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Article

Identification of Paracetamol Compound in Traditional Herbal Medicine as Muscle Reliever using Thin Layer Chromatography–Densitometry

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Abstract

Traditional medicine consists of substances or formulations derived from plants, animals, minerals, and galenic preparations or mixtures of these materials that have been used for generations as therapy and can be implemented according to the norms applicable in society. The pharmaceutical chemical that is often added to herbal medicine for muscle pain relief is paracetamol. This research aims to prove the presence or absence of the chemical drug paracetamol in the herbal medicine for muscle pain relief found in the Koja District. A total of 10 samples were selected through purposive sampling from herbal stores within the area. The analysis was conducted qualitatively using color reaction test and quantitatively using Thin Layer Chromatography-Densitometry. Color reaction tests indicated that two samples were positive for paracetamol. Analysis using thin-layer chromatography indicated that 3 samples had R_f values ranging from 0.3-0.35, close to the standard R_f value. Based on the analysis results using Thin Layer Chromatography-Densitometry, the herbal samples that tested positive for containing paracetamol were identified according to the maximum wavelength of the samples that matched the maximum wavelength of paracetamol at 247 nm in samples (S5), (S7), and (S9). The TLC-densitometry method can be used to analyze the presence of paracetamol in traditional herbal medicine for muscle pain relief.

Keywords: Traditional Herbal Medicine for Muscle Pain Relief; Medicinal Chemicals; Paracetamol; Thin Layer Chromatography-Densitometry

1. INTRODUCTION

Paracetamol is an analgesic and antipyretic medication that can be used to relieve pain due to arthritis [1]. Its mechanism of action is by inhibiting the synthesis of prostaglandins, primarily in the central nervous system (CNS). The use of paracetamol, if consumed regularly over a long period, can lead to severe kidney damage, liver disease, or hepatitis, which may lead to impaired liver and kidney function [2].

In Indonesia, herbal medicines are regulated by the Regulation of the Minister of Health of the Republic of Indonesia Number 006 of 2012 concerning the Industry and Business of Traditional Medicines, which states that traditional medicines are substances or preparations made from plant materials, animal materials, mineral materials, galenic preparations, or a mixture of these materials that have been used for treatment through generations and can be applied according to the norms prevailing in society [3].

Medicinal chemicals are chemical substances or drug compounds that are deliberately added to herbal medicines to provide effects or medicinal benefits more quickly than conventional drugs in general. However, this is very dangerous because the dose given is not appropriate and can affect patient safety. The proper way to determine the presence of pharmaceutical chemical compounds in traditional medicine is by observing consumer complaints. If the health status of the consumer improves and healing effects occur rapidly, it is likely that the traditional medicine used by the consumer contains pharmaceutical chemicals in relatively large doses [4]. Traditional medicine is prohibited from containing chemical substances that are the result of isolation or synthetic drugs that have medicinal properties according to the Minister of Health Regulation Number 007 of 2012 [5].

Previous research has proven the existence of samples that are positive for paracetamol adulteration in various herbal preparations [6-8]. This research was conducted by combining qualitative analysis (color reaction) and quantitative analysis (TLC-Densitometry).

2. MATERIALS AND METHODS

2.1. Material

The materials used are samples from the herbal shops in Koja district with purposive method, standard raw material of paracetamol (BPOM), ethanol (Merck), chloroform (Merck).

2.2. Instrument

Instruments used include a densitometer (Camag), analytical balance (Ohaus), chromatography chamber, GF254 silica gel (Merck), capillary tubes, pencil, ruler, filter paper, glass stirrers, measuring cylinders, glass beakers, volumetric flasks, Erlenmeyer flasks, and a UV lamp at 254 nm (Camag).

2.3. Method

2.3.1. Organoleptic Test

The organoleptic examination is carried out by directly observing using the five senses to determine the form of preparation, color, smell, and taste of the herbal medicine for muscle pain sample [9].

2.3.2. Sample Preparation

Each 500 mg sample is weighed and dissolved in methanol to a volume of 5 mL, macerated for 3 × 24 hours [10].

2.3.3. Preparation of Paracetamol Standard Solution

Ten mg of paracetamol is weighed, placed into a 5 mL volumetric flask, and then dissolved in 96% ethanol to the mark, after which it is shaken until homogeneous and transferred into a 5 mL vial [11].

2.3.4. Preparation of the Mobile Phase

The mobile phase used in this research is a mixture of chloroform-ethanol (9:1) [12].

2.3.5. Qualitative Test

Two drops of herbal extract were placed on a spot plate, followed by two drops of $FeCl_3$ solution. A positive result was indicated by a blue-violet-green color change [13].

