Identification of Prednison and Methyl Prednisolon in Weight-Gain Jamu from Online Marketplace Using TLC-Densitometry Method

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Abstract: Jamu or herbal medicine is an ingredient or mixture of ingredients derived from plant ingredients, animal ingredients, mineral ingredients, extract preparations (galenic), or a combination of these ingredients that have been used for generations for healing based on experience. Weight-gain jamu is a traditional medicine that is useful for increasing appetite and body weight. The product is widely used today and marketed through online platforms. This study aims to determine the corticosteroids Prednisone and Methyl Prednisolone that are present in weight-gain jamu using the TLC-Densitometry method. This study used 10 samples of weight-gain jamu purchased from the Shopee e-marketplace with inclusive criteria. Silica Gel GF 254 nm was used as the stationary phase. The mobile phases used are chloroform: ethyl acetate (1:9) for prednisone and water-saturated butanol: toluene: ether (5:10:85) for methyl prednisolone. Spiked samples were performed for confirmation of method validation. The results of this research showed that 2 jamu samples contained prednisone at 247 nm wavelength, and no jamu samples were positive for methyl prednisolone. This method could be applied to detect corticosteroids such as prednisone and methyl prednisolone in weight gain herbal medicine.

Keywords: Prednison, Methyl Prednisolon, Jamu, TLC

1. Introduction

Jamu is used by the people of Indonesia to treat diseases, maintain health and healing, improve physical fitness, and even beautify appearance. The field of medicine has reached a new method of understanding which is believed that all derived from nature can bring good properties for health, rather than chemical products or synthesis (Kementrian Kesehatan Republik Indonesia, 2012). Weight-gain jamu is a traditional medicine that is useful for increasing appetite and body weight. This product is widely used today and marketed through online platforms and prone to abuse for the addition of drug substances.

Drug substances are added to herbal medicine, with the aim of achieving the resulting effects more quickly than usual. Some medicinal chemicals that are often found in herbal medicine are Sildenafil Citrate, Prednisone, Dexamethasone, Phenylbutazone and Paracetamol. Traditional medicines that contain drug substances can be harmful to health or can result in death (Badan Pengawas Obat dan Makanan Republik Indonesia, 2010). Weight-gain herbal medicine

has been reported to contain sibutramine and phenylbutazone (Badan Pengawas Obat dan Makanan, 2017).

Ningsih, (2021) has identified the addition of dexamethasone in Fattening Herbs sold in the Shopee e-marketplace. Dexamethasone is a corticosteroid drug used as an anti-inflammatory agent. The other corticosteroid that are possibly added to jamu especially weight-gain jamu are prednisone and methyl prednisolone. Some research on the adulteration of prednisone in anti-rheumatic jamu, the results of 1 from 5 sample of anti-rheumatic herbs were identified of prednisone using the Thin Layer Chromatography-Densitometry method (Wirastuti et al., 2016). Sugiarti et al., (2017) found the presence of prednisone compounds analyzed using the High Performance Liquid Chromatography method. Identification of prednisone and methyl prednisolone in weight-gain jamu has not been reported recently.

Several methods can be used in analyzing medicinal chemicals (BKO) in herbal medicine such as Gas Chromatography, KCKT / MS, High Performance Liquid Chromatography (KCKT), and Thin Layer Chromatography-Densitometry. The chromatography method has been widely used, because it can separate analytes from the sample matrix which can interfere with analysis. The Thin Layer Chromatography-Densitometry method was used in this study to identify medicinal chemicals (BKO), because of its simple tools, accurate results, a widely used method, and besides that this method is easy and inexpensive (Gandjar, I.G & Rohman, 2009). Thin Layer Chromatography Densitometry commonly selected as identification method for corticosteroid because it uses simple equipment, accurate results, a standard method that is very widely used and this method is relatively easy and cheap (Dołowy et al., 2014; DołowY & Pyka, 2014).

The herbal medicine industry is constrained in the production process and sales of its products. The way of marketing is changing, one alternative way to increase sales capacity is through online digital marketing that focuses on digital marketing through websites used as e-commerce, social media, search engines, and forming a reseller team to sell their products [9]. The aim of this research is to identify the content of prednisone and methyl prednisolone in weight-gain jamu from e-marketplace using the Densitometric Thin Layer Chromatography method.

2. Materials and Methods

2.1.Materials

The tools used in this study were analytical balances (Ohauss), glassware, silica GF 254 (Merck), chambers, fume hoods, microliter syringes (Eppendorf), micropipettes (Eppendorf), pencils, rulers and UV 254 nm and Densitometer (Shimadzu). The materials used in this study were samples of weight-gain jamu obtained from the Shopee e-marketplace, analytical grade solvent : 96% ethanol (Merck), methanol (Merck), chloroform (Merck), ethyl acetate (Merck),

Butanol (Merck), Toluene (Merck), Ether (Merck), Prednisone (Infalabs), and Methyl Prednisolone (Infalabs).

