

Analysis of Sildenafil Citrate in Herbal for Men and Herbal for Stamina Enhancers Using High Performance Liquid Chromatography

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ABSTRACT: Jamu is a traditional Indonesian medicine that has been used for generations based on experience, using raw materials that have not been standardized. In accordance with existing laws and regulations, traditional medicines are prohibited from adding isolated chemicals or synthetic compounds with medicinal properties known as medicinal chemicals (BKO). Sildenafil citrate is one of drugs that are often added to herbal medicine samples. The purpose of adding sildenafil citrate for “jamu” for men and stamina enhancers is to improve the quality and duration of consumer erections. The purpose of this study was a qualitative and quantitative analysis of sildenafil citrate in samples of herbal for men and herbal for stamina enhancers circulating in the Magelang area, Central Java. The method chosen was thin layer chromatography with a stationary phase of silica gel F₂₅₄ with two different mobile phase systems, namely methanol: chloroform (1:1) and (4:1) and high-performance liquid chromatography for quantitative analysis with a stationary phase of a C18 column and a mobile phase of methanol: acetonitrile (70:30) with a flow rate of 1 ml/min. The results of the study showed that 1 in 3 herbal medicine samples contained sildenafil citrate with an average level of 0.75%.

Keywords: herbal for men; stamina; sildenafil citrate; HPLC; TLC

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INTRODUCTION

Jamu is one of the prides of the Indonesian people. In more detail, jamu or traditional medicine is an ingredient or mixture of ingredients in the form of plants, animal origin, mineral ingredients, in the form of juice (galenic) or a combination of these ingredients that have been used for generations for treatment based on experience (Anonim, 1986). In accordance with applicable laws in Indonesia, traditional medicines are prohibited from being added with isolated chemicals (isolates) or synthetic compounds with medicinal properties, which are often referred to as medicinal chemicals (BKO) (Anonim, 2012). BKO in the preparation of herbal medicine or traditional medicine is a gap for producers in marketing their products. One of the causes is the producers' ignorance of the dangers of consuming herbs containing excessive and uncontrolled BKO, both in terms of dosage and method of use or even solely for the sake of increasing sales, which is important to sell, because consumers like herbal products or traditional medicines to react quickly and "cespleng" for the body (Hung, 2020).

The government, in this case the Balai Besar Pengawas Obat dan Makanan (BBPOM) as an extension of the government, continues to educate local entrepreneurs, take samples for investigation and prosecution, but there are several obstacles that accompany it (Hung, 2020). According to Amsal, (2013) and Sudewi et al., (2020), legal protection for herbal medicine consumers has been carried out since the beginning of herbal medicine products in circulation, starting from making herbal medicine until finally herbal medicine is marketed, but the obstacle faced is the limited number of supervisory personnel owned by BBPOM as a regulator and supervisor. The limited number of supervisory personnel is not proportional to the number of hundreds or even thousands of herbal medicine products circulating in the community so that there are still many herbal medicines containing BKO circulating in the community. In addition, the low level of public knowledge about safe and efficacious traditional medicines and the low level of active participation of the community in product supervision also contribute to why BKO in herbal medicine is still widely found. The side effects of using herbal medicine or traditional medicine containing BKO will be seen over a long period of time due to the increasingly accumulated.

Sildenafil citrate is one of the classes of drugs that should be used based on a prescription issued by a doctor. Runtutahu et al., (2024) conducted research on 5 samples of jamu purchased in the Ungaran area, Central Java and identified that 3 of the 5 jamu samples contained the medicinal chemical sildenafil citrate. The purpose of adding sildenafil citrate for jamu producers is to improve the quality and duration of consumer erections (Umniyatul & Sambodo, 2019). Excessive use of sildenafil citrate can result in harmful unwanted effects such as visual and hearing impairment, stroke, heart attack and even death (Hakim et al., 2022).