2.3.6. Identification of Paracetamol Using Thin Layer Chromatography-Densitometry

Samples were spotted on an activated TLC plate using a capillary tube, with a distance of 1 cm between spots, then the TLC plate is eluted when the chamber is saturated. After elution is complete, the TLC plate is lifted and spots are observed under UV light at a wavelength of 254 nm. The TLC plate is placed into the densitometer for analysis, then reading is conducted on the computer. After obtaining the results, calculate the R_f value of the sample and then compare it with the standard R_f value of paracetamol [13].

2.3.7. Data Analysis

Data analysis was conducted with a theoretical approach. The analysis used Thin Layer Chromatography-Densitometry on the paracetamol reference standard and the muscle pain reliever herbal sample. Sample analysis was conducted by observing the visualization technique of the sample and reference standard spots, comparing the Rf values between the sample and the reference standard. In addition, identification can also be seen in the similarity of the densitometry spectra between the sample and the reference standard.

3. RESULT AND DISCUSSION

3.1. Organoleptic Test

Organoleptic tests were conducted using the five senses [9] including observing color, taste, shape, and smell of the muscle pain reliever herbal medicine as shown in Table 1.

Table 1. Result of Organoleptic Test

Sample	Color	Taste	Shape	Odor
S1	Yellow	Bitter	Powder	Characteristic herbal scent
S2	White	Bitter	Powder	Characteristic herbal scent
S3	Brown	Bitter	Powder	Characteristic herbal scent
S4	Yellow	Bitter	Powder	Characteristic herbal scent
S5	Yellow	Bitter	Powder	Characteristic herbal scent
S6	White	Bitter	Powder	Characteristic herbal scent
S7	White	Bitter	Powder	Characteristic herbal scent
S8	White	Bitter	Powder	Characteristic herbal scent
S9	White	Bitter	Powder	Characteristic herbal scent
S10	White	Bitter	Powder	Characteristic herbal scent

3.2. Qualitative Test

The $FeCl_3$ reaction test was conducted to observe color changes. A positive result was indicated by a greenish-blue color.

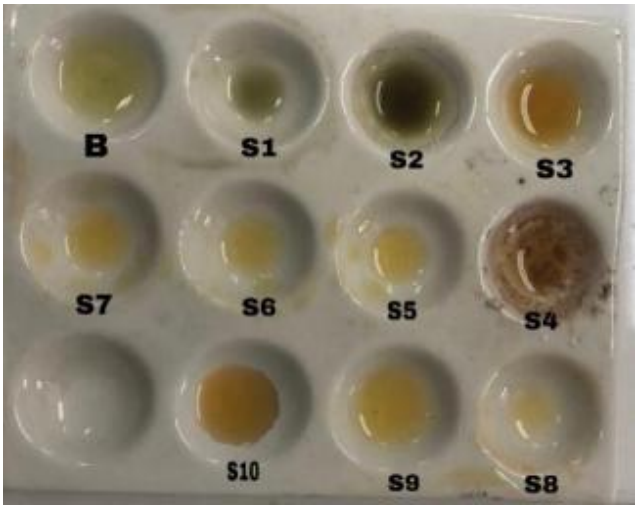

Figure 1. Color Reaction Test Results

The results of the $FeCl_3$ reaction test based on the table above. From 10 herbal samples suspected to contain paracetamol, showed that two samples, which are sample codes S1 and S2, changed color to green due to the formation of a reaction. When $FeCl_3$ is added to paracetamol, $FeCl_3$ will break the OH^- bond and be replaced with Fe that binds to 3 paracetamol molecules to form a complex compound, resulting in a blue-violet-green color (Figure 1 and Table 2).

Table 2. Color Reaction Test Results

Sample	Color Reaction Test Results
Standard Paracetamol	Green
S1	Green (+)
S2	Green (+)
S3	Orange (-)
S4	Brown precipitate (-)
S5	Yellow (-)
S6	Yellow (-)
S7	Yellow (-)
S8	Yellow (-)
S9	Yellow (-)
S10	Orange (-)

3.3. Results of Identification on Thin Layer Chromatography

In this test, there are 8 samples analyzed using Thin Layer Chromatography-Densitometry. Ethyl acetate:methanol (9:1) was used as the mobile phase, and silica gel was spotted with the sample as the stationary phase with a size of 10 x 10 cm, with a spotting distance of 1 cm [11]. The standard R_f value of paracetamol is compared with the R_f value from the muscle pain reliever herbal sample, stating that paracetamol is positively present if the herbal sample has the same or a similar R_f value to the standard R_f value of paracetamol.

