QUALITATIVE ANALYSIS OF DRUG SUBSTANCES IN RHEUMATIC JAMU SAMPLES USING THIN LAYER CHROMATOGRAPHY

Resma Dwi Putri Fitrianasari[1], Perdana Priya Haresmita[1*], Herma Fanani Agusta[1]

[1]*Departement of Pharmacy, Faculty of Health Sciences, Universitas Muhammadiyah Magelang*

Jl. Mayjen Bambang Soegeng, Glagak, Sumberrejo, Mertoyudan District, Magelang Regency, Central Java

**Email Corresponding : perdanapriyaharesmita@unimma.ac.id*

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ABSTRACT

Since ancient times, jamu have been used to treat various diseases. Recently, the number of jamu has increasing and this has led several manufacturers to commit fraud. They added drug substances (DS) into jamu for gaining the effect of jamu so that can raise the sales of jamu in market . The addition of DS into herbal medicine is a violation of the regulation issued by The Ministry of Health. Such practices can cause side effects that are very dangerous for health. The purpose of this research is to analyze the presence of DS in jamu samples that are frequently consumed and circulated in Kranggan, Temanggung and Magelang city. Thin-Layer Chromatography (TLC) was employed to detect the presence of prednisone, paracetamol, and mefenamic acid. The working principle of the separation of compounds with TLC is to separate samples based on the partition difference between samples and the solvent used. In this case, the stationary phase used is the silica gel plate GF_{254} with the mobile phase for prednisone using ethyl acetate:chloroform (4:1), mefenamic acid using chloroform:methanol (9:1),and paracetamol using chloroform:ethanol (8:1). As a result of qualitative analysis, jamu samples in those areas do not contain any drug substances that were suspected to be present.

Keywords: *jamu, prednisone, paracetamol, mefenamic acid*

INTRODUCTION

Jamu is one of sorts of traditional medicine that originates from plant or animal products. It commonly exists as powder, capsule, and liquid preparations (Kamar *et al.*, 2021). There are a lot of brands and claims of jamu that are traded in Indonesia, approximately 8,000 brands. The claims vary from relieving muscle aches, rheumatic disease, asthma, coughs and many others. It is not less than 50% of the Indonesian population that choose jamu to cure various diseases (Pratiwi *et al*., 2018), and 96% of those consumers claimed that they really got the benefits (Kemenkes RI, 2019).

High demand and consumption levels of jamu lead to merely profit-oriented competition among jamu sellers while quality and safety are neglected (Yastiara *et al.*, 2022). One of the fraud practices carried out by jamu manufacturers is adding drug substances to their products in order to convince consumers that the products give them immediate effects (Rusmalina *et al.*, 2020). However, in reality, the addition of drug substances in jamu will generate a bad effect to the body due to the presence of interactions between chemical constituents in jamu and drug substances. Such fraud, in addition, is a violation of Permenkes (Minister of Health Regulation) No. 07 Tahun 2012. According to the regulation about the Registration of Traditional Medicines article 7 which states that

traditional herbal medicines are prohibited from containing medicinal chemicals which are the result of isolation or synthetic medicinal properties (MENKES RI, 2012).

The most common substances that are added to jamu are prednisone, mefenamic acid, and paracetamol. These are frequently mixed with jamu for relieving muscle aches and rheumatic disease. Several investigations have been conducted to analyze the presence of such substances in jamu. In a study conducted by Yastiara *et al.* (2022), it was found that among samples of herbal medicine A─J purchased online and directly in Pontianak City, West Kalimantan Province, there were three samples of herbal medicine, including A, B, and G, which tested positive for containing the chemicals drug paracetamol.

Furthermore, according to another study conducted by Rusmalina *et al.* (2020), showing data from 27 samples circulating in the Pekalongan Region, there were three samples that were positively identified containing mefenamic acid. The positive result was characterized by the presence of a color change in the sample solution at the time of the color reaction test using phenolphthalein and potassium hydroxide, $FeCl_3$ and Vitali Morin.

In addition, a study by Fikayuniar *et al.* (2020), using 10 samples of jamu A─J circulating in Karawang City Region showed positive results against several samples with various types of comparison of eluents (ethyl acetate-chloroform (6:4), ethyl acetate-chloroform (9:1), and ethyl acetate-chloroform (8:2)). The results identified positively containing the chemical of the drug Prednisone as marked by the presence of the equation in the Rf value of the sample and standard.