Previous research found that herbal medicine samples taken from the market were positive for the medicinal chemical sildenafil citrate with a choice of TLC and TLC-densitometry methods (Setiawan et al., 2020; Triadisti & Heldawati, 2018; Umniyatul & Sambodo, 2019). Research conducted in West Bogor and Tanah Sareal Districts, West Java, showed that out of 32 herbal medicine samples, 5 samples were positive for sildenafil citrate (Sumiati et al., 2017). Other studies have shown that the determination of medicinal chemical levels in samples can be done by thin layer chromatography method followed by high performance liquid chromatography with good results. These two method options underlie the research to be conducted. Qualitative and quantitative analysis and validation

of methods have been carried out by many other researchers but for the herbal medicine samples studied, there have never been herbal medicine samples circulating in the Magelang Region. This study was conducted to do qualitative and quantitative analysis of sildenafil citrate suspected to be contained in samples of male strength and stamina enhancing herbs distributed in the Magelang Region, Central Java.

MATERIALS AND METHODS

Materials

Samples of “jamu” for men and stamina, glass laboratory equipment (Pyrex), water bath (Mettler), micropipette (Dragonlab), UV lamp, thin layer chromatography plate silica gel 60 F₂₅₄ (Merck), TLC chamber, filter paper, high performance liquid chromatography (Agilent 1220 Infinity II), C18 column (Zorbax), digital balance (Ohaus), vortex (K), ethanol 96% (Brataco Chemicals), methanol for HPLC (Sigma Aldrich), acetonitrile for HPLC (Sigma Aldrich), methanol p. a (Merck), ethanol p.a (Merck), chloroform p.a (SmartLab), methanol p.a (Merck), syringe filter, distilled water, and sildenafil citrate standard (Sigma Aldrich).

Research Process

Collection, organoleptic test and extraction of herbal samples

Samples of “jamu” for men and stamina enhancers were purchased from several areas of Magelang, Central Java. The samples amounted to 3 samples with various brands. Organoleptic test is an initial test conducted to examine the dosage form, color, taste and smell of herbal samples (Anonim, 2017). The next step is to extract the herbal medicine samples with 96% ethanol solvent. Herbal medicine samples weighing 1000 mg were weighed carefully and dissolved in a 50 ml volumetric flask with 96% ethanol solvent. The extraction method chosen was maceration for 24 hours. This sample was vortexed for two minutes while it was being macerated. The liquid extract obtained was then filtered with filter paper and evaporated using a water bath to obtain a thick ethanol extract according to Simaremare et al., (2018) with modifications.

Preparation of sildenafil citrate standard solution

Sildenafil citrate standard was used in pure powder form. Standard powder was carefully weighed as much as 10 mg and dissolved with 96% ethanol solvent. After the standard is completely dissolved, ethanol is added to the mark limit so that the concentration of sildenafil citrate standard solution is 1000 ppm according to (Fatmawati et al., 2023) with modifications.

Thin layer chromatography analysis

Samples of “jamu” for men and stamina enhancers and sildenafil citrate standard solution were blotted on silica gel F₂₅₄ plates for thin layer chromatography analysis with the two mobile and stationary phase systems below to ensure that the medicinal chemicals were indeed contained in the samples. Table 1 shows the thin layer chromatography systems used.

Thin layer chromatography analysis was performed visually by comparing the spots resulting from the elution process of both mobile phase systems. The spot parameters that were compared were the color of the spot and the distance of Retention Factor (R_f) with the spot of the result of the elution on the standard solution of sildenafil citrate viewed under UV₂₅₄ light.

Table 1. Mobile Phase Systems for Sildenafil Citrate Standard

Standard	System 1	System 2
Sildenafil citrate	Methanol: chloroform (1:1)	Methanol: chloroform (4:1)
Stationary Phase	Silica gel 60 F254	Silica gel 60 F254
Volume	10 μ l	10 μ l

Preparation of sildenafil citrate standard curve

Standard solution of sildenafil citrate at 1000 ppm was taken with a micropipette as much as 200; 400; 600; 800, and 1000 μ l, then put into a 10 ml volumetric flask and dissolved with 96% ethanol solvent. The standard solution was then added with ethanol up to the limit mark in the volumetric flask to produce levels of 20, 40, 60, 80 and 100 ppm respectively. These solutions were homogenized and then injected into the high-performance liquid chromatography to obtain the area under curve (AUC) so as to obtain the sildenafil standard curve and its linear regression equation can be determined. Linearity can be established through the linear regression equation $y = bx + a$. If the correlation factor has a value of 0.99 and $r \leq 1$, the linear regression equation of the research results can be used (Riyanto, 2014).

