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

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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, psidi, lelap, fitol 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 marginals 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:aquebdest (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 Cmax, 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 lipoxygenase 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.
### 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 $T_{max}$ 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.

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

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rest</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>rest</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>rest</td>
<td>A</td>
</tr>
</tbody>
</table>

### Table 2. Standard curve equation data.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>AUC (unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.102</td>
</tr>
<tr>
<td>4</td>
<td>1.801</td>
</tr>
<tr>
<td>6</td>
<td>3.348</td>
</tr>
<tr>
<td>8</td>
<td>5.314</td>
</tr>
<tr>
<td>10</td>
<td>7.1054</td>
</tr>
</tbody>
</table>

![Quercetin Standard Curve](image)

**Y = 0.876x – 1.7217**

**$R^2 = 0.9985$**

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

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Area (mAU*min)</th>
<th>Quercetin levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.00025</td>
<td>1.96569634</td>
</tr>
<tr>
<td>60</td>
<td>0.00005</td>
<td>1.9659817345</td>
</tr>
<tr>
<td>120</td>
<td>0.00145</td>
<td>1.96706583</td>
</tr>
<tr>
<td>240</td>
<td>0.00005</td>
<td>1.9659767345</td>
</tr>
<tr>
<td>360</td>
<td>0.00025</td>
<td>1.965696345</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Area (mAU*min)</th>
<th>Quercetin levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.0011</td>
<td>1.9666664885</td>
</tr>
<tr>
<td>60</td>
<td>0.00025</td>
<td>1.96786529</td>
</tr>
<tr>
<td>120</td>
<td>0.00425</td>
<td>1.96952051</td>
</tr>
<tr>
<td>240</td>
<td>0.00005</td>
<td>1.965981712</td>
</tr>
<tr>
<td>360</td>
<td>0.0001</td>
<td>1.965525114</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Area (mAU*min)</th>
<th>Quercetin levels (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.0001</td>
<td>1.959018255</td>
</tr>
<tr>
<td>60</td>
<td>0.0021</td>
<td>1.967808215</td>
</tr>
<tr>
<td>120</td>
<td>0.00025</td>
<td>1.977526139</td>
</tr>
<tr>
<td>240</td>
<td>0.00005</td>
<td>1.965753424</td>
</tr>
<tr>
<td>360</td>
<td>0.00015</td>
<td>1.96558219</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Product A</th>
<th>Product B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{max}$ (hours-1)</td>
<td>1,486454</td>
<td>1,29224019</td>
</tr>
<tr>
<td>Value $C_{max}$(µg/ml)</td>
<td>1,96898659</td>
<td>1,34912891251</td>
</tr>
<tr>
<td>Value AUC 0-6 (µg/mL/hour)</td>
<td>10, 2615291</td>
<td>11,30810501</td>
</tr>
</tbody>
</table>

Description: $T_{max}$: Peak time of the drug in plasma, $C_{max}$: 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 (ethylenediamine 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 (Ehlerl 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 (Ehlerl 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 T\text{max}, and C\text{p max} of the elimination constant and its AUC value. Because according to the literature, the ka parameter of a drug greatly affects the T\text{max} value of the drug, the smaller the KA value, the greater the T\text{max} 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 T\text{max} and C\text{p max} quercetin in B test animals, besides that the increase in C\text{p max} 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 T\text{max} and AUC value in each test animal given herbal medicine preparations containing combination and single guava leaf extract.

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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

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