The routine method for that analysis is thin-layer chromatography (TLC). This method is preferred based on the its ease, simplicity, low amount of required mobile phase, ability to analyze several samples simultaneously, and low cost (Wulandari, 2011).

Based on the problems in the previous research, researchers were interested in conducting research on the investigation of the presence of prednisone, paracetamol, and mefenamic acid in rheumatic jamu using the TLC method. This research was specifically carried out using samples purchased in the Kranggan Jamu Shop Area, Temanggung, and Rejowinangun Market Area, Magelang City.

RESEARCH METHODS

Research Design

This research is a type of experimental research by conducting an experimental qualitative analysis for samples from various jamu brands. The research was conducted in August 2022 ─ December 2022 at the Pharmaceutical Chemistry & Phytochemical Laboratory of the Pharmacy Study Program, Faculty of Health Sciences, University of Muhammadiyah Magelang. The samples were jamu that sold in the area of Kranggan, Temanggung and Magelang City. The samples used were purchased directly from Kranggan jamu Shops, Temanggung and Rejowinangun Market, Town Magelang. There are 6 samples of rheumatic jamu used in this research. Of the 6 samples, 3 samples were collected from the Kranggan Jamu Shop, Temanggung and the others from the Rejowinangun Market, Magelang City. Samples are taken using probability sampling techniques from rheumatic jamu group.

Equipment and Materials

The equipment used in this study was an analytical balance, Erlenmeyer flasks, Beaker glass, separating funnels, a measuring cylinder, volumetric flasks, rotary evaporator machines, a porcelain dish, waterbath, 254 nm UV lamps, chambers, and capillary tubes.The materials used in this study were prednisone standard, paracetamol standard, mefenamic acid standard, silica gel plate GF_{254}, ethyl acetate, chloroform, ethanol (p.a.), methanol, aluminum foil, and paper filters.

Research Procedure

1. **Organoleptic Identification of Jamu Samples**

   The samples are tested by performing organoleptic identification using several test parameters. Organoleptic test parameters include taste, texture, smell, and color. After identification, record the data obtained from the organoleptic test (D. D. Indriatmoko *et al.*, 2019).

2. **Extraction Samples of Jamu**

   One gram of each jamu powder was weighed, put in an Erlenmeyer, and dissolved with 50 mL of 96% ethanol. Next, the sample was macerated for 3x24 hours. After 3x24 hours, the sample was filtered using paper filter And the filtrate was collected. This filtrate was then evaporated in a water bath at temperature 70 ^{o}C. Then the residue from the sample that has been filtered, remacerated for 3x24 hours by placing the residue in an Erlenmeyer and dissolved by adding 25 mL of 96% ethanol. After remaceration, the filtration was repeated. The results of the maceration that has been evaporated and the results of remaceration are then evaporated again to obtain a fairly thick extract (D. D. Indriatmoko *et al.*, 2019).

3. **Qualitative Test with Thin Layer Chromatography Method**

   Each standard powder of medicinal chemicals weighed as much as 10 mg. Then, this powder was inserted into a 10 mL volumetric flask, dissolved with 96% ethanol, and added to the calibration mark. After being homogenized, the solution was further transferred into aluminum foil-covered vial and stored at temperature 5 ^{o}C in the (D. D. Indriatmoko *et al.*, 2019). The mobile phase/eluent for analysis of prednisone, mefenamic acid, and paracetamol was a mixture of ethyl acetate:chloroform (4:1), chloroform:methanol (9:1), and chloroform:ethanol (8:1), respectively (D. D. Indriatmoko *et al.*, 2019). Sample solutions and drug substances standard were spotted on the GF_{254} silica gel TLC plate. The eluent is in accordance with each standard drug substance that is introduced into the chamber. After the eluent reached the mark, TLC plates were taken and dried. The TLC plate after elution was illuminated under ultraviolet (UV) light with a wavelength of 254 nm, and marked on spots appeared. The Rf values of the sample and standard were later calculated.

