BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 12, Number 2, October 2023 | Pages: 593-599 | DOI: 10.14421/biomedich.2023.122.593-599 ISSN 2540-9328 (online)

Quercetin Bioavailability Evaluation on Standardized Herbal Medicine Containing Guava Leaf Extract with HPLC

Rizky Yulion*, Yulianis, Suntri
Department of Pharmacy, STIKes Harapan Ibu Jambi
Jl. Kol. Tarmizi Kodir No.71, Pakuan Baru, Kec. Jambi Sel., Kota Jambi, Jambi 36122, Indonesia.

Corresponding author*
rizkyyulionputra10@gmail.com

Manuscript received: 16 June, 2023. Revision accepted: 09 August, 2023. Published: 07 November, 2023.

Abstract

Standardized herbal medicines are classified as one of Indonesia's natural medicine ingredients in addition to herbal medicine and phytopharmaceuticals. The safety and efficacy of standardized herbal medicines are scientifically proven through preclinical trials, and raw materials and products have been standardized. One includes standardized herbal medicines is diapet, psidii, lelap, fitolac, and glucogarp. To determine the bioavailability of standardized herbal products containing guava leaf extract (*Psidium guajava* L.). which uses single and combined natural materials. The method used is experimental with a crossover design. Blood samples are taken from the marginalis vein of the rabbit ear at 0.5 hours; 1; 2; 4; and 6. The level of quercetin in the blood is determined by the reverse-phase HPLC method. The mobile phase used is methanol:aquabidest (59:41,v/v), stationary phase octadecyl silica (C18), flow rate 1 mL/min, UV-Vis detector 370 nm, and injection volume 20 µL. The value of bioavailability parameters obtained in the parameters Cpmax, Tmax, and AUC of product A is 1.486454 µg / ml; 1.4 hours and 10.2615291 µg/ml/h, product B is 1.29224019 µg/ml; 1.5 hours and 11.30810501 µg/ml/hour. Based on the results of this study, it can be concluded that the bioavailability profile of the two products is not much different, so it is expected that the effects caused are the same.

Keywords: HPLC; Quercetin; Bioavailability; Standardized Herbal Medicine.

INTRODUCTION

Indonesia is a tropical country with abundant natural resources. This abundant biodiversity is a source of natural materials that can be used to support the economy of the Indonesian people. Indonesian people use plants for traditional medicine, handicraft raw materials, industry, and natural dyes (Haryadi & Hidayati, 2018).

Until now, the myth that natural materials are safe has always been promoted by various parties. Some of the community, both practitioners and users of herbal medicines, have the assumption that herbal medicines are safe. This can be true because the use of herbal medicine has been a long time, there are even some plants that have been around for a long time, and there are even some plants that have been used for hundreds of years as traditional medicine (Wijayakusuma, 2002).

Many societies claim that the use of herbal remedies and chemical drugs does not cause side effects, and this statement is often combined with opinions that claim that herbal medicines do not cause harm. Of course, this is not true, which is supported by evidence that no drug is effective and directly free from side effects, let alone its use in conjunction with chemical drugs so that it can cause interactions and affect the bioavailability of the chemical drug (Britza et al., 2022; Fardin & Sarina, 2017a; Hussin, 2001).

For certain medicinal products bioavailability can be demonstrated by the fact that it is obtained in vitro which is carried out in such an environment as in vivo. Drugs' bioavailability mainly depends on the drug being in a dissolved state. The drug dissolution rate of the drug product is measured in vitro. The official dissolving tests are described in the United States Pharmacopeia (USP). The in-vitro dissolution rate data should relate to the data for the drug (Amidon et al., 1995; CDER/FDA, 2015; Fardin & Sarina, 2017b; Santos et al., 2019). One of the interactions that occur in herbal medicine is a pharmacokinetic interaction that affects the absorption, distribution, metabolism, or excretion of drugs. While pharmacodynamic interactions occur in drugs that work similar / or the same as herbal medicines, for example, concomitant administration between herbal drugs that have antiplatelet activity with anticoagulants, concomitant use of ephedrine with herbal medicines rich in caffeine (Amidon et al., 1995; Fardin & Sarina, 2017b).

Guava plants have been used to treat diarrhea, swollen gums, wound medicine, heart, and diabetes. The analgesic effect is thought to be because guava leaves

contain active substances such as essential oils, quercetin, and tannins that inhibit cyclooxygenase and lipooxygenase enzymes (Daud, 2002). Standardized herbal medicines are classified as one of Indonesia's natural medicine ingredients in addition to herbal medicine and phytopharmaceuticals. The safety and efficacy of standardized herbal medicines are scientifically proven through preclinical trials, and raw materials so the products have been standardized (BPOM, 2005; BPOM RI, 2005). One included standardized herbal medicines is diapet, lelap, fitolac, and glucogarp.

Based on this, research was conducted on quercetin bioavailability tests and standardized herbal products containing a single guava leaf extract and this combination aims to determine the bioavailability of quercetin as a compound combined from guava leaf extract in stranded herbal products containing single natural ingredients and combinations.

