Research Article

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Authentication of Medicinal Chemicals in Traditional Herbal Products (Jamu) by UV-Vis Spectrophotometry

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ABSTRACT

Traditional medicine is becoming very popular as an alternative treatment that can minimize side effects. However, it is profitable for manufacturers who are not responsible for adding medicinal chemicals (BKO) to traditional herbal products. The samples used in this study were traditional herbal medicine coded A1, A2; B1, B2; C1, C2; D1, D2; E1, E2; and F1, F2, which were analyzed qualitatively and quantitatively using thin-layer chromatography (TLC) and UV-Visible spectrophotometry. Based on the results obtained from the analysis using the TLC method, the herbal samples with code A1 and A2 had a staining profile, Rf value, and chromatographic peak that were almost the same as the comparison standard for sodium metamizole with levels of 51.99% (A1) and 53.06% (A2). The herbal medicine samples with codes B1, B2, and C1, C2 had stain profiles, Rf values in TLC, and spectrophotometric peaks that were almost the same as the comparison standard for paracetamol with paracetamol levels contained in the "Jamu" samples of 81.31% (B1), 81.66% (B2), 89.59% (C1) and 81.62% (C2). The results obtained from this study show that the samples of traditional herbal products with codes A1 and A2 contain sodium metamizole with levels of >50%. Samples of traditional herbal products B1, B2, C1, and C2 contain paracetamol with paracetamol levels contained > 50%, while the traditional herbal medicine samples with codes D1, D2, E1, E2, F1, and F2 have been identified as containing no medicinal chemicals in the traditional herbal medicine samples.

Keywords: traditional herbal medicine, jamu, medicinal chemicals, UV-Vis spectrophotometry

1. Introduction

In recent decades, the world has widely developed and used traditional medicine, with WHO estimating that around 65-80% of the world's population uses traditional medicine as alternative medicine [1]. This is because the price is more affordable and relatively safe, providing mild side effects than synthetic drugs [2]. However, irresponsible manufacturers often pollute the efficacy and purity of traditional medicine by adding medicinal chemicals to traditional medicine to provide faster efficacy and increase financial benefits. The uncontrolled addition of medicinal chemicals into traditional medicines can harm the body. It can also cause serious side effects such as nausea, vomiting, diarrhea, dizziness, headaches, visual disturbances, and chest pain, to severe organ damage such as liver damage, kidney failure, heart failure, visual impairment, and death. This problem is due to an interaction between medicinal chemicals and the content of compounds in traditional medicine when consumed by the public [2–4].

Based on the Indonesian Food and Drug Supervisory Agency [5,6] there are 53 traditional medicinal products and 1 health supplement product containing medicinal chemicals, and 18 types of cosmetic products containing hazardous or prohibited substances. Some traditional medicines are often contaminated with medicinal chemicals used for certain diseases such as rheumatism (phenylbutazone, methylprednisolone, diclofenac sodium, piroxicam, paracetamol, sodium metamizole, prednisone, and dexamethasone), slimming (sibutramine hydrochloride), stamina enhancer/male tonic (sildenafil), diabetes (glibenclamide), shortness of breath/asthma (theophylline) [7]. The Indonesian government has issued various regulations regarding traditional medicines containing medicinal chemicals, such as the Regulation issued by BPOM Number HM. Such as the registration of health supplements and Regulation of the Minister of Health of the Republic of Indonesia No. 007 Article 7 of 2012, that traditional medicines are prohibited from containing isolated and synthetic BKOs with medicinal properties and other materials based on health considerations. However, some irresponsible manufacturers have issued various regulations on traditional medicines and laws that the government has issued.

