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Identification of Ketoprofen Chemical Compounds in *Jamu* for Menstrual Pain Relief Using Thin-Layer Chromatography Method

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ABSTRACT/ ABSTRAK

ABSTRACT. *Jamu is a traditional form of medicine that should be free from the contamination of medicinal chemicals and only use organic materials from plants or animals. This study aims to detect the presence of Ketoprofen in Jamu for menstrual pain relief using Thin Layer Chromatography (TLC). In this study, TLC was used to identify the presence of medicinal chemicals through spots on the TLC plate. The mobile phase used was a mixture of chloroform and methanol (9:1), with the stationary phase being a silica gel GF 254 nm plate. Detection was performed at UV wavelengths of 254 nm and 365 nm. Six samples were tested, and the results showed that no Ketoprofen was detected in any of the samples, thus the study was not continued with UV-Vis spectrophotometry. The Rf value of the standard comparator was 0.53, while the Rf value of the samples was close to this standard, at 0.73. Based on these results, it can be concluded that all tested Jamu samples for menstrual pain relief do not contain Ketoprofen.*

ABSTRAK. Jamu merupakan salah satu bentuk obat tradisional, seharusnya bebas dari campuran bahan kimia obat (BKO) dan hanya menggunakan bahan organik dari tumbuhan atau hewan. Penelitian ini bertujuan untuk mendeteksi kandungan Ketoprofen dalam Jamu pereda nyeri haid menggunakan metode Kromatografi Lapis Tipis (KLT). Pada penelitian ini, metode KLT digunakan untuk mengidentifikasi keberadaan BKO melalui bercak pada plat KLT. Fase gerak yang digunakan berupa campuran kloroform : metanol (9: 1), dengan fase diam berupa plat silika gel GF 254 nm. Deteksi dilakukan pada panjang gelombang UV 254 nm dan 365 nm. Enam sampel diuji, dan hasil menunjukkan bahwa tidak ada kandungan Ketoprofen pada semua sampel, sehingga penelitian tidak dilanjutkan dengan spektrofotometri UV-Vis. Nilai Rf standar pembanding adalah 0,53, sedangkan nilai Rf pada sampel mendekati nilai standar tersebut, yaitu 0,73. Berdasarkan hasil ini, dapat disimpulkan bahwa semua sampel Jamu pereda nyeri haid yang diuji tidak mengandung Ketoprofen.

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INTRODUCTION

The use of herbal medicine has been practiced since ancient times, especially by ancestors in Asia, who pioneered the utilization and development of natural medicine. According to a report by the World Health Organization (WHO), about 65% of the population in developed countries use traditional medicine. WHO also recommends the use of traditional medicine to maintain health, as well as to prevent and treat various diseases, particularly chronic diseases such as degenerative conditions and cancer (Purwanto, 2016).

Indonesia has abundant natural resources that can be utilized for traditional healthcare services. One popular form of traditional medicine is *Jamu*, which has been passed down through generations to maintain health and treat various ailments (Ryansyah et al., 2022).

Jamu should be made from organic materials, either from plants or animals, without the addition of medicinal chemicals. The presence of medicinal chemicals in *Jamu* raises concerns, as some traditional *Jamu* products have been found to contain such substances. As a result, the Indonesian Food and Drug Authority (BPOM) has withdrawn these products from circulation (Febbyanto, 2019).

Medicinal chemicals are chemical compounds that are the main components in pharmaceutical drugs, often added to traditional medicines or *Jamu* to enhance their therapeutic effects. The use of medicinal chemicals in traditional medicine is prohibited because it can be harmful to health. If the consumption of traditional medicine leads to rapid reactions or symptoms such as dizziness, the possibility of medicinal chemicals should be considered. The addition of chemicals without dose supervision or without a doctor's recommendation may pose a risk of harmful side effects. Some medicinal chemicals that are often misused in traditional medicine include those for pain relief, gout, slimming, enhancing male endurance, appetite stimulation, and diabetes (Indriani, 2021).

To protect the public in the consumption of *Jamu*, the Indonesian government has issued Minister of Health Regulation No. 007 of 2012 concerning the Registration of Traditional Medicine. This regulation emphasizes that traditional medicines in circulation must not contain medicinal chemicals. Medicinal chemicals are chemical compounds that are often added to *Jamu* to enhance its effectiveness. However, the prolonged use of *Jamu* containing medicinal chemicals can potentially pose health risks, such as stomach disturbances, kidney failure, liver damage, and even the risk of death (Ryansyah et al., 2022).