MATERIALS AND METHODS

Research Design

This research design was carried out experimentally carried out in the laboratory (laboratory experimental research). Where the preparation of ingredients, administration of drugs to animals, blood draws, and blood obtained are centrifuged so that plasma is obtained, then analyte levels are determined using HPLC.

Tools and Materials

The ingredients used in this study include quercetin compounds (Sigma Aldrich), herbal medicines containing guava leaf extract containing quercetin (psidii and diapet), Ethylene Diamine Tetra Acetic Acid (Merck), Tri Chloro Acetic Acid (Loba Chemie), methanol (Merck), aquabidest (OneMed) and Na CMC. The tools used in this study were HPLC (Thermo Scientific Ultimate 3000), sonicator (Power), analytical balance (Shimazu), centrifuge (Hettich), syringe (OneMed), measuring flask (Iwaki), beaker (Iwaki), measuring cup (Pyrex), volume pipette (Iwaki), alcohol swab (OneMed), micropipette (Eppendorf), stirring rod, vial, cotton, drip pipette, pipette pump, tube rack, blood tube, feeding tube, and bite block.

Procedures

- ***Test Animal Preparation***

The test animals used were 3 male rabbits weighing 1.5 kg. Before treatment, test animals are acclimatized for 7 days and satisfied for 12 hours before the experiment (Mutiarahmi et al., 2021).

- ***Ethical Clearance***

Ethical clearance is a formal statement issued by the Research Ethics Commission for research involving living things that states that a research project can be carried out following the seven WHO standards. The Ethics Committee of the Health Service Poltekkes of the Ministry of Health, Jambi conducted the ethical review No.LB.02.06/2/183/2022.

- ***Grouping of Test Animals***

This study using the cross-over design method is an experimental design where each test animal receives more than one treatment at different periods (Yulion et al., 2023).

- ***Suspension Preparation of Na CMC 1%***

Na CMC 1% weighed as much as 1 gr then sealed with 5 ml of hot water while stirring until clear and shaped like jelly then add up to 100 ml (Yulion et al., 2023). Making quercetin suspension and standardized herbal medicines by weighing each ingredient and then sprinkling with 1% Na CMC little by little while stirring and add up to 5 ml (Usman & Fikifandry, 2019)

- ***Animal Preparations***

Satisfy test animals for 12 hours before oral administration. test animals get 3 treatments for the first-week Test animal A is given a dose of herbal medicine I with a dose of 0.24867 g, test animal B is given a dose of herbal drug II 0.351 g test animal C is given with pure quercetin compounds after which test animals are rested for 1 week (Usman & Fikifandry, 2019).

- ***Rabbit Blood Draw***

Blood is taken from rabbit ear veins at minutes 5, 60, 120, 240, and .360 as much as 1 ml and accommodated in a tube containing 2 drops of EDTA then centrifuged for 10 minutes at a speed of 3000 rpm will see a clear top layer (plasma), then separated using a micropipette. add 1 ml of 20% TCA then centrifuge at 3,000 rpm for 10 minutes so that a clear part is obtained (Siswanto et al., 2017).

- ***Preparation of Quercetin Raw Solution***

Weigh 10 mg of quercetin then put it in a 100 ml measuring flask then dilute it with methanol to the limit mark and homogenize it.

- ***Creation of Quercetin Calibration Curves***

Make several series of quercetin concentrations from the parent solution of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, by pickpocketing the parent solution of pm As 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1 ml, and dissolve into a 10 ml measuring flask using methanol solvent until the limit mark.

Table 1. Grouping test animals by cross-over design method.

Rabbit	Week		
	1	2	3
1	A	rest	B
2	B	rest	C
3	C	rest	A

Data Analysis

The method used in this study is an experimental method with data on the absorbance results of quercetin compounds. Plasma levels of quercetin in herbal products obtained regression equation. Research has been conducted on herbal medicines containing combined and single guava leaf extract, increasing bioavailability in local white rabbits can be shown by the increase in the absorption rate constant Tmax and AUC value in each test animal given herbal medicine preparations containing combination and single guava leaf extract.

RESULTS AND DISCUSSION

The results that can be obtained from the research that has been carried out are the measurement results of making quercetin calibration curves in methanol: water (59: 41) with a concentration of 2, 4, 6, 8, 10, wavelength 371nm and obtained the regression equation y = 0.876x - 1.7217 with a regression value of 0.9985. Results of quercetin level measurements carried out on local white rabbits at minutes 5, 60, 120, 240, and 360 minutes. The value of the result of the calculation of bioavailability parameters.

Tabel 2. Standard curve equation data.

