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Identification and Quantification of Paracetamol Adulteration in *Jamu Pegal Linu* by TLC-Densitometry

Zunita Puspita Sari[1*], Shania Chrisnatasha Putri[1], Fita Sari[1], Dyah Aryantini[1]

[1] *Department of Pharmacy, Faculty of Health Sciences, Bhakti Wiyata Institute of Health Sciences, Kediri 64114, Indonesia*

** Corresponding author. Email: zunita.puspitasari@iik.ac.id*

BSTRACT

Jamu is a traditional Indonesian herbal medicine that has long been used for health maintenance, disease prevention, and treatment. With the increasing public preference for natural products, the circulation of *jamu* in the market has expanded significantly. However, some products have been reported to contain illegally added pharmaceutical substances, including paracetamol, to provide rapid therapeutic effects. The undeclared presence of paracetamol in herbal medicine may pose serious health risks, especially when consumed repeatedly or without proper dosage control. Therefore, this study aimed to identify and quantify paracetamol in *jamu pegal linu* samples using thin-layer chromatography (TLC) combined with densitometry. Sample preparation was carried out using 96% ethanol as the extraction solvent. Chromatographic separation was performed on silica gel 60 F254 plates using chloroform:ethyl acetate (9:1, v/v) as the mobile phase. Qualitative identification was based on retention factor (Rf) values and spot profile comparison with paracetamol standard under UV light at 254 nm. Quantitative analysis was conducted using TLC–densitometry by constructing a calibration curve from standard paracetamol solutions. Each sample was prepared and analyzed in triplicate (n=3), and results are expressed as mean ± standard deviation (SD). The TLC results showed that sample S2 exhibited a chromatographic spot with an Rf value comparable to that of the paracetamol standard, confirming the presence of paracetamol. The calibration curve demonstrated acceptable linearity with the regression equation of $y = 1 \times 10^{-5}x + 0.002$ and the calibration showed acceptable linearity for screening purposes (R^2=0.9615). Based on densitometric measurement, the concentration of paracetamol in sample S2 was 3563 µg/mL 3563 ± 2 µg/mL, corresponding to 35.63 ± 0.06% w/w (n=3). This relatively high concentration indicates intentional adulteration rather than accidental contamination. In conclusion, TLC–densitometry is a rapid, simple, precise, and cost-effective method for detecting and quantifying paracetamol in *jamu* products. These findings emphasize the need for stricter quality control and regulatory monitoring to ensure the safety, quality, and authenticity of herbal medicines marketed to the public.

Keywords:
Jamu; Paracetamol; Adulteration; TLC-densitometry; Herbal medicine

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1. Introduction

Herbal medicine (*jamu*) is one of the traditional forms of natural product utilization that has long been used by Indonesian communities for health maintenance,

disease prevention, and traditional treatment. The use of *jamu* has been passed down from generation to generation and has become an integral part of the traditional healthcare system based on local wisdom [1]. In addition, the existence of *jamu* reflects the enormous potential of Indonesia's biodiversity, which is rich in medicinal plants containing various bioactive compounds [2], such as flavonoids, alkaloids, saponins, and tannins, all of which are known to possess diverse pharmacological activities.

Along with the increasing public awareness of natural products and the growing "back to nature" trend, the consumption of *jamu* has risen significantly. *Jamu* is generally perceived as safer than synthetic medicines because it is derived from natural ingredients and is believed to cause fewer side effects when used appropriately. This perception has contributed to the growing market demand for *jamu*, both in traditional preparations and modern packaged forms [3]. However, this increasing demand has also been accompanied by serious issues related to product quality, safety, and authenticity. One of the most frequently reported problems is the illegal adulteration of *jamu* with pharmaceutical substances or medicinal chemical compounds (*Bahan Kimia Obat*, BKO) [4].BKO refers to synthetic active compounds that should only be used in pharmaceutical preparations under strict supervision, yet they are intentionally added to *jamu* products to provide rapid and noticeable therapeutic effects, such as analgesic, antipyretic, or anti-inflammatory actions.

