities. The most common antihypertensive regimens are angiotensin-converting-enzyme inhibitors (ACEI)/angiotensin II receptor blockers (ARB) with thiazide (Johansen *et al*., 2020). A significant increase in hypertension among people on polytherapy regimenshas shown to meet their blood pressure goal (Gu *et al*., 2012). In hypertensive patients, comorbidities, complex drug treatment plans, and polypharmacy have raised the risk of incidence of drug-drug interaction (DDI) (Akbar *et al*., 2021).

DDI incidence is defined as a different pharmacological or clinical response to administering two or more drugs than one drug alone. This interaction can lead to a decrease or increase in the drug effects. The combination of drugs was considered potential drug-drug interactions (pDDIs) when the theoretical interactions in the prescription are evaluated rather than actual occurrence (Rodrigues *et al.*, 2017). Identification and management of pDDIs are important to prevent the associated risk (Ismail *et al*., 2013). A study of pDDIs in ambulatory patients from the internal medicine department has shown 292 (83.42%) prescriptions, with at least one identifiable pDDIs (Rana *et al*., 2014). Because of the differences in the study population, environment, design and drug interaction software platform used in these studies, the prevalence of pDDIs in various studies varied from 16% to 91% (Mistry *et al*., 2017; Al-Qerem *et al*., 2018; Ismail *et al.*, 2018;). In Indonesia, the study of antihypertensive drugs' problems held from March to May 2012 at Geriatric Department RSUD Dr. Soetomo reported 62% pDDIs incidence (Suprapti *et al*., 2014).

Therefore, this study was conducted to provide information on the frequency levels of discovered pDDIs and a list of frequently relevant interactions among the ambulatory patients of the Cardiology Department at Universitas Airlangga Teaching Hospital. Information about potential drug-drug interaction in any clinical setting will help the healthcare providers to improve patients' therapeutic outcomes.

MATERIALS AND METHODS

The study was designed as an observational retrospective study by observing the pDDIs of hypertensive patients with or without comorbidities. This study aims to identify pDDIs among patients with hypertension at the outpatient cardiology department, Universitas Airlangga Teaching Hospital Surabaya, Indonesia, during March 2021.

Data collection procedures

A retrospective observational was carried out from electronic prescriptions received by the patients that met the inclusion criteria and recorded on the data collection sheet, including demographic data, diagnosis, the profile of antihypertensive drug use, and pDDIs. Electronic prescriptions were obtained for a total of 704 patients in March 2021. The inclusion criteria included patients with hypertensive diagnosis and antihypertensive agents. This study observed pDDIs among all prescribed drugs. The board has approved the methodology of this study of ethics of Universitas Airlangga Teaching Hospital number 139/KEP/2021.

Data analysis

Identification of pDDIs was carried out based on data recorded on the data collection sheet. pDDIs in each prescription were identified by severity (minor, moderate, and major) using Lexicomp Drug Interaction Checker (Application) (Lexicomp, 2021). Data were analyzed descriptively using SPSS version 25. Furthermore, univariate logistic regression analysis was used to find associated factors of major pDDIs. A p-value less than 0.05 (≤ 0.05) was considered statistically significant.

RESULTS AND DISCUSSION

The demographic and clinical characteristics of the patients are listed in Table 1. About 704 hypertensive patients included in this study, the mean age of patients was 63.15 ± 10.57 years. The majority of patients were females (n = 380, 54%), ≥ 60 years old (n = 483, 68.6%), hypertensive heart disease (HHD) (n = 608, 86,4%), and

P-ISSN: 2406-9388
E-ISSN: 2580-8303

polytherapy hypertensive regiment (n = 659, 93.6%). Hypertension is attributed to one-fifth of the deaths of US women and is a more significant burden for women than men. Moreover, around 60% of the population has hypertension by 60 years of age, and about 65% of men and 75% of women acquire high blood pressure by 70 years (Franklin et al., 2001; Lloyd-Jones et al., 2009; Mozaffarian *et al*., 2016). A study conducted in Malang District, East Java Province, Indonesia, demonstrated that 55.8% CVD risk factor was hypertension (Maharani *et al*., 2019). The seventh JNC guideline showed that more hypertensive patients are on a polytherapy hypertensive regimen than a decade ago. Polytherapy is defined as a person using > 1 antihypertensive drug (Chobanian *et al*., 2003; Gu *et al*., 2012).

The study revealed that 89.06% of pDDIs in hypertensive patients were considerably higher than reported studies in the other countries (Sharma *et al*., 2014; Kovačević et al., 2017; Muhammad & Afridi, 2017; Diksis *et al*., 2019). Few studies have evaluated the frequency and severity level of pDDIs among CVD patients in Indonesia. This study raises the awareness of pDDIs in pharmacy and hospital services, especially for ambulatory services. Moreover, the observed findings were likely the differences in the pattern of prescriptions between different countries and the patient population included in these studies. Our study found 89.4% of moderate pDDIs and 9.8% of major pDDIs levels in hypertensive patients (Table 2). Other studies also reported t that the most common types of pDDIs in CVD patients were moderate level. A survey conducted in the cardiology departments of two tertiary care teaching hospitals in the Quetta, Balochistan indicated 74.06% of moderate pDDIs (Akbar *et al*., 2021). Another study found a higher frequency of severe pDDIs (45%) than our study (Murtaza *et al*., 2016). Drug combinations involved major pDDIs and their potential consequences are listed in Table 3.

Table 1. Sociodemographic and clinical characteristics of study participants

Gender	**n (%)**
Female	380 (53.98)
Male	324 (46.02)
Age (years)	
18 – 30	7 (0.99)
31 – 40	12 (1.70)
41 – 50	54 (7.67)
51 – 60	148 (21.02)
> 60	483 (68.61)
Type of CVD	
Hypertensive Heart Disease without (congestive) heart failure (HHD)	608 (86.36)
HHD + Coronary Artery Disease (CAD)	84 (11.93)
HHD + Atrial Fibrillation (AF)	6 (0.85)
Hypertensive Heart Disease with (congestive) heart failure (HHF)	2 (0.30)
HHD + CAD + AF	2 (0.28)
Hypertension + Valvular Heart Disease (VHD)	1 (0.14)
HHD + Peripheral Artery Disease (PAD)	1 (0.14)
Number of hypertensive drugs prescribed	
Monotherapy	45 (6.39)
Polytherapy	659 (93.61)

Table 2. Category of pDDIs

Category	**pDDIs n (%)**
Severity	
Minor	11 (0.8)
Moderate	1210 (89.4)
Major	133 (9.8)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. Drug combination involved in class major pDDIs and their potential consequences

Category	Drug combination	Frequency	Potential consequence
Major	Candesartan-Spironolakton	110	Increased risk of hyperkalemia
	Lisinopril-Spironolakton	8	Increased risk of hyperkalemia
	Ramipril-Spironolakton	7	Increased risk of hyperkalemia
	Lisinopril-Allopurinol	3	Increased potential for hypersensitivity
	Diltiazem-Bisoprolol	2	Increased risk of bradycardia
	Diltiazem-Simvastatin	2	Increased potential for rhabdomyolysis and myopathy
	Ramipril-Allopurinol	1	Increased potential for hypersensitivity

Table 4. Associated factors of major class pDDIs

Variables	Major class pDDIs	Univariate analysis	
	No. (%)	OR (95% Cl)	*p-value*
Gender			
Female	71 (56.80)	Referent	
Male	54 (43.20)	1.149 (0.778 – 1.696)	0.485
Age			
< 60	41 (32.80)	Referent	
≥ 60	84 (67.20)	1.082 (0.716 – 1.635)	0.708
Number of hypertensive drug prescribed			
Polytherapy			
2 - 3	40 (32.00)	Referent	
4 - 5	85 (68.00)	24.586 (15.143 – 39.919)	0.000

Table 4 showed that 125 (9,8%) patients had a significant severity of pDDIs. In univariate analysis, patients receiving 4-5 antihypertensive drug regimens (odd ratios (OR) 24.586, p-value – 0.000) were statistically associated with major pDDIs level. This study showed a significant association between an increased number of antihypertensive drugs prescribed (4 - 5) and the presence of major pDDIs levels is congruent with studies conducted in some countries and correlated with other studies which reported polypharmacy as a risk factor of the pDDIs prevalence (Murtaza *et al.*, 2016; Kovačević *et al*., 2017; Shakeel *et al.*, 2018; Diksis *et al*., 2019; Akbar *et al*., 2021). This present study also showed similarities to Subramanian et al. (2018) study in which hypertensive patients are vulnerable to DDI. The limitation of this study was conducted retrospectively and only in one institution. Prospective studies are required in the future to determine more associated factors of pDDIs.

CONCLUSION

There was a high prevalence of pDDIs in hypertensive ambulatory patients, and moderate severity was the most frequent pDDIs. Multiple antihypertensive drug regimens were demonstrated as associated factors of the significant pDDIs occurrence.

ACKNOWLEDGMENT

We would like to acknowledge Universitas Airlangga Teaching Hospital for permission to conduct this research.

AUTHOR CONTRIBUTIONS

Conceptualization, B.S., M.Y.A., S.S., M.D.S.; Methodology, B.S., M.Y.A., S.S., M.D.S.; Software, M.D.S., S.M.A.; Validation, B.S., M.Y.A., S.S.; Formal Analysis, M.D.S., K.F.H.; Investigation, M.D.S., S.M.A., K.F.H.; Resources, S.M.A., K.F.H.; Data Curation, M.D.S., S.M.A.; Writing - Original Draft, B.S., M.D.S., K.F.H.; Writing - Review & Editing, B.S., M.D.S.; Visualization, B.S., M.D.S.; Supervision, B.S., M.Y.A., S.S.; Project Administration, M.D.S., S.M.A.; Funding Acquisition, B.S.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Akbar, Z., Rehman, S., Khan, A., Khan, A., Atif, M. & Ahmad, N. (2021). Potential Drug–Drug Interactions in Patients with cardiovascular Diseases: Findings from a Prospective Observational Study. *Journal of Pharmaceutical Policy and Practice*; *14*; 1-9. doi: 10.1186/s40545-021-00348-1.

Al-Qerem, W., Jarrar, Y. B., Al-Sheikh, I. & El Maadani, A. (2018). The Prevalence of Drug-Drug Interactions and Polypharmacy Among Elderly Patients in Jordan. *Biomedical Research*; *29*; 2561-2569. doi: 10.4066/biomedicalresearch.29-18-618.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A. & Izzo, J. L Jr. (2003). Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. National Heart, Lung, and Blood Institute; National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*; *42*; 1206 –1252. doi: 10.1161/01.HYP.0000107251.49515.c2.

Diksis, N., Melaku, T., Assefa, D. & Tesfaye, A. (2019). Potential Drug–Drug Interactions and Associated Factors Among Hospitalized Cardiac Patients at Jimma University Medical Center. Southwest Ethiopia: SAGE Open Medicine.

Franklin, S. S., Larson, M. G., Khan, S. A., Wong, N. D., Leip, E. P., Kannel, W. B. & Levy, D. (2001). Does the Relation of Blood Pressure to Coronary Heart Disease Risk Change with Aging? the Framingham Heart Study. *Circulation*; *103*; 1245-1249. doi: 10.1161/01.cir.103.9.1245

Gu, Q., Burt, V. L., Dillon, C. F. & Yoon, S. (2012). Trends in Antihypertensive Medication Use and Blood Pressure Control Among United States Adults with Hypertension: the National Health and Nutrition Examination Survey, 2001 to 2010. *Circulation*; *126*; 2105–2114. doi: 10.1161/CIRCULATIONAHA.112.096156.

Indonesia Ministry of Health. (2013). Basic Health Research (RISKESDAS) 2013. Jakarta: Agency for Health Research and Development.

Indonesia Ministry of Health. (2018). Basic Health Research (RISKESDAS) 2018. Jakarta: Agency for Health Research and Development.

Ismail, M., Iqbal, Z., Khattak, M. B., Khan, M. I., Arsalan, H., Javaid, A., Gul Q. & Khan, F. (2013). Potential Drug–Drug Interactions in Internal Medicine Wards in Hospital Setting in Pakistan. *International Journal of Clinical Pharmacy*; *35*; 455–462. doi: 10.1007/s11096-013-9764-1.

Ismail, M., Noor, S., Harram, U., Haq, I., Haider, I., Khadim, F., Khan, Q., Ali, Z., Muhammad, T. & Asif, M. (2018). Potential Drug-Drug Interactions in Outpatient Department of a tertiary Care Hospital in Pakistan: a Cross-Sectional Study. *BMC Health Services Research*; *18*; 1-7. doi: 10.1186/s12913-018-3579-7.

Johansen, M. E., Yun, J., Griggs, J. M., Jackson, E. A. & Richardson, C. R. (2020). Anti-Hypertensive Medication Combinations in the United States. *The Journal of the American Board of Family Medicine*; *33*; 143–146. doi: 10.3122/jabfm.2020.01.190134.

Kovačević, M., Vezmar Kovačević, S., Miljković, B., Radovanović, S. & Stevanović, P. (2017). The Prevalence and Preventability of Potentially Relevant Drug–Drug Interactions in Patients Admitted for Cardiovascular Diseases: a Cross Sectional Study. *International Journal of Clinical Practice*; *71*; 1-9. doi: 10.1111/ijcp.13005.

Lexicomp. (2021). Lexicomp: Drug Interaction Checker (app). Alphen aan den Rijn: Wolters Kluwer.

Lloyd-Jones, D., Adams, R., Carnethon, M., Simone, G. D., Ferguson, T. B., Flegal, K., Ford, E., Furie, K., Go, A., Greenlund, K., Haase, N., Hailpern, S., Ho, M., Howard, V., Kissela, B., Kittner, S., Lackland, D., Lisabeth, L., Marelli, A., McDermott, M., Meigs, J., Mozaffarian, D., Nichol, G., O'Donnell, C., Roger, V., Rosamond, W., Sacco, R., Sorlie, P., Stafford, R., Steinberger, J., Thom, T., Wasserthiel-Smoller, S., Wong, N., Wylie-Rosett, J. & Hong, Y. (2009). Heart Disease and Stroke Statistics—2009 Update: a Report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*; *119*; 480-486. doi: 10.1161/CIRCULATIONAHA.108.191261.

Maharani, A., Sujarwoto, Praveen, D., Oceandy, D., Tampubolon, G. & Patel, A. (2019). Cardiovascular Disease Risk Factor Prevalence and Estimated 10-Year Cardiovascular Risk Scores in Indonesia: the SMART Health Extend Study. *PLOS ONE*; *14*; 1-13. doi: 10.1371/journal.pone.0215219.

Mistry, M., Gor, A., Ganguly, B. (2017). Potential Drug-Drug Interactions among Prescribed Drugs in Paediatric Outpatients Department of a Tertiary Care Teaching Hospital. *Journal of Young Pharmacists*; *9*; 371-375. doi:10.5530/jyp.2017.9.74.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M. (2016). Heart Disease and Stroke Statistics—2016 Update: a Report from the American Heart Association.