Concentration (µg/ml)	AUC (unit)
2	0,102
4	1,801
6	3,348
8	5,314
10	7,1054

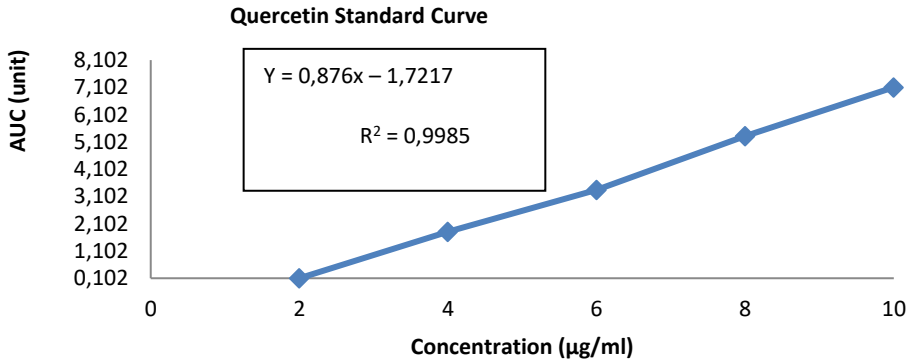

Figure 1. Quercetin Standard Curve.

Table 3. Determination of quercetin levels in rabbit plasma (quercetin).

Time (minutes)	Area (mAU*min)	Quercetin levels (µg/ml)
5	0,00025	1,96569634
60	0,0005	1,9659817345
120	0,00145	1,96706583
240	0,0005	1,9659767345
360	0,00025	1,965696345

Table 4. Determination of quercetin levels in rabbit plasma (Product A).

Time (minutes)	Area (mAU*min)	Quercetin levels (µg/ml)
5	0,0011	1,9666664885
60	0,00205	1,96786529
120	0,00425	1,969520515
240	0,0005	1,965981712
360	0,0001	1,965525114

Table 5. Determination of quercetin levels in inch plasma (Product B).

Time (minutes)	Area (mAU*min)	Quercetin levels (µg/ml)
5	0,0001	1,959018255
60	0,0021	1,967808215
120	0,00025	1,977526139
240	0,0003	1,965753424
360	0,00015	1,96558219

Table 6. The value of the result of the calculation of bioavailability parameters.

Pharmacokinetic parameters	Product A	Product B
Tmax value (hours-1)	1,486454	1,29224019
Value Cpmax(µg/ml)	1,96898659	1,349128791251
Value AUC 0-6 (µg/mL/hour)	10, 2615291	11,30810501

Description: T_{max} : Peak time of the drug in plasma, Cp_{max}: Peak levels of the drug in plasma, AUC: Area Under Curve

Discussion

This research began by preparing samples including 2 products containing guava leaf extract and pure quercetin, products purchased at one of the nearest minimarkets. After making preparations in sampling, then preparing three test animals aimed to facilitate carrying out this activity, as for the test animals are prepared in the form of healthy white rabbits weighing 1.5 kg and aged ± 9 months. Animals are acclimatized for 1 week in the laboratory to adjust to their new environment (Afriani et al., 2020; Fithriyah et al., 2013; Mutiarahmi et al., 2021; Yulion et al., 2023)

After that, making a master solution to measure wavelength, calibration curve, and retention time aims to find out the time interval required by the analyte from the time of injection until it exits the column and the signal is maximally captured by the detector. The results obtained in this treatment are in the form of wavelengths and quercetin calibration curves (Kopjar et al., 2022; Phattanaphakdee et al., 2022; Rosydiati & Saleh, 2019)

After knowing the wavelength and calibration curve of quercetin (F. & A. B. Husnia, 2021; Maesaroh et al., 2018) then conducted a trial of recognition on test animals by taking rabbit blood in the Auricularis Vein (Voigt et al., 2006) This blood draw is usually done in animals that have large blood vessels in the ears, usually in rabbits and pigs (Usman & Fikifandry, 2019). This blood collection uses a 1 cc syringe of as much as 1 ml accommodated in a centrifugation tube (Sebayang et al., 2020a) that has been dripped EDTA (ethylene diamine tetraacetic acid).

EDTA is an anticoagulant (Nur Ramadhani et al., 2019) that is widely used in the form of sodium salts or potassium salts that function to convert calcium ions from the blood into non-ionic forms. Calcium itself is blood clotting so without calcium blood clotting would not occurred (Aziz et al., 2019; Devi et al., 2016; Oikonomidis et al., 2021). After being drip EDTA is centrifugated again for 10 minutes at a speed of 3000rpm, it will be sliced between plasma and blood (Burak et al., 2017a; Svennebring, 2016). Plasma is located at the top and blood is located at the bottom. The difference between plasma and blood is that blood consists of blood components in the form of liquid and solid liquid parts called plasma made of salt, water, and protein. Meanwhile, the solid part of the blood is blood cells known as white blood cells, red blood cells, and platelets (Saleh et al., 2019).