The herbal product “Jamu” is a traditional medicinal preparation derived from ingredients in plants, animals, mineral materials, and galenical preparations that have been processed from generation to generation based on practical use for treatment by many people [8]. Jamu is still very popular in rural as well as in urban areas. Based on its traditional use Jamu is being developed into a rational form of therapy, by herbal practitioners and in the form of phytopharmaceuticals. Jamu has acquired a potential benefit, both economically and clinically. We surveyed the most frequently used plants in Jamu that have also been investigated regarding their constituents and pharmacological effects. The Indonesian government has divided the preparation of medicinal plants into three categories, i.e. Jamu, standardized herbal medicines and fitofarmaka (phytomedicines). Until now, the public’s use of traditional medicine is very much in demand, with the assumption that traditional medicine has minimal side effects. However, herbal medicine production by several manufacturers has been intentionally added in the form of medicinal chemicals (BKO) to increase the efficacy and turnover in the market [8–10] as the consumption of herbs increased into 50%. This research is particularly important to analyze health quality of society through herbs as alternative of modern medicine to lower, middle, upper-social class group. The research problem addressed is how is the acceptance level of herb as alternative of modern medicine to middle, lower and upper class society in East Java. After finding the acceptance level of lower, middle, and upper class group toward herb usage, further analysis was focused on herbs management model in order to enhance society’s health quality. Analysis unit in this research are society and bureaucracy. Informant was chosen by purposive sampling method (for key informants).

The analysis carried out to determine the presence of medicinal chemicals in traditional medicine (Jamu) can be done using the thin layer chromatography method to see the stain profile at UV 254 and UV 366 and UV-Vis spectrophotometry [1,11,12]. These two methods often analyze medicinal chemicals in traditional medicinal products qualitatively and quantitatively based on the absorbance read in spectrophotometry. This method has several advantages, such as the results obtained are fast and inexpensive. Still, it has drawbacks, such as what you want to measure, namely the sample with a chromophore group to provide absorbance absorption in the form of peak chromatography. Therefore, in this study, researchers wanted to explore and determine the levels of

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medicinal chemicals in traditional herbal medicine samples using thin-layer chromatography (TLC) and UV-Vis spectrophotometry using dexamethasone ibuprofen, paracetamol, and sodium metamizole as the standard as comparisons.

2. Material and Methods

2.1. Material

The materials used in this study were acetaminophen, aqua demineralization (OneMed, Indonesia), dexamethasone, ethanol pro analysis (Merck, Germany), ethyl acetate (Merck, Germany), ibuprofen, sodium metamizole, methanol (Merck, Germany), and herbal product samples (A1, A2; B1, B2; C1, C2; D1, D2; E1, E2; and F1, F2). There are 6 types of herbal products obtained from herbal sellers in traditional markets in Gorontalo Regency, Gorontalo Province (6°74'63." S; 122°35'83'05" E) altitude of 122 m, Indonesia. Each herb was taken twice with different batch numbers.

2.2. Identification by Thin Layer Chromatography (TLC)

2.2.1 Eluent setup

The elution solvent (eluent) was prepared as much as 50 mL by mixing ethyl acetate: methanol (8:2) solvent. The elution solvent was then saturated for a while before being used on thin-layer chromatography (TLC) plate.

2.2.2 Identification by TLC

Identification of BKO content in Jamu products was carried out using the thin layer chromatography (TLC) method [1,11]. Prepared a TLC plate with a size of 10 cm x 10 cm and made a lower limit on the TLC with 1.5 cm on the bottom edge and an upper limit with a distance of 1 cm on the top edge of the TLC plate. Each comparison and sample solution were spotted on a TLC plate and dried. The plate was then put into a 20 x 15 cm chamber that contained the eluent—further expanded until the eluent reached the upper boundary of the plate. The plates were dried, and the spots were identified under UV 254. Spots with similarities were marked, and their Rate of flow (Rf) values were calculated.

2.3. Preparation of Standard Solution

The preparation of working standard solutions and calibration curves for paracetamol and sodium metamizole were carried out according to procedures [7,13] with minor modifications. Each standard of Sodium Metamizole (M), Dexamethasone (D), Ibuprofen (I), and Paracetamol (PC) was weighed 10 mg and dissolved in absolute ethanol pro analysis up to 10 mL to obtain a standard solution with a concentration of 1000 ppm. Each standard solution was pipetted as much as 1 mL, and the volume was sufficient to obtain a final concentration of 100 ppm. The standard solution is then stored in a closed glass container for further tests.

2.4. Preparation of Paracetamol Standard Curve Solution

100 ppm paracetamol (PC) stock solution was taken with a volume of 0.1 mL; 0.2 mL; 0.3 mL; 0.4 mL, and 0.5 mL and put into a 5 mL volumetric flask. Then, each solution was made up to 5 mL in volume with ethanol pro analysis to obtain a series of concentrations of the standard solution of paracetamol 2, 4, 6, 8, and 10 ppm. Absorption of each concentration of the standard solution was measured using a UV-visible spectrophotometer (Shimadzu UV-1900) at a maximum wavelength of 249 nm.

