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ORIGINAL ARTICLE

Detection of glibenclamide adulteration in herbal remedies for diabetes mellitus using TLC

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ABSTRACT

The widespread use of *jamu*, a traditional Indonesian herbal medicine, has been accompanied by a surge in adulteration with synthetic drugs to enhance efficacy and sales. This study aimed to detect the presence of glibenclamide, a common adulterant, in antidiabetic jamu product using TLC. This study employed three herbal medicine samples marketed for lowering blood glucose levels, with glibenclamide BPFI serving as a reference standard. Thin-layer chromatography, employing a mobile phase consisting of chloroform, cyclohexane, ethanol, and glacial acetic acid (9:9:1:1), was utilized for the analysis. Thin-layer chromatography (TLC) analysis revealed a standard Rf value of 0.4 for glibenclamide, producing a distinct blue-violet spot when visualized with 10% sulfuric acid. The results indicated that the tested herbal medicine samples did not contain the pharmaceutical substance glibenclamide. This conclusion was drawn from the absence of a corresponding Rf value for glibenclamide in the herbal samples when compared to the reference standard.

Keywords: medicinal chemicals, glibenclamide, herbal medicine, thin-layer chromatography

Introduction

Traditional medicine, as defined by Indonesian Health Ministry Regulation No. 12 of 2014, refers to substances or formulations derived from animal, plant, or mineral sources, galenical preparations, or combinations thereof, which have been traditionally used for medicinal purposes based on empirical knowledge.[1] Traditional medicine is categorized into three types: jamu, standardized herbal medicine, and phytopharmaceuticals, according to the provisions of Indonesian Food and Drug Authority (BPOM) Regulation No. HK.00.05.4.2411.[2] *Jamu* is a traditional medicinal preparation derived from plant or animal materials, presented in the form of powders, pills, capsules, liquids, or infusions.[3]

The public's perception of *jamu* as being relatively safer than synthetic drugs has contributed to a decline in the demand for synthetic pharmaceuticals. The widespread use of *jamu* has also led to a surge in market demand, prompting some unscrupulous producers to intentionally adulterate their products with medicinal chemicals (BKO) to boost sales.[3] This practice is in direct violation of the Indonesian Minister of Health Regulation No. 007 of 2012 on Traditional Medicine Registration, which explicitly prohibits traditional medicines from containing isolated or synthetic chemical substances with medicinal properties.[4]

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Despite the existence of these regulations, the Indonesian Food and Drug Monitoring Agency (BPOM) continued to detect medicinal chemicals in traditional medicine and health supplements as recently as December 2023.

The deliberate adulteration of traditional herbal medicines with medicinal chemicals is commonly motivated by the desire to achieve rapid symptomatic relief, thereby increasing market demand. The uncontrolled use of pharmaceutical drugs within *jamu*, without adherence to prescribed dosages and administration guidelines, poses significant risks to human health. Prolonged consumption of traditional herbal medicines contaminated with medicinal chemicals can lead to adverse effects due to potential interactions between the chemical constituents of the pharmaceutical drug and the herbal components.[5]

Mu et al. identified the presence of the medicinal chemical glibenclamide in three samples of Wei Yi Wang antidiabetic jamu using thin-layer chromatography (TLC) and further confirmed this finding with spectrophotometric analysis. Based on this previous research, this study aimed to qualitatively identify the presence of the pharmaceutical ingredient glibenclamide in antidiabetic *jamu* products distributed in Medan Petisah district using TLC. TLC was selected due to its simplicity compared to other chromatographic techniques. The advantages of TLC include the ability to analyze multiple samples simultaneously on a single plate, requiring only a small amount of mobile phase (eluent) and simple equipment, thereby reducing analysis time and cost.[7]

Method

This study aims to identify the presence of glibenclamide in herbal medicinal products marketed as blood glucose-lowering agents in powder, pill, and capsule formulations, circulating in Medan Petisah District. This descriptive qualitative study utilized herbal medicine samples that were previously analyzed at the Universitas Prima Indonesia laboratory in November 2023.

