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QUALITATIVE AND QUANTITATIVE ANALYSIS OF MEDICINAL CHEMICALS OF "JAMU REMATIK DAN PEGAL LINU" IN MAGELANG REGION

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Abstract: Jamu for rheumatism and "pegal linu" are two of the most commonly used traditional medicine products in the community, so it is a type of traditional medicine preparation that is prone to the addition of medicinal chemicals. Any type of medicinal chemical should not be added to herbal preparations. The purpose of this study was to determine is there any medicinal chemicals in traditional medicine samples for "pegal linu" and rheumatism in the Magelang area. The total number of samples used is nine. Thin layer chromatography as qualitative analysis was used to analyze medicinal chemicals in traditional medicine, and preliminary organoleptic test was performed. The next step is a quantitative analysis using the UV-Visible spectrophotometric method. Prednisone, mefenamic acid, and ibuprofen were used as standards. The stationary phase was silica gel 60 F_{254}, and the mobile phase was ethyl acetate: chloroform (4:1) for the prednisone standard, chloroform:methanol (9:1) for the mefenamic acid standard, and chloroform : ethanol (8:1) for the ibuprofen standard. Thin layer chromatography analysis showed that three of the nine traditional medicine samples tested positive for prednisone, but none contained mefenamic acid or ibuprofen. Herbs A, D, and E contained prednisone in their samples. Prednisone concentrations in the three samples were 0.252%; 0.754%, and 2.005%, respectively.

Keywords: *Ibuprofen, mefenamic acid, prednisone, thin layer chromatography, UV-Visible spectrophotometry*

1. Introduction

Jamu or traditional medicine is a material or combination of materials in the form of plant materials, animal materials, mineral materials, galenic preparations, or a mixture of these materials that have been used for generations for treatment based on experience (Anonim, 2016). In accordance with applicable laws and regulations, isolated or synthetic chemicals with medicinal properties, which are often referred to as medicinal chemicals, are prohibited from

being added to traditional medicines in any form and at any size (Anonim, 2017b). These medicinal chemicals “Bahan Kimia Obat” (BKO) in traditional medicine preparations are used by manufacturers to defraud customers. This might happen due to the manufacturer's ignorance of the dangers of consuming medicinal chemicals uncontrollably and excessively, both in terms of dose and method of use, or it might have been accomplished solely for the objective of increasing sales because consumers would rather traditional medicine products that are effective, safe if used rationally and have an instantaneous impact on the body (Ermawati *et al.,* 2022; Yuliati, 2009).

Furthermore, according to Amsal (2013), the limited number of human resources of the supervisory staff at the “Balai Besar Pengawas Obat dan Makanan” (BBPOM) as a regulatory and supervisory authority for drugs and traditional medicines, as well as the low level of public knowledge about traditional medicines containing medicinal chemicals or failure to meet requirements and the limited active role of the community in implementing oversight mean that medicinal chemicals are still widely available on the market. The side effects of using traditional BKO-containing medicines become visible in the long term due to the increasing dose. Steroid-derived drugs, such as prednisone and prednisolone, may accelerate osteoporosis. The drugs mentioned above are usually added to herbal medicine or traditional medicine with claims to help reduce pain due to sciatica and rheumatism. The larger the dose and the longer the use, the greater the risk of osteoporosis. Prednisone at high or long-term doses can cause bone loss. The most major side effect of steroid treatment is bone loss.

People who used prednisone at a daily dose of 7.5 mg or above had a fivefold increased risk of vertebral fracture compared to those who did not take steroids. Synthetic corticosteroids have a major impact on reducing the effects of inflammation, skin diseases and allergies. Corticosteroid use is restricted to pregnant women. Corticosteroids are thought to interfere with fetal development and the health of pregnant women (Cosman, 2009; Mahardika *et al.*, 2022). The addition of medicinal chemicals increases the risk of cardiovascular disease, hepatotoxicity, gastric ulcer and moon face (Syahfitr *et al.,* 2021). Previous research found that out of 33 jamu samples purchased in Yogyakarta, Kediri, Wonosobo and Kendari, 13 jamu samples contained the chemical drug prednisone (Harimurti *et al.,* 2020; Maharani *et al.,* 2023; Permatasari *et al*., 2021; Sahumena *et al*., 2020; Sukmawati and Sembiring, 2021).

