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Analysis of sildenafil citrate in male stamina herbal medicines from the Magelang region, Central Java using thin-layer chromatography and UV spectrophotometry

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ABSTRACT

Background: Male stamina herbal medicines are widely consumed in Indonesia, often believed to enhance sexual performance. However, the illegal addition of medicinal chemicals (BKO) such as sildenafil citrate in traditional herbal products poses health risks.

Objective: To identify and quantify sildenafil citrate in selected herbal medicine samples using thin-layer chromatography (TLC) and UV spectrophotometry.

Method: Three samples of male stamina herbal medicine were purchased and subjected to organoleptic tests. Ethanol (96%) was used for extraction, followed by TLC analysis using two mobile phases (methanol: chloroform at 4:1 and 1:1 ratios). UV spectrophotometry was employed to determine the maximum absorbance wavelength and quantify sildenafil citrate.

Results: TLC analysis revealed that two samples (K and L) contained sildenafil citrate, with R_f values consistent with the standard. UV spectrophotometry confirmed the presence of sildenafil citrate at a maximum wavelength of 292.5 nm. Sample K contained 0.96%, while sample L had 13.766% sildenafil citrate.

Conclusion: The presence of sildenafil citrate in two samples violates Indonesian regulations prohibiting medicinal chemicals in herbal products, raising significant safety concerns.

Keywords: sildenafil citrate, male stamina herbal medicine, thin-layer chromatography, UV spectrophotometry, BKO

Introduction

According to the Indonesian Ministry of Health Regulation No. 003/Menkes/Per/1/2010, *jamu* refers to natural remedies made from plant, animal, mineral, or galenic substances used for medicinal purposes. Many Indonesians consume *jamu* due to its traditional reputation for preventing illness, maintaining overall health, and enhancing physical stamina. Male stamina herbal medicine, a specific category of *jamu*, are

formulated from natural ingredients and are believed to enhance sexual performance in men [1]. Importantly, the use of medicinal chemicals (*bahan kimia obat* or BKO) is prohibited in herbal medicine by Indonesian law.

BKOs are chemical substances often added to *jamu* to increase their therapeutic effects. However, their use, particularly in products such as phenylbutazone, paracetamol, sildenafil citrate, dexamethasone, prednisone, glibenclamide, and theophylline, poses significant health risks, despite the immediate efficacy they may offer. These risks include serious side effects that may jeopardize consumer health [2].

Sildenafil citrate (Figure 1), a common BKO found in male stamina herbal medicine still marketed in

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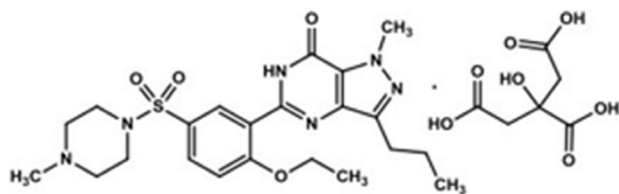


Figure 1. Chemical structure of sildenafil citrate

Indonesia, belongs to the phosphodiesterase inhibitor class and is used for treating erectile dysfunction [3]. It has the chemical formula $C_{22}H_{30}N_6O_4S$ and appears as an odorless white crystalline powder, freely soluble in solvents such as dimethylformamide, water, methanol, and ethanol [4]. While sildenafil citrate can improve sexual performance, it is also associated with dangerous side effects, such as hearing and vision loss, chest pain, dizziness, and in severe cases, even death, particularly when consumed improperly [5].

Previous research has demonstrated the presence of BKO in traditional herbal medicines, with one study revealing that four out of five tested samples contained BKO [6]. Although research on male stamina herbal medicine containing BKO has been conducted in various regions, no studies have specifically investigated products circulating in the Magelang region of Central Java. Therefore, this study aims to analyze samples of male stamina herbal medicine available in Magelang to identify the presence of sildenafil citrate using the thin-layer chromatography (TLC) method. TLC offers several advantages, including ease of use and shorter processing times compared to column chromatography [7]. The findings of this research will provide important insights into the safety of male stamina herbal medicine in the Magelang area and inform public awareness regarding their potential risks.