High performance liquid chromatography analysis

The ‘jamu’ samples that proved positive for sildenafil citrate based on the thin layer chromatography method were then analyzed quantitatively. Quantitative analysis of sildenafil citrate in the ‘jamu’ samples was carried out with a reversed-phase high-performance liquid chromatography instrument. The stationary phase selected was a 150 mm x 3.9mm and 5 μ m C18 Zorbax column. For the mobile phase, a solvent mixture of methanol: acetonitrile (70:30) was selected with a flow rate set at 1mL/min and an injection volume of 20 μ l. The detector wavelength used was 290 nm. The pump pressure was 1.0 mL/min and the injection volume was 20 μ L according to Hakim et al., (2022) with modifications. The data of area under the curve (AUC) values were entered into a linear regression equation $y = bx + a$ to obtain data concentration.

RESULT AND DISCUSSION**Organoleptical test and extraction**

A total of 3 samples of “jamu” for men and stamina enhancers were used (M, N and O) with different product names. Sample M contains ginseng extract, “pasak bumi” extract, ginger extract and areca nut extract. Sample N contains nutmeg extract, ginseng extract, yohimbe extract, ginger extract, “temulawak” extract and “pasak bumi” extract while sample O contains ginseng extract, “pasak bumi” extract, Java chili extract and “tapak liman” extract. Organoleptical test is an initial test conducted to examine the dosage form, color, taste and smell of herbal medicine samples (Anonim, 2017). This is done to illustrate that the sample used is a true sample of herbal medicine, seen from the color, taste, shape and smell of the sample. The results of organoleptical testing are shown in Table 2.

Table 2. Results of Organoleptic Test

Organoleptic Sample	Color	Taste	Aroma	Dosage Forms
M	White	Bitter taste	Vanilla	Capsule
N	Cream	Bitter taste	Ginseng	Capsule
O	Yellowish Brown	Spicy	Ginseng	Capsule

The three samples of “jamu” for men and stamina enhancers examined in this study were presented in capsule form. Each sample consisted of fine powder and exhibited a characteristic herbal aroma. In terms of flavour, two samples were noted for their bitterness, while one sample was identified as spicy. The flavour profile of the samples is influenced by their constituent ingredients. Variations in taste can include bitter, cold bitter, spicy bitter, slightly bitter, sweet, and tasteless (Permatasari et al., 2021; Rusmalina et al., 2020). The color of herbal medicine samples varies from green, brown, light brown, yellow and greenish brown. The color of this sample certainly shows the color of the simplisia in the sample (Haresmita et al., 2023).

Extraction and qualitative analysis by thin layer chromatography

“Jamu” samples were dissolved in 96% ethanol solvent to obtain ethanolic extracts of herbal samples. The solvent used is a universal solvent, namely ethanol. Ethanol is polar so that the compounds that are extracted are non-polar compounds to polar compounds in “jamu” samples. Three ethanolic extracts of herbs were photographed on a thin layer chromatography plate and compared not only the distance but also the color of the samples spot and spot of sildenafil citrate standard solution. Silica gel 60 F₂₅₄ was selected for the stationary phase and a mixture of two organic solvents as the mobile phase was the chromatographic system used. Silica gel 60 F₂₅₄ thin layer chromatography plate was used as stationary phase which is polar in nature.

A mixture of two mobile phases was used for easy optimization so that the separation was as desired. The mobile phase mixtures used were methanol: chloroform (1:1) and (4:1). These two systems were used to ensure that there were medicinal chemicals added in the “jamu” for men and stamina enhancer samples. This mixture of 2 systems was also chosen because it has shown differences in the elution profile results by producing different standard R_f, namely 0.65 for system 1 with the mobile phase methanol: chloroform (1: 1) and R_f 0.43 for system 2 with the mobile phase methanol: chloroform (4: 1). The standard used was pure sildenafil citrate in powder form. The elution results of thin layer chromatography method of three samples and standard sildenafil citrate can be seen in Figure 1.