Magelang is one of the towns where numerous regions are known as jamu villages because the majority of locals manufacture and sell jamu concoctions. The presence of jamu dealers implies that jamu consumption in Magelang remains high (Kusuma *et al.,* 2020). Based on this, a qualitative analysis using thin layer chromatography was performed on several herbal preparations of sciatica, followed by a quantitative analysis using a UV-Vis spectrophotometric instrument, so that it can be used as a reference for the community about the safety of several herbal preparations of sciatica and rheumatism circulating in the market, particularly in the Magelang area.

2. Materials and Methods

2.1 Materials

Glassware (Pyrex), vortex (K), silica gel 60 GF_{254} plate (Merck), thin layer chromatography chamber, micropipette (Dragonlab), UV lamp, UV-Visible spectrophotometer (Cecil Aureus CE 7400), cuvette (Starna), analytical balance (Ohaus), ethanol 96% (Bratachem), methanol (Merck), ethanol pro analysis (Merck), chloroform pro analysis (SmartLab), ethylacetate pro analysis (Merck), prednisone standard (Sigma Aldrich), mefenamic acid standard (Sigma Aldrich) and ibuprofen standard (Sigma Aldrich).

2.2 Sampling, Extraction and Organoleptic Test of Herb Samples

The total number of samples used was nine (A, B, C, D, E, F, G, H, and I) from various brands. The first test was an organoleptic test, which included the fragrance of the preparation as well as the form, color, and taste of the herbal medicine sample (Anonim, 2017a). After that, nine herbal medicine samples as weighed of 1000mg were extracted with a 96% ethanol solvent. Each herbal medicine sample was weighed, then dissolved in 50 cc of 96% ethanol and macerated for 1x24 hours. The maceration process will be accompanied by 2 minutes of vortex stirring. The liquid extract obtained is then filtered and then evaporated to obtain thick ethanol extract according to Simaremare et al., (2018) with modifications.

2.3 Preparation of Standard Solution

Prednisone, mefenamic acid, and ibuprofen in powder form were used as standards. Each standard powder was carefully weighed up to 10 mg and then placed in a 10 ml measuring flask. The standard powder was dissolved in 96% ethanol up to the limit mark, resulting in a standard solution with a level of concentration of 1000 ppm sesuai Haresmita & Pradani, (2022) dengan modifikasi.

2.4 Thin Layer Chromatography Analysis of Herb Samples

Thin layer chromatography was the method of choice for qualitative analysis. The sample and reference solutions were taken on a thin layer chromatography plate using a mobile phase and stationary phase according to Fikayuniar and Abriyani (2020) and Mustarichie *et al.*, (2017) with slight modification (Table 1)

Table 1. Mobile Phase System of Each Standard Chemical Drug

Standard	Prednisone	Mefenamic Acid	Ibuprofen
Mobile phase	Ethyl acetate: chloroform (4:1)	chloroform: methanol (9:1)	chloroform: ethanol (8:1)
Stationary phase	Silica gel 60 F_{254}	Silica gel 60 F_{254}	Silica gel 60 F_{254}
Spots	10µl	10µl	10µl

2.5 Determination of Maximum Wavelength for Prednisone

The 1000 ppm prednisone stock solution was diluted to 1.5 mL in a 10 mL volumetric flask, and ethanol was added to the limit mark to achieve a level of 150 ppm. A visible light spectrophotometer was used to measure the absorbance of the 150 ppm solution at a wavelength of 200-400 nm.

2.6 Determination of the Prednisone Standard Curve

The 1000 ppm prednisone stock solution was transferred into a 10 ml volumetric flask in quantities of 100, 120, 140, 160, 180, and 200 µl using a micropipette. The stock solution was then added to ethanol until the limit mark was attained, obtaining levels of 10, 12, 14, 16, 18, and 20 ppm. This solution was homogenized, and the absorbance was measured using a visible light spectrophotometer at the maximum absorption wavelength of prednisone. The next step is to generate a calibration curve in order to determine the linear regression equation

2.7 Data Analysis

a. Standard Curve Linearity Calculation

The linear regression equation $y = a + bx$ is used to determine linearity. This regression equation can be used if the correlation factor has a value of 0.99 and $r \leq 1$ (Riyanto, 2014).

b. Determination of Prednisone Level in Samples

The absorbance data of the samples were entered into the linear regression equation obtained from the prednisone standard curve, namely $y = a + bx$ so that the calculated levels were obtained as the prednisone levels in the samples.