Tool used in this study included a chamber, filter paper, analytical balance, beakers, Erlenmeyer flasks, graduated cylinders, dropping pipettes, micropipettes, capillary tubes, aluminum foil, water bath, spatula, silica gel TLC plates, sonicator, centrifuge, UV lamp, and mortar and pestle. Materials employed comprised glibenclamid BPFI (British Pharmacopoeia for Indonesia) as a standard, herbal samples for blood sugar reduction from brands R, S, and T, and various solvents including chloroform, cyclohexane, ethanol, methanol, glacial acetic acid, and 10% sulfuric acid.

This study involved a multi-step process. Initially, organoleptic evaluation was conducted on herbal medicine samples for lowering blood sugar levels from brands "R," "S," and "T," involving visual and sensory observations. Subsequently, 6-gram portions of powdered samples from each brand were subjected to maceration in 30 mL of ethyl acetate for 30 minutes using an orbital shaker. The resulting mixtures were centrifuged at 4000 rpm for 15 minutes. The filtrates were then filtered using filter paper, and the extracts were evaporated to dryness using a water bath at 80°C. The residues were reconstituted in 5 mL of methanol and stored in aluminum foil.

To prepare the reference standard solution, 10 mg of glibenclamide BPFI was accurately weighed and quantitatively transferred to a 5 mL volumetric flask. Methanol was added up to the 2 mL mark, and the flask was agitated until complete dissolution. The volume was then adjusted to the mark with methanol. For the preparation of the spiked solution, 100 µL aliquots of both the reference standard solution and the herbal medicine sample were transferred into a beaker glass using a calibrated micropipette. The contents were thoroughly mixed to ensure homogeneity.

Subsequently, thin-layer chromatography (TLC) was employed for analysis. TLC plates measuring 11 cm x 9 cm were prepared with 2 cm and 1 cm margins at the top and bottom, respectively. A TLC chamber was saturated with a mobile phase consisting of chloroform: cyclohexane: ethanol: glacial acetic acid (9:9:1:1). Standard reference solutions, herbal sample, and spiked samples were spotted onto the TLC plate, maintaining a 1 cm distance from the bottom margin. The plate was then immersed in the pre-saturated chamber. Upon completion of the elution process, the plate was removed and allowed to dry. The developed chromatogram was visualized under UV light at 254 nm. The Rf values of the individual spots were calculated and compared to those of standard medicinal chemical reference compounds.

Results and Discussion

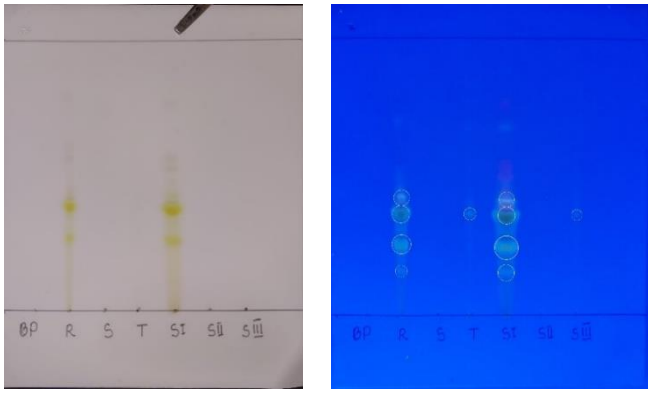
This study utilized three herbal medicinal product samples from different brands, all purported to lower blood glucose levels. An organoleptic evaluation was conducted, encompassing observations of color,

form, odor, expiration date, registration number, and logo on each product's packaging. The results of these observations are presented in Table 1. All three samples exhibited a registration number and logo, indicating that the products have undergone the necessary registration processes. Physically, sample "R" was a bright yellow powder, sample "S" was a brownish-white capsule, and sample "T" was a dark brown pill. A characteristic herbal aroma and a bitter taste, common in herbal products, were detected in all samples.