Data Analysis

The data obtained from this analysis is the Rf (retardation factor) value of the samples. Those values were compared to the Rf of the standard (D. D. Indriatmoko *et al.*, 2019). The calculation of the data is done with the Rf value (ratio of sample spot distance from start line to the distance reached by the eluent)

Based on the results of the Rf data obtained later, the results can be stated with the following conditions:

a. If the Rf value of the sample solution is close to the standard Rf values and both of the spots colors are the same, then the presence of an analyte has been confirmed. Otherwise, the sample does not contain analytes.
b. The range of the Rf value is from 0 to 1.0.

RESULTS AND DISCUSSION

In order to guarantee the accuracy of these results, a qualitative study was carried out with an initial examination of the sample through an organoleptic tests, followed by a thin layer chromatography (TLC) test.

1. **Organoleptic Testing**

   Organoleptic testing is carried out by looking at the shape, color, smell, and taste of each sample used as a test material. The results of the preliminary examination with organoleptic tests can be seen in **Table I**, below.

Table I. Organoleptic Sample Results

Sample	Dosage Form	Color	Smell	Taste
Sample A	Fine powder	Yellow 	Typical aromatic	Bitter
Sample B	Fine powder	Dark yellow 	Typical aromatic	Bitter
Sample C	Capsule (fine powder)	Dark yellow 	Clove scent	Bitter
Sample D	Fine powder	Brownish yellow 	Typical aromatic	Bitter
Sample E	Fine powder	Faded brown 	Typical aromatic	Bitter
Sample F	Fine powder	Yellow 	Typical aromatic	Bitter

2. Thin Layer Chromatography Analysis

Previously, the analysis of the sample was prepared using the maceration method with the aim of separating other compounds from the samples so that the result of the elusion would not be disturbed by the existence of other unseparated compounds. The solvent used in this process is 96% ethanol. The use of ethanol is based on the easy evaporation of the solvent during the sampling process, so it can affect the movement of

compounds in the sample during the elution process (Kemenkes RI, 2020). The elution process will be carried out using a stationary phase of silica gel plates GF_{254} and eluent according to the polarity of the substances being analyzed. The selection of the mobile phase will greatly affect the separation, since the closeness in polarity governs whether the interaction between analyte and eluent is stronger or between analyte and silica.

Table II. TLC Results of Sample Solutions

	Samples	Distance (cm)	Rf
Prednisone (Rf= 0.475)	**A**	6.5	0.8125
	B	6.5	0.8125
	C	6.4	0.8
	D	6.9	0.8625
	E	6.9	0.8625
	F	6.9	0.8625
Paracetamol (Rf= 0.2875)	**A**	5.9	0.7375
	B	5.8	0.725
	C	5.7	0.7125
	D	5.7	0.7125
	E	4.8	0.6
	F	5.7	0.7125
Mefenamic Acid (0.8125)	**A**	6.5	0.8125
	B	6.5	0.8125
	C	6.5	0.8125
	D	5.5	0.6875
	E	5.6	0.7
	F	5.7	0.7125

1) Prednisone

Prednisone is usually used to relieve pain and commonly found in jamu fortreating rheumatic and asthmatic conditions . The addition of this substance leads to cause osteoporosis (Wirastuti *et al.*, 2016).

To determine the presence of a drug substance i.e., prednisone in jamu, analysis was carried out using Thin Layer Chromatography (TLC) with the stationary phase consisting of Silica gel GF_{254} and the mobile phase consisting of a mixture of ethyl acetate:chloroform (4:1)**.** After the elution process, the prednisone standard obtained an Rf value of 0.475. **Table II** shows the Rf result of the sample. The Rf value of the sample has a difference from the standard Rf. The result of the calculation of the Rf value was obtained after looking at the spot standard under UV. The spots under UV 254 light can be seen in the following **Figure 1.** At 254 nm UV light, prednisone standard appeared as a purple spot. Whereas for samples A, C, D, E, and F, they had almost the same spot color, namely faded brown, and for sample B, they had almost the same spot color as the standard, namely faded purple. When compared with the prednisone standard for all

samples A, B, C, D, E, and F, it can be said that the sample did not contain prednisone. This can be inferred from the difference in the Rf value and the difference in the color of the spot between the standard and the samples.