Sample prednisone levels were calculated with the equation =

$$\frac{\text{sample concentration (ppm) X solvent volume (L) X dilution factor}}{\text{Mass of sample (mg)}} \text{ X } 100\%$$

(Anonim, 2017a)

3. Results and Discussion

3.1 Extraction and Organoleptic Test of Herbal Samples

The total number of samples used was nine (A, B, C, D, E, F, G, H, and I) with different brands. This is done to demonstrate that the samples utilized are genuine herbal medicine samples based on their color, taste, form, and fragrance. All of the samples were in the shape of fine powder and reeked of herbal medicine. For taste, all taste bitter and for color, it varies from yellow, greenish yellow, dark yellow, to brownish yellow. Organoleptic tests of pain and rheumatic herbal medicine samples range from pale yellow to light brown, with additional colors of yellow, gray, brown, greenish yellow, and greenish brown, and tastes ranging from bitter, sweet, spicy bitter, cold bitter, and tasteless (Permatasari *et al.*, 2021; Rusmalina *et al.*, 2020). The organoleptic test findings indicate that the herbal medicine samples utilized contain a variety of herbs. Table 2 shows the results of organoleptic tests on herbal medicine samples.

Table 2: Herbal Sample Organoleptic Test Results

Sample	Odor	Taste	Organoleptic	Color
A	typical herbal medicine	Bitter	Fine powder	Greenish Yellow
B	typical herbal medicine	Bitter	Fine powder	Yellow
C	typical herbal medicine	Bitter	Fine powder	Yellow
D	typical herbal medicine	Bitter	Fine powder	Dark Yellow
E	typical herbal medicine	Bitter	Fine powder	Yellow
F	typical herbal medicine	Bitter	Fine powder	Yellow
G	typical herbal medicine	Bitter	Fine powder	Brownish Yellow
H	typical herbal medicine	Bitter	Fine powder	Yellow
I	typical herbal medicine	Bitter	Fine powder	Yellow

Maceration was chosen because this method is simple, fast, no special instruments are needed but effective. Maceration does not require any external heat and is simple to perform. Stirring using a vortex is intended to improve the efficiency of the maceration process. The solvent chosen is ethanol with a concentration of 96%. Ethanol is a universal solvent, and compounds with extremely varied polarity, ranging from non-polar to polar, will dissolve in it. Ethanol 96% is a solvent that is much safer than other solvents and with 96% content will be easily evaporated by heating (Anonim, 2000). The result of the extraction process is a concentrated extract that is ready to be spotted on a thin layer chromatography plate.

3.2 Thin Layer Chromatography Analysis of Herbal Samples

The thin layer chromatography method is used for the following analysis. Chromatography is defined as a method of separating solutes using a dynamic differential displacement process in a two-phase system (mobile phase and stationary phase). Solubility, adsorption, vapor pressure, partition, molecular size, and ion charge density differ amongst compounds that move in both phases. These factors will influence compound mobility in the two-phase system described above. In the thin layer chromatography method, estimates and conclusions are generated by comparing the spot of the sample with the spot of the standard of the medicinal compounds utilized (Anonim, 2017a). The thin layer chromatography system chosen was silica gel 60 F254 as stationary phase and as mobile phase was a mixture of two organic solvents. Silica gel 60 was chosen as the polar stationary phase. The combination of two mobile phases was designed so that the separation was able to be easily optimized. Prednisone, mefenamic acid, and ibuprofen were used as controls. The three pain relievers are three types of pain relievers that are widely used by the community so it is likely that the three types of drugs above are added to herbal medicine samples. Three of the nine herbal medicine samples tested positive for prednisone, while all tested negative for mefenamic acid and ibuprofen. Table 3 summarizes the outcomes of the qualitative analysis of herbal medicine samples using three BKO standards.