Table 1. Organoleptic observations of *jamu* samples

Observation	Sampel		
	Jamu "R"	*Jamu* "S"	*Jamu* "T"
Form	Powder	Capsule	Pill
Color	Bright yellow	Off-white	Dark brown
Odor	Aromatic	Aromatic	Aromatic
Taste	Bitter	Bitter	Bitter
Product logo	Present	Present	Present
Exp. Date	December 9, 2025	February 2, 2027	February 1, 2026
Registration Number	POM TR. 183214571	POM TR. 082380811	POM TR. 173 406 361

This qualitative analysis employed Thin-Layer Chromatography (TLC) to detect the presence of the drug substance glibenclamide in herbal samples purported to lower blood glucose levels. Identification was achieved by comparing the Rf values of the herbal samples to those of a glibenclamide standard on the chromatogram. The identification procedure involved spotting the TLC plate, pre-saturated with the mobile phase, with solutions of the glibenclamide standard, sample, and a spiked sample. The chamber was lined with filter paper to ensure optimal spot development and uniform distribution of the eluent vapor.[8] TLC plates measuring 11 cm x 9 cm, with application lines 2 cm from the bottom and 1 cm from the top, were used. Spots were applied 1 cm apart. After spotting, the plate was placed in the developing chamber. The TLC results for all three herbal samples are depicted in Figure 1.



(a) (b)

Figure 1. (a) TLC chromatogram of a hypoglycemic *jamu* sample using a mobile phase of chloroform: cyclohexane: ethanol: glacial acetic acid (9:9:1:1) and (b) Visualization of glibenclamide impurity under UV 254 nm light in the hypoglycemic *jamu* sample.

Visual inspection of the glibenclamide standard and samples 'S' and 'T' initially revealed no spots. Subsequent analysis was conducted under UV light at 254 nm, given the silica gel 254 plates' strong fluorescence at this wavelength and the presence of chromophores in glibenclamide that can produce colored spots.[9] Herbal samples 'R' and 'T' exhibited spots, while the glibenclamide standard and sample 'S' remained invisible. To enhance spot visibility, the TLC plate was sprayed with 10% sulfuric acid in methanol. Post-spraying and re-examination under UV light at 254 nm, distinct spots and corresponding Rf values for the glibenclamide standard were obtained, as shown in the table below.

The reference standard glibenclamide exhibited an Rf value of 0.41 and a blue-violet color when visualized under UV 254 nm. TLC analysis of the three herbal samples revealed varying Rf values. Sample "R" showed multiple spots, while sample "S" produced no spots at all. The presence of multiple spots in some samples could be attributed to the presence of secondary metabolites such as flavonoids, tannins, alkaloids, and steroids, or possibly other medicinal chemicals.[10]

The glibenclamide reference standard used was pure glibenclamide obtained from the Indonesian Food and Drug Monitoring Agency (BPOM). Glibenclamide is a second-generation sulfonylurea oral antidiabetic agent used to lower blood glucose levels in type 2 diabetes mellitus patients.[11] Common side effects of

glibenclamide include hypoglycemia, manifested by symptoms such as pallor, sweating, weakness, palpitations, and constipation.[12]

Table 2. Nilai RF glibenklamid pada sampel jamu penurun kadar gula darah

Sample	RF value	Interpretation
Glibenclamide standard	0,41	-
Jamu "R"	0,13	negative
	0,26	negative
	0,36	negative
	0,58	negative
Jamu "S"	-	-
Jamu "T"	0,35	negative
Spike I	0,13	negative
	0,26	negative
	0,362	negative
	0,58	negative
Spike II	-	-
Spike III	0,35	negative

Based on the results, herbal samples "R" and "T" tested negative for the presence of the API glibenclamide, as no sample spot exhibited an Rf value identical to the reference standard. The presence or absence of glibenclamide in sample "S" remains inconclusive due to the lack of visible spots and, consequently, an Rf value. Several factors can influence TLC separation and Rf values, including the chemical structure of the compounds, the nature and activity of the adsorbent, the thickness and uniformity of the adsorbent layer, experimental technique, solvent purity, saturation level, sample amount, temperature, and equilibrium.[13]

Conclusion

TLC analysis of herbal medicinal products 'R' and 'T' for the treatment of hyperglycemia, currently distributed in Medan Petisah district, yielded negative results for the presence of the glibenclamide. This conclusion is supported by the discrepancy in Rf values between the herbal samples and the glibenclamide standard. However, TLC analysis of herbal product 'S' was inconclusive regarding the presence of glibenclamide due to the absence of any detectable spots on the chromatogram.

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