2.2.Method

Samples Collection

This sampling (S1-S10) is carried out by purposive sampling, which is a sampling technique with certain considerations (Lenaini, 2021). The selection of herbal medicine sellers taken as samples have the inclusion criteria such as Jamu sellers who sell their product through the e-marketplace are Shopee with keyword searching "Jamu Penggemuk, Jamu Gemuk, Kapsul Penggemuk", the coverage area is from all of the city in Indonesia. Product claims of efficacy for weight gain are listed on the packaging. The product do not have an Indonesian FDA (BPOM) registration number or a fake registration number. The product is in capsule, not powder or liquid.

Reference Solution

Prednisone is weighed 10 mg and dissolved with 10 mL ethanol 96%. Methyl prednisolone is weighed 10 mg then dissolved and 10 mL methanol. The concentration of reference solutions are 1000 ppm.

Samples extraction

The weight-gain jamu sample weighed approximately 1 gram into a beaker, then 96% ethanol is added for prednisone and methanol for methyl prednisolone approximately 20 mL, then sonicated for 20 minutes, filtered and collected liquid extract from the herbal sample (Wisnuwardhani et al., 2013).

Simulation Sample and Spiked Sample

The simulation sample used jamu that have BPOM registration and used as negative control. Positive controls (spiked sample) by adding extracted negative control with Prednisone or Methyl prednisolone reference solution 1000 ppm.

Identification using Thin Layer Chromatography – Densitometry

Samples extract, reference solution and spiked sample were placed on the Silica GF 254 plate with a size of 20 x 20 cm. On the plate, each solution was placed on about 5 μ L using a capillary pipe with a distance of 1 cm. The plate then put into a saturated chamber containing chloroform : Ethyl acetate (1: 9 v/v) for prednisone and water-saturated butanol: toluene: ether (5:10:85 v/v/v) for methyl prednisolone. Once the eluent reaches the limit of the marked line, remove and dry the plate. The chromatogram result was observed under ultraviolet 254 nm UV light. The stains are compared from reference with samples extracts and note whether there are similarities in the appearance of the stain and calculate the Rf value (Firdaus & Utami, 2009). The scan is performed using Densitometer ranging in wavelength 200 nm - 400 nm.

3. Results and Discussion

In this study, sampling was conducted by buying in an e-marketplace (Shopee) at randomly selected stores. The number of samples is 10 products with different brands (different logos, stamps and packaging). The 10 samples selected were based on inclusion criteria suspected of containing drug substance such as prednisone or methyl prednisolone. All samples are checked on the registration number on the BPOM website. There are 5 samples that list fake registration numbers and 5 samples that do not have registration number. The following samples are marked with numbering from S1 to S10.



Figure 1. Collected Weight-Gain Jamu Samples from Online Marketplace Shopee

Samples that have been selected as mentioned above are extracted using the maceration method with 96% ethanol solvent for prednisone and methanol for methyl prednisolone. This maceration method is able to extract compounds that have thermolabile properties. In addition, this method is simple and fast. Ethanol and methanol are a versatile solvent and very good for extraction. Prednisone has better solubility in ethanol than methanol (Hu et al., 2011). After the screening process, a sample extract is obtained which will be placed on the TLC plate. The best separation of methylprednisolone and prednisolone is in non-water environment with chromatographic system that includes silica gel as a stationary phase and a mixture of acetonitrile and DMSO (Nowakowska et al., 2013). The mobile phase that used in this study are chloroform : Ethyl acetate (1: 9 v/v) (Fitrianasari et al., 2023 with modification) for prednisone and water-saturated butanol: toluene: ether (5:10:85 v/v/v) for methyl prednisolone (Council of Europe, 1999).

С)	0	0		C)	0	С)				
BP	SN	SS	S 1	82	S 3	S4	85	S6	87	S8	S9	S10	

Figure 2. TLC Plate of Prednisone in Weight-Gain Jamu



Figure 3. Densitogram of Prednisone in weight-Gain Jamu Samples (BP=Black, SS=Green, S1=Red, S2=Pink, S5=Yellow, S6=Orange)

Table 1. T	LC and	Densitometric	Result	of Ic	dentification	of Pre	dnisone ir	ı Weight-	Gain
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			Jaillu		
Code	Replication	Manual Rf	Densitogram	λ (nm)	Conclusion
			Rf		
BP	1	0.58	0.72	247 nm	-
(reference)	2	0.65	0.79	248 nm	-
SN	1	-	-	-	Not detected
(Negative	2	-	-	-	
control)					
SS	1	0.58	0.71	248 nm	Positive control
(Positive	2	0.65	0.79	248 nm	