In a study conducted by Wirastuti *et al.* (2016), the results of five samples showed that one sample tested positive for prednisone. The sample that tested positive for prednisone was herbal medicine sample A. The prednisone level contained in herbal sample A was 475.421 µg/mL with a percentage of 4.754%. Prednisone levels in the herbal medicine were obtained after conducting qualitative analysis using the Thin Layer Chromatography (TLC) method with a chloroform:ethyl acetate (1:9) mobile phase and quantitative analysis using a TLC-Densitometer at a maximum wavelength of 254 nm. In addition, from another study conducted by Ningrum *et al*, (2018), the results showed that from samples of rheumatic jamu GI and MT taken from the Pekalongan area, there was one sample of jamu GI positive for containing prednisone. The analysis was proven after carrying out organoleptic tests, microscopic tests, and Thin Layer Chromatography (TLC) tests with a mixture of chloroform:ethyl acetate (9:1) as the mobile phase.

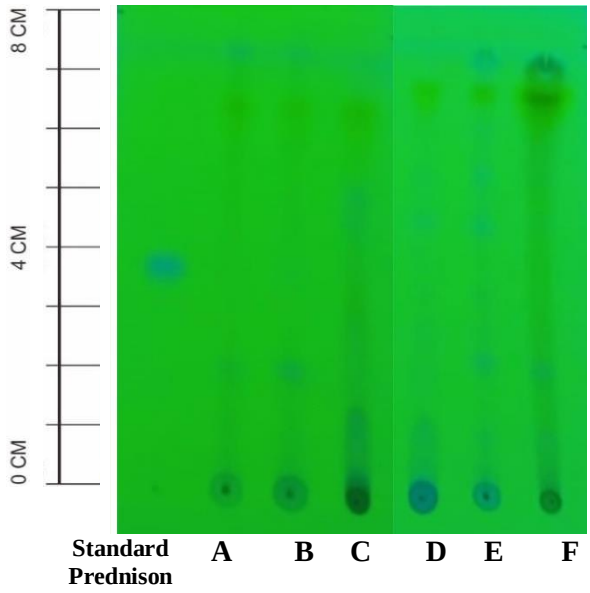

Figure 1. Visualization of standard prednisone and jamu sample spots after elution with mobile phase ethyl acetate:chloroform (4:1)

There are some differences between previous research and recent research. The difference lies in the number of samples used, the sampling location, the methods used, and the results obtained. Previously, the sample used as research material was a sample of rheumatic jamu circulating in Makassar, with a total of 5 herbal samples of herbal medicine. For the method used, the previous researchers used two analyses, namely qualitative with Thin Layer Chromatography (TLC) and quantitative analysis using a TLC-densitometer, and the results obtained were one positive sample containing the medicinal chemical prednisone. Then the results of another study conducted by qualitative analysis by taking samples in the Pekalongan area showed that one sample of GI rheumatic herbal medicine tested positive for prednisone. The two studies both stated positive results based on the results of one sample. Whereas in a recent study, after conducting qualitative tests on herbal samples taken in the Kranggan Jamu Shop Area, Temanggung, and Rejowinangun Market, Magelang City, using Thin Layer Chromatography (TLC) the results showed that all samples were negative for the chemical content of prednisone.

2) Paracetamol

Paracetamol, or acetaminophen, is an analgesic and antipyretic class of drugs used to relieve mild to moderate pain such as headaches, aches, and menstrual pain. The long-term consumption of herbal medicines containing paracetamol can cause hepatotoxicity. In order to avoid the occurrence of fraud, an analysis was carried out by looking at the presence or absence of paracetamol in herbal medicine preparations circulating at the Kranggan herbal medicine Shop, Temanggung, and Rejowinangun Market, Magelang City. The test was carried out qualitatively using the thin layer chromatography method with chloroform:ethanol (8:1) as the mobile phase.

Based on the analysis results with standard paracetamol, an Rf value of 0.1875 was obtained for the standard paracetamol. **Table II** shows the Rf results of the samples. Samples A and B has an Rf value of 0.7375; sample C and F have an Rf value of 0.725; sample D has an Rf value of 0.7125; and sample E has an Rf value of 0.5625. There is a significant difference in the Rf value between the standard and the sample, indicating that the sample is devoid ofparacetamol content. The results of the Thin Layer Chromatography (TLC) test on each comparison standard and jamu samples showed the color of the spots produced when viewed at 254 nm UV light. The spots result under UV 254 light can be seen in the following **Figure 2**.