The elution results show that there is one sample that contains sildenafil citrate, namely sample N because the sample has an R_f that is parallel to the standard R_f in both mobile phase mixtures with the same R_f difference or less than 0.02 cm. In addition, the color of the spot on sample N is also the same as the color of the sildenafil citrate standard spot. R_f calculation if it has an R_f value equal to or equal to 0.02 means that the sample contains a predetermined standard (Syamsul et al., 2018). Sample N has an R_f in system 1 of 0.65 while the standard R_f is 0.64. For system 2, the sample R_f is 0.43 and the standard R_f is also 0.43. Both R_f of sample N had a difference with the standard R_f of 0.02 at most so sample N was suspected to contain sildenafil citrate. R_f calculation data of standard and sample can be seen in Table 3.

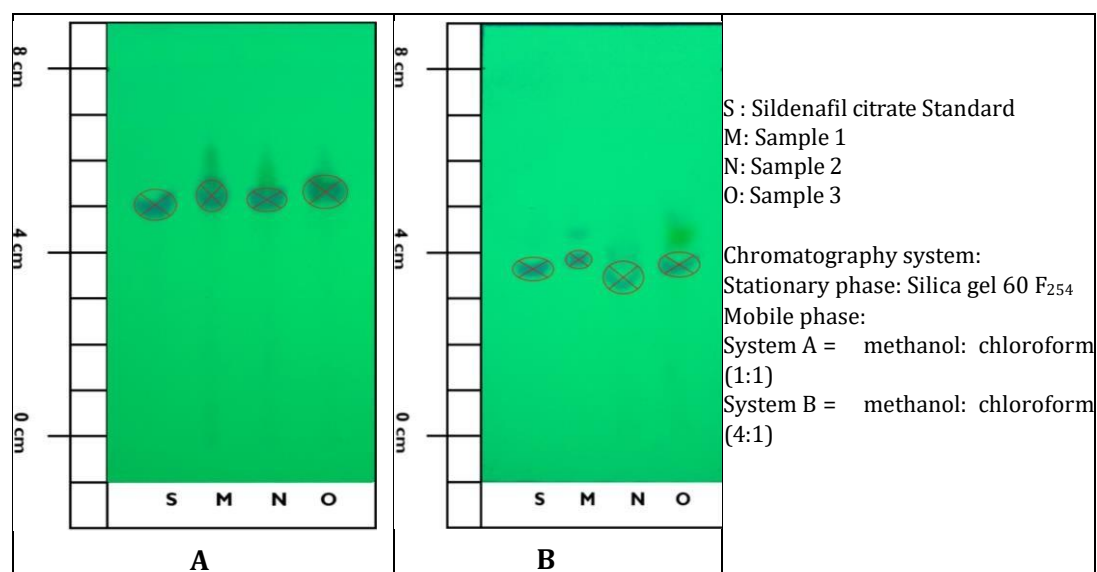


Figure 1. Elution Results of “Jamu” Samples and Sildenafil Citrate Standards with 2 Chromatography Systems

Table 3. Standard and Sample Rf Data

System A	A	B	Rf	Difference with standard Rf	($\leq \pm 0.02$)	System B	A	B	Rf	Difference with standard Rf	($\leq \pm 0.02$)
S	5.1	8	0.64	0.00	+	S	3.4	8	0.43	0.00	+
M	5.3	8	0.66	0.02	+	M	3.8	8	0.48	0.05	-
N	5.2	8	0.65	0.01	+	N	3.4	8	0.43	0.00	+
O	5.4	8	0.68	0.04	-	O	3.8	8	0.48	0.05	-
System A		= methanol: chloroform (1:1)									
System B		= methanol: chloroform (4:1)									
A		= sample distance									
B		= total distance of TLC plate									
S		= Standard									

High performance liquid chromatography analysis

“Jamu” samples that have been qualitatively analyzed to contain sildenafil citrate, namely sample N, are then further analyzed by the high-performance liquid chromatography (HPLC) method. The stationary phase used was a C-18 non-polar column, while the selected mobile phase was a mixture of methanol: acetonitrile (70:30) solvent. The flow rate was set at 1 mL/min with an injection volume of 20 μ L. The detector wavelength used was 290 nm. The system used was a reverse phase system. HPLC is a separation method that can also be used for quantitative analysis.