The addition of BKO into *jamu* is commonly carried out by irresponsible manufacturers to enhance product attractiveness in the market. This practice is intended to ensure that consumers experience fast and obvious therapeutic effects, thereby encouraging repeat purchases. One of the most commonly misused compounds is paracetamol, which is frequently added to herbal remedies for muscle aches or fever reduction [5]. In addition, other BKO substances such as nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and sildenafil have also been reported in various *jamu* products circulating in the community [6].

Paracetamol is a compound with analgesic and antipyretic activities [**7**], and its inclusion in *jamu* aims to provide rapid pain-relieving effects [**8**]. However, the addition of paracetamol is usually not declared on the product label, leaving consumers unaware of its presence. This condition results in uncontrolled use, particularly when consumed repeatedly or over a long period, which may lead to serious adverse effects such as hepatotoxicity due to the accumulation of toxic metabolites in the liver [9].Furthermore, concurrent use with other medications containing paracetamol may increase the risk of unintentional overdose.

In the context of natural product chemistry, studies on *jamu* products are not only focused on identifying natural active compounds responsible for pharmacological effects, but also on detecting contamination and adulteration practices, including the addition of pharmaceutical compounds [10]. The presence of synthetic substances in the *jamu* matrix may affect product quality, safety, and authenticity, thereby posing potential health risks to consumers. Moreover, adulteration creates discrepancies between the composition stated on the label and the actual product content. Therefore, selective, sensitive, and accurate analytical methods are required to provide both qualitative and quantitative information regarding the compounds present in *jamu*, including the detection of paracetamol as a BKO adulterant.

One of the analytical methods that can be applied is thin-layer chromatography (TLC) combined with densitometry. This method is widely used in the analysis of traditional medicines because of its simple procedure, cost efficiency, and ability to separate compounds within complex matrices [11]. Compared to HPLC and LC-MS,

TLC–densitometry does not require expensive instrumentation or complex sample preparation, making it particularly applicable in resource-limited regulatory laboratories [12,8]. A key advantage is its capacity to analyze multiple samples simultaneously in a single elution run which is important feature for large-scale product surveillance [12]. When coupled with densitometry, the method gains quantitative capability while maintaining simplicity, serving as a reliable first-line screening tool [13]. In addition, TLC-densitometry enables quantitative analysis based on the measurement of spot area or absorbance intensity at specific wavelengths. Compound identification is performed based on the retention factor (Rf) values and the similarity of chromatographic profiles between samples and standard solutions, while quantitative determination is carried out by constructing calibration curves using linear regression analysis [14].

Using this approach, the identification and quantification of compounds such as paracetamol in *jamu* can be conducted in a more systematic and measurable manner. Therefore, this study aims to investigate the chemical profile and detect the presence of paracetamol as a pharmaceutical adulterant in *jamu pegal linu* using the TLC-densitometric method. The findings of this study are expected to provide scientific information regarding the safety, quality, and authenticity of *jamu* products available in the market, as well as to serve as a basis for regulatory supervision and consumer protection

2. Methods

Materials

The materials used in this study included paracetamol standard, herbal medicine (*jamu*) samples, 96% ethanol (Merck), chloroform, and ethyl acetate. All chemicals used were of analytical grade.

The instruments used included an analytical balance, micropipettes, volumetric glassware, a vortex mixer, a thin-layer chromatography (TLC) chamber (10 × 10 cm), silica gel 60 GF254 aluminum TLC plates (50 × 100 mm, Merck), a UV lamp (254 nm and 366 nm), and a TLC Scanner 3 equipped with a UV detector and winCATS software version 1.4.8.2012 (CAMAG).

Sample Collection

Three commercially available *jamu pegal linu* products were randomly purchased from traditional herbal medicine vendors in Bandar, Kediri, East Java, Indonesia. Samples were selected based on their indication as *jamu pegal linu*. The products were coded as S1, S2, and S3. Product identities, brands, and registration information were not disclosed to maintain confidentiality and prevent commercial bias.

Preparation of Standard Solution

A stock solution of paracetamol was prepared by accurately weighing 10 mg of paracetamol standard and dissolving it in ethanol to a final volume of 10 mL in a volumetric flask to obtain a concentration of 1000 ppm. The stock solution was further diluted with ethanol to prepare working standard solutions at concentrations of 100, 200, 300, and 400 ppm.