2.5 Preparation of Sodium Metamizole Standard Curve Solution

100 ppm sodium metamizole (M) stock solution was taken with a volume of 0.2 mL; 0.4 mL; 0.6 mL; 0.8 mL, and 1.0 mL and put into a 5 mL volumetric flask. Next, the volume of each solution was made up to 5 mL with ethanol pro analysis to obtain a series of concentrations of the standard solution of paracetamol 4, 8, 12, 16, and 20 ppm. Absorption of each concentration of the standard solution was measured using a UV-visible spectrophotometer (Shimadzu UV-1900) at a maximum wavelength of 263.8 nm.

2.6 Sample Solution Preparation

Each herbal preparation was weighed as much as 100 mg and dissolved in absolute ethanol. Then it was filtered, and the volume was made up of absolute ethanol up to 10 mL.

2.7 Determination of BKO level in the Sample Solution

A total of 10 µL (1g/100 mL in ethanol p.a.) of the herbal sample solution was put into a 10 mL volumetric flask, and then the absorbance was measured

at the maximum wavelength. This study is equipped with an analysis of precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ) based on the criteria for acceptance of measurements carried out according to the protocol [14].

3. Results and Discussion

The herbal product "Jamu" is a traditional medicinal preparation derived from ingredients in plants, animals, mineral materials, and galenical preparations that have been processed from generation to generation based on practical use for treatment by many people [8]. Jamu is still very popular in rural as well as in urban areas. Based on its traditional use jamu is being developed into a rational form of therapy, by herbal practitioners and in the form of phytopharmaceuticals. Jamu has acquired a potential benefit, both economically and clinically. We surveyed the most frequently used plants in Jamu that have also been investigated regarding their constituents and pharmacological effects. The Indonesian government has divided the preparation of medicinal plants into three categories, i.e. Jamu, standardized herbal medicines and fitofarmaka (phytomedicines). Until now, the public's use of traditional medicine is very much in demand, with the assumption that traditional medicine has minimal side effects. However, herbal medicine production by several manufacturers has been intentionally added in the form of medicinal chemicals (BKO) to increase the efficacy and turnover in the market [8–10] as the consumption of herbs increased into 50%. This research is particularly important to analyze health quality of society through herbs as alternative of modern medicine to lower, middle, upper-social class group. The research problem addressed is how is the acceptance level of herb as alternative of modern medicine to middle, lower and upper class society in East Java. After finding the acceptance level of lower, middle, and upper class group toward herb usage, further analysis was focused on herbs management model in order to enhance society's health quality. Analysis unit in this research are society and bureaucracy. Informant was chosen by purposive sampling method for (key informants). The prohibition of BKO content in herbal medicine has been regulated by the Minister of Health of the Republic of Indonesia No. 007 of 2012. Traditional medicine is prohibited from containing isolated and synthetic BKO with medicinal properties and other ingredients based on health considerations. Therefore, research has been carried out to identify BKO content in herbal packaging preparations circulating in the market.

In this study, identification was carried out regarding the profile of compounds containing BKO contained in herbal preparations using comparison standards in the form of Metamizole (M), Dexamethasone (D), Ibuprofen (I), and Paracetamol (PC). This comparison was used because several references have investigated that the average BKO is often found in herbal preparations, especially in the herbal products used for aches and pains. BKO in sodium metamizole, dexamethasone, ibuprofen, and paracetamol are often added to Jamu products that claim to be analgesic and anti-inflammatory drugs [15,16]. The herbal medicine samples used were 6 (A, B, C, D, E, F) packaged preparations obtained from the market where each brand of packaging samples was taken from two different locations so that they were coded (A1, A2; B1, B2; C1, C2: D1, D2: E1, E2: and F1, F2).

The BKO tracing method was carried out qualitatively using the Thin Layer Chromatography (TLC) method to separate the compounds produced, making it easier to identify similarities based on Rf values between samples and comparison standards [1,17]. The results of the BKO search that showed positive results were followed by identification by UV spectrophotometry to confirm the results obtained and then continued with the determination of the levels of BKO compounds using the UV spectrophotometric method.