Circulation; *133*; e38-e360. doi: 10.1161/CIR.0000000000000350.

Muhammad, N. & Afridi, R. (2017). Prevalence, Types and Predictors of Potential Drug-Drug Interactions in an Internal Medicine Ward of the Mardan Medical Complex, Mardan, Kpk, Pakistan. *International Journal of Basic Medical Sciences and Pharmacy*; *6*; 28–32.

Murtaza, G., Khan, M. Y. G., Azhar, S., Khan, S. A. & Khan, T. M. (2016). Assessment of Potential Drug–Drug Interactions and Its Associated Factors in the Hospitalized Cardiac Patients. *Saudi Pharmaceutical Journal*; *24*; 220–225. doi: 10.1016/j.jsps.2015.03.009.

Raebel, M. A., McClure, D. L., Simon, S. R., Chan, K. A., Feldstein, A., Andrade, S. E., Lafata, J. E., Roblin, D., Davis, R. L., Gunter, M. J. & Platt, R. (2006). Laboratory Monitoring of Potassium and Creatinine in Ambulatory Patients Receiving Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers. *Pharmacoepidemiology and Drug Safety*; *16*; 55–64. doi: 10.1002/pds.1217.

Rana, D., Suthar, J., Malhotra, S., Patel, V. & Patel, P. (2014). A Study of Potential Adverse Drug-Drug Interactions Among Prescribed Drugs in Medicine Outpatient Department of a Tertiary Care Teaching Hospital. *Journal of Basic and Clinical Pharmacy*; *5*; 44-48. doi: 10.4103/0976-0105.134983.

Rodrigues, A. T., Stahlschmidt, R., Granja, S., Pilger, D., Falcao, A. L. E. & Mazzola, P. G. (2017). Prevalence of Potential Drug-Drug Interactions in the Intensive Care Unit of a Brazilian Teaching Hospital. *Brazilian Journal of Pharmaceutical Sciences*; *53*; 1–8. doi: 10.1590/s1807-59322011000100003.

Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C. & Abbasi, N. (2018). Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries and Territories, 1980–2017: a Systematic Analysis for the Global Burden of Disease Study 2017. *The Lancet*; *392*; 1736–1788. doi: 10.1016/S0140-6736(18)32203-7.

Schepkens, H., Vanholder, R., Billiouw, J. M., Lameire, N. (2001). Lifethreatening Hyperkalemia During Combined Therapy with Angiotensin-Converting Enzyme Inhibitors and Spironolactone: an Analysis of 25 Cases. *The American Journal of Medicine*; *110*; 438–441. doi: 10.1016/s0002-9343(01)00642-8.

Shakeel, F., Khan, J. A., Aamir, M., Hannan, P. A., Zehra, S. & Ullah, I. (2018). Risk of Potential Drug–Drug Interactions in the Cardiac Intensive Care Units: a Comparative Analysis Between 2 Tertiary Care Hospitals. *Saudi Medical Journal*; *39*; 1207–1212. doi: 10.15537/smj.2018.12.23430.

Sharma, S., Chhetri, H. P. & Alam, K. (2014). A Study of Potential Drug–Drug Interactions Among Hospitalized Cardiac Patients in a Teaching Hospital in Western Nepal. *Indian Journal of Pharmacology*; *46*; 152–156. doi: 10.4103/0253-7613.129303.

Subramanian, A., Adhimoolam, M. & Kannan, S. (2018). Study of drug–Drug interactions among the Hypertensive Patients in a Tertiary Care Teaching Hospital. *Perspectives in Clinical Research*; *9*; 9–14. doi: 10.4103/picr.PICR_145_16.

Suprapti, B., Nilamsari, W. P. & Hapsari, P. P. (2014). Permasalahan Terkait Obat Antihipertensi pada Pasien Usia Lanjut di Poli Geriatri RSUD Dr. Soetomo Surabaya. *Jurnal Farmasi dan Ilmu Kefarmasian Indonesia*; *1*; 36-41

World Health Organization (WHO). (2019) Hypertension. https://www.who.int/news-room/fact-sheets/detail/hypertension. Accessed: 20 Januari 2021.

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 75-81
DOI: 10.20473/jfiki.v9i12022.75-81
Available online at https://e-journal.unair.ac.id/JFIKI/

Molecular Docking of Active Compound of *Lavandula angustifolia* Mill Essential Oil against N-methyl-D-aspartate (NMDA) Receptor

Baiq Risky Wahyu Lisnasari[1], Aniek Setiya Budiatin[2], Chrismawan Ardianto[2], Junaidi Khotib[2]*
[1]Undergraduate Program of Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: junaidi-k@ff.unair.ac.id

Submitted: 19 November 2021
Accepted: 2 February 2022
Published: 26 April 2022

Abstract

Background*: Lavender oil is widely known to possess a relaxant effect to relieve stress, anxiety, and depression. Linalyl acetate, linalool, geranyl acetate, and β-caryophyllene were the major constituents of lavender oil that potentially act on NMDAR (N-methyl-d-aspartate receptors), and emerging targets in the treatment of depression.* ***Objective****: This study aims to predict the binding of lavender compounds to NMDA receptors using an in silico model.* ***Methods****: The ligands of the docking study were four major chemical compounds of lavender oil, i.e., linalyl acetate, linalool, geranyl acetate, and β-caryophyllene. 5YE was defined as a native ligand, while memantine, an NMDAR antagonist, was used as a reference ligand. The NMDAR structure was taken from Protein Data Bank (ID 5H8Q), while the lavender compound was sketched in Chem3D. Autodock 4.2 was used to perform the docking analysis.* ***Results****: The result showed that beta-caryophyllene had the most potent interaction with NMDAR (free binding energy was -8.02 kcal/mol and inhibitory constant was 1.32 µM).* ***Conclusion****: The docking results suggest that beta-caryophyllene could be an NMDAR antagonist and be developed as a treatment for depression.*

Keywords*: depression, lavender oil, molecular docking, NMDAR*

How to cite this article:
Lisnasari, B. R. W., Budiatin, A. S., Ardianto, C., & Khotib, J. (2022). Molecular Docking of Active Compound of *Lavandula angustifolia* Mill Essential Oil against N-methyl-D-aspartate (NMDA) Receptor. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 75-81. http://doi.org/10.20473/jfiki.v9i12022.75-81

INTRODUCTION

Essential oil from black seed (*Nigella sativa*), Japanese rush (*Acorus gramineus*), lavender (*Lavandula angustifolia*), blue gum (*Eucalyptus globulus*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sambac jasmine (*Jasminum sambac*), black pepper (*Piper nigrum*) and several other plants have been reported to have neuroprotective effects *(*Ayaz *et al*., 2017). Lavender (*Lavandula angustifolia*) is the most widely used essential oil for aromatherapy (Wells *et al*., 2018). Lavender oil is known for its delicate aroma and is commonly used in the perfume, flavoring, and cosmetic industries. Lavender has a long history of medicinal use and is reported to have antidepressants, anti-anxiolytic, sedative, analgesics, and calming effects (Wells *et al.*, 2018; Kang *et al.*, 2019; Lizarraga-Valderrama, 2021). Aromatherapy is considered therapeutically effective because of the inhaled volatile compounds' psychological effects, which are believed to act through the limbic system, particularly the amygdala and hippocampus (Fung *et al.*, 2021). While the exact cellular mechanism of action is unknown, it is predicted that lavender (based on studies on *L. angustifolia*) may have an effort similar to benzodiazepines and may enhance the effects gamma-aminobutyric acid (Lizarraga-Valderrama, 2021). In their publication, López *et al*. (2017) reported that the main constituents of lavender essential oil analyzed by GC-MS were linalyl acetate (52.1%), linalool (37.4%), geranyl acetate (5.4%), and β-caryophyllene.

Stress is one of the most common psychological disorders and is generally the beginning of other psychiatric disorders, such as anxiety, insomnia, and depression (López *et al.*, 2017). Currently, drugs for depression exert their effects by increasing the levels of biogenic amines, i.e., norepinephrine (NE), dopamine (DA), and serotonin (5HT) by various mechanisms, such as inhibiting the degradation or reuptake of neurotransmitters. However, these drugs have serious side effects, including sexual dysfunction, weight gain, sleep disturbance, etc., leading to an effective and better-tolerated antidepressant. On the other hand, the NMDA receptor family (N-methyl-D-aspartate) has received particular attention in psychiatric disorders, especially major depressive disorder (Pochwat *et al.*, 2019). Therefore, direct targeting of the NMDA receptor may yield alternative strategies for treating depression.

Docking is the process of interaction between two molecules in three-dimensional space. Molecular docking is a valuable tool in structural molecular biology and is a potential field for new drug discovery. This method is widely used to predict the binding mode of the ligand to the protein. Applications of docking techniques include the prediction of new drug binding modes and the screening of active compounds. This study aims to predict the binding of lavender compounds to the receptors using an in silico model.

MATERIALS AND METHODS

Protein preparation

The crystal structure of the NMDA with ID 5H8Q was downloaded in PDB file format from the RCSB protein data bank and imported into AutoDock 4.2. Polar hydrogens and Kollman charges are then added to the protein. Considering that the water molecule is not involved in the ligand-receptor bonding process, the water molecule is removed. This step of removing water molecules may also significantly improve computations and prevent distortions that might occur.

Ligand preparation

The molecular structures of four major compounds from lavender oil are given in Figure 1. The structures of linalyl acetate, linalool, geranyl acetate, and β-caryophyllene were drawn using Chem3D. Next, energy minimization of each ligand was carried out using the MM4 force field, and then the ligands were stored in mol2 format. Finally, the ligands are imported into the AutoDock workspace. The nonpolar hydrogens were merged for each ligand, and the gasteiger charges were computed.

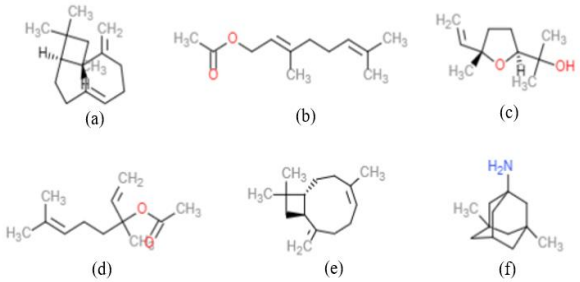


Figure 1. The molecular structure of (a) beta-caryophyllene, (b) geranyl acetate, (c) linalool, (d) linalyl acetate, (e) 5_YE, and (f) memantine

Docking

Molecular docking was performed using Autodock 4.2. Before the docking study was performed, the docking parameters and algorithm were validated by re-docking the native ligand to the target receptor. According to the native ligand, a grid box of 14.595 Å x -14.301 Å x -25.15Å along x, y, and z was defined as a binding site. The docking parameters were left as

P-ISSN: 2406-9388
E-ISSN: 2580-8303

default, except the docking run was set to 30 for each compound. Finally, the Lamarckian Genetic Algorithm generated a molecular docking study. Root mean standard deviation (RMSD) lower than 2 Å suggested that the method could consistently predict the natural conformation of the ligand-receptor. Favorable conformation was selected based on the lowest energy binding and inhibitory constant (*K*i).

Statistical analysis

The quantitative relationship between the chemical structure in lavender oil and its biological activity was determined by correlation analysis in the SPSS program. Analysis of the structure-activity relationship was carried out using the LFER Hansch model. In this study, lipophilic parameters involved were partition coefficient (logP), electronic parameter, i.e., ligand-receptor bond energy (binding energy), and steric parameter, i.e., molar refraction (MR). Linear and nonlinear regression analysis was performed between lipophilic, steric, and electronic parameters with antidepressant activity. The equation with the best relationship significance was selected based on the values of r, F, and SD.

RESULTS AND DISCUSSION

The docking protocol was validated through a re-docking experiment using the native ligand. A root means square deviation (RMSD) value of less than 2 Å was observed, suggesting that the ligand-receptor conformation has a high docking accuracy. Another method to validate the docking parameters and algorithm is docking decoy ligands to the receptor's binding site (Huang *et al*., 2006). Decoys are compounds that share similar physical properties with the reference ligand but may not bind to the target.

Table 1. Results of molecular docking of *Lavandula angustifolia* Mill compound with NMDA1/2 (5H8Q) receptors

No	Ligand	Binding Energy (kcal/mol)	Estimated Binding Constant (Ki) (µM)	Hydrogen bond	Interacting Amino Acid	Number of Binding Site Similar to Native Ligand
1	Beta-caryophyllene	-8.02	1.32	-	THR A: 242, GLU A: 132, PHE A: 130, VAL A: 131, GLY B: 250, PRO B: 141, PRO A: 129, TYR B: 144, ILE B: 128	9
2	Geranyl acetate	-6.49	17.60	-	PHE A: 130, VAL A: 131, GLU A: 132, PRO A: 129, TYR B: 144, THR A: 241, THR A: 242, PRO B: 141, GLY A: 243, HIS B: 273, LEU B: 270, LYS B: 140	12
3	Linalool	-5.91	46.83	THR A: 242	ILE B: 269, GLU A: 132, TYR B: 144, LYS B: 143, LEU B: 270, THR A: 241, LYS B: 140, HIS B: 273, THR A: 242, PRO A: 129, GLY A: 243, ILE A: 116, PRO B: 141	13
4	Linalyl acetate	-6.37	21.33	-	GLU A: 131, PHE A: 130, VAL A: 131, PRO A: 129, GLY A: 243, LYS B: 140, THR A: 242, LYS B: 143, PHE B: 142, THR A: 241, HIS B: 275, THR B: 144,	9
5	5YE (native ligand)	-10.43	22.75	THR A: 242	PHE B: 142, LYS B: 143, THR A: 241, THR A: 242, HIS B: 273, LYS B: 140, ILE A: 116, LEU B: 270, GLY A: 243, PRO B: 141, PHE A: 130, VAL A: 131, VAL A: 266, TYR B: 144, PRO A: 129, GLU A: 132, GLY B: 250	All
6	Memantine	-8.72	407.81	THR A: 133, THR A: 242, GLU A: 132	THR A: 133, THR A: 242, THR A: 241, GLU A: 132, PRO A: 129, HIS B: 273, TYR B: 144, PRO B: 141, GLY A: 243	9