Preparation of Sample Solution

A total of 100 mg of herbal medicine sample suspected to contain paracetamol was accurately weighed and transferred into a 10 mL volumetric flask. Ethanol was added and the volume was adjusted to the mark to obtain a sample solution with a concentration of 1000 ppm.

TLC-Densitometric Analysis

The analysis was carried out using thin-layer chromatography combined with densitometry. Chromatographic evaluation was carried out on a 50 × 100 mm TLC plate. Scanning was performed at a speed of 40 mm/s with a data resolution of 200 µm/step and a slit dimension of 8.00 × 0.20 mm (macro). Silica gel 60 GF254 plates were used as the stationary phase, while chloroform:ethyl acetate (9:1, v/v) was used as the mobile phase. Before development, the TLC chamber was saturated with the mobile phase for 20–30 min.

Standard and sample solutions were applied onto the TLC plate as bands at a distance of 1.5 cm from the lower edge of the plate, with an application volume of 30 µL for each spot. The plate was developed until the solvent front reached a migration distance of 8 cm. After development, the plate was dried at room temperature and observed under UV light at 254 nm and 366 nm for spot identification.

Densitometric scanning was then performed using TLC Scanner 3. The peak area obtained from each standard concentration was used to construct a calibration curve by linear regression analysis. The paracetamol content in the sample was calculated using the regression equation obtained from the calibration curve.

Data Analysis

Quantitative analysis was performed by constructing a calibration curve using paracetamol standard concentrations versus peak area responses obtained from densitometric scanning. Linear regression analysis was applied to generate the regression equation, which was then used to calculate the paracetamol concentration in the sample.

Ethical Approval

Not applicable, as this study did not involve human participants or experimental animals.

3. Results and Discussion

Validation Method Analysis

Similarity and Selectivity

The results showed that the paracetamol peak in the standard solutions, at concentrations ranging from 100–400 µg/mL, exhibited a relatively consistent Rf value. The peak detected in the jamu samples also appeared at the same Rf value as the standard solution, indicating similarity between the two. The Rf values of the paracetamol standard (100–400 µg/mL) ranged from 0.826 to 0.832, closely matching the Rf value of 0.844 observed in the jamu sample, with a percentage difference of only 1.44%. This close correspondence confirms the identity of the detected compound as paracetamol and supports the selectivity of the method. These findings confirm the presence of paracetamol in the jamu samples based on Rf similarity and chromatogram profile (**Figure 1**).

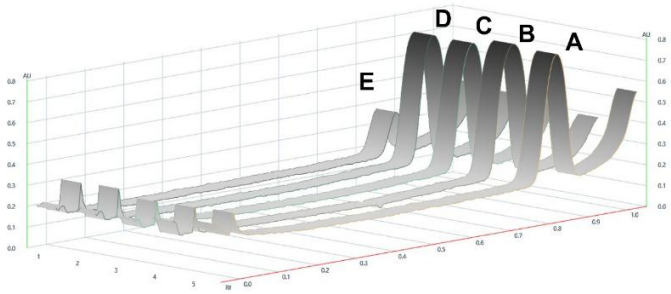

Figure 1. Chromatogram of Paracetamol A: Standard Solution 100 µg/ml; B: Standard Solution 200 µg/ml; C: Standard Solution 300 µg/ml; D: Standard Solution 400 µg/ml; E: Sample Solution

Linearity

Linearity was assessed based on the relationship between standard solution concentrations and the corresponding analytical responses, expressed through the correlation coefficient (r) or coefficient of determination (R^2). An analytical method is considered to exhibit good linearity when the R^2 value approaches 1, indicating a strong relationship between concentration and response.

The results showed that the calibration curve of paracetamol followed the linear regression equation $y = 1 \times 10^{-5}x + 0.002$, with a coefficient of determination (R^2) of 0.9615. This value indicates a strong relationship between concentration and analytical response, suggesting that the method exhibited acceptable linearity within the concentration range used [15].