In this study, a sample of herbal medicine was prepared by dissolving the herbal medicine powder using absolute ethanol. The solvent used is ethanol intended to dissolve BKO, which may be present in each herbal medicine. Comparative Standards A, B, C, and D have good solubility with ethanol. Each reference standard and Jamu sample was dissolved in absolute ethanol, spotted on a TLC plate (10 x 10 cm), and developed with a solvent mixture of Ethyl Acetate: Methanol (8:2). Tracing the profiles of BKO compounds in herbal medicine can be seen in Figure 1 and Table 1.

Tracing the profile of BKO content using the Thin Layer Chromatography method showed that there were samples of Jamu suspected of containing BKO. This tracing can be observed from the profile of the spots produced under UV light at 254 nm, which

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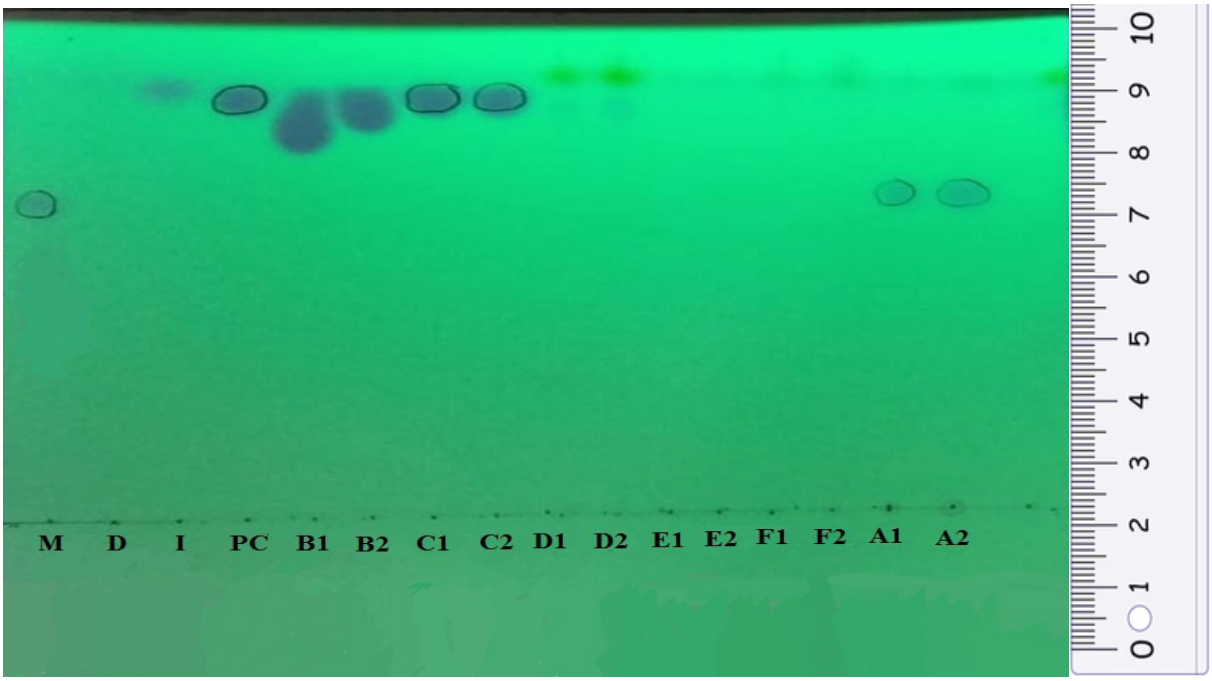

Figure 1. Spot profiles from the chemical standard (M, D, I, PC) and herbal samples (A to F). M is Sodium metamizole, D is Dexamethasone, I is Ibuprofen, and PC is Paracetamol. A to F is the herbal product "Jamu."