Table 3. Qualitative Analysis Results of Herbal Samples with 3 Medicinal Chemical Standards

Standard / Sample	Prednisone	Mefenamic Acid	Ibuprofen
A	+	-	-
B	-	-	-
C	-	-	-
D	+	-	-
E	+	-	-
F	-	-	-
G	-	-	-
H	-	-	-
I	-	-	-

Description: (+) = positive for the chemical substance of the drug
(-) = negative contains the chemical drug

Herbal medicine samples that showed positive results for prednisone were herbal medicine samples A, D and E. Samples A, D, and E of medicinal products had the same Rf value as the prednisone standard, which was Rf 0.54. Furthermore, the color of the spot in the three samples matches the color of the prednisone standard spot. The thin layer chromatography method was chosen because this method is simple, cheap and easy (Haresmita *et al.*, 2023). Figure 2 depicts the chromatogram of sample elution data and the prednisone standard.

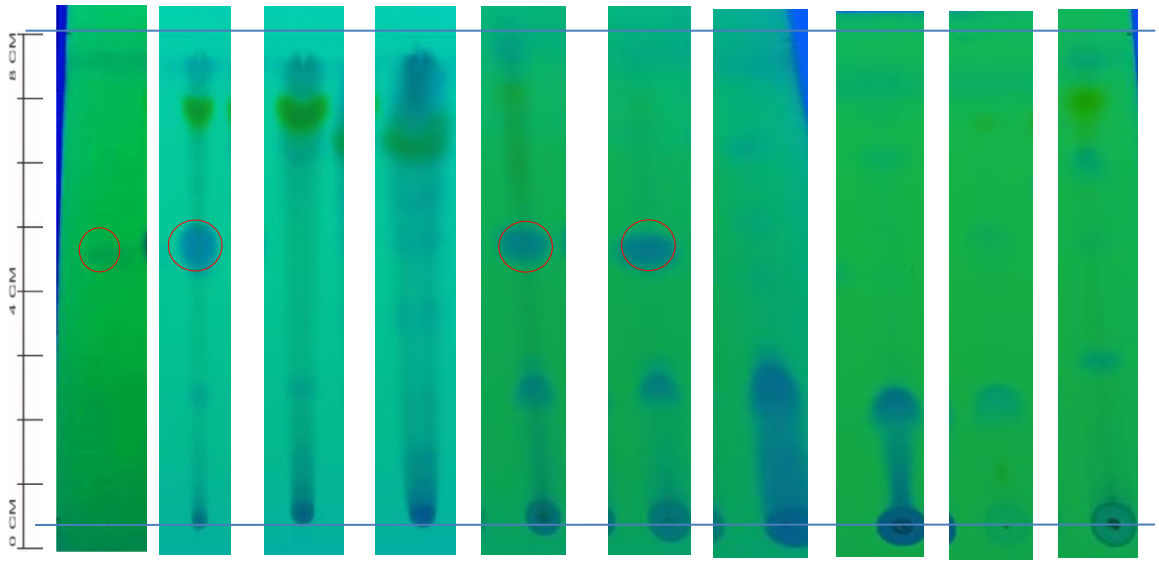

Figure 2. Chromatogram of Sample and Prednisone Standard Elution Results

3.3 Determination of the Maximum Absorbance Wavelength and Prednisone Standard Curve

Herbal medicine samples that tested positive for the chemical substance prednisone were subjected to quantitative analysis. The uv-visible spectrophotometry method was applied for the quantitative analysis. The first step was to determine the maximum absorbance wavelength of prednisone. The maximum absorbance wavelength was determined to be 150 ppm, whereas the maximum absorbance wavelength of prednisone was 237.5 nm. The maximum absorbance wavelength was used to determine the prednisone standard curve and determine the prednisone content in herbal medicine samples. The prednisone standard curve was determined using prednisone standard solution with levels of 10, 12, 14, 16, 18 and 20 ppm. The absorbance of a series of prednisone solution levels was then measured at a wavelength of 237.5 nm. Measurement of the standard curve obtained the results of a linear regression equation is y = 0.0354x - 0.017 with a value of R2 = 0.9972. The ability of an analytical method to respond directly or indirectly through good and proportional mathematical transformations to the levels of analytes in a sample is usually referred to as linearity. A linear relationship is called ideal if it has a value of b = 0 and r = 1 or -1 depending on the direction of the line (Harmita, 2004). Figure 3 depicts the standard curve indicating the correlation between prednisone levels and absorbance.