LOD and LOQ

The limit of detection (LOD) is defined as the lowest analyte concentration that can be detected by the method, but not necessarily quantified with adequate precision. The limit of quantification (LOQ), in contrast, is defined as the lowest analyte concentration that can be quantitatively measured with acceptable accuracy and precision. LOD and LOQ values were calculated based on statistical parameters derived from the calibration curve. The results showed an LOD of 592.68 mg/L and an LOQ of 1796 mg/L for paracetamol. These values indicate that the method possesses adequate detection capability within the concentration range used, although its sensitivity is relatively limited for the analysis of very low analyte concentrations [15].

Range

The method's range refers to the interval of analyte concentrations within which the analytical method provides a response proportional to the analyte concentration while meeting the required analytical performance criteria. The range was determined by constructing a calibration curve using several standard solution concentrations.

In this study, the calibration curve for paracetamol was constructed within a concentration range of 0.1–0.5 g/L. The evaluation results showed an acceptable relationship between concentration and analytical response within this range, indicating that it can be used as the working range of the method for the analysis of paracetamol in jamu samples [15].

Accuracy

Accuracy reflects the closeness of agreement between the test result obtained using the analytical procedure and the true value (USP, 2022). The accuracy test was performed using the standard addition method. A 100 mL volume of standard solution was added at concentrations of 80%, 100%, and 120% of the paracetamol sample

concentration in the preparation (1:1). Each concentration level was analyzed in triplicate. Accuracy was considered acceptable if the percentage recovery fell within the range of 98.0–102.0% [15,16].

Table 1. Accuracy Test Results of Paracetamol Using the Standard Addition Method

Level (%)	Mean Recovery ± SD (%)	RSD (%)
80	99.20 ± 0.63	0.64
100	99.75 ± 0.80	0.80
120	100.10 ± 0.89	0.89
	99.68 ± 0.78	0.78

The accuracy test at standard addition levels of 80%, 100%, and 120% yielded mean recovery values of 99.20%, 99.75%, and 100.10%, respectively (**Table 1**). All recovery values were within the acceptance range of 98–102%. The RSD values ranged from 0.64% to 0.89%, indicating good accuracy with low measurement variability.

TLC Identification of Paracetamol in *Jamu* Samples

Jamu is one of Indonesia's traditional medicinal products prepared from natural ingredients and has been used for generations based on empirical knowledge [17]. Various *jamu* products are widely available in the market and are highly consumed by the community. In this study, paracetamol content in *jamu* samples was analyzed using thin-layer chromatography (TLC), followed by TLC-densitometry. Sample preparation was carried out using 96% ethanol, which was selected for its ability to dissolve polar to semipolar compounds, including paracetamol, a relatively polar compound. The extraction process was continued until a homogeneous solution was obtained to ensure even distribution of analytes within the sample.

Preliminary identification was performed using TLC with silica gel 60 F254 as the stationary phase and chloroform:ethyl acetate (9:1, v/v) as the mobile phase. This solvent system was selected based on its suitability for separating compounds according to polarity differences. The chromatographic results are presented in **Figure 2**. Spots were observed in both the standard solution and *jamu* samples under UV light at 254 nm. The spots appeared as dark bands against a bright fluorescent background, which is characteristic of compounds capable of absorbing UV radiation at this wavelength and causing quenching of the fluorescence indicator on the plate.

The paracetamol standard produced a distinct single spot with an Rf value of 0.65, corresponding to the spot observed in sample S2. As shown in **Figure 2**, samples S1 and S3 did not show spots corresponding to the standard, whereas sample S2 exhibited a spot at the same migration distance with a comparable Rf value of 0.65. The similarity in spot position, migration distance, and chromatographic appearance strongly suggested the presence of paracetamol in sample S2.

The dark and compact spot observed under UV 254 nm indicates that paracetamol has sufficient UV absorbance due to the presence of its aromatic chromophore structure. The clear spot shape without tailing also suggests that the selected mobile phase system provided adequate separation efficiency and good interaction balance between the stationary and mobile phases.