Table 1. TLC profile and UV spectra of each comparison and herbal medicine samples

Sample Code	Spot Color	Rate of Flow	λ_{max} (in ethanol)	Information
M	Dark Blue	5,2	263,8	
D	Dark	0,97	-	
I	Blue	1	-	
PC	Light blue	6,9	249,25	
A1	Dark Blue	5,2	264,4	+
A2	Dark Blue	5,2	264,2	+
B1	Light blue	6,7	249,4	+
B2	Light blue	6,8	249,3	+
C1	Light blue	6,9	249,45	+
C2	Light blue	6,9	249,35	+
D1	Yellow	0,99	-	-
D2	Yellow	0,99	-	-
E1	Pale yellow	0,99	-	-
E2	Pale yellow	0,99	-	-
F1	Pale yellow	0,99	-	-
F2	Pale yellow	0,99	-	-

Note: + indicate the sample containing BKO
- Indicate the BKO-free sample

have the same color and rate of flow (Rf) values from the comparison and Jamu samples [7]. From Figure 1, it is shown that the Jamu samples A1 and A2 have similar spots with P1 (Sodium metamizole), which are dark blue, while the Jamu samples B1, B2, C1, and C2 have similar spots with P4 (Paracetamol) which shows a bright blue color. The Rf value of each spot can be seen in Table 1.

The data in Table 1 shows the similarity of the Rf values between the samples of Herbal Medicine A1 and A2 with the comparator Sodium Metamizole (M) with an Rf value of 5.2. At the same time, samples C1 and C2 have the same Rf value with the comparison standard of paracetamol, which is 6.9. In samples of herbal medicines B1 and B2, characteristically, the spots formed to indicate the presence of tailings so that it gives a slight difference to the Rf value, where the comparison of paracetamol obtained an Rf value of 6.9 while in the samples of herbal medicine B1 and B2 it was 6.7 and 6.8. However, the herbal medicine samples B1 and B2 showed a similarity in spot color with the paracetamol comparison. Therefore, a confirmatory test for identification was carried out using a UV spectrophotometer. The comparison of standards M and PC can be identified by looking at the maximum wavelength in the UV region. M and PC are chemical compounds containing aromatic molecules that are good chromophores to be identified using a UV spectrophotometer [12]. The search results can be seen in Figure 2 and Figure 3.

The results of the identification by spectrophotometer between Herbal Samples A, B, and C with comparison P1 and P4 showed a shift in wavelength that was similar or the same. The maximum wavelength results between the sample and the comparator can be seen in Table 1. Figure 2 shows that PC and Jamu samples gave the same max, namely a max of 249 nm, so it can be ascertained that samples B1, B2, C1, and C2 were positive for BKO in the form of paracetamol. Figure 3 shows the similarity between the UV (M) spectrum pattern and the A1 and A2 Jamu samples. In standard (M), the maximum absorption is obtained at a wavelength of 263.8 nm, and in samples A1 and A2, it is obtained that the maximum absorption is in the range of wavelengths of 264.4 and 264.2 respectively, and has similarities with the maximum wavelength of the standard. The maximum wavelength absorption of sodium metamizole, at a wavelength of 263 nm in ethanol media, obtained similar results to research from [13] that the maximum wavelength of sodium metamizole was in the range of 260-270 nm. This result follows