P-ISSN: 2406-9388
E-ISSN: 2580-8303

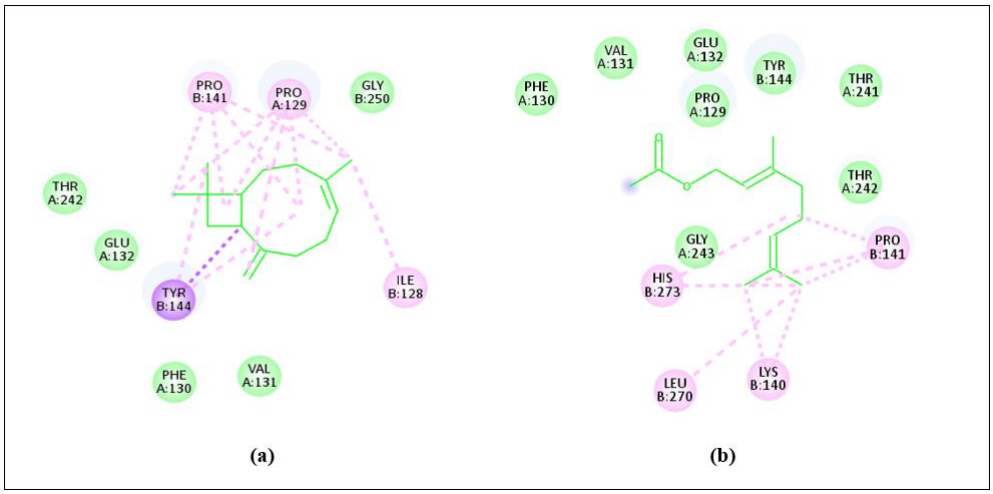


Figure 2. Interaction between (a) beta-cayophyllene and (b) geranyl acetate with N-methyl D-aspartate (NMDA) receptors

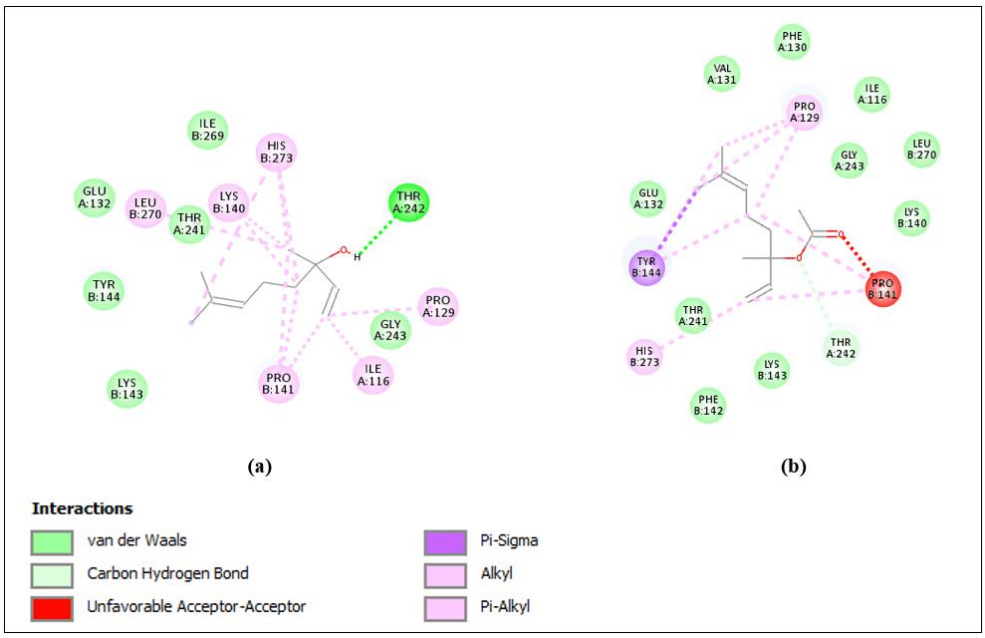


Figure 3. Interaction between (a) linalool and (b) linalyl acetate with N-methyl D-aspartate (NMDA) receptors

The molecular docking of the Lavandula angustifolia Mill compound components against the NMDA1/2 (5H8Q) receptor obtained using Autodock are presented in Table 1, while the 2D interactions are visualized in Figure 2 and 3. The docking results showed that all ligands from lavender oil were docked on the same binding site as the native ligand. Similar interactions with binding site residue indicate that the compound might exhibit inhibitory activity toward the receptor. Based on the docking data, we found that beta-caryophyllene has the lowest energy required to bind to the targeted receptor compared to other compounds. The binding energy is -8.02 kcal/mol, and Ki is 1.32 µM.

Furthermore, the low binding energy value indicated the stability of the ligand-receptor interaction. Interestingly, beta-caryophyllene has no hydrogen bond with the target receptor. The interaction formed is mainly hydrophobic via Pro B: 141, Pro A: 129, Tyr B: 144, and Ile B: 128 residues. Meanwhile, 5YE (native ligand) had binding energy of -10.43 kcal/mol and made one hydrogen bond, one pi-pi stacked, and five hydrophobic interactions (Figure 4a). Memantine, an NMDAR antagonist, had binding energy of -8.72 kcal/mol and formed three hydrogen bonds, one carbon-hydrogen bond, and four hydrophobic interactions (Figure 4b). Therefore, by comparing the binding energy of the three complexes, it is suggested that the contribution of hydrophobic interaction to binding affinity is comparable to electrostatic interaction.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

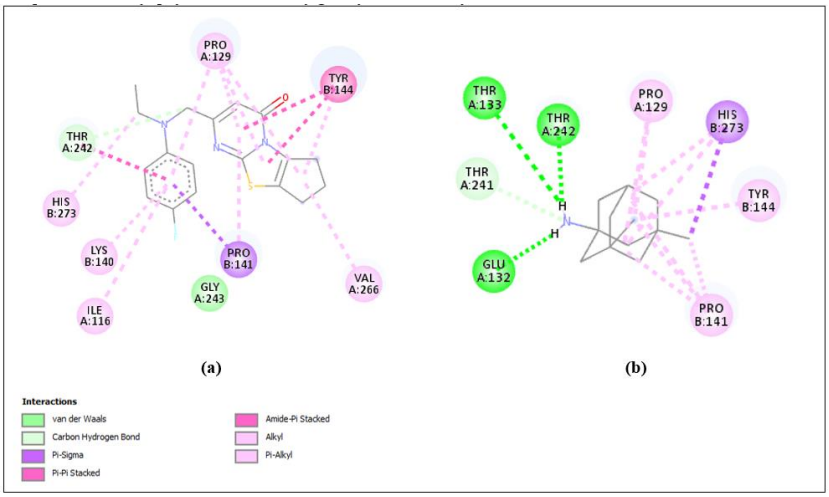


Figure 4. Interaction between (a) 5YE (native ligand) and (b) memantine (reference ligand) with N-methyl D-aspartate (NMDA) receptors

Table 2. The value of electronic properties (σ), lipophilic properties (π), steric properties (RM), and log activity of each lavender compound

Ligand	σ	π	π^2	RM	Log A
Beta-caryophyllene	-8.02	4.32	18.662	67.452	-0.12
Geranyl acetate	-6.49	3.25	10.563	60.334	-1.245
Linalool	-5.91	2.55	6.503	50.206	-1.67
Linalyl acetate	-6.37	3.189	10.170	59.357	-1.33

Linalool is the only compound that forms a hydrogen bond with the targeted receptor, but this compound has the highest energy to bind to the receptor (-5.91 kcal/mol) and the highest *K*i (46.83 µM). Meanwhile, the ester of linalool, linalyl acetate, has a slightly higher binding affinity, evidenced by the free binding energy of -6.37 kcal/mol and *K*i of 21.33 µM. The in-vitro study supports this result reporting the monoterpenes (linalool and linalyl acetate) can inhibit NMDA receptor, in which linalyl acetate has the greater inhibition against the receptor (0.54 mM and 2.3 mM for linalyl acetate and linalool, respectively) (López *et al*., 2017). Indeed, the presence of the acetate group is predicted to increase the compound's acts on the NMDA receptor. This may explain the higher affinity of geranyl acetate geranyl acetate's affinity toward the receptor (binding energy -6.49 kcal/mol and *K*i 17.60), compared to linalool. To summarize the findings, the lavender compound with the best affinity against NMDA receptors is beta-caryophyllene, followed by geranyl acetate, linayl acetate, and linalool.

The value of electronic, lipophilic, and steric properties of each lavender compound is given in Table 2. Statistical analysis was conducted to examine the correlation between the compound structure and its activity. It was found that the electronic property (binding energy) is the parameter that is most related to compound activity, as indicated by the values of r, r^2, F, and S. The closer to 1 the value of r, the higher the value of r^2 and F, and the smaller the value of S, the greater the significance of the relationship between physical chemistry parameters and compound activity. The linear regression equation of electronic parameters with antidepressant activity is Log A = 0.992 σ + 2.229 (r = 0.992; r^2 = 0.984; F = 124.658; S = 0.2; Sig 0.008), while the linear equation of lipophilic parameters is Log A = 0.418 π - 10.457 (r = 0.418; r^2 = 0.175; F = 0.424; S = 16.067; Sig = 0.582), and the equation of steric parameters is Log A = 0.411 RM - 1.065 (r = 0.411; r^2 = 0.169; F = 0.406; S = 1.672; Sig = 0.589). These results indicate that the binding energy value is the most significant parameter in influencing the antidepressant activity of compounds in lavender.

A recent systematic review and meta-analysis of randomized controlled studies reported that lavender oil significantly reduced depressive scores compared to the control group (Firoozeei *et al*., 2021). As lavender oil is mainly used in the inhalation method, it can cross the blood-brain barrier, has a faster onset, and limited side effects. Inhaled essential oil is thought to have better bioavailability than oral drugs, ascribed to its high lipid solubility and minimal to zero hepatic metabolisms (Ayaz *et al*., 2015). The molecular pathway of lavender oil in exerting its antidepressant and antianxiety effect is

P-ISSN: 2406-9388
E-ISSN: 2580-8303

reported through the inhibition of NMDA receptor, $5HT_{1A}$ receptor, serotonin transporter, voltage-gated calcium channel, and neurotoxic agent as hydrogen peroxide (López *et al*., 2017; Firoozeei *et al*., 2021). Linalool is the most studied lavender oil compound for its anxiolytic effect. Linalool was reported to have a relaxant effect, increased social interaction and reduced aggressive behavior in the animal model (Ayaz *et al*., 2017). Previously, it was reported that beta-caryophylline could improve rat depressive-like behavior and suppress the altered hippocampal expression of BNDF, COX-2, and CB2 receptors (Hwang *et al*., 2020).

Targeting the classic monoaminergic receptor, the current antidepressants on the market, such as selective serotonin reuptake inhibitor (SSRI) or serotonin-norepinephrine reuptake inhibitor (SNRI), have become unfavorable to the lack of efficacy and prominent side effects. This limitation leads to finding an alternative antidepressant with better efficacy and tolerability. In recent years, the glutamate receptor has been emerging as a key target of antidepressants, owing to the rapid and robust antidepressant effect of ketamine, an NMDA receptor antagonist (Machado-Viera *et al*., 2017). Glutamate is the main excitatory neurotransmitter in the brain and is involved in neurotoxicity by activating the NMDA receptor. Thus, targeting the NMDA receptor may exert a neuroprotective effect that improves depressive symptoms. In this study, we find that the primary compound of lavender oil has an affinity to bind to the NMDA receptor and act as an inhibitor. Beta-caryophyllene has the best binding affinity, making it a promising compound for further development as an NMDA receptor antagonist and a new candidate of antidepressant.

Beta-caryophyllene is a component of *L. angustifolia* with a molecular weight of 204.357 g/mol and a logP of 4.73. Geranyl acetate has high absorption in the gastrointestinal tract, where it is predicted that 94.85% will be absorbed through the intestine. This compound also has good permeability to the blood-brain barrier, with a predictive value of 0.733 logBB. In addition, geranyl acetate is not a substrate for P-gp, a transporter that acts as a biological barrier that removes toxins and xenobiotics from cells (Pires *et al*., 2015).

Despite its wide application, molecular docking also has a limitation, including the wrong binding site to the target, the choice of docking pose, the uncertainty of whether a compound is a true antagonist or agonist, and the nonlinearity of the docking and molecular dynamic simulation results (Chen, 2014). The molecular dynamic simulation is warranted to further investigate the stability of ligand binding poses to protein (Liu *et al*., 2017).

CONCLUSION

In silico molecular docking, the analysis revealed that beta-caryophyllene has the lowest binding energy (-8.02 kcal/mol) to NMDA receptor, suggesting its potential as an inhibitor of the NMDA receptor its future use as an antidepressant agent. Analysis of the structure-activity relationship indicates that the ligand-receptor bond energy was the parameter that most influenced the compound's activity. Further preclinical research of the compound in lavender oil needs to be carried out to validate the in silico results.

ACKNOWLEDGMENT

We would like to thank Prof Siswandono for guiding the Moldoc Program in drug design and development.

AUTHOR CONTRIBUTIONS

Conceptualization, B.R.W.L., A.S.B., J.K.; Methodology, B.R.W.L., A.S.B., C.A.; Software, B.R.W.L.; Validation, C.A.; Formal Analysis, B.R.W.L.; Investigation, B.R.W.L.; Resources, J.K.; Data Curation, B.R.W.L., C.A., J.K.; Writing - Original Draft, B.R.W.L.; Writing - Review & Editing, J.K.; Visualization, B.R.W.L.; Supervision, C.A.; Project Administration, B.R.W.L.; Funding Acquisition, J.K.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Khan, M. A., Ahmad, W., Shah, M. R., Imran, M. & Ahmad, S. (2015). Comparative Chemical Profiling, Cholinesterase Inhibitions and Anti-Radicals Properties of Essential Oils from *Polygonum hydropiper* L: a Preliminary Anti-Alzheimer's Study. *Lipids in Health and Disease*; *14*; 1–12. doi: 10.1186/s12944-015-0145-8.

Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Subhan, F. & Ahmed, J. (2017). Neuroprotective and Anti-Aging Potentials of Essential Oils from Aromatic and Medicinal Plants. *Frontiers in Aging Neuroscience*; *9*; 1–16. doi: 10.3389/fnagi.2017.00168.

Chen, Y. C. (2014). Beware of docking!. *Trends in Pharmacological Sciences*; *36*; 78–95. doi: 10.1016/j.tips.2014.12.001.

Firoozeei, T. S., Feizi, A., Rezaeizadeh, H., Zargaran, A., Roohafza, H. R. & Karimi, M. (2021). The Antidepressant Effects of Lavender (*Lavandula angustifolia* Mill.): A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials. *Complementary Therapies in Medicine*; *59*; 1-13. doi: 10.1016/j.ctim.2021.102679.

Fung, T. K. H., Lau, B. W. M., Ngai, S. P. C. & Tsang, H. W. H. (2021). Therapeutic Effect and Mechanisms of Essential Oils in Mood Disorders: Interaction between the Nervous and Respiratory Systems. *International Journal of Molecular Sciences*; *22*; 1-17. doi: 10.3390/ijms22094844.

Huang, N., Shoichet, B. K. & Irwin, J. J. (2006). Benchmarking Sets for Molecular Docking. *Journal of Medicinal Chemistry*; *49*; 6789–6801. doi: 10.1021/jm0608356.

Hwang, E. S., Kim, H. B., Lee, S., Kim, M. J., Kim, K. J., Han, G., Han, S. Y., Lee, E. A., Yoon, J. H., Kim, D. O., Maeng, S. & Park, J. H. (2020). Antidepressant-Like Effects of β-caryophyllene on Restraint Plus Stress-Induced Depression. *Behavioural Brain Research*; *380*; 1-8. doi: 10.1016/j.bbr.2019.112439.