Control)					
S 1	1	0.60	0.74	247 nm	Positive
	2	0.66	0.83	247 nm	
S2	1	-	-	-	Not detected
	2	-	-	-	
S 3	1	0.6	0.73	247 nm	Positive
	2	0.67	0.82	247 nm	
S 4	1	-	-	-	Not detected
	2	-	-	-	
S5	1	0.58	0.73	281 nm	Negative
	2	0.67	0.82	282 nm	C
S 6	1	0.6	0.73	281 nm	Negative
	2	0.67	0.82	281 nm	C
S 7	1	-	-	-	Not detected
	2	-	-	-	
S 8	1	-	-	-	Not detected
	2	-	-	-	
S9	1	-	-	-	Not detected
	2	-	-	-	
S10	1	-	-	-	Not detected
	2	-	-	-	

From the results of separation using the KLT method, there are 4 samples that have the same Rf value as prednisone, meaning that the 4 samples are positive for prednisone. After being identified using KLT-Densitometer, 4 out of 10 samples obtained Rf values equal to or close to prednisone, which is 0.6 (Figure 1). From 10 samples that were measured, there were 2 samples identical to the standard wavelength of prednisone comparison, which is 247 nm (Figure 2), meaning that both samples positively contain prednisone, while the other 2 samples do not contain prednisone, but other substances that have the same Rf value as the prednisone comparison standard.



Figure 4. TLC Plate of Methyl Prednisolone in Weight-Gain Jamu



Figure 5. Densitogram of Methyl Prednisolone in weight-Gain Jamu Samples BP=Black, SS=Green, S1=Red, S2=Pink, S5=Yellow, S6=Orange)

Four jamu samples had the same Rf as the standard of methyl prednisolone but none of the samples detected on the same wavelength as reference. We assume that the spot of the 4 samples are from Prednisone because the wavelength result 249 nm (Table 2) is near with the Prednisone at 247 nm (Table 1). The results obtained from this study show that eventhough using different mobile phases, prednisone and methyl prednisolone provide almost the same Rf. This phenomenon can cause a doubt regarding the positive results of the identified samples so that

further analysis such as densitometry is needed to provide confirmation of the positive results obtained.

Densitometry are challenging for analyzing natural products. Because raw materials contain multicomponent composition that will significantly affect the analysis process, starting from sample preparation techniques that must separate each component well (Renger et al., 2011; Xie et al., 2006). The variability factor in the composition of chemical compounds, influenced by environmental and plant genetic factors, also affects the analysis results (Kusumawati, 2021). TLC densitometry methods can be used for quantitative and qualitative analyses to determine the concentrations of active ingredients in pharmaceutical products (Ramadhani et al., 2023). TLC method is simple, fast, and the operating costs are inexpensive. Hence, it can be used in small laboratories to control adulterated drugs in herbal medicines. Despite the benefit of TLC, the selectivity of TLC is not sufficient for confirmation of illegal adulteration. The selectivity and sensitivity of TLC can be improved by selecting an appropriate detection for analysis such as densitometer (Pratiwi et al., 2021).

Gain Jainu							
Code	Replication	Manual Rf	Densitogram	λ (nm)	Conclusion		
	-		Rf				
BP	1	0.46	0.53	253 nm	-		
(reference)	2	0.47	0.57	253 nm	-		
SN	1	-	-	-	Not detected		
(Negative	2	-	-	-			
control)							
SS	1	0.45	0.54	254 nm	Positive control		
(Positive	2	0.46	0.56	252 nm			
Control)							
S 1	1	0.50	0.61	249 nm	Negative		
	2	0.51	0.62	249 nm			
S2	1	-	-	-	Not detected		
	2	-	-	-			
S 3	1	0.50	0.61	249 nm	Negative		
	2	0.50	0.61	249 nm			
S4	1	-	-	-	Not detected		
	2	-	-	-			
S5	1	0.50	0.61	249 nm	Negative		
	2	0.52	0.62	249 nm			
S6	1	0.51	0.60	249 nm	Negative		
	2	0.52	0.62	249 nm			
S 7	1	-	-	-	Not detected		
	2	-	-	-			
S 8	1	-	-	-	Not detected		
	2	-	-	-			
S9	1	-	-	-	Not detected		

 Table 2. TLC and Densitometric Result of Identification of Methyl Prednisolone in Weight

 Gain Jamu

	2	-	-	-	
S10	1	-	-	-	Not detected
	2	-	-	_	

4. Conclusion

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Two jamu samples contained prednisone at 247 nm wavelength, and none of jamu samples were positive for methyl prednisolone. This method could be applied to detect corticosteroids such as prednisone and methyl prednisolone in weight gain herbal medicine.

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Conflict of Interest

All Authors declare no conflict of interest and agree with the content of the manuscript.

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