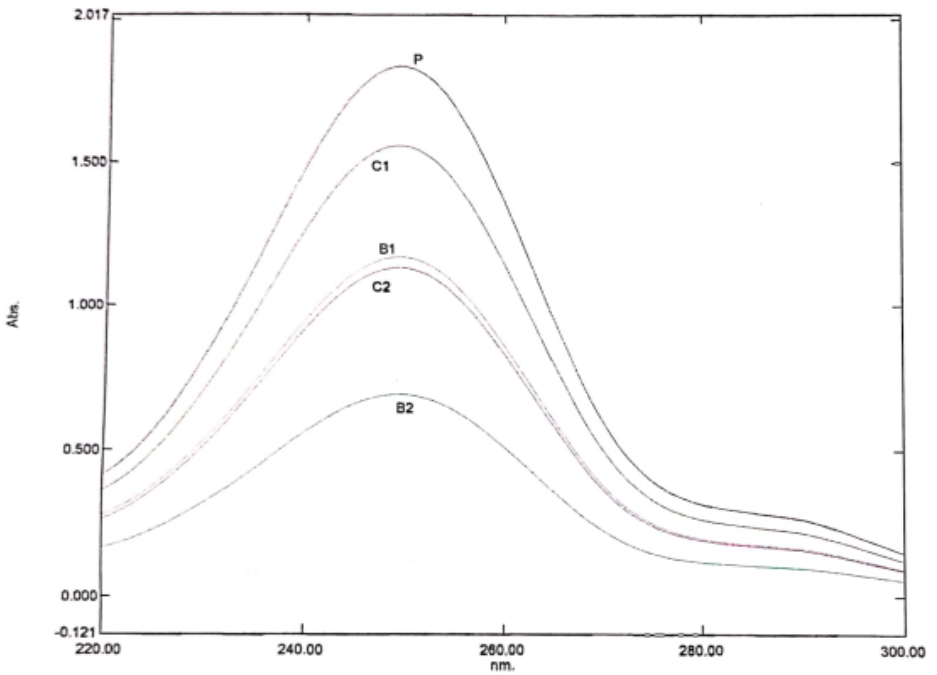

Figure 2. UV spectra profile of Paracetamol (PC) and Herbal Medicine Samples (B1, B2, C1, and C2)

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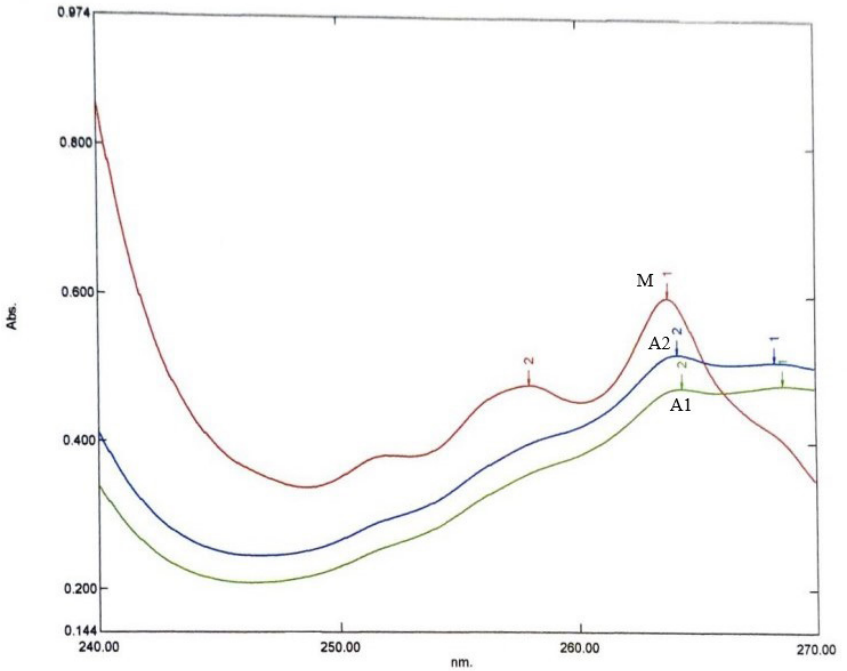

Figure 3. Spectra Profile of UV of Sodium Metamizole (M) and Herbal Medicine Samples (A1 and A2)

the identification results in Figure 1, which shows the similarity of spots and their Rf values. It can be concluded that samples A1 and A2 are positive for Sodium Metamizole. Qualitative test results from the herbal medicine samples and BKO content determine the levels of these medicinal compounds in the herbal medicine samples.

Determination of BKO levels in Jamu samples was carried out using a UV spectrophotometer. The standard for comparing M and PC is a calibration curve to obtain the standard curve equation. Figure 4 shows the calibration curves of the chemicals standards M and PC.

The resulting calibration curve shows a good correlation (R^2=0.996), so it can be used as an equation to determine the levels of each sample. Table 2 shows the BKO levels of each Jamu sample obtained from the standard curve equation. The acceptance criteria for precision RSD are not more than 2% [18] which is in line with the RSD results of each sample (<2%).

To ensure precise and accurate results, the analysis of BKO levels in Jamu preparations was replicated (n = 3) and analyzed on the same day (intraday) and different days (inter-days). In intraday analysis, the Table 2 shows that samples A1 and A2 have sodium metamizole levels of 50.87±0.69% and 58.19±0.69%, while samples B1, B2, C1, and C2 have paracetamol levels in the herbal medicine samples 83.25±1.13%, 81.59±0.43%, 89.40±0.29%, and 81.22±1.27% respectively. The analysis results on inter-days showed no significant difference in paracetamol or sodium metamizole content levels in each sample analyzed on the intraday. It is stated that the analytical method that has been carried out provides valid precision results.