Sildenafil citrate standards were injected at 20, 40, 60, 80 and 100 ppm to create a standard curve and determine the linear regression equation. The 50ppm level was also injected into the high-performance liquid chromatography instrument for the initial injection to determine the retention time of sildenafil citrate. From the initial injection of 50 ppm, the retention time of sildenafil citrate was 1.88 minutes. The injection results of the 20-100 ppm range produced area under the curve (AUC) values of 545.2; 1000.2;

1575.2; 1951.2; and 2578.9 with a linear regression equation $y = 25.029x + 24.63$ with R^2 values of 0.9952 and R is 0.997. A linear and proportional relationship is considered ideal if it has a value of $R = +1$ or -1 depending on the direction of the line formed (Harmita, 2004). The standard curve of sildenafil citrate can be seen in Figure 2.

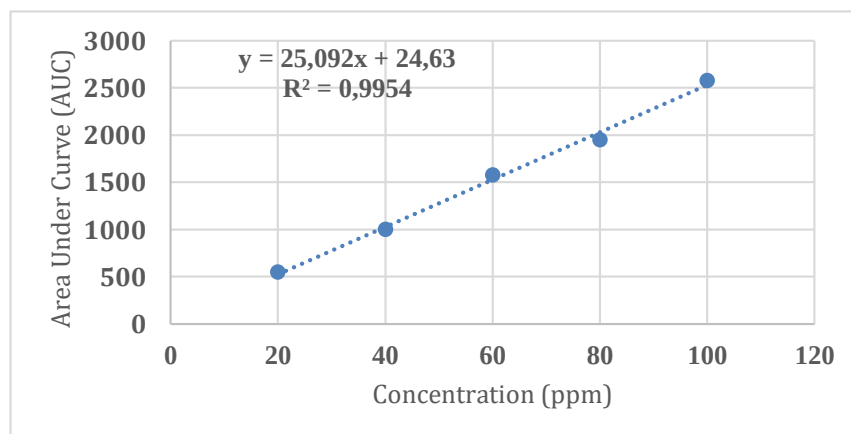


Figure 2. The standard curve of sildenafil citrate

The results of calculating the area under the curve using high-performance liquid chromatography showed consecutive data of 407.70; 428.43 and 456.83 and resulted in sildenafil citrate levels in sample N of 0.05%; 0.35%; and 1.85% with an average level of 0.75%. The retention time obtained was 1.723 minutes.

Sildenafil citrate is an oral medication used for erectile dysfunction by functioning as an inhibitor of the phosphodiesterase type 5 (PDE5) enzyme. Some side effects commonly reported when taking this drug include skin flushing, headache, dyspepsia and visual impairment. Sildenafil citrate has characteristics as a vasodilator but has no effect on heart rate (Cruz-Burgos et al., 2021; Verma et al., 2019). Research conducted in Ungaran, Central Java using the UV-Vis spectrophotometric method showed that 3 out of 5 samples of male herbal medicine and male stamina contained sildenafil citrate with consecutive levels of 0.53%; 0.23%, and 0.16% (Elsan & Minarsih, 2022).

Another study from Jayapura City, Papua Province stated that 3 out of 4 herbal medicine samples were also positive for sildenafil citrate with levels of 0.11%; 0.10 and 0.02% which were also determined by TLC and UV-Vis spectrophotometry methods (Simaremare et al., 2018). Another study in Bumiayu, Central Java also revealed that of the 7 samples studied all proved positive for sildenafil citrate with the same method used in this study, namely TLC for qualitative analysis methods and HPLC for quantitative analysis methods. The sildenafil citrate levels in the samples varied from 33.44 to 104.78 mg/capsule (Hakim et al., 2022). Previous research plus this study showed that there are still strong male herbs and enhancers containing medicinal chemicals circulating in the community, both in Central Java, West Java and other regions. The public as consumers need to be wise in consuming herbal products and ensuring that herbal products are safe (Sudewi et al., 2020).

CONCLUSION

One of 3 samples of jamu marketed in Magelang area, Central Java based on qualitative analysis using thin layer chromatography (KLT) method was positive for sildenafil citrate and based on quantitative analysis using high performance liquid chromatography (HPLC) contained sildenafil citrate with an average level of 0.75%. We recommend for the future research to do the method validation for sildenafil citrate analysis.

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AUTHOR CONTRIBUTION

PPH : Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.

AKW : Definition of intellectual content; literature search; experimental studies; data analysis.

MPKP : Experimental studies; manuscript editing; manuscript review.

CONFLICT OF INTEREST

None to declare.

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