The numerous discoveries of medicinal chemicals in traditional medicine sales outlets in the Riau Archipelago have raised concerns, particularly in Batam City, where some market vendors are still distributing *Jamu* containing medicinal chemicals. One type of *Jamu* suspected of containing medicinal chemicals is *Jamu* for menstrual pain relief. Based on this background, the researchers aim to investigate whether *Jamu* for menstrual pain relief in circulation contains the medicinal chemical ketoprofen.

RESEARCH METHOD

Tools and Materials

The *Jamu* samples to be tested, ketoprofen ($C_{16}H_{14}O_3$), silica gel G 60 F254nm, 96% ethanol (C_2H_6O), chloroform ($CHCl_3$), methanol (CH_3OH), and distilled water (aquadest).

Chamber, aluminum foil, filter paper, UV lamps (254 nm and 365 nm), capillary tubes, spatula, pestle, silica plates, 5 ml volumetric flask, 10 ml volumetric flask, 100 ml volumetric flask, analytical balance, 50 ml beaker, 500 ml beaker, 10 ml measuring cylinder, volume pipette, dropper pipette, funnel, micropipette, and stir bar.

Procedure

Sample Collection

The samples for this study were obtained from several markets across Batam City. Six samples of *Jamu* for menstrual pain relief were collected from different locations.

Sample Preparation

The samples were extracted by evaporation using a water bath to reduce the moisture content in the *Jamu*. After this process, 96% ethanol was added. The addition of 96% ethanol was performed because ethanol is a polar solvent that can extract both polar and non-polar compounds.

Organoleptic Test

The form, color, and taste of each *Jamu* sample for menstrual pain relief were tested.

Stationary Phase Preparation

A silica gel GF 254 plate was cut into two parts. The TLC plate was activated by heating it in an oven at 105°C for 30 minutes. After that, a development line was drawn 8 cm from the base, and marks were made with a pencil, leaving a 1 cm distance from the top and bottom edges. The distance between each sample point was set to 1 cm. The sample solution was then applied to the plate using a capillary tube, positioned 1 cm from the bottom of the plate.

Mobile Phase Preparation

In previous studies, ketoprofen tablet content analysis was performed using the TLC-Densitometry method, with the best mobile phase being a mixture of chloroform and methanol in a 9:1 ratio (Mandela, 2015).

Identification Using TLC Method

The required tools and materials for this research were prepared. The stationary phase used was Silica GF 254, and the mobile phase consisted of a mixture of chloroform and methanol (9:1), which had been saturated with filter paper. A 15 µL sample was applied to the TLC plate with a migration distance of 8 cm. The resulting spots were observed under UV light at wavelengths of 254 nm and 366 nm. The Rf values obtained from each sample were calculated and compared with the Rf value of the positive control.

RESULTS

Organoleptic Test Results

In this study, the form, color, and taste of each *Jamu* sample for menstrual pain relief were tested.

Qualitative Analysis

The analysis of the medicinal chemical ketoprofen in 6 types of *Jamu* for menstrual pain relief circulating in Batam City was conducted. The qualitative analysis aimed to identify the presence of additional medicinal chemicals in the *Jamu* products. The qualitative analysis used the TLC method with a mobile phase of chloroform and methanol (9:1). The results were as follows:

Table 1. Qualitative Analysis Results on TLC Plate (UV 254 nm)

Sample	Distance of Spot Migration (cm)	Rf Value ($Rf=\frac{x}{y}$)	Rf Value Difference (Standard-Sample)	Conclusion
Standard Reference	-	-	-	No spots were detected under UV 254 nm, therefore no Rf value for the standard reference
Positive control (+)	4,6	0,76	-	A yellow spot was observed with UV 254 nm, and the Rf value of the positive control is 0.8.
Sample A	4.4	0,73	0,2	Negative (-)
Sample B	4,4	0,73	0,2	Negative (-)
Sample C	4,4	0,73	0,2	Negative (-)
Sample D	4,5	0,75	0,22	Negative (-)
Sample E	4,5	0,75	0,22	Negative (-)
Sample F	4,6	0,76	0,23	Negative (-)

Table 1 results show the presence of yellow spots in all 6 samples when using a UV lamp (254 nm), and the Rf values were obtained; however, no identification was observed for the standard reference.