Based on the results of determining BKO levels in the herbal medicine samples, Paracetamol and Sodium Metamizole levels were quite high,> 50%. According to the Indonesian Minister of Health (No. 7 in 2012), traditional medicine is prohibited from containing medicinal chemicals derived from isolation or synthetic, medicinal properties [19]. According to previous studies [6, 20], excessive use of paracetamol or not by the rules of drug use can lead to the formation of metabolic products N-acetyl-p-benzoquinone imine (NAPQI), which has an effect in damaging the liver. The use of paracetamol together with curcumin in the long term can cause toxic effects on cells, namely by inhibiting the cytochrome

Table 2. BKO levels (precision) in herbal preparations

Rep.	Intraday						Inter-Day					
	A1	**A2**	**B1**	**B2**	**C1**	**C2**	**A1**	**A2**	**B1**	**B2**	**C1**	**C2**
1	51.99	57.17	84.63	81.15	89.46	83.74	51.12	57.26	84.31	81.01	89.66	81.91
2	50.14	59.89	81.31	81.91	89.46	80.92	50.71	59.37	81.80	81.80	89.50	81.12
3	50.75	58.11	83.56	81.91	89.86	80.21	51.37	58.08	83.69	80.98	89.67	80.25
4	50.82	57.83	83.98	80.94	88.93	80.75	51.05	57.79	83.88	81.59	89.30	80.65
5	51.27	57.39	83.08	81.72	89.38	81.07	50.96	57.25	83.11	81.26	89.36	81.86
6	50.25	58.75	82.95	81.89	89.32	80.65	50.61	58.08	82.63	81.81	89.28	80.83
Average	50.87	58.19	83.25	81.59	89.40	81.22	50.97	57.97	83.24	81.41	89.46	81.10
SD	0.69	1.01	1.13	0.43	0.29	1.27	0.28	0.78	0.92	0.37	0.17	0.67
RSD	**1.34**	**1.72**	**1.36**	**0.53**	**0.33**	**1.56**	**0.55**	**1.34**	**1.10**	**0.45**	**0.19**	**0.82**

Description: A1 and A2 samples contained sodium metamizole, while the B1, B2, C1, and C2 samples contained paracetamol. SD is the standard deviation, and RSD is the relative standard deviation (%).