Kang, H. J., Nam, E. S., Lee, Y. & Kim, M. (2019). How Strong is the Evidence for the Anxiolytic Efficacy of Lavender?: Systematic Review and Meta-analysis of Randomized Controlled Trials. *Asian Nursing Research*; *13*; 295–305. doi: 10.1016/j.anr.2019.11.003.

Liu, K., Watanabe, E. & Kokubo, H. (2017). Exploring the Stability of Ligand Binding Modes to Proteins by Molecular Dynamics Simulations. *Journal of Computer-Aided Molecular Design*; *31*; 201–211. doi: 10.1007/s10822-016-0005-2.

Lizarraga-Valderrama, L. R. (2021). Effects of Essential Oils on Central Nervous System: Focus on Mental Health. *Phytotherapy Research*; *35*; 657–679. doi: 10.1002/ptr.6854.

López, V., Nielsen, B., Solas, M., Ramírez, M. J. & Jäger, A. K. (2017). Exploring Pharmacological Mechanisms of Lavender (*Lavandula angustifolia*) Essential Oil on Central Nervous System Targets. *Frontiers in Pharmacology*; *8*; 1–8. doi: 10.3389/fphar.2017.00280.

Machado-Viera, R., Henter, I. D. & Zarate Jr., C. A. (2017). New Targets for Rapid Antidepressant Action. *Progress in Neurobiology*; *152*; 1-37. doi: 10.1016/j.pneurobio.2015.12.001.

Pires, D. E. V., Blundell, T. L. & Ascher, D. B. (2015). pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry*; *58*; 4066–4072. doi: 10.1021/acs.jmedchem.5b00104.

Pochwat, B., Nowak, G. & Szewczyk, B. (2019). An Update on NMDA Antagonists in Depression. *Expert Review of Neurotherapeutics*; *19*; 1055–1067. doi: 10.1080/14737175.2019.1643237.

Wells, R., Truong, F., Adal, A. M., Sarker, L. S. & Mahmoud, S. S. (2018). Lavandula Essential Oils: a Current Review of Applications in Medicinal, Food, and Cosmetic Industries of Lavender. *Natural Product Communications*; *13*; 1403–1417. doi: 10.1177/1934578X1801301038.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 82-91
DOI: 10.20473/jfiki.v9i12022.82-91
Available online at https://e-journal.unair.ac.id/JFIKI/

Detection of Potentially Inappropriate Medication in Elderly Outpatient Based on The Beer's Criteria 2019

Nurmainah*, Rindu Astuti, Ressi Susanti
Pharmacy Study Program, Faculty of Medicine, Tanjungpura University, Pontianak, Indonesia

*Corresponding author: nurmainah@pharm.untan.ac.id

Submitted: 6 April 2021
Accepted: 9 February 2022
Published: 26 April 2022

Abstract

Background*: Elderly patients generally have more than one disease, so they need several drugs to treat the condition and Potentially Inappropriate Medication (PIM).* ***Objective:*** *This study aimed to examine the inaccuracy of prescription drugs in the elderly using Beer's criteria 2019.* ***Methods****: The research method used an observational study with a descriptive cross-sectional design. Beer's Criteria 2019 was compiled by The American Geriatrics Society (AGS) and grouped PIM into five categories. A total of 138 prescriptions met the inclusion criteria at the Internal Medicine Clinic.* ***Results****: The results showed that the elderly who received the prescription was in the age range of 60-69 years (73.2%), male gender (54%), and had comorbidity (77.5%). Of the 138 prescriptions for elderly patients, 117 prescriptions for elderly patients experienced PIM incidence (84.78%). The percentage of PIM incidents was based on categories; namely, category 1 was 74.2%, category 2 was 3.5%, 3 was 16.7%, category 4 was 3%, and category 5 was 2.5%. Medicines that include PIM in category 1 are lansoprazole, glimepiride, glibenclamide, alprazolam, diazepam, amitriptyline, diclofenac sodium, ibuprofen, meloxicam. PIM in category 2 are cilostazol, pioglitazone, and diclofenac sodium. Category 3 PIMs include furosemide, spironolactone, and hydrochlorothiazide (HCT), category 4 PIMs include dexamethasone-sodium diclofenac, methylprednisolone-ibuprofen, and alprazolam-codeine, and category 5 PIMs include spironolactone, ciprofloxacin, and ranitidine..* ***Conclusion****: This study concludes that the elderly are at risk for receiving inappropriate drug prescriptions. Patients received more drugs belonging to category 1 PIM than category 2, 3, 4, and 5.*

Keywords*: elderly, beer's criteria 2019, PIM*

How to cite this article:
Nurmainah, Astuti, R., & Susanti, R. (2022). Detection of Potentially Inappropriate Medication in Elderly Outpatient Based on The Beer's Criteria 2019. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, 9(1), 82-91. http://doi.org/10.20473/jfiki.v9i12022.82-91

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

The elderly are susceptible to various physical complaints due to natural factors and disease factors (Kementerian Kesehatan Republik Indonesia, 2014). The aging process occurs due to changes in various organs, including the gastrointestinal system, genito-urinary system, central nervous system, and others (Astuti *et al*., 2017). As a result, the morbidity rate in the elderly in Indonesia is relatively high in which there are 27 out of 100 elderly who experience pain (Badan Pusat Statistik, 2018). Degenerative diseases dominate the main disease pattern in the elderly. This health problem requires the elderly to take medication (Rahmawati *et al*., 2019; Tamher, 2009).

Drug selection for the elderly group is a complex process. Thus, the elderly are vulnerable to potentially inappropriate medication (PIM). Besides, generally, the elderly have comorbidities and experience changes in physiological conditions that can affect pharmacokinetics and pharmacodynamic sensitivity to certain drugs (Abdulah & Barliana, 2015). Therefore, the selection of medications for elderly patients should be made carefully by considering the benefits and risks involved (Walsh, 1997).

Beer's Criteria is one of the explicit criteria that can improve the selection of drugs for the elderly, reduce the incidence of adverse drugs, and function as a tool to evaluate the quality of care, costs, and patterns of drug use in the elderly (American Geriatrics Society, 2019). Based on some research results, it was found that the use of unrecommended drugs in the elderly such as non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPI), digoxin, antihistamine two blockers, and psychotropics (Mulyani & Rukminingsih, 2020; Negara *et al.*, 2016). The use of this drug is risky for the condition of elderly patients. For example, NSAIDs are very risky in elderly patients with heart problems as this drug can inhibit prostaglandins and worsen heart failure conditions. Besides, NSAIDs are risky for gastrointestinal bleeding or peptic ulcers (Mulyani & Rukminingsih, 2020). Furthermore, psychotropic drugs can cause prolonged sedation and an increased risk of falls and fractures (Setyowati *et al.*, 2011).

The detection using the Beer's Criteria method found the pattern of potentially inappropriate medication in every hospital. For example, at the Semarang City hospital, the prescription of NSAID PIM class reached 25.05% (Mulyani & Rukminingsih, 2020). Besides, Purwokerto City Hospital tended to prescribe diazepam at 31.0% (Setyowati *et al.*, 2011). Based on these data, there are differences in PIM patterns in each hospital. Therefore, the researcher is interested in describing the percentage of PIM incidence in Sultan Syarif Mohamad Alkadrie Hospital, Pontianak City. Research on PIM prescription incidents for elderly patients at this hospital has never been carried out using the Beer's Criteria 2019 method so far.

Beer's Criteria 2019 includes five categories for determining the prevalence of PIM in elderly patients. Drugs in category one should be avoided in elderly patients; drugs in category two should be avoided if a patient has a history of certain diseases; drugs in category three can still be used with caution; drugs in category four cause drug-disease interactions; and drugs in category five should be reduced in dose or avoided if a patient has a history of certain diseases (American Geriatrics Society, 2019).

MATERIALS AND METHODS

This observational study used a descriptive cross-sectional design. Data were collected retrospectively using an electronic prescription and medical record database for elderly patients at Sultan Syarif Mohamad Alkadrie Hospital, Pontianak, from January-June 2020.

The study was conducted on outpatient elderly at the Internal Medicine Clinic with inclusion criteria of 1) age > 60 years, 2) having laboratory data for serum creatinine, and 3) at least taking one drug. The exclusion criteria were the patient's identity which was not recorded in the prescription and medical record data. The variables of this study were the type of drugs, medical history, and laboratory data.

Drug use and laboratory data obtained for the elderly were identified as potentially inappropriate medication (PIM) using Beer's Criteria 2019. Beer's Criteria 2019 was compiled by The American Geriatrics Society (AGS). These guidelines aim to improve drug selection, educate clinicians and patients, reduce adverse drug events, and serve as a tool for evaluating older adults' quality of care, cost, and drug use patterns. Beer's criteria include five types of drugs, which are as follows:

Category 1) potentially inappropriate medication use in older adults. Example: anticholinergic, antiparkinsonian agents, benztropine (oral), trihexyphenidyl, antithrombotics, anti-infective, etc.

Category 2) potentially inappropriate medication use in older adults due to drug-disease or drug-syndrome interactions exacerbate the disease or syndrome. Example: Drugs that act on the cardiovascular system, syncope, delirium, a history of falls or fractures,

P-ISSN: 2406-9388
E-ISSN: 2580-8303

gastrointestinal, kidney, or urinary tract problems, and urinary incontinence (all types) in women are examples.
Category 3) potentially inappropriate medications: drugs to be used with caution in older adults. Example: aspirin for primary prevention of cardiovascular disease and colorectal cancer, dabigatran, rivaroxaban, prasugrel, antipsychotics, carbamazepine, diuretics, mirtazapine, oxcarbazepine, SNRIs, SSRIs, TCAs, Tramadol, dextromethorphan/ quinidine, trimethoprim-sulfamethoxazole.
Category 4) potentially clinically critical drug-drug interactions should be avoided in older adults. Example: RAS inhibitor-RAS inhibitor, opioids-benzodiazepines, anticholinergic-anticholinergic, corticosteroid-NSAID, etc.
Category 5) medications that should be avoided or have their dosage reduced with varying levels of kidney function in older adults. Example: anti-infective (ciprofloxacin, trimethoprim-sulfamethoxazole), cardiovascular and hemostasis (amiloride, dabigatran, edoxaban, spironolactone), SSP drugs and analgesics, gastrointestinal.

PIM was identified in samples containing one or more drugs from Beer's Criteria 2019 drug list. Based on the criteria of each, the sample in the PIM category is divided into five categories of Beer's Criteria 2019. The categorizing of PIM from data on drug use in the elderly was done by the research team without the involvement of clinicians in this study. The investigation also included secondary data from prescription and medical records of older patients.The data obtained will be used to calculate the percentage of the sample that contains PIM and which drugs contain the most PIM. Using Microsoft Excel, the data were descriptively analyzed.

RESULTS AND DISCUSSION

A total of 138 elderly outpatients at the Internal Medicine Clinic of Sultan Syarif Mohamad Alkadrie Hospital, Pontianak City, met the inclusion criteria from January to June 2020. This study involved more patients in the age group of 60-69 years old or young elderly. Based on Badan Pusat Statistik (2020), the population was dominated by this age group (64.29%). The percentage of elderly patients in the 60 - 69 year age group was 73.2% higher than the 70 - 79 year age group (25.4%) and the > 80 year age group (1.4%). The number of elderly patients (54%) was higher than that of female elderly patients (46%). Male patients risk degenerative diseases two times higher than female patients. Degenerative diseases tend to occur in male patients because of bad lifestyles, such as smoking, drinking alcohol, diet, lack of physical activity, and obesity. However, female patients also have a risk of PIM, especially those who begin to experience a decrease in estrogen. Menopause women experience a reduction in the production of estrogen and progesterone. The decline in hormone production affects body fat distribution, interfering with the metabolic system (Handajani *et al*., 2010). A total of 107 (77.5%) elderly patients tend to have comorbidities. The most common disease in elderly patients was Type II diabetes mellitus (68.84%), as presented in Table 1. A study by the World Health Organization (2002) revealed that the most common diseases suffered by the elderly worldwide are cardiovascular disease, hypertension, diabetes mellitus, stroke, chronic obstructive pulmonary disease (COPD), and musculoskeletal conditions (such as arthritis and osteoporosis). Decreased physiological function due to aging causes the elderly to suffer from more than one disease or multi-pathology. On the other hand, decreased body resistance in the elderly is the cause of degenerative problems (Kementerian Kesehatan Republik Indonesia, 2013).

Table 2 shows identifying potentially inappropriate medication prescriptions in elderly patients at the Internal Medicine Clinic of Sultan Syarif Mohamad Alkadrie Hospital, Pontianak, using the Beer's Criteria 2019. Beer's Criteria 2019 was met by 117 prescriptions (84.78 %), while 21 prescriptions (15.22 %) did not meet the criteria. The Beer's Criteria 2019 list contained 198 drug items out of 117 prescriptions. According to a study conducted by Mulyani & Rukminingsih (2020), 265 elderly patients (88.33 %) received prescriptions, with 487 drug items (23.98 %) meeting Beer's Criteria. Drugs listed in Beer's Criteria should be administered with caution, if not avoided, because the risks and side effects outweigh the benefits when used in elderly patients (American Geriatrics Society, 2019). This study supports previous research conducted by Yuliawati *et al*. (2020) using the Beer's Criteria 2015, which revealed that 79.4% of prescriptions met Beer's Criteria. This study includes several drug items in the Beer's Criteria, similar to previous studies Yuliawati *et al*. (2020). Alprazolam, amitriptyline, diazepam, ibuprofen, lansoprazole, meloxicam, diclofenac sodium, ranitidine, and spironolactone are examples of these medications. The similarity of results with a previous study in Beer's Criteria 2019 can be attributed to an update of the Beer's Criteria 2015, indicating some similarities in the category and type of drug that has the potential to PIM.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Based on Table 3, PIM drugs for the elderly reached 74.2% for category 1, 3.5% for category 2, 16.7% for category 3, 3% for category 4, and 2.5% for category 5.

Category 1

In general, drugs in Category 1 should be avoided in elderly patients. Lansoprazole, glimepiride, glibenclamide, alprazolam, diazepam, amitriptyline, diclofenac sodium, ibuprofen, and meloxicam were some of the drugs in this category used in the elderly at Sultan Syarif Mohamad Alkadrie Hospital Pontianak City

Lansoprazole

Lansoprazole is a proton pump inhibitor (PPI) drug. In this study, lansoprazole is a PIM drug of category 1, which is most widely used by the elderly. The percentage of lansoprazole use reached 36.9%. Based on the Beer's Criteria 2019 recommendations, this drug should not be used for more than eight weeks in high-risk patients, patients with esophagitis, and pathological hypersecretory conditions (American Geriatrics Society, 2019). PPI drugs can cause magnesium deficiency if taken for a long time as these drugs inhibit the absorption of magnesium (Food and Drug Administration, 2011). Alternative treatment therapy that can be used is H2-receptor antagonist drugs such as cimetidine and famotidine. These drugs do not cause fractures (Yu *et al.*, 2011).