Methods

Organoleptic test and extraction of herbal medicine samples

Samples of herbal medicine marketed for male strength and stamina were purchased from an herbal medicine shop in Magelang, Central Java. Three samples from different brands were selected for analysis. The first step involved conducting an organoleptic test to evaluate the dosage form, color, smell, and taste of the herbal medicines as outlined by standard procedures [8].

Following the organoleptic test, the samples were extracted using 96% ethanol as the solvent. Each

sample was weighed to 1000 mg and dissolved in a 50 ml volumetric flask with 96% ethanol. The mixture was macerated for 24 hours, during which the solution was shaken for 15 minutes at 100 rpm using a shaker. After 24 hours of maceration, the liquid extract was filtered and then subjected to evaporation in a water bath to obtain a concentrated ethanol extract [9].

Qualitative analysis using thin-layer chromatography (TLC)

For the thin-layer chromatography (TLC) analysis, sample solutions and a standard solution of sildenafil citrate (1000 ppm) were applied onto silica gel 60 F_{254} plates (Merck®). Two mobile phase systems were used: system I consisted of methanol and chloroform (4:1), and system II consisted of methanol and chloroform (1:1). A 10 μ l volume of each sample and standard solution was applied to the TLC plates for separation and analysis.

Determination of maximum wavelength

To determine the maximum wavelength, 1 ml of each herbal medicine filtrate was diluted with 96% ethanol to a total volume of 10 ml. The solution was thoroughly mixed and placed in a cuvette for spectrophotometric analysis. The wavelength was set between 200–400 nm using a UV spectrophotometer (Cecil Aureus CE 7400) to identify the maximum absorbance wavelength [10].

Preparation of standard curve for sildenafil citrate

A standard solution of sildenafil citrate was prepared by diluting the 1000 ppm stock solution to five concentrations: 2, 4, 6, 8, and 10 ppm. The absorbance of each solution was measured using UV spectrophotometry to establish a standard curve and assess linearity (10).

Measurement of sample absorbance

For samples testing positive for sildenafil citrate, 1 ml of the filtrate was taken and diluted to 10 ml with 96% ethanol. The solution was homogenized and placed in a cuvette for absorbance measurement at the previously determined maximum wavelength using UV spectrophotometry. Each measurement was performed in triplicate to ensure accuracy.

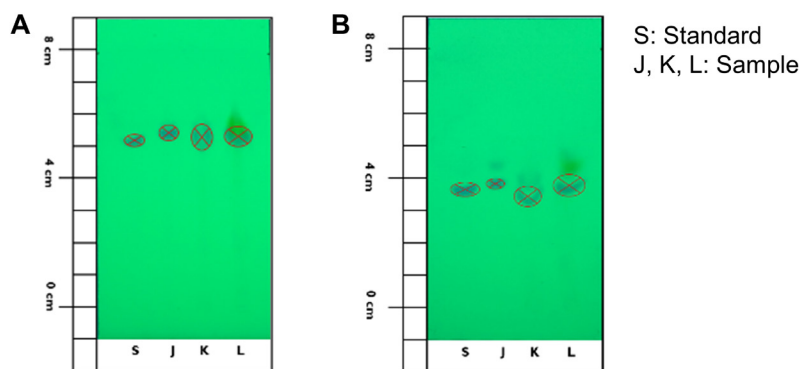


Figure 2. TLC results of male stamina herbal medicine samples. (A) Mobile phase methanol: chloroform (4:1), (B) Mobile phase methanol: chloroform (1:1)

Table 1. Organoleptic test

Sample	Dosage form	Color	Smell	Taste
J	Powder in capsul	White	Ginseng	Slightly bitter
K	Powder in sachet	Beige	Ginseng	Sweet
L	Powder in capsul	Brownish yellow	Ginseng	Bitter