After separation, separate the blood plasma using micropipettes little by little after separating add 1 ml of TCA (Tri Chloro Acetic Acid) (Mukhtiar et al., 2018; Rahim et al., 2016). The addition of TCA aims to separate/precipitate the proteins contained in the filtrate so that pure protein isolates are obtained, and centrifuged again for 10 minutes at a speed of 3000rpm (Sebayang et al., 2020b). After all the preparation processes were carried out, the sample was measured using HPLC with a wavelength of 370nm (Nugraha et al., 2011; Sahu et al., 2013; Shebeko et al., 2018; Valerio et al., 2009). In obtaining a good analysis method, it is also necessary to consider the chromatographic conditions between the columns, mobile phases, elution systems, flow rates, and detectors used. The HPLC system is divided into two, namely normal phase HPLC and reverse phase HPLC (Chitra et al., 2020; Hermes et al., 2021a, 2021b; Nugraha et al., 2011). The difference between the two systems lies in the stationary phase and the mobile phase. For normal phase HPLC, the stationary phase is polar and the mobile phase is nonpolar and in reverse HPLC, the stationary phase is nonpolar and the mobile phase is polar (Angraini & Desmaniar, 2020).

The reverse-phase HPLC system has the advantage of producing good chromatograms on less polar substances such as quercetin. The column that is often used is column C18 (Angraini & Desmaniar, 2020; Chitra et al., 2020). In this quercetin separation, the methanol mobile phase is often used as nonpolar and water as a polar solvent. Methanol is a commonly used mobile phase in systems (F. Husnia & Budiarti, 2021). Another chromatographic condition that needs to be considered is the elution system.

The elution system is divided into two, namely isocratic and gradient (Ehlert et al., 2010; Mitrović et al., 2020; Shrivastava & Gupta, 2012). The isocratic system is an elution system where the strength of the mobile phase is consistent from beginning to end while the gradient system is an elution system in which the strength of the mobile phase changes from the beginning to the end of the process. Gradient elution systems are commonly used for the separation of large amounts of compounds in samples (Ehlert et al., 2010; Shrivastava & Gupta, 2012). In addition to the elution system, the flow rate is also one of the chromatographic conditions that need attention. The flow rate is the speed of flow when passing through the stationary phase. Determination of flow rate is included in the optimization process of the HPLC method that needs to be done before analysis. flow rates that are too fast and too slow can result in imperfect separation. The flow rate reviewed in this study was 1 mL/min (Moldoveanu & David, 2022; Petrásková et al., 2020; Stojanović et al., 2021).

From these results, there is a value of the area obtained and can be calculated quercetin levels in each sample and a single quercetin and quercetin combination log level curve is made in the blood over time from the 2 groups have different curve shapes where the combined quercetin log curve is higher than the peak of the single quercetin log curve. This can be explained because the combined dose of quercetin is greater than a single so it affects the profile of quercetin levels in the blood (Çelebier et al., 2018; Phengvongsone et al., 2022; Rudraraju et al., 2014).

3 kinds of parameters can be used to explain the pharmacokinetic profile of drugs in the body, namely

primary, secondary, and derivative parameters. Primary parameters include KA, VD, and clearance parameters (Hailat et al., 2022; Hasler et al., 1997; Li et al., 2021; Tikhomirov et al., 2021). The KA parameter can explain the absorption kinetics of quercetin. It is known that there is an increase in the value of KA in the combination test animal group when compared to single-product test animals, this is because the increase in quercetin absorption rate causes the quercetin absorption rate to increase, therefore the value of the quercetin absorption rate constant in quercetin combination test animals increases. While test animals are given single quercetin the value of the absorption rate constant is smaller than those of combination herbal products.

By increasing the value of the absorption rate constant of this herbal product, it can influence, the value of Tmax, and Cp max of the elimination constant and its AUC value. Because according to the literature, the ka parameter of a drug greatly affects the Tmax value of the drug, the smaller the KA value, the greater the Tmax value, and vice versa. The decrease in KA shows that the drug is absorbed slowly by the body, this is what causes a decrease in Tmax and Cmax quercetin in B test animals, besides that the increase in Cpmax can also be caused by the addition or homogeneous mixing of an active ingredient (Billah et al., 2014; Larochelle et al., 1982; Metwally et al., 1995; Stanczyk et al., 1983).

The AUC parameter Is a parameter that reflects the total amount of over-the-counter drugs reaching systemic circulation. The AUC parameter value is closely related to the distribution volume parameter, the greater the distribution volume of a drug, the greater the AUC volume of the drug (Kusuma & Rosalina, 2016; Puranik et al., 2020). From the results of research that has been done that the AUC value of single quercetin is greater than the value of a quercetin combination. As has been explained in the outline the research obtained has been following the hypothesis that administering standardized herbal medicines containing guava leaf extract can increase bioavailability in local white rabbits (Burak et al., 2017b).

CONCLUSIONS

Research has been conducted on herbal medicines containing combined and single guava leaf extract, increasing bioavailability in local white rabbits can be shown by the increase in the absorption rate constant Tmax and AUC value in each test animal given herbal medicine preparations containing combination and single guava leaf extract.

Acknowledgements: Thanks are given to all colleagues who have helped in the process of this research.