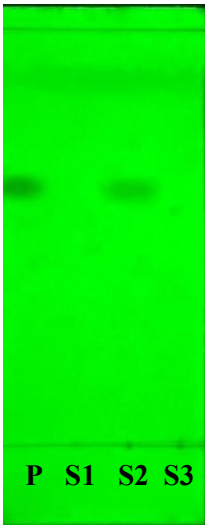

Figure 2. TLC profile of paracetamol standard (P) and *jamu* samples (S1–S3) using silica gel GF254 as the stationary phase and chloroform:ethyl acetate (9:1, v/v) as the mobile phase, observed at 254 nm

Quantitative Determination of Paracetamol by TLC–Densitometry

The TLC analysis of the herbal medicine (*jamu*) sample showed that sample S2 exhibited a chromatographic spot with an average Rf value comparable to that of the paracetamol standard under the same chromatographic conditions. The similarity in Rf values indicates that the detected spot in sample S2 corresponds to paracetamol. This result confirms the presence of paracetamol as an adulterant in the analyzed herbal product. In this study, quantitative determination of paracetamol in the *jamu* sample was carried out using the TLC–densitometric method. This analytical technique offers several advantages, including good specificity, operational simplicity, relatively short analysis time, and low analytical cost [18,19,20]. In addition, TLC allows flexibility in the selection of mobile phase composition, enabling optimization of compound separation in complex herbal matrices.

To obtain accurate quantitative results, the optimal detection wavelength for paracetamol was selected prior to densitometric analysis. The paracetamol standard solution was scanned in the ultraviolet region, and the maximum absorbance was observed at 254 nm. This finding is in accordance with previous studies reporting that paracetamol exhibits strong UV absorption at approximately 254 nm due to the presence of an aromatic chromophore system which undergoes $\pi \rightarrow \pi^*$ electronic transitions in the UV region. Therefore, 254 nm was selected as the analytical wavelength because it provided the highest sensitivity and clearer peak response for densitometric measurement [21].

Method linearity was subsequently evaluated by analyzing a series of paracetamol standard solutions at different concentrations using TLC–densitometry. The relationship between standard concentration and the corresponding peak area (area under the curve, AUC) showed a linear response. The calibration curve produced a linear regression equation of $y = 1 \times 10^{-5}x + 0.002$, with a correlation coefficient (R^2) of 0.9615 (**Figure 3**). Although this value is below the $R^2 \geq 0.99$ criterion generally expected for definitive pharmaceutical quantitative analysis [22]. This R^2 of 0.9615 remains acceptable for semiquantitative screening purposes, consistent with comparable

correlation coefficients reported in previous adulterant screening studies using TLC (e.g., r = 0.9783) [23]. It is important to note that the R^2 value reflects the linearity of the standard calibration curve, which is distinct from the precision of sample measurement. The replicate analysis of sample S2 yielded an RSD of 0.06%, indicating excellent repeatability and low variability in the densitometric response for the actual sample measurement. This high level of precision in replicate sample analysis supports the reliability of the reported paracetamol concentration in S2, despite the comparatively lower linearity observed across the calibration standard series. This result demonstrates that the analytical method is suitable for quantitative determination of paracetamol in herbal samples.