Table 2. Rf values in the mobile phases of system I and II

Sample	Methanol : Chloroform (4:1)			Methanol : Chloroform (1:1)		
	Rf (cm)	Difference between Rf and standard	Interpretation	Rf (cm)	Difference between Rf and standard	Interpretation
Standard	0.45	0.00	+	0,66	0.00	+
J	0.48	0.03	-	0,69	0.03	-
K	0.43	0.02	+	0,68	0.02	+
L	0.46	0.01	+	0,68	0.02	+

Results

Organoleptic test

This study analyzed three brands of male stamina herbal medicine, labeled as sample codes J, K, and L. These samples were selected based on their popularity and frequent demand among consumers. Some of the herbal medicine products provided information such as the producer's name, claimed efficacy, intended use, net weight, and the herbal medicine notification number. However, details like the distribution permit number were still missing from some product packaging (11).

Organoleptic tests were conducted to evaluate the dosage form, color, smell, and taste of each sample, with the results summarized in Table 1. The taste of these herbal medicines varies depending on their ingredients, which commonly include ginger, turmeric, and ginseng (12).

TLC analysis

In the TLC analysis using two different mobile phase systems, the spot patterns on the TLC plates indicated that two samples contained the sildenafil citrate. This was confirmed by the presence of spots at the same location as the sildenafil citrate standard in both mobile phase systems: methanol: chloroform (4:1) (Figure 2A) and methanol: chloroform (1:1) (Figure 2B).

The Rf values for the two samples, coded K and L, were found to be consistent with the Rf value of the sildenafil citrate standard in both mobile phase systems. The Rf values for these samples are presented in Table 2.

Concentration of sildenafil citrate in samples

The maximum absorbance wavelength for sildenafil citrate was determined to be 292.5 nm (Figure 3A). The

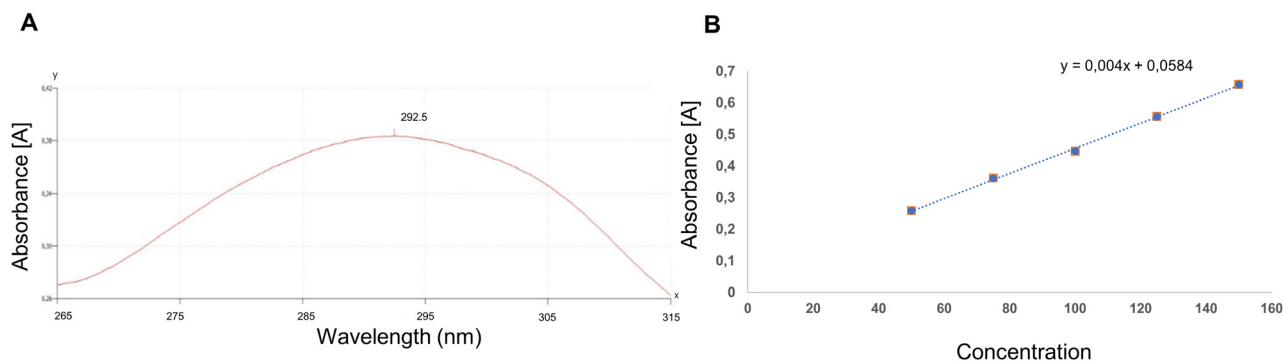


Figure 3.

Table 3. Sildenafil citrate concentrations in herbal medicine samples K and L

Sample	Replication	Absorbance	Concentration % (b/v)	Mean \pm SD
K	1	0.210	1.01	0.96 \pm 0.05
	2	0.205	0.96	
	3	0.197	0.91	
L	1	0.244	12.7	13.766 \pm 0.929
	2	0.263	14.2	
	3	0.266	14.4	

standard curve for sildenafil citrate, generated using UV spectrophotometry, demonstrated a high degree of linearity, with a correlation coefficient (r) of 0.9956. The equation of the standard curve was $y = 0.026x + 0.0784$ (Figure 3B).

The absorbance values and sildenafil citrate concentrations in the two male stamina herbal medicine samples (codes K and L) are shown in Table 3.

Discussion

Male stamina herbal medicines are formulated from natural ingredients intended to enhance male sexual performance. This study aimed to identify the presence of medicinal chemicals (BKO), specifically sildenafil citrate, in male stamina herbal medicines available in the Magelang region, using TLC and UV spectrophotometry methods.