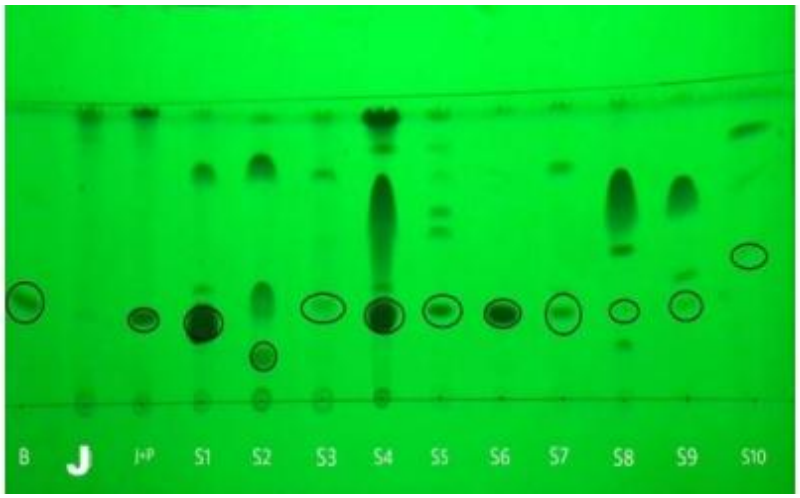

Figure 2. Results of TLC

3.4. R_f Value Results

The R_f value is calculated by dividing the distance traveled by the spot from the starting point of the compound's application to where the compound stops by the distance of elution. In this study, an R_f value of 0.35 was obtained using manual calculations, where there were 3 samples that were identical with this R_f value. For the calculation of the R_f value on TLC-Densitometry, an R_f value of 0.36 was obtained with 3 samples that were identical with this R_f value [15].

3.4.1. Results of Manual R_f Value Calculation

Table 3. Results of Manual R_f Value Calculation

Sample	Replication	Rf	Average Rf
B (paracetamol)	1	0.36	0.35
	2	0.34	
J (curcumin)	1	0.31	0.31
	2	0.31	
J + B	1	0.30	0.30
	2	0.30	
Sample 1 (S1)	1	0.28	0.28
	2	0.29	
S2	1	-	-
	2	0.25	-

S3	1	0.35	0.30
	2	0.25	
S4	1	0.31	0.26
	2	0.22	
S5	1	0 32	0.28
	2	0.25	
S6	1	0.31	0.27
	2	0.24	
S7	1	0.31	0.28
	2	0.26	
S8	1	0.32	0.30
	2	0.29	
S9	1	0.34	0.32
	2	0.30	

3.4.2. Results of R_f Value TLC-Densitometry

The obtained R_f value is compared with the standard comparison R_f value obtained, which indicates that the R_f value in the sample and the standard comparison R_f is the same or close. A difference between the sample R_f and the comparison R_f of ≤ 0.05 indicates that the sample is positive for containing chemical drugs, but if the difference between the sample R_f and the comparison R_f is > 0.05, then the sample is negative for containing chemical drugs [14,16]. From the R_f values, it can be concluded that 3 samples are suspected of containing paracetamol because their Rf values are close to the standard R_f value of 0.35.

Table 4. Results of R_f Value Calculation for TLC-Densitometry

Sample	Replication	Rf	Average Rf	λ max (nm)	Result
B	1	0.36	0.35	247 nm	-
	2	0.34		247 nm	
J	1	0.31	0.31	200 nm	Negative
	2	0.31		200 nm	Negative
J + P	1	0.30	0.3	247 nm	Positive
	2	0.30		247 nm	Positive
Sample 1 (S1)	1	0.28	0.285	249 nm	Negative
	2	0.29		249 nm	Negative
S2	1	-	-	-	Negative
	2	0.25	-	248 nm	Negative
S3	1	0.35	0.3	321 nm	Negative
	2	0.25		321 nm	Negative

S4	1	0.31	0.26	250 nm	Negative
	2	0.22		254 nm	Negative
S5	1	0 32	0.28	247 nm	Positive
	2	0.25		247 nm	Positive
S6	1	0.31	0.27	249 nm	Negative
	2	0.24		249 nm	Negative
S7	1	0.31	0.28	247 nm	Positive
	2	0.26		247 nm	Positive
S8	1	0.32	0.30	200 nm	Negative
	2	0.29		200 nm	Negative
S9	1	0.34	0.3	247 nm	Positive
	2	0.30		247 nm	Positive
S10	1	-	-	-	Negative
	2	-	-	-	Negative

3.5. Results of TLC-Densitometry Dimension

The results from the TLC-Densitometry dimension are in the form of spectral patterns that vary from each sample. The spectral patterns generated from the TLC-Densitometry dimension are used to compare the patterns produced by the standard reference and the patterns produced by the traditional herbal medicine samples for muscle pain relief.