Glimepiride dan glibenclamide

Sulfonylurea drugs such as glimepiride and glibenclamide were mostly prescribed to elderly patients. Glimepiride was used by 19.2 % of elderly patients, while glibenclamide was used by 3.5 %. According to Beer's Criteria 2019, the use of glimepiride and glibenclamide should be avoided. They have a high risk of causing long-term hypoglycemia in the elderly (American Geriatrics Society, 2019). Sulfonylureas drugs work by lowering blood glucose levels quickly. Besides, these drugs increase HbA1c, causing hypoglycemia, which reduces patients' awareness (Gumantara & Oktarlina, 2017). Short-acting sulfonylureas such as glipizide are recommended to treat diabetes mellitus in the elderly as they have the lowest risk of hypoglycemia (Marinda *et al*., 2016).

Table 1. Characteristics of elderly outpatients at Sultan Syarif Mohamad Alkadrie Hospital Pontianak

Characteristics	**N = 138**	
	Number	**Percentage (%)**
Age		
a. 60-69	101	73.2
b. 70-79	35	25.4
c. ≥80	2	1.4
Gender		
a. Male	74	54
b. Female	64	46
Comorbidities		
a. Yes	107	77.5
b. No	31	22.5
Main diagnosis:		
a. Type II Diabetes Mellitus	95	68.84
b. Hypertension	11	7.97
c. BPH	2	1.45
d. Dyspepsia	11	7.97
e. Angina Pectoris	2	1.45
f. Hyperuricemia	1	0.72
g. Hemorrhoids	3	2.17
h. Cholelithiasis	2	1.45
i. Heart failure	4	2.9
j. Asthma	2	1.45
k. Osteoarthritis	2	1.45
l. Tuberculosis	2	1.45
m. Kidney failure	1	0.72

Table 2. Distribution of drugs in the beer's criteria 2019

Distribution of Sample based on the Beer's Criteria	N= 138	
	Number	Percentage (%)
Drug prescription included in the Beer's Criteria 2019	117	84.78
Drug prescription outside the Beer's Criteria 2019	21	15.22

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Table 3. Profile of potentially inappropriate medication based on the beer's criteria 2019

PIM Category	Name of Drugs	N= 198			
		QE	SR	Number	Percentage (%)
Category 1	Lansoprazole	Moderate	Strong	73	36.9
	Glimepiride	High	Strong	38	19.2
	Glibenclamide	High	Strong	7	3.5
	Alprazolam	Moderate	Strong	4	2
	Diazepam	Moderate	Strong	4	2
	Amitriptyline	High	Strong	12	6.1
	Diclofenac Sodium	Moderate	Strong	2	1
	Ibuprofen	Moderate	Strong	2	1
	Meloxicam	Moderate	Strong	5	2.5
Subtotal				**147**	**74.2**
Category 2	Cilostazol – Heart failure	Moderate	Strong	3	1.5
	Pioglitazone – Heart failure	High	Strong	1	0.5
	Diclofenac Sodium – Kidney failure	Moderate	Strong	3	1.5
Subtotal				**7**	**3.5**
Category 3	Furosemide	Moderate	Strong	21	10.6
	Spironolactone	Moderate	Strong	10	5.1
	Hydrochlorothiazide (HCT)	Moderate	Strong	2	1
Subtotal				**33**	**16.7**
Category 4	Dexamethasone – Diclofenac Sodium	Moderate	Strong	2	1
	Methylprednisolone – Ibuprofen	Moderate	Strong	1	0.5
	Alprazolam – Codeine	Moderate	Strong	3	1.5
Subtotal				**6**	**3**
Category 5	Spironolactone	Moderate	Strong	3	1.5
	Ciprofloxacin	Moderate	Strong	1	0.5
	Ranitidine	Moderate	Strong	1	0.5
Subtotal				**5**	**2.5**
Total				**198**	**100**

Notes: QE = quality of evidence; SR = strength of recommendation

Alprazolam dan diazepam

Alprazolam and diazepam are benzodiazepine drugs in category 1 of the Beer's Criteria 2019 with the same percentage of 2%. Alprazolam and diazepam should be avoided based on Beer's Criteria as they can increase the risk of cognitive impairment, delirium, falls, and fractures in the elderly (American Geriatrics Society, 2019).

The risk of falls and fractures in the elderly is caused by the side effects of benzodiazepine drugs, such as dizziness, weakness, and drowsiness, which can reduce the patient's concentration and balance (Sukmawati *et al.*, 2016). Benzodiazepine drugs can be replaced with trazodone and low-dose doxepin alternatives. If benzodiazepines must be used, it is necessary to reduce the dose by half and shorten the duration of therapy. During these drugs, side effects should be monitored (Holt *et al*., 2010).

Amitriptyline

The s of amitriptyline in the elderly has a high risk. The percentage of amitriptyline prescriptions in the elderly reached 6.1% for category 1. Based on Beer's Criteria 2019, amitriptyline should be avoided. Amitriptyline can cause anticholinergic solid effects, sedation, and orthostatic hypotension (American Geriatrics Society, 2019). Side effects of anticholinergics on the central nervous system cover cognitive impairment, confusion, hallucinations, and delirium. Anticholinergics also affect the peripheral nervous system, including causing dry mouth, dry eyes, and constipation (Rudolph *et al.*, 2008). If amitriptyline needs to be used in the elderly, it should be administered in low doses to reduce the side effects (Suga *et al.*, 2019).

Diclofenac Sodium, Ibuprofen, and Meloxicam

NSAIDs (Non-Steroid Anti-Inflammatory Drugs) are often prescribed to the elderly. NSAIDs included in the PIM category 1 were 1% diclofenac sodium, 1% ibuprofen, and 2.5% meloxicam. The mechanism of action of the NSAID group is to inhibit the cyclooxygenase-1 and 2 (COX-1 and COX-2) enzymes. The inhibition of the COX enzyme works results in a decrease in the production of prostaglandins (PGE2) and prostacyclin (PGI2), which are inflammatory mediators

P-ISSN: 2406-9388
E-ISSN: 2580-8303

(Lovell & Ernst, 2017). Based on Beer's Criteria 2019, NSAIDs should be avoided for long-term use in the elderly as they can cause perforation. Perforation is a hole or wound in the wall of organs such as the stomach, esophagus, small intestine, and large intestine. This drug may increase the risk of gastrointestinal bleeding or gastric ulcers in high-risk age groups (> 75 years old). Elderly patients are advised to take gastroprotective drugs such as misoprostol to reduce the risk of gastrointestinal bleeding if taking NSAID drugs (American Geriatrics Society, 2019).

Category 2

Drugs in category 2 should be avoided if there is a history of certain diseases or if they can cause drug-disease interactions that can worsen the disease's condition. Some of the drugs used in the elderly at the Sultan Syarif Mohamad Alkadrie Hospital in Pontianak in this category included cilostazol, pioglitazone, and diclofenac sodium

Cilostazol – heart failure

The incidence of PIM category 2 in the use of cilostazol in heart failure patients reached 1.5%. Cilostazol works by inhibiting phosphodiesterase 3, causing an increase in cAMP concentration, resulting in platelet aggregation inhibition prostate (Shinohara, 2010). Beer's Criteria 2019 recommends avoiding using cilostazol in heart failure patients with a reduced ejection fraction. The use of cilostazol can increase the risk of death in the elderly with heart failure (American Geriatrics Society, 2019). An alternative therapy that can be used is pentoxifylline which can improve blood flow by reducing blood viscosity (Utami *et al*., 2016).

Pioglitazone – heart failure

Pioglitazone is a drug in category 2 used in the elderly with heart failure. The use of pioglitazone in elderly with heart failure reached 0.5%. Based on the Beer's Criteria 2019, this drug should be used with "caution" in asymptomatic heart failure patients and should be avoided in symptomatic heart failure patients (American Geriatrics Society, 2019). Pioglitazone works by binding to PPAR-gamma to increase sensitivity to insulin (Tjokroprawiro, 2015). The use of pioglitazone in patients with heart failure can increase fluid retention, which can worsen heart failure (American Geriatrics Society, 2019).

Diclofenac sodium – kidney failure

Prescription of NSAIDs is included in category 2 as these drugs can worsen the condition of kidney failure in elderly patients. NSAIDs can worsen kidney failure due to the inhibition of prostaglandin synthesis, resulting in the renal medulla. The use of NSAIDs can also increase sodium retention. In this study, the prescription of the NSAID group for elderly patients with kidney failure reached 1.5% for diclofenac sodium. Paracetamol is the safer alternative for elderly patients with kidney failure (Pham *et al*., 2009; Supadmi & Hakim, 2012).

Category 3

Category 3 is drugs that should be used with "caution". Prescriptions for the elderly in category 3 are diuretic drugs. A study conducted by Lutfiyati showed that diuretics were widely prescribed in elderly patients (21.18%) (Lutfiyati et al., 2017). Diuretic drugs in PIM incidence in this category reached 10.6% for furosemide, 5.1% for spironolactone, and 1% for hydrochlorothiazide (HCT).

The diuretic drugs work to increase the excretion of sodium, water, and chloride, which then reduce blood volume and extracellular fluid. The diuretic drugs have to be administered with 'caution' as they can cause hyponatremia or SIADH (Syndrome of Inappropriate Antidiuretic Hormone Secretion) (Suryatenggara & Astrawinata, 2018). The use of diuretic drugs in the elderly should be followed by monitoring sodium levels (American Geriatrics Society, 2019).

Category 4

Drugs in category 4 should be avoided because they can cause drug-disease interactions. Dexamethasone, diclofenac sodium, methylprednisolone, ibuprofen, alprazolam, and codeine were among the drugs in this category 4 used in elderly patients at Sultan Syarif Mohamad Alkadrie Hospital in Pontianak.

Corticosteroids - NSAIDs

The incidence of PIM in the corticosteroid-NSAID group reached 1% for dexamethasone-diclofenac sodium and 0.5% for methylprednisolone-ibuprofen. Based on Beer's Criteria 2019, corticosteroids–NSAIDs should be avoided. Concomitant use of corticosteroids–NSAIDs can increase the risk of gastrointestinal adverse events such as peptic ulcer, dyspepsia, gastrointestinal bleeding, and gastritis (American Geriatrics Society, 2019). The potential for gastrointestinal disturbances due to the combined use of NSAIDs with corticosteroids was only caused by the COX-1 enzyme. This effect can occur due to the corticosteroids' mechanism, which inhibits arachidonic acid by phospholipases so that prostaglandins are not formed. Prostaglandins function as gastrointestinal protectors, so if the formation of prostaglandins is inhibited, it can increase the potential for gastrointestinal disorders. Prescription of a COX-2 selective NSAID is recommended if the prescription must be combined with corticosteroids (Moore *et al.*,

P-ISSN: 2406-9388
E-ISSN: 2580-8303

2015). If the use of corticosteroids-NSAIDs cannot be avoided, it is recommended to take drugs that can protect the gastrointestinal tract (American Geriatrics Society, 2019).

Opioids - benzodiazepine

The incidence of PIM in the opioid–benzodiazepine class reached 1.5% for alprazolam–codeine 3. Based on the Beer's Criteria 2019, the combinations of opioid-benzodiazepine drugs should be avoided as they can increase the risk of overdose (American Geriatrics Society, 2019). Research conducted by Darke *et al*. (2003) showed that 62% of patients had overdosed due to consuming more than one class of drugs. Overdose due to opioid-benzodiazepine drugs causes respiratory depression, leading to death (Jones *et al*., 2013).

Category 5

Category 5 is drugs that should be avoided, or the dose should be reduced according to creatinine clearance (CrCl) value. Some drugs in category 5 used in the elderly at the Sultan Syarif Mohamad Alkadrie Hospital, Pontianak were spironolactone, ranitidine, and ciprofloxacin.

Spironolactone

Spironolactone is prescribed for the elderly with creatinine clearance of < 30 mL/min. The prescription of spironolactone in category 5 reached 1.5%. Spironolactone is a potassium-sparing diuretic that acts on the renal tubules. Spironolactone maintains sodium levels in the blood from getting too low, so it can lower blood pressure. Based on Beer's Criteria 2019, the use of spironolactone in the elderly can increase potassium levels or hyperkalemia. A safer alternative is to combine with a thiazide (hydrochlorothiazide) to reduce the loss of potassium ions. If spironolactone should be used, it requires monitoring of the patient's renal function during use (creatinine clearance should not be < 30 mL/min), and dosage adjustments (Fakultas Kedokteran Universitas Sriwijaya, 2004; American Geriatrics Society, 2019).

Ranitidine

The prescription for ranitidine in category 5 reached 0.5% in patients with creatinine clearance of < 50 mL/min. The mechanism of ranitidine is by inhibiting the histamine H2 receptor which results in inhibition of gastric acid secretion (Fakultas Kedokteran Universitas Sriwijaya, 2004). The use of ranitidine with a conventional dose of 150 mg every 12 hours in the elderly can cause reactions in the central nervous system. The central nervous system reactions cover lethargy, confusion, and drowsiness (Pahwa *et al.*, 2016). The use of ranitidine in elderly patients with creatinine clearance of < 50 mL/min is recommended at 150 mg every 24 hours (Food and Drug Administration, 2009).

Ciprofloxacin

Ciprofloxacin, a fluoroquinolone antibiotic in the elderly with creatinine clearance of < 30 mL/min, reached 0.5%. Bird *et al.* (2013) showed that ciprofloxacin was the most widely prescribed fluoroquinolone antibiotic in elderly patients (44.5%). Ciprofloxacin causes the risk of acute kidney failure 2.18 times higher than those who do not use ciprofloxacin (Bird *et al*., 2013). The use of ciprofloxacin can increase the risk of seizures and confusion, so dose adjustments are needed for patients with creatinine clearance of < 30 mL/minute (American Geriatrics Society, 2019). The recommended oral dose of ciprofloxacin in patients with creatinine clearance of < 30 min/mL is 500 mg every 24 hours, and for elderly patients with the creatinine clearance of 30-50 mL/min is 250-500 mg every 12 hours (Lacy *et al*., 2009).

This study has both strengths and weaknesses. The instrument for assessing drugs in the elderly is vital because it employs Beer's Criteria 2019. Beer's Criteria 2015 has been superseded by this assessment tool. Furthermore, PIM drugs in this study were discussed based on each category in the Beer's Criteria 2019, and treatment recommendations were made. However, this study has several limitations, including the use of laboratory data in clinical assessments and drug use in elderly patients and the use of only serum creatinine data. Other laboratory data, such as sodium, potassium, and albumin protein levels that could lead to PIM risk in elderly patients, were not found in the medical record. Because this study only uses prescription medication from outpatient medical records, the doctor's considerations in prescribing medicine cannot be discovered. Furthermore, the researcher conducted the PIM assessment in this study with no involvement from the doctor who wrote the prescription.

In the future, interviews with doctors could be conducted to determine the factors to consider when prescribing drugs to identify PIM incidence in elderly patients. Other laboratory data such as sodium, potassium, and albumin protein levels can also be used as supporting data in identifying PIM incidence in elderly patients, so the research does not only use creatinine serum patient data.