Ethanol (96%) was used as the extraction solvent due to its strong absorption properties and its ability to minimize interference from other substances [13]. The extraction process was critical to separating any BKOs, such as sildenafil citrate, from other herbal components [14]. In the TLC analysis, two mobile phase systems—methanol: chloroform in ratios of 4:1 (system I) and 1:1 (system II)—were used. The stationary phase consisted of silica gel 60 F₂₅₄, which is polar and ideal

for separating chemical components via adsorption, desorption, and elution processes [15]. Proper chamber saturation was essential to optimize the development process, reduce solvent evaporation, and produce clear spot results [16].

The use of two mobile phases allowed for a more reliable and reproducible analysis. The similarity in retention factor (R_f) values between the samples and the sildenafil citrate standard indicated that samples K and L were positive for BKO sildenafil citrate. The difference in R_f values between the two mobile phases was minimal, as expected [17]. According to previous research [18], a sample is considered positive for sildenafil citrate if its R_f value is within ± 0.02 of the standard. Thus, samples K and L were confirmed to contain sildenafil citrate based on these criteria. Other unidentified spots suggest the possible presence of additional BKOs, such as tadalafil or other compounds [19]. This is a violation of Indonesian regulations, which prohibit the addition of synthetic medicinal chemicals to traditional herbal products (Indonesian Minister of Health Regulation No. 007/2012; BPOM Per-KA No.12/2014).

UV spectrophotometry was employed for further analysis due to its advantages of rapid testing and proven accuracy in similar studies. Sildenafil citrate contains

a chromophore group, making it ideal for detection through spectrophotometry, as the chromophore system absorbs light in the ultraviolet range [20]. Proper sample preparation, including the use of a blank solution, ensured the accuracy and validity of the measurements. The maximum absorption wavelength for sildenafil citrate was determined to be 292.5 nm, which aligns with the literature value of 291 ± 2 nm [10]. Accurate determination of the maximum wavelength is crucial for improving absorbance accuracy [10].

The sildenafil citrate standard curve, generated using UV spectrophotometry, demonstrated a high correlation value ($r = 0.9956$), consistent with Lambert-Beer's Law, which states that absorbance values between 0.2 and 0.8 yield the most accurate and reliable results [20]. The results confirmed that the standard curve met the requirements for quantitative analysis, allowing for precise calculation of sildenafil citrate concentrations in the herbal medicine samples.

Two herbal medicine samples, K and L, tested positive for sildenafil citrate using UV spectrophotometry at the wavelength of 292.5 nm. Sample K had a mean \pm SD content of $0.96 \pm 0.05\%$, indicating a relatively low concentration, which could suggest unintentional contamination or adherence to stricter manufacturing practices. In contrast, sample L contained a significantly higher concentration of sildenafil citrate ($13.766 \pm 0.929\%$), implying deliberate adulteration with BKO to enhance its effectiveness or appeal.

Previous studies on male stamina herbal medicines have similarly detected sildenafil citrate, with reported levels ranging from 0.15% to 0.53% [1]. The relatively low levels of sildenafil citrate found in some herbal products may suggest lower risks, but these products still pose a potential threat to consumers if they contain unlisted medicinal chemicals.

Conclusion

This study, using TLC and UV spectrophotometry, confirmed the presence of BKO sildenafil citrate in two herbal medicine samples marketed for male stamina. Sample K contained 0.96% sildenafil citrate, while sample L had a significantly higher concentration of 13.766%. These findings indicate a clear violation of Indonesian regulations prohibiting the addition of medicinal chemicals to traditional herbal medicines. Further monitoring and regulation enforcement are

essential to ensure the safety of these products for consumers.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

IFS: investigation, data curation, writing – original draft; PPH: conceptualization, methodology, supervision, writing – review & editing, funding acquisition; PSD: formal analysis, validation, visualization, writing – review & editing; EK: resources, software, writing – review & editing. All authors have read the final manuscript.

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