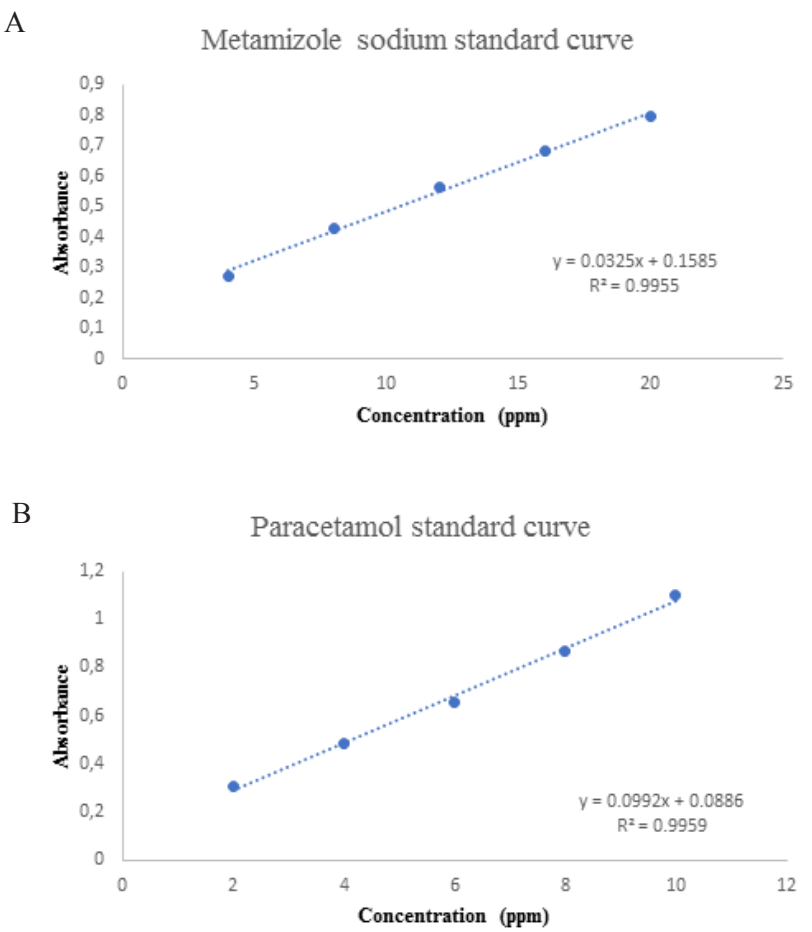

Figure 4. Calibration Curves of Comparative Standards M (Sodium metamizole) and PC (Paracetamol). The data were analysis triplicate (n=3)

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P450 enzyme, which plays a role in paracetamol metabolism, which can cause an increase in the number of paracetamol metabolites so that it has a toxic effect on cells [21, 22].

In addition to the precision parameter, the accuracy method is also applied in this study. The accuracy method applied in this study is the internal standard spike method at levels 80, 100, and 120%, which is added to the sample with a ratio of 70:30 (Jamu sample: standard). The accuracy parameters' results obtained the average BKO levels of paracetamol and sodium metamizole in each Jamu sample that met the recovery requirements of >90-110% [23] (Table 3). The accuracy test results on the inter-day also showed no significant differences with intraday analysis. Test results on different days still provide paracetamol and metamizole sodium recovery percentage in herbal preparations between 90-110% and still meet the requirements.

The limit of detection and limit of quantification analysis is also reported in this study. Based on the detection limit test results, the LOD values for paracetamol and sodium metamizole were 0.698 ppm and 1.472 ppm, respectively, while the quantity limit test results obtained LOQ values of 2.329 ppm and 4.91 ppm, respectively. The LOD test was carried out to see the smallest amount of analyte in the sample that can be detected and still gives a significant response compared to the blank. Meanwhile, the LOQ test was carried out to see the smallest analyte in the sample that can still meet the criteria of being careful and thorough [23].

Based on the analysis of paracetamol and metamizole sodium content in Jamu products shows that the spectrophotometer method can provide valid test results. This can be strengthened by the results of testing on the parameters of precision, accuracy, LOD, and LOQ, which are by predetermined requirements, so that in the future, it can be applied to the analysis of BKO levels in Jamu preparations.

4. Conclusions

Based on the results obtained from this study, it can be concluded that the samples of Jamu coded A1 and A2 containing sodium metamizole with levels of >50% and samples of traditional herbal medicine products B1, B2, C1, and C2 contain paracetamol with paracetamol levels contained >50%. In contrast, Jamu samples with codes D1, D2, E1, E2, F1, and F2 have been identified using TLC and spectrophotometry; there were no medicinal chemicals in the Jamu samples. Based on the analysis of BKO level in Jamu products, the UV-Visible spectrophotometer method has met the criteria of accuracy, precision, LOD, and LOQ. It can be concluded that the UV-Visible spectrophotometry method can provide valid measurement results on herbal products.

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Conflict of Interest

The authors declare that there is no conflict of interest in this study

Table 3. Accuracy (% recovery) measurement results from each Jamu sample

Sample	Intraday at level (%)			Inter-Day at level (%)		
	80	100	120	80	100	120
A1	99.97±0.82	101.37±1.50	98.42±0.10	98.05±0.71	100.7±1.50	99.37±1.58
A2	99.01±1.57	100.24±1.53	98.78±10	99.18±1.16	99.5±2.11	99.62±2.11
B1	98.15±0.48	97.97±0.86	101.37±97	98.48±4.62	98.31±0.40	100.71±0.40
B2	100.14±0.40	99.9±0.35	100.30±99	99.85±1.57	100.88±1.26	100.13±1.26
C1	99.69±0.37	101.27±1.04	99.91±10	100.10±1.71	100.4±1.52	99.57±1.50
C2	97.47±1.70	100.45±1.18	102.47±10	99.04±0.12	99.83±1.71	100.58±1.71

Statement of Contribution of Researchers

Data Collection and/or Processing – F.H., A.N.A.; Analysis and/or Interpretation – I.I., R.D.; Writing – F.H., A.N.A., I.I., R.D., A.I., S.N.; Critical Reviews – S.N.

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