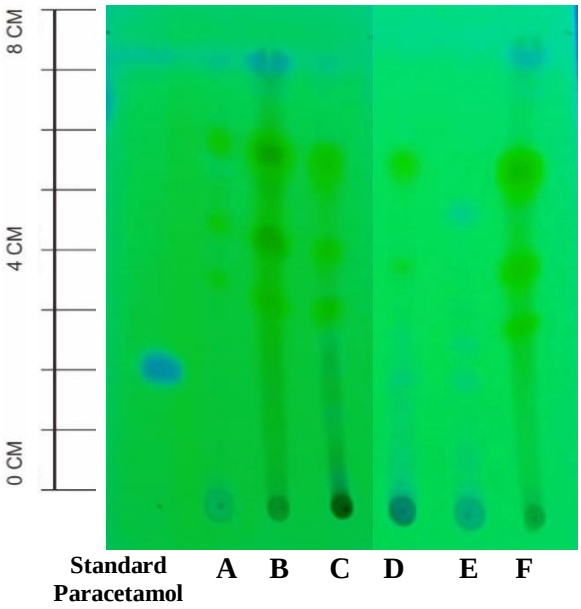

Figure 2. Visualization of standard paracetamol and jamu sample spots after elution with mobile phase Chloroform: ethanol (8:1)

When viewed under UV light 254, the spots for the comparison standard have a purple color; Sample A has a faded brown color; Sample B and C are brown; Sample D is yellow; Sample E is purple; and Sample F is brownish yellow. From the outcome of the elution, there is the same color of spots between the standard and sample E, however, the sample could not be considered to be positive for paracetamol because it has an Rf value that is different from the Rf value for the paracetamol standard, so in the identification of paracetamol there are no medicinal chemicals in the herbal medicine for muscle pain and rheumatic.

In a study conducted by Rahmadani R *et al.* (2021), the identification of the paracetamol content in five traditional herbal medicine preparations (samples A, B, C, D, and E) rheumatic pain in the Banjarmasin City Night Market Area, South Kalimantan, showed positive results containing the medicinal chemical paracetamol on herbal medicine samples C and D. These results were demonstrated by two qualitative analyses

using TLC and a quantitative analysis using a UV-Vis spectrophotometer. On the qualitative results, sample C has an Rf value of 0.75, and sample D has an Rf value of 0.75. The value of Rf is equal to the standard Rf value of 0.75. Other evidence indicates that the positive paracetamol sample is found in the color of the spot, which has a similar purple color between the standard and samples C and D. Then, in the results of quantitative analysis using the UV-Vis spectrophotometry method, the level of paracetamol in sample C is 8.13 mg/kg and in that sample D is as much as 6.28 mg/kg. Then, in a study conducted by Harimurti *et al.*(2020), regarding the identification of paracetamol samples for rheumatic and gout jamu circulating in the Special Region of Yogyakarta using the KLT-Densitometer method, the results of 14 herbal medicine samples rheumatic pain and gout showed that 3 samples of herbs number 3 (SM), 7 (AS), and 10 (JE) were positive for containing paracetamol with concentrations of 0.04% (w/v), 0, 30% (w/v), and 0.13% (w/v), respectively.

According to the identification, there is a difference between the results of previously conducted research and recent research. The two previous studies showed positive results for paracetamol, while the latest study showed the results of the five samples tested negative for paracetamol. Another difference is in the analysis carried out by previous researchers using qualitative and quantitative analysis, whereas the latest research only carried out a qualitative analysis by determining the color of the sample spot stains on the TLC plate and calculating the Rf value of each spot (comparison between standard and the sample).