CONCLUSION

This study concludes that the elderly are at risk of receiving potentially inappropriate drug prescriptions.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Optimizing drug prescribing for the elderly is very necessary. Health care providers must integrate clinical and pharmacological conditions based on Beer's criteria before prescribing to elderly patients. A study implementing the application of the Beer's criteria in healthcare service during hospital admission is warranty.

ACKNOWLEDGMENT

The researcher highly appreciates the Pharmacy Study Program, Universitas Tanjungpura, and Sultan Syarif Mohamad Alkadrie Hospital, Pontianak City, for their support.

AUTHOR CONTRIBUTIONS

Conceptualization, N.N.; Methodology, N.N.; Software, N.N., R.A.; Validation, N.N., R.A.; Formal Analysis, N.N., R.A.; Investigation, N.N., R.A.; Resources, N.N., R.A.; Data Curation, N.N., R.A.; Writing - Original Draft, N.N., R.A., R.S.; Writing - Review & Editing, N.N.; Visualization, N.N.; Supervision, N.N.; Project Administration, N.N.; Funding Acquisition, N.N.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abdulah, R. & Barliana, M. I. (2015). Potentially Inappropriate Medication Use for Geriatric Population in Bandung City. *Indonesian Journal of Clinical Pharmacy; 4*; 226–233. doi: 10.15416/ijcp.2015.4.3.226.

American Geriatrics Society. (2019). American Geriatrics Society 2019 Updated AGS Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. *Journal of the American Geriatrics Society*; *67*; 1-21. doi: 10.1111/jgs.15767.

Astuti, S. D., Lubis, N. D. & Kurniasari, F. (2017). Evaluasi Ketidaktepatan Pemilihan Obat Berdasarkan Kriteria STOPP Pada Pasien Geriatri. *Jurnal Farmasi Indonesia*; *14*; 182–190. doi: 10.31001/jfi.v14i2.347.

Badan Pusat Statistik. (2018). Statistik Penduduk Lanjut Usia. Jakarta: Badan Pusat Statistik.

Badan Pusat Statistik. (2020). Statistik Penduduk Lanjut Usia 2020. Jakarta: Badan Pusat Statistik.

Bird, S. T., Etminan, M., Brophy, J. M., Hartzema, A. G. & Delaney, J. A. C. (2013). Risk of Acute Kidney Injury Associated with the Use of Fluoroquinolones. *Canadian Medical Association Journal*; *185*; 475–482. doi: 10.1503/cmaj.121730.

Darke, S. G., Ross, J. & Teesson, M. L. M. (2003). Health Service Utilization And Benzodiazepine Use Among Heroin Users: Findings From the Australian Treatment Outcome Study (ATOS). *Drug Alcohol Depend*; *98*; 1129–1135. doi: 10.1046/j.1360-0443.2003.00430.x.

Fakultas Kedokteran Universitas Sriwijaya. (2004). Kumpulan Kuliah Farmakologi. Jakarta: EGC.

Food and Drug Administration. (2009). Food Safety. Silver Spring: FDA.

Food and Drug Administration. (2011). Drug Safety Communication: Low Magnesium Levels Can be Associated with Long-term Use of Proton Pump Inhibitor drugs (PPIs). Silver Spring: FDA.

Gumantara, M. P. B. & Oktarlina, R. Z. (2017). Perbandingan Monoterapi dan Kombinasi Terapi Sulfonilurea-Metformin terhadap Pasien Diabetes Melitus Tipe 2. *Majority*; *6*; 55–59.

Handajani, A., Roosihermiatie, B. & Maryani, H. (2010). Faktor-Faktor yang Berhubungan dengan Pola Kematian pada Penyakit Degeneratif di Indonesia. *Buletin Penelitian Sistem Kesehatan*; *13*; 42–53.

Holt, S., Schmiedl, S. & Thürmann, P. A. (2010). Potentially Inappropriate Medications in the Elderly: The PRISCUS List. *Deutsches Ärzteblatt International*; *107*; 543–551. doi: 10.3238/arztebl.2010.0543.

Jones, J. D., Mogali, S. & Comer, S. D. (2013). Polydrug Abuse: a Review of Opioid and Benzodiazepine Combination Use. *Drug Alcohol Depend*; *125*; 8–18. doi: 10.1016/j.drugalcdep.2012.07.004.

Kementerian Kesehatan Republik Indonesia. (2013). Gambaran Kesehatan Lanjut Usia di Indonesia. Jakarta: Kementerian Kesehatan Republik Indonesia.

Kementerian Kesehatan Republik Indonesia. (2014). *Situasi dan Analisis Lanjut Usia.* Jakarta: Pusat Data dan Informasi Kementrian Kesehatan Republik Indonesia.

Lacy, C. F., Armstrong, L. L., Goldman, M. P. & Lance L. L. (2009). Drug Information Handbook, 17th Ed. Washington, DC: American Pharmacists Association.

Lovell, A. R., & Ernst, M. E. (2017). Drug-Induced Hypertension: Focus on Mechanisms and Management. *Current Hypertension Reports*; *19*; 1–12. doi: 10.1007/s11906-017-0736-z.

Lutfiyati, H., Yuliastuti, F. & Khotimah, A. (2017). Pola Pengobatan Hipertensi Pada Pasien Lansia Di Puskesmas Windusari Kabupaten Magelang. *Jurnal Farmasi Sains dan Praktis*; *3*; 14–18. doi: 10.31603/pharmacy.v3i2.1726.

Marinda, F. D., Suwandi, J. F. & Karyus, A. (2016). Pharmacologic Management of Diabetes Mellitus Type 2 in Elderly Woman with Uncontrolled Blood Glucose. *Jurnal Medula Unila*; *5*; 26–32.

Moore, N., Pollack, C. & Butkerait, P. (2015). Adverse Drug Reactions And Drug–Drug Interactions with Over-The-Counter NSAIDs. *Therapeutics and Clinical Risk Management*; *11*; 1061–1075. doi: 10.2147/TCRM.S79135.

Mulyani, T., & Rukminingsih, F. (2020). Evaluasi Peresepan pada Pasien Geriatri di Klinik Penyakit Dalam Instalasi Rawat Jalan RSUD K.R.M.T Wongsonegoro Semarang. *Jurnal Riset Kefarmasian Indonesia*; *2*; 89-96. doi: 10.33759/jrki.v2i2.82.

Negara, Y. R., Machlaurin, A., & Rachmawati, E. (2016). Potensi Penggunaan Obat yang Tidak Tepat pada Peresepan Pasien Geriatri Rawat Jalan di RSD dr. Soebandi Jember Berdasarkan Beers Criteria. *e-Jurnal Pustaka Kesehatan*; *4*; 14–19.

Pahwa, R., Sharma, S., Kumar, V. & Kohli, K. (2016). Ranitidine Hydrochloride: An Update On Analytical, Clinical And Pharmacological Aspects. *Journal of Chemical and Pharmaceutical Research*; *8*; 70–78.

Pham, P. C. T., Toscano, E., Pham, P. M. T., Pham, P. A. T., Pham, S. V. & Pham, P. T. T. (2009). Pain Management In Patients With Chronic Kidney Disease. *NDT Plus*; *2*; 111–118. doi: 10.1093/ndtplus/sfp001.

Rahmawati, F., Mustafidah, N. & Annisa, L. (2019). Prevalensi Penggunaan Fall Risk Medicine pada Pasien Lanjut Usia di Instalasi Rawat Jalan Rumah Sakit Madiun. *Journal of Management and Pharmacy Practice; 9*; 135–142. doi: 10.22146/jmpf.45206.

Rudolph, J. L., Salow, M. J., Angelini, M. C. & McGlinchey, R. E. (2008). The Anticholinergic Risk Scale and Anticholinergic Adverse Effects in Older Persons. *Archives of Internal Medicine*; *168*; 508–513. doi: 10.1001/archinternmed.2007.106.

Setyowati, D., Sudarso., & Utaminingrum, W. (2011). Evaluasi Pola Peresepan Berdasarkan *Beers Criteria* Pada Pasien Geriatri Rawat Jalan Pada Poli Penyakit Dalam di RSUD Prof. Dr. Margono Soekarjo Purwokerto Periode Agustus 2010-Maret 2011. *Pharmacy*; *8*; 26–27. doi: 10.30595/pji.v8i03.1127

Shinohara, Y. (2010). Factors Affecting Health-Related Quality of Life Assessed with The SF-36v2 Health Survey In Outpatients with Chronic-Stage Ischemic Stroke In Japan - Cross-Sectional Analysis of the OASIS Study. *Cerebrovascular Diseases*; *29*; 361–371. doi: 10.1159/000281834.

Suga, T., Takenoshita, M., Watanabe, T., Tu, T. T. H., Mikuzuki, L., Hong, C., Miura, K., Yoshikawa, T., Nagamine, T. & Toyofuku, A. (2019). Therapeutic Dose of Amitriptyline for Older Patients with Burning Mouth Syndrome. *Neuropsychiatric Disease and Treatment*; *15*; 3599–3607. doi: 10.2147/NDT.S235669.

Sukmawati, S., Kosman, R. & Damayanti, I. (2016). Identifikasi Penggunaan Obat Pada Pasien Hipertensi Usia Lanjut Dengan *Beer's Criteria* di Instalasi Rawat Inap Rumah Sakit Ibnu Sina Makassar Periode Tahun 2012. *As-Syifaa Jurnal Farmasi*; *8*; 52–58. doi: 10.33096/jifa.v8i1.151.

Supadmi, W. & Hakim, L. (2012). Kaitan Penggunaan Obat Analgetik dan Anti Inflamasi Non Steroid dengan Kejadian Gagal Ginjal Kronik pada Pasien Hemodialisis di RSU PKU Muhammadiyah Yogyakarta. *Jurnal Ilmiah Farmasi*; *9*; 1-8. doi: 10.20885/jif.vol9.iss2.art2.

Suryatenggara, A. N., & Astrawinata, D. A. W. (2018). Sindrom Hormon Antidiuretik Berlebih. *Indonesian Journal of Clinical Pathology and Medical Laboratory*; *18*; 134–140. doi: 10.24293/ijcpml.v18i2.1013.

Tamher, S. N. (2009). Kesehatan Usia Lanjut dengan Pendekatan Asuhan Keperawatan. Jakarta: Salemba Medika.

Tjokroprawiro, A. (2015). Buku Ajar Ilmu Penyakit Dalam. Surabaya: UNAIR Press.

Utami, P., Cahyaningsih, I., & Setiawardani, R. M. (2016). Identification of Drug Related Problems in Congestive Heart Failure Patients in Hospital During January to June 2015. *Jurnal Farmasi Sains dan Praktis*; *4*; 37–43.

Walsh, T. (1997). Kapita Selekta Penyakit dan Terapi. Jakarta: Penerbit Buku Kedokteran EGC.

World Health Organization. (2002). Active Ageing, a Policy Framework. Madrid: World Health Organization.

Yu, E. W., Bauer, S. R., Bain, P. A. & Bauer, D. (2011). Proton Pump Inhibitors and Risk of Fractures: a Meta-Analysis of 11 International Studies.

American Journal of Medicine; *124*; 519–526. doi: 10.1016/j.amjmed.2011.01.007.

Yuliawati, Krisdawati, Sutrisno, D. & Yulion, R. (2020). Potentially Inappropriate Medication Use in Geriatric Patients Based on 2015 Beers Criteria. *SCIENTIA Jurnal Farmasi dan Kesehatan*; *10*; 70–76. doi: 10.36434/scientia.v10i1.217.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Published by Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol. 9 No. 1 April 2022, 92-100
DOI: 10.20473/jfiki.v9i12022.92-100
Available online at https://e-journal.unair.ac.id/JFIKI/

Antidiabetic Activity of Ethanolic Extract of Kale (*Brassica oleracea* var. *sabellica*)

Afdrian Kusumawardianingrum*, Novena Yety Lindawati
Pharmacy Study Program, National College of Health Sciences (STIKES Nasional), Surakarta, Indonesia

*Corresponding author: afdrianafaf@gmail.com

Submitted: 16 August 2021
Accepted: 2 March 2022
Published: 26 April 2022

Abstract

Background*: Diabetes Mellitus is a disease caused by a disruption of the pancreas in producing insulin. The International Diabetes Federation (IDF) organization identified ten countries that have the highest cases of Diabetes Mellitus. Indonesia is ranked 7th out of 10 countries and is the only Southeast Asian country included in the list. Prevention of diabetes mellitus is a solution that must be done to reduce cases of diabetes mellitus in Indonesia., one of which is by consuming vegetables that have antidiabetic activity.* ***Objective****: This study was conducted to determine the activity of kale (Brassica oleracea var. sabellica) extract in reducing glucose levels.* ***Methods****: The qualitative test results showed that the kale extract contained positive flavonoids, triterpenoids, tannins and phenols. The antidiabetic activity test was carried out using the UV-Vis spectrophotometer and the Nelson Somogyi method. This test is carried out at the 25th minute operating time and a maximum λ of 745 nm.* ***Results****: The decrease in glucose levels in kale extract concentration of 4 ppm was 2.23% ± 0.46, 6 ppm of 16.47% ± 0.27.8 ppm of 30.62% ± 0.46, 10 ppm of 41.88 ± 0.27, 12 ppm of 55.50 ± 0.20. The concentration of kale extract in reducing glucose up to 50% (EC_{50}) was 11.13 ppm.* ***Conclusion****: Kale extract (Brassica oleracea var. sabellica) can reduce glucose levels or have antidiabetic activity.*

Keywords*: activity antidiabetic, kale, nelson-somogyi*

How to cite this article:
Kusumawardianingrum, A., & Lindawati, N. Y. (2022). Antidiabetic Activity of Ethanolic Extract of Kale (*Brassica oleracea* var. *sabellica*). *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia,* 9(1), 92-100. http://doi.org/10.20473/jfiki.v9i12022.92-100

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels due to impaired insulin production or use by body cells. Symptoms experienced by patients with diabetes mellitus are frequent eating (polyphagia), frequent drinking (polydipsia) and frequent urination (polyuria) (WHO, 2018). Diabetes mellitus is often associated with inflammation. The inflammatory response to an infection or virus is influenced by blood glucose levels in a person's body. Hyperglycemia in type 2 diabetes will stimulate macrophages to secrete the proinflammatory cytokine TNF-α. When patients with type 2 diabetes have high levels of TNF-α this will lead to more severe insulin resistance resulting in endothelial dysfunction resulting in disease complications (Yuniarti, 2017).

Prevention and control of diabetes mellitus is carried out to keep individuals healthy and people who already suffer or have risk factors for this disease can control their disease so as not to cause complications or death. The International Diabetes Federation Organization (IDF) projects the number of people with diabetes in the population aged 20 - 79 years in several countries in the world in 2019. Research from the IDF is able to show a list of 10 countries with the highest number of people with diabetes mellitus. Indonesia is ranked 7th among ten countries, with10.7 millions of sufferers. Indonesia is the only country from Southeast Asia on the list, so it can be estimated that Indonesia's contribution to the prevalence of diabetes cases in Southeast Asia can be estimated (Kemenkes RI, 2020). With the high number of sufferers in Indonesia, it is necessary to prevent and control the disease preventing and controlling the disease is necessary.