Authors' Contributions: Rizky Yulion designed the study. Rizky Yulion, Yulianis, and Suntri work together in research work in the laboratory. Yulianis processed the data using statistics, Suntri was prepared the samples used in the research. Rizky Yulion wrote the final script of the article.

Competing Interests: There was no conflict of interest in this study

Funding: There is no cost support from any party for this research. The study was carried out at the own expense of the researchers.

REFERENCES

Afriani, T., Yulion, R., Andriani, M., Syufyani, F., & Fadri, D. (2020). *The Effect of Ginger (Zingiber Officinale Roscoe) Fractionation in Decreasing Uric Acid Level of Hyperuricemic White Mice*. 467–474. https://doi.org/10.5220/0009838404670474

Amidon, G. L., Lennernäs, H., Shah, V. P., & Crison, J. R. (1995). A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. *Pharmaceutical Research: An Official Journal of the American Association of Pharmaceutical Scientists*, *12*(3). https://doi.org/10.1023/A:1016212804288

Angraini, N., & Desmaniar, P. (2020). Optimasi penggunaan High Performance Liquid Chromatography (HPLC) untuk analisis asam askorbat guna menunjang kegiatan Praktikum Bioteknologi Kelautan. *Jurnal Penelitian Sains*, *22*(2), 69. https://doi.org/10.56064/jps.v22i2.583

Aziz, N., Butch, A. W., Ryner, T. C., Martinez-Maza, O., & Detels, R. (2019). The influence of EDTA Vacutainer blood collection tube on the level of blood interleukin-1 receptor antagonist. *Journal of Immunological Methods*, *464*. https://doi.org/10.1016/j.jim.2018.10.009

Billah, M. M., Rana, S. M. M., Hossain, M. S., Saifuddin, A. K. M., Islam, S. K. M. A., Naim, Z., & Barua, S. (2014). Determination of the presence and pharmacokinetic profile of ciprofloxacin by TLC and HPLC method respectively in broiler chicken after single oral administration. *Journal of Antibiotics*, *67*(11). https://doi.org/10.1038/ja.2014.56

BPOM. (2005). Kriteria dan Tata Laksana Pendaftaran Obat Tradisional, Obat Herbal Terstandar dan Fitofarmaka. In *Bpom Ri*.

BPOM RI. (2005). Peraturan Kepala BPOM RI No HK.00.05.41.1384 tentang Kriteria Dan Tata Laksana Pendaftaran Obat Tradisional, Obat Herbal Terstandar dan Fitofarmaka. *Badan Pengawas Obat Dan Makanan*.

Britza, S. M., Byard, R. W., & Musgrave, I. F. (2022). Traditional Chinese medicine-associated nephrotoxicity and the importance of herbal interactions – An overview. In *Pharmacological Research - Modern Chinese Medicine* (Vol. 3). https://doi.org/10.1016/j.prmcm.2022.100099

Burak, C., Brüll, V., Langguth, P., Zimmermann, B. F., Stoffel-Wagner, B., Sausen, U., Stehle, P., Wolffram, S., & Egert, S. (2017a). Higher plasma quercetin levels following oral administration of an onion skin extract compared with pure

quercetin dihydrate in humans. *European Journal of Nutrition*, *56*(1), 343–353. https://doi.org/10.1007/s00394-015-1084-x

Burak, C., Brüll, V., Langguth, P., Zimmermann, B. F., Stoffel-Wagner, B., Sausen, U., Stehle, P., Wolffram, S., & Egert, S. (2017b). Higher plasma quercetin levels following oral administration of an onion skin extract compared with pure quercetin dihydrate in humans. *European Journal of Nutrition*, *56*(1), 343–353. https://doi.org/10.1007/s00394-015-1084-x

CDER/FDA. (2015). Guidance for Industry, Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system. *Center for Drug Evaluation and Research*, *May*.

Çelebier, M., Koçak, E., Doğan, A., Altinöz, S., & Başci, N. E. (2018). Investigating the physicochemical properties of phenazopyridine hydrochloride using high-performance liquid chromatography and uv-visible spectrophotometry. *Marmara Pharmaceutical Journal*, *22*(4). https://doi.org/10.12991/jrp.2018.94

Chitra, J., Mohamed Yacoob, S. A., Senthil Kumar, S., Venkataraman, A., Vijayaraghavan, R., & Nagarajan, Y. (2020). HPLC characterization, acute and sub-acute toxicity evaluation of bark extract of Rhizophora mucronata in Swiss Albino mice. *Heliyon*, *6*(1), e03108. https://doi.org/10.1016/j.heliyon.2019.e03108

Daud, E. al. (2002). Pengaruh Perbedaan Metode Ekstraksi Terhadap Aktivitas Antioksidan Ekstrak Etanol Daun Jambu Biji (Psidium guajava L .). *Prosiding SNaPP2011 Sains, Teknologi, Dan Kesehatan*, *ISSN:2089-3582*.