3) Mefenamic Acid

Mefenamic acid is a drug in a group of nonsteroidal anti-inflammatory drugs (AINS) that have an analgesic effect (Supardi, *et al.*, 2017). In order to determine the presence of mefenamic acid in samples of rheumatic pain herbal medicine, identification of the mefenamic acid content was carried out using the TLC method. The test results with TLC will show qualitative data with the Rf values obtained from calculating the distance of the spots that appear on the TLC plate. The results of the Rf value obtained after conducting the analysis can be seen in **Table II.**

The Rf value of the sample is different from the standard Rf of the drug substance. When compared with the Rf values of samples A, B, C, D, E, and F, it can be seen that the Rf values of the samples do not have the same values as the standards.Thus,from the Rf results it can be confirmedthat the jamu samples did not contain mefenamic acid. The results of the spot color of the standard and the sample can be seen on a TLC plate that irradiated with UV 254 light. From Figure 3, it can be seen that the spots for the mefenamic acid comparison standard have a purple color, while for samples A, B C, and D they have yellow spots, for sample E they have purple spots, and for sample F they have brownish yellow spots. There is a similarity between the spot stains of the comparison standard and sample E, but this similarity cannot be used to confirmed that sample E is positive for containing mefenamic acid. This is because the Rf value has a different value from sample E.

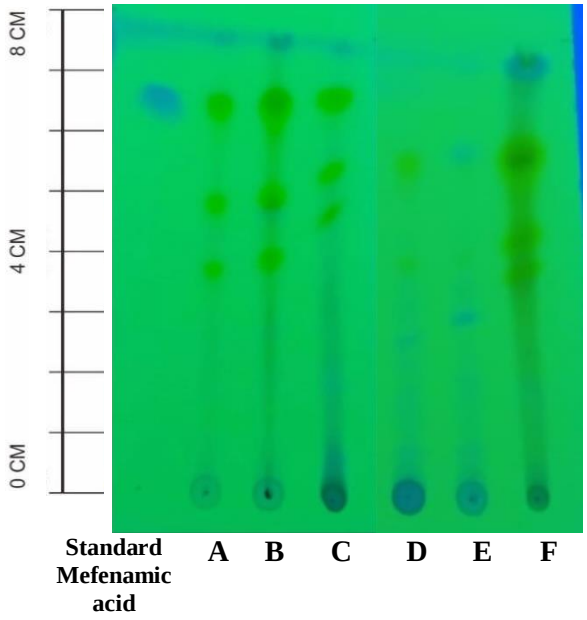

Figure 3. Visualization of standard Mefenamic acid and jamu sample spots after elution with mobile phase chloroform:methanol (9:1)

In a study conducted by Rusmalina *et al*, (2020), showed that the results of 27 samples that had been replicated, there were three types of samples J, O, and Y, which were said to be positive for mefenamic acid. Analysis of the samples was carried out using the TLC method with a mixed mobile phase of ethyl acetate:methanol:ammonia (80:10:10). The results of TLC were then detected by looking at the stains under UV-254 light. From the detection results, this would later be followed by testing the color reactions of PP+KOH, $FeCl_3$, and Vitalin Morin for positive sample results shown in the TLC plate. The positive results from the qualitative analysis show that the Rf of sample J is 0.33 and the Rf of the standard is 0.32; for sample O, the sample Rf is 2.3 and the standard Rf is 2.3; and for sample Y, it has an Rf of 1.91 and the Rf standard is 1.95. Each of these tests is then followed by testing the color reagent to ensure the presence of ingredients in the herbal medicine samples. The color test showed the same color results in the J, O, and Y samples, namely that the PP+KOH color reaction test turned pink, the $FeCl_3$ color reaction test became purple, and the Vitalin morin color reaction test turned red. According to research by Supardi *et al* (2017), after analysis using the UV spectrophotometry method, the results obtained from the five samples contained 2 samples, namely sample 1 and sample 4 which had positive results with levels exceeding the stipulations of the Farmakope Indonesia Herbal dan Peraturan Menteri Kesehatan Nomor 07 Tahun 2012.

Both studies differ from recent research. Previous research stated positive results, with 3 positive samples from the results of research conducted by Rusmalina *et al.* (2020) and 2 positive samples from results conducted by Supardi *et al.* (2017). Whereas in a recent study conducted by qualitatively testing the color of the spot and calculating the Rf value of the samples, the results showed that none of the samples tested positive for mefenamic acid.

CONCLUSION

Based on the data obtained from qualitative analysis using TLC, the results showed the absence of prednisone, paracetamol, and mefenamic acid in jamu samples. It provides information that the jamu samples in Kranggan Jamu Shop Area, Temanggung, and Rejowinangun Market Area, Magelang City could be said to be safe for consumption

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