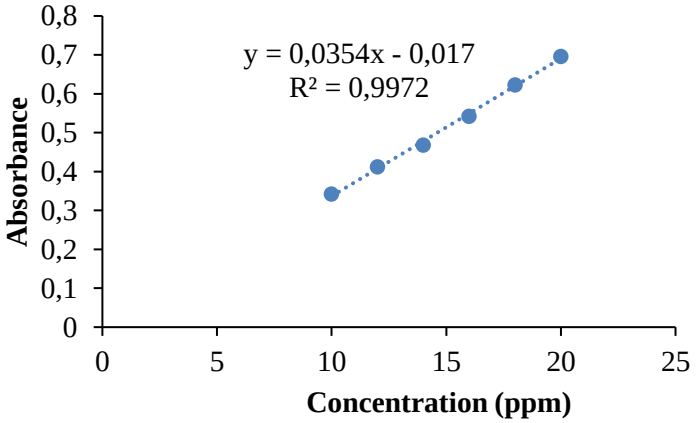

Figure 3: Standard Curve for Prednisone Level vs. Absorbance

3.4 Prednisone Level Determination in Herbal Samples

Further quantitative analysis was conducted in order to determine the amounts of prednisone medicinal compounds detected in herbal medicine samples A, D, and E. The extracts were processed, and their absorbance values were determined using UV-Vis spectrophotometry. To determine prednisone levels in herbal medicine samples, the absorbance outcomes were incorporated into a linear regression equation. The absorbance of each sample was 0.879; 0.867; 0.869 (sample A), 0.249; 0.253; 0.248 (sample D) and 0.753; 0.663; 0.662 (sample E). The measurement results of prednisone levels in herbal medicine samples were 0.252%; 0.754% and 2.005% with values for herbal medicine A, D and E, respectively. Table 4 shows the findings of qualitative and quantitative analysis of medicinal compounds in herbal medicine samples.

According to a study conducted in Jayapura City, Papua Province, one of the six samples of pain-relieving herbal medicine examined contained paracetamol and three other samples contained mefenamic acid (Simaremare *et al.,* 2018). Ibuprofen and paracetamol were found in 16 of 20 jamu samples tested in Banjarmasin City, South Kalimantan Province (Kumalasari *et al.,* 2018; Rahmadani and Alawiyah, 2021). Research conducted in Mataram, West Nusa Tenggara showed that, of the 10 herbal medicine samples studied, 2 herbal medicine sample contained prednisone chemicals with a level of 0.249 and 0,197mg/sample (Lestari, 2019). Researchers conducting herbal medicine adulteration study in West Karawang, West Java Province discovered that all seven samples tested positive for prednisone pharmaceutical ingredients, with the highest level being 0.0783% (Fikayuniar, 2021). Herbal medicine samples acquired from Semarang region were also found to contain medicinal chemicals at a rate of 60% (3 samples out of 5 samples were positive for medicinal chemicals) (Padanun and Minarsih, 2021). The amount of medicinal chemicals in herbal medicine samples varies, yet it is still illegal because herbal medicine should not contain medicinal chemicals. For further research, existing herbal medicine samples can be tested with other medicinal chemicals and using other methods.

Table 4. Results of Qualitative and Quantitative Analysis of Medicinal Chemicals in Herbal Samples

Sample	Qualitative test	Rf	Prednisone (% w/v)
Mefenamic Acid	Standar	0,81	
Prednisone	Standar	0,54	
Ibuprofen	Standar	0.86	
A	+	0,54	0,252
B	-	-	-
C	-	-	-
D	+	0,54	0,754
E	+	0,54	2,005
F	-	-	-
G	-	-	-
H	-	-	-
I	-	-	-

4. Conclusion

According to the findings of qualitative and quantitative analyses on nine samples of herbal medicine circulating in Magelang District, three of the samples had prednisone chemicals, whereas none of the samples included mefenamic acid or ibuprofen chemicals. The three prednisone-containing samples were A, D, and E, with average values of 0.252%, 0.754%, and 2.005%, respectively. From the above data, the public should be vigilant and careful in consuming herbal medicine circulating in the Magelang region.

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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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