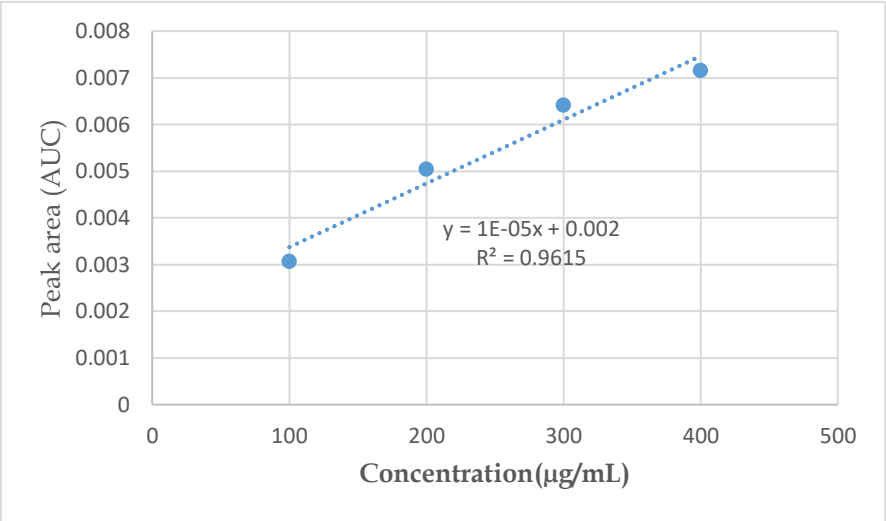

Figure 3. Calibration curve of paracetamol standard

Based on the TLC–densitometric analysis, the peak area obtained from sample S2 corresponded to a calculated concentration of 3563 µg/mL. Considering that 100 mg of sample was diluted to a final volume of 10 mL during sample preparation, the total paracetamol content in the analyzed sample was calculated to be 35.63 mg per 100 mg sample, equivalent to 35.63% w/w. Nevertheless, the detected level was substantially higher than could reasonably be attributed to accidental contamination, providing strong evidence of intentional paracetamol adulteration**.** The detailed quantitative results are presented in **Table 2**.

Table 2. Determination of paracetamol content in *jamu* sample

Sample	Volume (mL)	Weight (mg)	Peak Area	Concentration (µg/mL)	Content (mg/100 mg)	Content (%)
R1	10	100	0.03761	3561	35.61	35.61
R2	10	100	0.03763	3563	35.63	35.63
R3	10	100	0.03765	3565	35.65	35.65
Mean ± SD	-	-	**0.03763 ± 0.00002**	**3563 ± 2.00**	**35.63 ± 0.02**	**35.63 ± 0.02**

The quantitative analysis showed that the paracetamol content detected in the sample was considerably higher than trace contamination levels, suggesting intentional addition rather than accidental cross-contamination during manufacturing. Such adulteration is commonly performed to provide rapid analgesic and antipyretic effects [24], thereby increasing consumer satisfaction [25] and encouraging repeat purchases [26]. However, the undeclared presence of paracetamol in herbal medicine poses serious health risks, particularly when consumed repeatedly or together with other paracetamol-containing medicines. Excessive intake may result in hepatotoxicity due to the accumulation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI) in the liver, which can lead to liver damage, liver failure, and even death [27,28,29].

The close agreement between the Rf value of sample S2 and that of the paracetamol standard further supports the reliability of the identification process. In addition, the linear calibration curve and clear densitometric response demonstrate that the TLC–densitometric method can be effectively applied for both qualitative and quantitative determination of paracetamol in herbal medicine samples. Overall, the results indicate that TLC–densitometry provides a rapid, simple, precise, and cost-effective analytical approach suitable for routine screening, quality control, and regulatory monitoring of herbal products suspected of containing pharmaceutical adulterants.

This study has limitations that should be considered when interpreting the findings. Although three jamu pegal linu samples were analyzed, paracetamol was detected only in sample S2, limiting the ability to draw broader conclusions regarding the prevalence of paracetamol adulteration in herbal products available on the market. Additionally, confirmation using complementary techniques such as HPLC or LC-MS/MS would further strengthen the certainty of the quantitative findings, particularly for regulatory or legal applications.

4. Conclusion

This study successfully identified and quantified paracetamol in jamu pegal linu samples using TLC–densitometry. Qualitative analysis showed that sample S2 exhibited a chromatographic spot and Rf value comparable to the paracetamol standard, confirming its presence as an undeclared adulterant. Quantitative analysis revealed a paracetamol content of 35.63% w/w, indicating a significant level of adulteration that may pose serious health risks to consumers, particularly with prolonged or uncontrolled use. The TLC–densitometric method proved effective, rapid, simple, and economical for routine screening of paracetamol adulteration in herbal products. However, as this method is primarily intended for screening purposes, confirmatory analysis using HPLC or LC–MS/MS is recommended when regulatory or legal decisions depend on the analytical results. Continuous surveillance and stricter regulatory control are therefore strongly recommended to ensure the safety, quality, and authenticity of jamu products in the market.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this paper.

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