Devi, E., Krishariyani, D., & Wahyuni, S. (2016). Sitrat Estimasi Perhitungan Jumlah Trombosit Sampel Darah Terhadap Sampel Darah Ethylene Diamine TetraaceticAcid(EDTA). *Analis Kesehatan Sains*, *5*(2).

Ehlert, S., Trojer, L., Vollmer, M., Van De Goor, T., & Tallarek, U. (2010). Performance of HPLC/MS microchips in isocratic and gradient elutionmodes. *Journal of Mass Spectrometry*, *45*(3). https://doi.org/10.1002/jms.1719

Fardin, F., & Sarina, S. (2017a). Pengaruh Pemberian Ekstrak Daun Oregano (Origanum vulgare) terhadap Bioavailabilitas Tablet Diazepam pada Mencit. *Majalah Farmasi Nasional*, *14*(1).

Fardin, & Sarina. (2017b). Pengaruh Pemberian Ekstrak Daun Oregano (Origanum vulgare) Terhadap Bioavailabilitas Tablet Diazepam Pada Mencit. *Majalah Farmasi Nasional*, *14*(1), 52–58.

Fithriyah, N., Arifin, S., & Santi, E. (2013). Lumatan Daun Sirih Merah (Piper crocatum) Terhadap Lama Penyembuhan Luka Bakar Derajat II pada Kulit Kelinci (Cavia cobaya). *Dunia Keperawatan*, *1*(1), 24–31.

Hailat, M., Zakaraya, Z., Al-Ani, I., Al Meanazel, O., Al-Shdefat, R., Anwer, M. K., Saadh, M. J., & Dayyih, W. A. (2022). Pharmacokinetics and Bioequivalence of Two Empagliflozin, with Evaluation in Healthy Jordanian Subjects under Fasting and Fed Conditions. *Pharmaceuticals*, *15*(2). https://doi.org/10.3390/ph15020193

Haryadi, I., & Hidayati, N. (2018). Ekstraksi Zat Warna Dari Daun Jambu Biji Australia (Psidium Guajava L). *Indonesia Journal of Halal*, *1*(2). https://doi.org/10.14710/halal.v1i2.4180

Hasler, F., Bourquin, D., Brenneisen, R., Bär, T., & Vollenweider, F. X. (1997). Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man. *Pharmaceutica Acta Helvetiae*, *72*(3). https://doi.org/10.1016/S0031-6865(97)00014-9

Hermes, L., Römermann, J., Cramer, B., & Esselen, M. (2021a). Quantitative Analysis of β-Asarone Derivatives in Acorus calamus and Herbal Food Products by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, *69*(2), 776–782. https://doi.org/10.1021/acs.jafc.0c05513

Hermes, L., Römermann, J., Cramer, B., & Esselen, M. (2021b). Quantitative Analysis of β-Asarone Derivatives in Acorus calamus and Herbal Food Products by HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*, *69*(2), 776–782. https://doi.org/10.1021/acs.jafc.0c05513

Husnia, F. & A. B. (2021). Pengembangan metode analisis kuersetin dalam ekstrak etanol buah leunca (. *Media Farmasi*, *17*(2).

Husnia, F., & Budiarti, A. (2021). Pengembangan metode analisis kuersetin dalam ekstrak etanol buah leunca (Solanum nigrum L.) Menggunakan Kromatografi Cair Kinerja Tinggi. *Media Farmasi*, *17*(2), 108–115.

Hussin, A. H. (2001). Adverse Effects Of Herbs And Drug-Herbal Interactions. *Malaysian Journal of Pharmacy*, *1*(2).

Kopjar, M., Buljeta, I., Ćorković, I., Pichler, A., & Šimunović, J. (2022). Adsorption of Quercetin on Brown Rice and Almond Protein Matrices: Effect of Quercetin Concentration. *Foods*, *11*(6). https://doi.org/10.3390/foods11060793

Kusuma, A. S. W., & Rosalina, G. (2016). Analisis Kadar Kapsaisin dari Ekstrak "Bon Cabe" dengan Menggunakan Kromatografi Cair Kinerja Tinggi (KCKT). *Farmaka*, *14*(2).

Larochelle, P., Du Souich, P., Hamet, P., Larocque, P., & Armstrong, J. (1982). Prazosin plasma concentration and blood pressure reduction. *Hypertension*, *4*(1). https://doi.org/10.1161/01.HYP.4.1.93

Li, W., Wang, Y., Pei, Y., & Xia, Y. (2021). Pharmacokinetics and bioequivalence evaluation of two montelukast sodium chewable tablets in healthy chinese volunteers under fasted and fed conditions. *Drug Design, Development and Therapy*, *15*. https://doi.org/10.2147/DDDT.S298355

Maesaroh, K., Kurnia, D., & Al Anshori, J. (2018). Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC Terhadap Asam Askorbat, Asam Galat dan Kuersetin. *Chimica et Natura Acta*, *6*(2). https://doi.org/10.24198/cna.v6.n2.19049