Prevention and control of diabetes mellitus that everyone can do is to eat vegetables that contain polyphenols. Polyphenols, in particular flavonoids can be suggested as a better treatment therapy in diabetes mellitus as well as chronic complications associated with the disorder. Research from Arjadi & Susatyo (2010) states that flavonoid compounds can lower blood sugar levels by stimulating pancreatic ß cells to produce more insulin. One of the vegetables in Indonesia that contains flavonoids is kale (*Brassica oleracea* var. *sabellica*). Kale is a vegetable with curly leaves that are dark green and belongs to the cabbage family. This vegetable kale abroad is nicknamed the Queen of Vegetable (Superfood) because it has many benefits, but some Indonesian people are not familiar with this vegetable. Based on research by Krumbein *et al.* (2010), kale contains flavonoids, namely quercetin, kaempferol and isorhamnetin. Study from Cieslik *et al.* (2008) states that the polyphenol content in kale is higher than brocoli, brussels sprouts, and cauliflower, namely 773 mg/100 g.

Based on the data obtained from kale vegetables and the antidiabetic effects of kale that have not been studied the researchers were interested in testing the antidiabetic activity of kale extract (*Brassica oleracea* var. *sabellica*) using the Nelson-Somogyi method. The principle of the Nelson somogyi method is that Nelson's reagent in a sample that has been added to standard glucose will reduce Cu^{2+} ions to form gluconic acid and cuprous oxide precipitates. The copper oxide precipitate will react with the arsenomolybdate reagent to form a turquoise-blue copper and molybdate complex. The color created is then measured in its absorbance with a UV-Vis spectrophotometer at the maximum wavelength (Advistasari *et al.,* 2019). This study of glucose-lowering activity is expected to provide an initial study of the use of kale (*Brassica oleracea* var. *sabellica*) as an antidiabetic vegetable.

MATERIALS AND METHODS

Materials

The materials used in this study were kale vegetables from Pasar Gedhe Harjonagoro, Jebres District, Surakarta, Nelson reagent (Merck), 96% ethanol, glucose pa (Merck), arsenomolybdate (Merck), concentrated HCl (32% Technical HCl 500 mL), Mg powder Merck (Catalog 1.05815.1000), Technical HCl 2N (Colorless), aquadest, gelatin solution, 10% NaCl, Mayer reagent HgI_4K_2 25 mL, Dragendroff reagent 10 mL, Wagner reagent 25 mL, glacial CH_3COOH 100% Merck, concentrated H_2SO_4 95 - 97% pa (Merck), Technical $FeCl_3$ 5% 100 mL.

Tools

The tools used analytical scales (Ohaus Pioneer with a sensitivity of 0.0001 g and a minimum weighing of 0.1000 g), rotary evaporator (IKA RV 10 Digital V), waterbath WNB14RING 6 holes, volumetric flask 100 mL 50 mL 10 mL (pyrex), beaker glass 50 mL and 100 mL (pyrex), measuring cup (Merck IWAKI), measuring pipette 1 mL (Pyrex), test tube 17 mL Pyrex and test tube rack 12 hole, UV-Visible Spectrophotometer (Shimadzu UV mini-1240), Hellma Analytic cuvette type No. 100.600 QG Light parh lotum.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Methods

Sample determination

Identification of kale (*Brassica oleracea* var. *sabellica*) was carried out at the Research and Development Center for Medicinal and Traditional Medicinal Plants in Tawangmangu with the letter-number YK.01.03/2/545/2021.

Preparation of kale powder

The kale vegetables collected from the Pasar Gedhe Harjonagoro are carried out wet sorting, which aims to separate the dirt from the kale, then continue washing it using running water to facilitate the loss of dirt is still attached to the kale. After that, it was chopped to speed up the drying time. Drying is carried out in an oven at 40ºC until dry so that the moisture content in the material is reduced so that it can prevent the growth of microbes in the sample, then mashed using a blender and sieve no. 40 to get a fine kale powder. The kale powder obtained is stored in a tightly closed container.

Preparation of extract

A total of 200 g of kale powder put into a maceration container, then (1 : 7.5) dissolved in 1500 mL of 96% ethanol. The solution left to stand for 3 days and stirred occasionally. After 3 days, the kale sample was filtered, the first filtrate was obtained. Furthermore, the remaceration was carried out with 500 mL of 96% ethanol solvent (1 : 2.5), left to stand for two days. Then the sample is filtered until a second filtrate is obtained. The results of the first and second filtrate that have been collected are subjected to a concentration process with the help of a rotary evaporator at a temperature of 40ºC, then continued with concentration with an electric water bath at 40°C. After that, the yield was calculated, and phytochemical screening was carried out on kale extract. The extract obtained is a thick extract with a constant weight when weighed.

Antidiabetic activity test

The antidiabetic activity test was carried out using the Nelson Somogyi method and the UV-Vis Spectrophotometry instrument. This test starts from determining the operating time, maximum wavelength, positive glucose control, and decreasing the glucose levels of kale extract.

The operating time was determined by pipetting 1 mL of glucose 100 ppm standard working solution, 1 mL of nelson reagent, and putting it in a test tube closed with a cotton swab. Then, heating the solution for 10 minutes in boiling water and cooling for 5 minutes, then transferred to a 5 mL volumetric flask. The solution was added with 1 mL of arsenomolybdate reagent and diluted with distilled water to mark the limit and shake. Absorbance measured at the theoretical maximum wavelength until optimum time was stable (Aprizayansyah *et al.*, 2016).

The maximum wavelength and positive control of 20 ppm glucose were determined with the same treatment as the operating time. The two tests differ only in wavelength measurements. In the test, the maximum wavelength was suspended during operational time and measured at a 700 - 780 nm wavelength. Meanwhile, the treatment on the positive control test for glucose carried out by measuring the maximum wavelength of 745 nm.

The test for lowering the glucose level of kale extract was carried out by pipetting 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL from the 100 ppm working sample solution. The sample was pipetted into a test tube, added with a standard solution of 100 ppm glucose and 1 mL Nelson's reagent, and then covered with cotton. It was heated for 10 minutes in boiling water,, cooled for 5 minutes, and then transferred to a 5 mL volumetric flask. 1.0 mL of arsenomolybdate reagent was added, diluted with distilled water to the mark, shaken and deferred for an operational time. The measurement results of the sample are read at the maximum wavelength, and the percentage of glucose reduction is calculated.

Data analysis

Calculation of the percent decrease in glucose levels

The reduction in glucose levels is measured by the difference between the positive controls for glucose and residual glucose, then compared with intact glucose to get a percent decrease in glucose levels. The formula for this value is stated as follows:

$$A = \frac{B-C}{B} \times 100\%$$

Note:

A = % decrease in glucose levels

B = absorbance of glucose positive control

C = residual glucose absorbance

EC_{50} calculation

The EC_{50} value (Effective Concentration) is a value that describes 50% of the maximum effect of kale extract in reducing glucose levels. This calculation uses a linear equation from the relationship between the concentration of kale extract and the percent decrease in glucose levels.

$$Y = bx+a$$

Note :

y = percent decrease in glucose levels (50)

P-ISSN: 2406-9388
E-ISSN: 2580-8303

b = slope (slope)
a = intercept
x = concentration of kale extract

RESULTS AND DISCUSSION

Determination

The results showed that the vegetables taken from Pasar Gedhe were kale with the name simplisia *Brassica oleracea* var. *sabellica*. Vegetable kale is shown in Figure 1.

Figure 1. Kale

Kale extraction

Kale is carried out by making powder first before the extraction process is carried out. The kale powder was made using a blender and sieve no. 40 to get a fine kale powder. The use of sieve no. 40 is based on research by Nurhasnawati *et al.* (2017) which states that the mesh size of 40 on tiwai onion bulbs has a higher yield than mesh 20, 60, and 80.

Dry powder of kale is remacerated with 96% ethanol while stirring once every 8 hours. Remaceration is carried out to optimize the process of withdrawing the active compounds contained in kale powder because there is a possibility that in the previous maceration process, the active substance was still left in the residue and stirring aims to maintain the difference in concentration inside and outside the cell so the filter fluid can still filter substances according to its properties polarity (Anggraini & Damayanti, 2019).

The maceration method is a cold extraction method, this method is appropriate to use in research, where this test will take flavonoid compounds that have heat insoluble properties at temperatures > 50ºC. The choice of 96% ethanol solvent was chosen because based on Bintang et al. (2014), it showed that the total flavonoids were more dissolved in 96% ethanol compared to 70% ethanol and water. This solvent also has the ability to extract compounds better because the high solvent concentration influences the amount obtained from the extraction. The extraction results obtained a thick brownish green color extract with 15.3%.

Phytochemical screening

Phytochemical screening is carried out to determine the presence of active compounds in kale extract which are expected to act as antidiabetic. The results of phytochemical screening on kale extract (*Brassica oleracea* var *sabellica*) can be seen in Table 1. The table shows that kale extract contains flavonoids, tannins, triterpenoids and phenols.

Flavonoids

Figure 2 below shows the flavonoid reactions occur because Magnesium and HCl powder will reduces flavonoids that have hydrogen bonds (-OH), resulting in a red or orange color (Agustina *et al*., 2017).

Table 1. The phytochemical test of kale extract

Compound Group	Positive result based on reference	Test Result	Note
Flavonoids	Red or orange	Red	Positive flavonoids
Saponins	A stable foam is formed with a height of 1.5 cm	No foam is formed	Negative saponin
Tannins	Yellowish white precipitate	Yellowish white precipitate	Positive tannins
Alkaloids Mayer	White precipitate	No precipitate is formed	Negative alkaloids
Alkaloids Dragendroff	Orange red precipitate		
Alkaloids Wagner	Chocolate precipitate		
Steroids	Blue or green	Sample color does not change	Negative steroids
Triterpenoids	Red or purple	Purple	Positive triterpenoids
Phenols	Green, purple, blue, and black	Black	Positive phenols

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Tannins

Kale extract also contains tannins because the sample is able to form a yellowish white precipitate. This happened because of the nature of tannins that can tanner proteins (gelatin) so as to form a water-insoluble precipitate. Protein is more difficult to dissolve at high salt concentrations, the addition of sodium chloride, a salt that aims to optimize gelatin deposition (Esteti, 2008).

Triterpenoids

Triterpenoids which are hydrolyzed with concentrated sulfuric acid will produce hydroxyl groups and react with acetic anhydride (Souhoka et al., 2019). In the identification of triterpenoids, the testing of this group of compounds was carried out with the reagent Liebermann-Burchard (acetic anhydrous-H_2SO_4). Figure 3 shows the reaction of triterpenoid with H_2SO_4.

Phenols

Kale extract contains phenols. Positive results are shown in black after adding reagent. This happens because $FeCl_3$ is able to react with the hydroxyl groups present in phenol (Susanti *et al*., 2017). The color change reaction is shown in Figure 4, where the reaction of $FeCl_3$ with phenols produces a black complex.

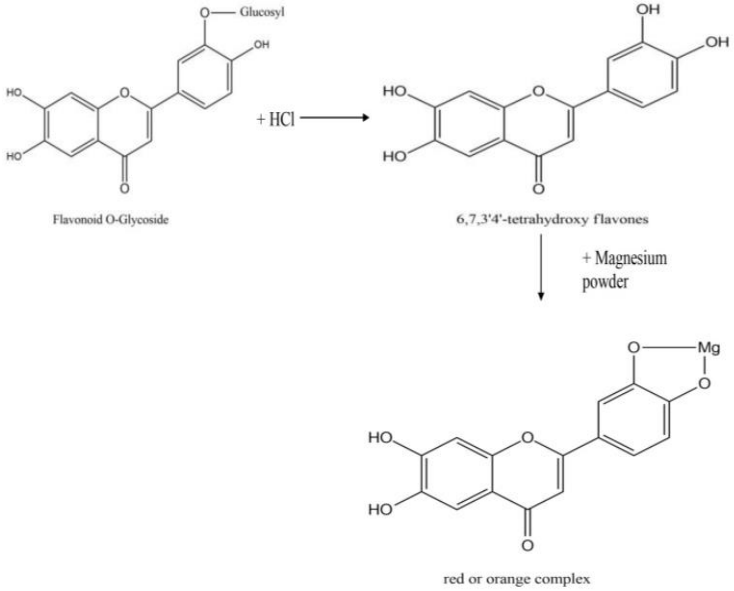


Figure 2. Flavonoid reaction with magnesium powder and concentraced HCl

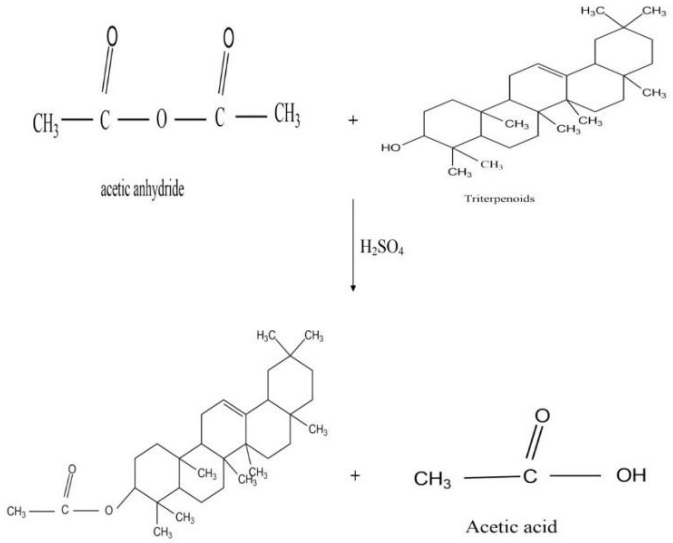


Figure 3. Reaction of triterpenoids with H_2SO_4

P-ISSN: 2406-9388
E-ISSN: 2580-8303

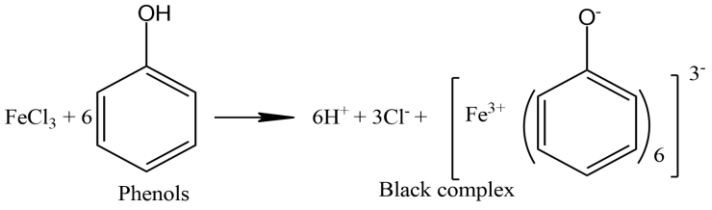


Figure 4. Reaction of phenol with $FeCl_3$

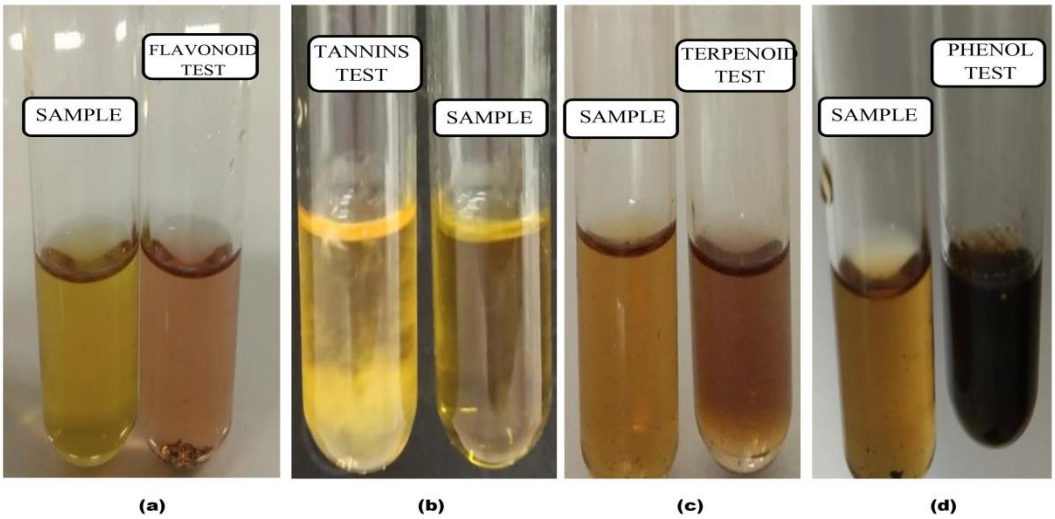


Figure 5. Results of phytochemical screening of flavonoids (a), tannins (b), triterpenoids (c) and phenols (d)