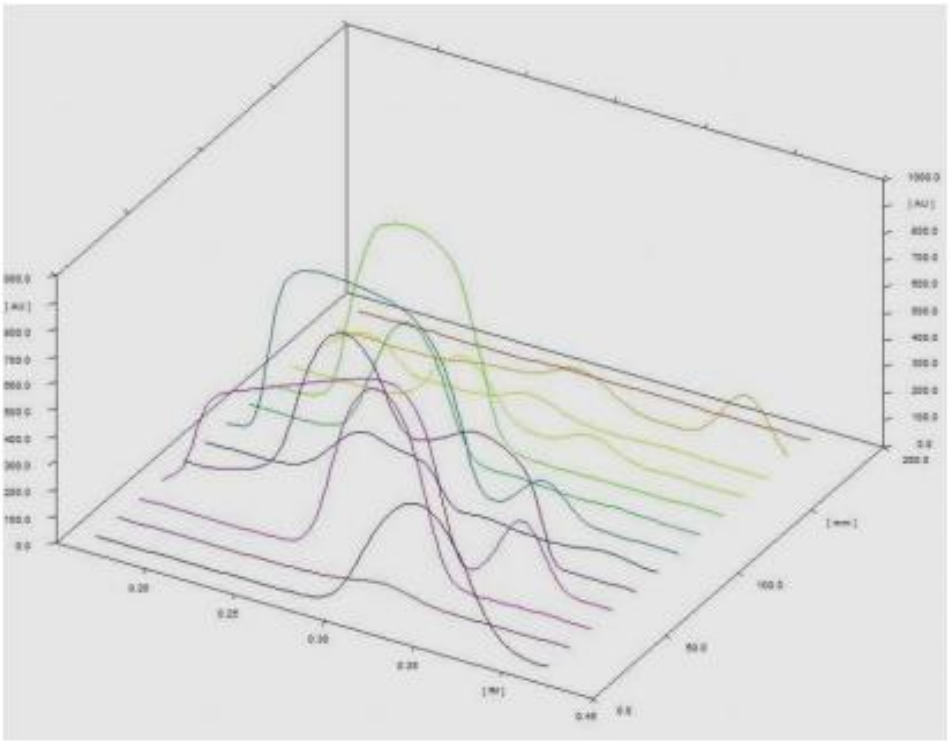

Figure 3. Results of TLC-Densitometry Dimension

According to the dimensions produced from both plates, the samples suspected to contain paracetamol are samples 5, 7, and 9 which have the same spectral pattern [17,18].

3.6. Results of Maximum Wavelength Measurement

Based on the maximum wavelength measurement obtained from the TLC-Scanner, the maximum wavelength of paracetamol is 247 nm.

Table 5. Results of Maximum Wavelength Measurement

Sample	Replication	Maximum Wavelength
B	1	247 nm
	2	247 nm
J	1	200 nm
	2	200 nm
J + B	1	247 nm
	2	247 nm
Sample 1 (S1)	1	249 nm
	2	249 nm
S2	1	-
	2	248 nm
S3	1	321 nm
	2	321 nm
S4	1	250 nm
	2	254 nm
S5	1	247 nm
	2	247 nm
S6	1	249 nm
	2	249 nm
S7	1	247 nm
	2	247 nm
S8	1	200 nm
	2	200 nm
S9	1	247 nm
	2	247 nm

3.1.7. Results and Conclusions of Analysis Using TLC-Densitometry

Based on the results of the analysis using TLC-Densitometry from eight samples of slimming herbal medicine, five samples were found to contain furosemide positively. It was declared positive for containing paracetamol based on the maximum wavelength of the herbal sample that is identical to the maximum wavelength of 247 nm. The results of the TLC-Densitometry analysis can be seen in table 4.

Based on the test using TLC-Densitometry, the result from the paracetamol reference standard showed a TLC Rf value of 0.35, and the results from TLC-Densitometry gave an Rf value of 0.36 at a wavelength of 247 nm. From the obtained results, it was found that samples 5, 7, and 9 were positively identified to contain paracetamol due to having a wavelength that is identical to the standard wavelength of paracetamol [19].

4. CONCLUSION

The results obtained from the study should be considered as preliminary data for future studies and may be used to establish a pharmacognosic parameter. This parameter has been documented for the first time concerning this particular plant. In

addition to its function in establishing parameters for the identification of raw materials and the preparation of a plant monograph, the project will also assist in the development of future studies.

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CONFLICT OF INTEREST: The author declares no conflict of interest.

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