Metwally, A., Bennett, J., Botros, S., Ebeid, F., & El Attar, G. E. D. M. (1995). Impact of drug dosage and brand on bioavailability and efficacy of praziquantel. *Pharmacological Research*, *31*(1). https://doi.org/10.1016/1043-6618(95)80048-4

Mitrović, J., Nikolić, N., Karabegović, I., Danilović, B., Lazić, M., & Nikolić, L. (2020). Nettle (Urtica dioica L.) seeds as a source of free and bound phenolics: The antioxidant, antimicrobial activity and the composition. *Advanced Technologies*, *9*(1). https://doi.org/10.5937/savteh2001013m

Moldoveanu, S., & David, V. (2022). Essentials in Modern HPLC Separations. In *Essentials in Modern HPLC Separations*. https://doi.org/10.1016/C2020-0-04542-1

Mukhtiar, M., Jan, S. U., IhsanUllah, Gul, R., Hussain, A., Ali, E., IzharUllah, Jabbar, A., Akram, M., Mansoor, S., & Khan, M. F. (2018). Interaction of palladium inorganic salt and organic complex with glutathione content of liver homogenate. *Pakistan Journal of Pharmaceutical Sciences*, *31*(2).

Mutiarahmi, C. N., Hartady, T., & Lesmana, R. (2021). Use of Mice As Experimental Animals in Laboratories That Refer To the Principles of Animal Welfare: a Literature Review.

Indonesia Medicus Veterinus, *10*(1), 134–145. https://doi.org/10.19087/imv.2020.10.1.134

Nugraha, A., Ghozali, M., Farmasi FKIK, D., & Muhammadiyah Yogyakarta, U. (2011). Penetapan Kadar Flavonoid Kuersetin Ekstrak Kulit Buah Apel Hijau (Pyrus Malus L.) Dengan Menggunakan Metode Kromatografi Cair Kinerja Tinggi Mahasiswa Farmasi Fkik, Universitas Muhammadiyah Yogyakarta 2). *Jurnal Penelitian*, *18*.

Nur Ramadhani, Q. A., Garini, A., Nurhayati, N., & Harianja, S. H. (2019). Perbedaan Kadar Glukosa Darah Sewaktu Menggunakan Serum Dan Plasma Edta. *JPP (Jurnal Kesehatan Poltekkes Palembang)*, *14*(2), 80–84. https://doi.org/10.36086/jpp.v14i2.407

Oikonomidis, I. L., Milne, E., & Piccinelli, C. (2021). Differential white blood cell counts in rabbits: a comparison of the Advia 2120 and a manual method. *Journal of Veterinary Diagnostic Investigation*, *33*(4). https://doi.org/10.1177/10406387211007877

Petrásková, L., Káňová, K., Biedermann, D., Kren, V., & Valentová, K. (2020). Simple and rapid HPLC separation and quantification of flavonoid, flavonolignans, and 2,3-dehydroflavonolignans in silymarin. *Foods*, *9*(2). https://doi.org/10.3390/foods9020116

Phattanaphakdee, W., Ditipaeng, C., Uttayarat, P., Thongnopkoon, T., Athikomkulchai, S., & Chittasupho, C. (2022). Development and Validation of HPLC Method for Determination of Quercetin in Hydrogel Transdermal Patches Loaded with Red Onion Peel Extract. *Tropical Journal of Natural Product Research*, *6*(8). https://doi.org/10.26538/tjnpr/v6i8.8

Phengvongsone, X., Thamrongyoswittayakul, C., Sukon, P., Aimsaard, J., & Mektrirat, R. (2022). Antibacterial effect of ethanolic Morus alba Linn. leaf extract against mastitis-causing Escherichia coli and Staphylococcus aureus in vitro. *Veterinary Integrative Sciences*, *20*(3). https://doi.org/10.12982/VIS.2022.039

Puranik, M., Shambharkar, S., Nimbalkar, S., & Mahapatra, D. K. (2020). Comparison of UV-spectrophotometric and RP-HPLC methods for estimation of deflazacort in solid dosage form. *Journal of Applied Pharmaceutical Science*, *10*(7). https://doi.org/10.7324/JAPS.2020.10711

Rahim, N., Naqvi, S. B. S., Alam, M., Rasheed, A., & Khalique, U. A. (2016). Comparative bioavailability and pharmacokinetic study of Cefadroxil capsules in male healthy volunteers of Pakistan. *Pakistan Journal of Pharmaceutical Sciences*, *29*(2).

Rosydiati, & Saleh, E. K. (2019). Karakterisasi puncak kromatogram dalam High Performance Liquid Chromatography (HPLC) terhadap perbedaan fase gerak, laju alir, dan penambahan asam dalam analisis Indole Acetic Acid (IAA). *Kandaga*, *1*(2), 65–73.