Figure 5 contains two tube names, where the tube labeled as sample contains kale extract without reagents and the second tube is sampled with each reagent. The positive result of favonoid in the test is shown in Figure 5 (a) where the kale sample that has not been added with the reagent is greenish yellow and after testing the tube II sample becomes red. Figure 5 (b) shows the difference in tube II (Sample) which was not tested and tube I (tannins) which after being tested with gelatin and NaCl formed a yellowish white precipitate. The test results in Figure 5 (c) there is a difference in the color, tube II is purple because the sample has been added Lieberman-Burchard reagent, which means kale extract contains triterpenoids. Tube II (phenols) in Figure 5 (d) shows that the kale sample that has been added with $FeCl_3$ can turn black, which means that the kale sample contains phenol.

Antidiabetic activity test

Operating time

Determination of the operating time is done with the aim of knowing the time required for the test solution to achieve a constant absorbance. This solution is in the form of a complex compound formation reaction between a whole glucose solution, Nelson's reagent and arsenomolybdate reagent which forms a greenish blue color. The operational time used in this study is at 25 minutes in accordance with previous research by Hamdani *et al.* (2017).

Maximum wavelength

This determination aims to determine the maximum absorption area that can be produced from the 20 ppm glucose standard solution. According to the theoretical results, the measurement results obtained a maximum wavelength of 745 nm with an absorbance of 0.72, according to the theoretical results, namely 745 nm.

Positive glucose control

Positive control testing for glucose is very important in this test. This determination is used to determine the% value of glucose reduction so that we can find out what concentration of the sample has an effective value in reducing glucose levels (50%). In this determination, positive glucose control obtained an average absorbance of 0.75.

Antidiabetic activity

The antidiabetic activity test was carried out by Nelson Somogyi method. This method was chosen because the Nelson Somogyi method has specific properties for reducing sugars. The principle in this method remaining glucose which is not bound by the active compound in the kale extract will react with Nelson's reagent to form a brick-red precipitate which is then added with arsenomolybdate to form

molybdenum blue which is turquoise. Glucose reaction with Nelson can be seen in Figure 6.

The kale extract which added with glucose and Nelson's reagent subjected to a heating process for 10 minutes to accelerate the reaction of copper ions to form a brick red copper oxide precipitate. The cooling process for 5 minutes in this method is carried out so that the reaction can run stably, because there may be compounds that are not heat-resistant and volatile.

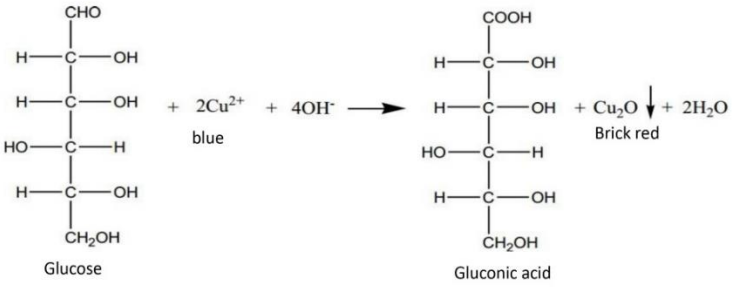
The brick-red precipitate will react with arsenomolybdate to form a turquoise color. This color results from the reduction of cuprooxide to cuprioxide. The smaller the residual glucose level, the smaller the absorbance value, because the color that is formed has a low concentration. A small absorbance value results in a large decrease in glucose levels.

The results of study in Table 2 start from the calculation of the decrease in glucose levels. The difference in positive control of glucose and absorbance of kale samples was calculated for each concentration, then the results of the difference were compared with the positive control of glucose. The final result is a percentage. The higher the concentration of added kale extract, the greater the percent decrease in glucose levels, and vice versa. The low concentration of kale extract contains glucose which is still high, so the absorbance value of the sample read on uv-vis spectrophotometer is almost close to the value of the positive control of glucose.

The EC_{50} value is obtained from the linear equation for the average decrease in glucose levels with the concentration of kale extract so that the value is 11.13 ppm. The value of this test shows that with a concentration of 11.13 ppm kale extract has produced a 50% maximum effect in reducing glucose levels. This ability is thought to be obtained from the flavonoid compounds identified in kale extract, where these compounds can react with glucose to form a glucose-flavonoid complex. The reaction for forming the glucose-flavonoid complex is shown in Figure 7.

The hydroxyl group on the flavonoids in kale extract will bind glucose so that the glucose level in the sample solution will decrease. The higher the kale extract concentration will make the flavonoids in it bind more glucose so that the remaining glucose is less, and the decrease in glucose levels in the kale extract gets bigger.

Flavonoids are compounds that have the ability to lower glucose through inhibition of metabolic enzymes, increase insulin secretion, reduce apoptosis, and reduce insulin resistance (Azzahra *et al*., 2020). Kaempferol and quercetin found in kale also have a working mechanism: protecting beta cells from damage, supporting glycogen synthesis, and preventing alpha glucosidase (Adams *et al*., 2015).



$$(NH_4)_6Mo_7O_{24}.4H_2O + 3H_2SO_4 \longrightarrow 7\,H_2MoO_4 + 3(NH_4)_2SO_4$$

$$12MoO_4^{2-} + AsO_4^{3-} \longrightarrow [AsMo_{12}O_{40}]^{3-} + 12H_2O$$

$$[AsMo_{12}{}^{V}O_{40}]^{3-} + 4Cu_2O \longrightarrow [AsMo4{}^{V}Mo_8{}^{VI}O_{44}]^{7-} + 8Cu^{2-}$$

Figure 6. Glucose reaction with Nelson's reagent (Anggraini & Damayanti, 2019)

Table 2. Results of the percent decrease in glucose levels

Concentration (ppm)	% Decrease in Glucose Levels			Mean ± SD	Regression Equations	EC50 (ppm)
	Test I	Test II	Test III			
4	2.67 %	2.27 %	1.74 %	2.23% ± 0.46		
6	16.56 %	16.69 %	16.15 %	16.47% ± 0.27		
8	31.11 %	30.57 %	30.17 %	30.62% ± 0.46	y = 6.59x	11.13
10	42.19 %	41.79 %	41.66 %	41.88% ± 0.27	- 23.44	
12	55.54 %	55.27 %	55.67 %	55.50% ± 0.20		

P-ISSN: 2406-9388
E-ISSN: 2580-8303

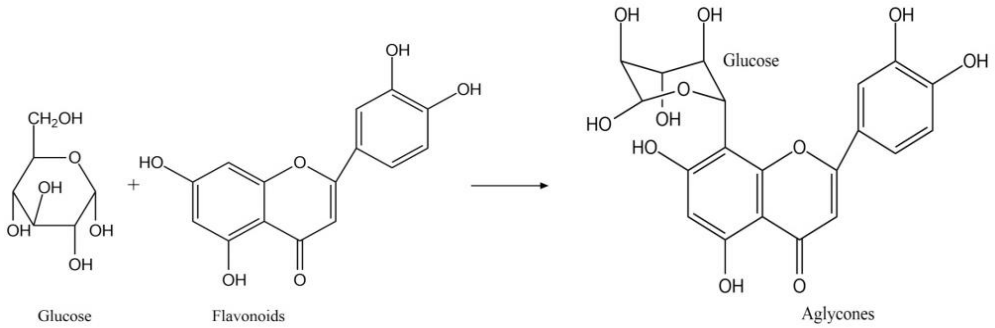


Figure 7. Reactions of the formation of glucose complex compounds with flavonoids

CONCLUSION

Phytochemical results show kale extract (*Brassica oleracea* var. *sabellica*) contains flavonoids, tannins, terpenoids, and phenols. Kale extract can reduce glucose levels or has antidiabetic activity with an EC_{50} of 11.13 ppm.

AUTHOR CONTRIBUTIONS

Conceptualization, A.K., N.Y.L.; Methodology, A.K., N.Y.L.; Software, A.K.; Validation, A.K., N.Y.L.; Formal Analysis, A.K., N.Y.L.; Investigation, A.K.; Resources, A.K.; Data Curation, A.K., N.Y.L.; Writing - Original Draft, A.K.; Writing - Review & Editing, A.K., N.Y.L.; Visualization, A.K.; Supervision, N.Y.L.; Project Administration, A.K., N.Y.L.; Funding Acquisition, A.K., N.Y.L.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adams, A., Chen, J., Kimpe, N. D., Li, W., Mangelinckx, S. & Wang Z. (2015). Natural Flavonoids as Potential Herbal Medication for the Treatment of Diabetes Mellitus and its Complications. *Natural Product Communications*; *10*; 187-200. doi: 10.1177/1934578X1501000140.

Advistasari, Y. D., Vifta, R. & Wilantika. (2019). In Vitro Study of Antioxidant Potential and Antidiabetic Activity of the Ethyl Acetate Fraction of Parijoto Fruit (*Medinilla speciosa* B.). *Indonesian Journal of Medicinal Plants*; *12*; 93-102. doi: 10.22435/jtoi.v12i2.1160.

Agustina, W., Handayani, D. & Nurhamidah. (2017). Screening Phytochemicals and Antioxidant Activities Some Fractions of Distance Skin (*Ricinus communis* L.). *Journal of Education and Chemistry*; *1*; 117-122.

Anggraini, D. I. & Damayanti D. (2019). Antidiabetic Study of Combination of Cabbage (*Brassica oleracea* L.) and Tomato (*Solanum lycopersicum* L.) Extract by In Vitro. *As-Syifaa Journal of Pharmacy*; *11*; 30-37. doi: 10.33096/jifa.v11i1.503.

Aprizayansyah, A., Wiendarlina, I. Y. & Wardatun, S. (2016). Activity of In Vitro Decreasing Glucose Levels of Breadfruit Leaf Extract (*Artocarpus altilis* (Park.) Fosberg) and Its Correlation to Flavonoid Content. *Phytopharmaca*; *6*; 1-11.

Arjadi, F. & Susatyo, P. (2010). Regeneration of Langerhans Island Cells in Diabetic White Rats (*Rattus norvegicus*) Boiled Mahkota Dewa Meat (*Phaleriamacrocarp* (scheff.) Boerl. *Medica Journal of Medicine and Health*; *2*; 1-11.

Azzahra R., C., Nurmala, S., Risnanda H. D. & Yanuar, A. (2020). The Antidiabetic Potential of Orange Peels Flavonoids: A Review Regarding the Mechanism. Jakarta: Faculty of Pharmacy University of Indonesia.

Bintang, M., Falah S. & Syafitri, N.E. (2014). Phytochemical Content, Total Phenol, and Total Flavonoids of Harendong Fruit Extract (*Melastoma affine* D. Don). *Current Biochemistry*; *1*; 105–115.

Cieslik, E., Florkiewicz, A. F., Leszczynska, T., Pisulewski, P. M. & Sikora, E. (2008). The Antioxidant Activity of Selected Cruciferous Vegetables Subjected to Aquathermal Processing. *Food Chemistry*; *107*; 55–59. doi: 10.1016/j.foodchem.2007.07.023.

Esteti, P. (2008). Isolation and Identification of Tannins in Purslane Herbs (Portulaca oleracea L.). *Thesis*; Faculty of Pharmacy University of Sanata Dharma, Yogyakarta.

Hamdani, L. S., Wardatun, S. & Miranti, M. (2017). Sugar Level Reduction Activity and Antioxidant Potential of Dayak Onion (*Eleutherine palmifolia* (L.) Merr) Extract. Bogor: Faculty of

Mathematics and Natural Science. Pakuan University.

Kemenkes RI. (2020). InfoDATIN: Stay Productive, Prevent and Overcome Diabetes Mellitus. Jakarta: Kementerian Kesehatan Republik Indonesia.

Krumbein, A., Kroh, L.W., Rohn, S., Schmidt, S., Schreiner, M. & Zietz, M. (2010). Identification of Complex, Naturally Occurring Flavonoid Glycosides in Kale (*Brassica oleracea* var. Sabellica) By High-Performance Liquid Chromatography Diode-Array Detection/Electrospray Ionization Multi-Stage Mass Spectrometry. *Rapid Communications in Mass Spectrometry*; *24*; 2009–2022. doi: 10.1002/rcm.4605.

Nurhasnawati, H., Putri, M. & Supriningrum, R. (2017). Determination of Flavonoid Levels of Ethanol Extract of Tiwai Onion (*Eleutherine palmifolia* (L.) Merr) Based on Simplicia Powder Size. Samarinda: Academy of Pharmacy Samarinda.

Souhoka, A., Sunarty & Tulus, L.F. (2019). Utilization of Moringa Leaf Extract (*Moringa oleifera*, Lam) as Antioxidant in Coconut Oil. *Molluca Journal of Chemistry Education*; *9*; 18-30.

Susanti, N. M. P., Dewi, L. P. M. K.., Harlina, S. M. & Wirasuta, I. M. A. G. (2017). Identification of Phenol Group Compounds from Ethanol Extract of Green Betel Leaf (*Piper betle* Linn) Using TLC-Spectro Photo Densitometry Method. *Metamorphosis Journal*; *4*; 108-113.

WHO. (2018). World Health Organization Diabetes mellitus. *World Health Organization*. http://www.who.int/mediacentre/factsheets/fs138/en/.

Yuniarti, E. (2017). Differences in Tumor Necrosis Factor - Alpha Levels Between Controlled and Uncontrolled Type 2 Diabetes Mellitus. Padang: Faculty of Mathematics and Natural Sciences State University of Padang.

P-ISSN: 2406-9388
E-ISSN: 2580-8303