Table 2. Qualitative Analysis Results on TLC Plate (UV 366 nm)

Sample	Distance of Spot Migration (cm)	Rf Value ($Rf=\frac{x}{y}$)	Rf Value Difference (Standard-Sample)	Conclusion
Standard Reference	3,2	0,53	0,53	The Rf value of the standard reference under UV 366 nm is 0.53
Positive Control (+)	3,2	0,53	0,53	The Rf value of the positive control matches the standard reference, with a value of 0.53
Sample A	4,8	0,8	0,27	Negative (-)
Sample B	4.8	0,8	0,27	Negative (-)
Sample C	4.8	0,8	0,27	Negative (-)
Sample D	4.8	0,8	0,27	Negative (-)
Sample E	-	-	-	No fluorescence observed under UV 366 nm

Sample F	-	-	-	No fluorescence observed under UV 366 nm

In Table 2, after using the UV lamp (366 nm), 4 samples were identified, while 2 samples (E and F) showed no spots.

DISCUSSION

Upon observing the TLC plate under UV 254 nm, all six samples and the positive control showed yellow spots. This yellow spot appeared because the samples could absorb UV light at this wavelength, while the standard reference did not exhibit UV 254 nm absorption. This indicates that ketoprofen may not have significant absorbance at this wavelength. However, when examined under UV 366 nm light, blue spots were observed in both the standard reference and positive control. This suggests that ketoprofen present in the standard reference and positive control can absorb UV light at the 366 nm wavelength.

In sample A, the Rf value obtained under UV 254 nm was 0.73, and the Rf value under UV 366 nm was 0.8. For sample B, the Rf value obtained under UV 254 nm was 0.73, and the Rf value under UV 366 nm showed a purple spot with an Rf value of 0.8. Similarly, in sample C, the Rf value under UV 254 nm was 0.73, and the Rf value under UV 366 nm showed a purple spot with an Rf value of 0.8. In sample D, the Rf value under UV 254 nm was 0.75, and the Rf value under UV 366 nm also showed a purple spot with an Rf value of 0.8. For sample E, the Rf value obtained from UV 254 nm was 0.75, but sample E did not show a purple spot under UV 366 nm. For sample F, the Rf value obtained from UV 254 nm was 0.76, but sample F did not show a purple spot under UV 366 nm. The positive control exhibited a yellow spot at 0.8 under UV 254 nm, while both the standard reference and the positive control had an Rf value of 0.53 under UV 366 nm. According to Husna et al. (2020), the difference in Rf values is considered positive if the difference is ≤ 0.05, and negative if the difference is > 0.05. Based on this analysis, it can be concluded that all six *Jamu* samples for menstrual pain relief did not have Rf values that closely match the standard reference ketoprofen. Therefore, it can be stated that none of these samples contain the medicinal chemical ketoprofen. This is in line with the findings of Irawan (2023), who used thin-layer chromatography (TLC) to analyze the contents of samples, with the Rf value indicating the movement of the compound relative to the solvent. The testing was conducted three times for each sample, and the average Rf values obtained for sample A were 0.67, 0.77, and 0.86; for sample B (residue) was 0.19; for sample C was 0.68, 0.77, and 0.86; for sample D was 0.55, 0.63, and 0.72; and for sample E was 0.54, 0.62, and 0.72. Based on these results, it can be concluded that all the samples tested had Rf values different from those of the anti-inflammatory drug, indicating that these samples did not contain that drug.

The Rf value can be used as an indicator in the process of compound identification. If two compounds have the same Rf value, they can be considered to have similar or identical properties to the reference compound. The Rf value is calculated as the ratio of the distance traveled by the compound to the distance traveled by the mobile phase on the TLC plate. In analysis, this value serves as a relative measure to compare different samples. Compounds with higher Rf values indicate lower polarity, while compounds with lower Rf values indicate higher polarity. If the Rf value is too high, the polarity of the eluent needs to be reduced, while if the value is too low, the polarity of the eluent must be increased (Gandjar & Rohman, 2007). According to Lipsy (2010) in Muzdhalifah et al. (2019), the Rf value can serve as

evidence in identifying a compound. If the obtained Rf value is the same, the compound can be considered to have similar or identical characteristics. On the other hand, if the Rf values differ, the compounds can be stated as different compounds.

CONCLUSION

The analysis of Medicinal Chemicals in the menstrual pain relief herbal samples using thin-layer chromatography (TLC) showed that none of the samples tested positive for Medicinal Chemicals. This was due to the absence of a difference in Rf values that would approach the Rf value of the ketoprofen standard. Therefore, the herbal products tested can be considered safe for consumption by the public.

It is recommended to conduct further examination of the herbal products to identify the possible presence of other Medicinal Chemicals. To obtain more accurate results in determining the content of Medicinal Chemicals, it is advisable to use High-Performance Liquid Chromatography (HPLC).

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