Rudraraju, A. V., Amoyaw, P. N. A., Hubin, T. J., & Khan, M. O. F. (2014). Determination of log P values of new cyclen based antimalarial drug leads using RP-HPLC. *Pharmazie*, *69*(9). https://doi.org/10.1691/ph.2014.4019

Sahu, S., Saraf, S., Kaur, C. D., & Saraf, S. (2013). Biocompatible nanoparticles for sustained topical delivery of anticancer phytoconstituent Quercetin. *Pakistan Journal of Biological Sciences*, *16*(13), 601–609. https://doi.org/10.3923/pjbs.2013.601.609

Saleh, R., Dwiyana, A., & Parno. (2019). Pengaruh Variasi Waktu Centrifugasi Terhadap Hasil Pemeriksaan Hematokrit Metode Makro Pada Mahasiswa Program Studi D-Iii Analis Kesehatan. *Jurnal Media Laboran*, *9*(2), 39–43.

Santos, A. C., Pereira, I., Pereira-Silva, M., Ferreira, L., Caldas, M., Collado-González, M., Magalhães, M., Figueiras, A., Ribeiro, A. J., & Veiga, F. (2019). Nanotechnology-based formulations for resveratrol delivery: Effects on resveratrol in vivo bioavailability and bioactivity. In *Colloids and Surfaces B: Biointerfaces* (Vol. 180). https://doi.org/10.1016/j.colsurfb.2019.04.030

Sebayang, R., Idawati, Y., & Sinaga, H. (2020a). Analisis Lactat Dehydrogenase dalam Serum Darah Menggunakan Sentrifugasi. *Jurnal Keperawatan Silampari*, *4*(1), 274–280. https://doi.org/10.31539/jks.v4i1.1450

Shebeko, S. K., Zupanets, I. A., Popov, O. S., Tarasenko, O. O., & Shalamay, A. S. (2018). Effects of quercetin and its combinations on health. In *Polyphenols: Mechanisms of Action in Human Health and Disease* (2nd ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813006-3.00027-1

Shrivastava, A., & Gupta, V. B. (2012). Review Article HPLC : Isocratic or Gradient Elution and Assessment of Linearity In Analytical Methods. *Journal of Advanced Scientific Research*, *3*(2).

Stanczyk, F. Z., Mroszczak, E. J., Ling, T., Runkel, R., Henzl, M., Miyakawa, I., & Goebelsmann, U. (1983). Plasma levels and pharmacokinetics of norethindrone and ethinylestradiol administered in solution and as tablets to women. *Contraception*, *28*(3). https://doi.org/10.1016/0010-7824(83)90065-3

Stojanović, J., Krmar, J., Protić, A., Svrkota, B., Đajić, N., & Otašević, B. (2021). Experimental design in HPLC separation of pharmaceuticals. *Arhiv Za Farmaciju*, *71*(4). https://doi.org/10.5937/arhfarm71-32480

Svennebring, A. (2016). The connection between Plasma Protein Binding and Acute Toxicity as Determined by the LD50 Value. *Drug Development Research*, *77*(1), 3–11. https://doi.org/10.1002/ddr.21286

Tikhomirov, M., Poźniak, B., & Śniegocki, T. (2021). High-performance liquid chromatography-tandem mass spectrometry for buprenorphine evaluation in plasma—application to pharmacokinetic studies in rabbits. *Molecules*, *26*(2). https://doi.org/10.3390/molecules26020437

Usman, S., & Fikifandry. (2019). Uji Efek Hepatotoksik Ekstrak Etanol Buah Takokak (Solanum torvum Swartz) Dengan Parameter Bilirubin Darah Pada Kelinci (Oryctolagus cuniculus). *Majalah Farmasi Nasional*, *16*(01), 21–28.

Valerio, D. A., Georgetti, S. R., Magro, D. A., Casagrande, R., Cunha, T. M., Vicentini, F. T. M. C., Vieira, S. M., Fonseca, M. J. V, Ferreira, S. H., & Cunha, F. Q. (2009). *Quercetin Reduces Inflammatory Pain: Inhibition of Oxidative Stress and Cytokine Production*. *1*, 1975–1979.

Voigt, C. C., Peschel, U., Wibbelt, G., & Frölich, K. (2006). An alternative, less invasive blood sample collection technique for serologic studies utilizing Triatomine bugs (Heteroptera; Insecta). *Journal of Wildlife Diseases*, *42*(2). https://doi.org/10.7589/0090-3558-42.2.466

Wijayakusuma, M. H. (2002). *Tumbuhan berkhasiat obat Indonesia : rempah, rimpang dan umbi*. Jakarta : Milenia Populer.

Yulion, R., Perawati, S., Hartesi, B., Anggresani, L., Andriani, L., & Indriani, L. (2023). Acute Toxicity LD 50 Fraction Ethyl Acetate Aquilaria Malaccensis , Ficus Benjamina , Mikania Micrantha , and Fraction Water Cinnamomum Burmanii in Mus Musculus. *Biology, Medicine, & Natural Product Chemistry*, *12*(1), 55–60. https://doi.org/10.14421/biomedich.2023.121.55-60

THIS PAGE INTENTIONALLY LEFT BLANK