

Spectrophotometry UV-Vis Technique Combined with

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Spectrophotometry UV-Vis Technique Combined with Chemometrics for Determination Phenylbutazone and Acetaminophen in Herbal Medicines

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Abstract

Objective: Indonesia is known with its natural resources, one of them is abundant herbs. The herbs are basic ingredients of herbal medicine which is called jamu. Jamu started to develop since hundred years ago and passed through generation which is trusted to cure many diseases without giving any side effect. Lately, there are many cases of combining jamu with some chemical substances. The most common chemical substances used in jamu are acetaminophen and phenylbutazone. **Methods:** This research develops method to detect acetaminophen and phenylbutazone by using spektrofotometric UV-Vis which is combined with chemometrics. The most common chemometrics multivariate calibration used is Partial Least Square (PLS) and Principal Component Analysis (PCA). From this method, the data analysis used is R Studio software. This research generates a regression equation. **Results:** Based on this research, the regression equation indicates that the result is significance which can be seen from the value of $P < 0.05$. Besides that, the value of correlation coefficient of acetaminophen as 0.9889, and phenylbutazone as 0.9728. **Conclusion:** This method can be considered as qualified and meet the requirement.

Keywords: *Jamu, Acetaminophen, Phenylbutazone, Spektrofotometric UV-Vis, chemometrics.*

Introduction

Indonesia is famous for its natural resources, one of which is abundant spices. Since colonial times, Indonesia is known as one of the largest spice producing countries in the world [1]. Spices are the basic ingredients of making traditional medicines, such as in herbal medicine, cosmetics, and antimicrobials. Indonesia is still known as traditional medicine from herbal plants. This traditional medicine is commonly called to as "jamu".

Jamu began to develop in hundreds of years ago and carried out from generation to generation which is believed to relieve various diseases without giving effect to the body. Jamu come from natural plants, namely roots, stems and leaves. In recent years, there have been many cases of mixing herbal medicine with medicine. Herbs in general can not immediately cure or give effect to the body quickly, if this happens then the possibility of herbs can contain drugs [2].

This can have a bad effect on the body. Mixing drugs is usually done when brewing herbal medicine is ready to drink. So consumers sometimes don't know that. One of the drugs used is acetaminophen and phenylbutazone [3]. Acetaminophen is one of the commercially available drug compounds for relieving headaches or analgesics. While phenylbutazone is a non-steroidal anti-inflammatory drug as a pain reliever. Mixing the two drugs in herbal medicine can cause some bad effects.

Some of the bad effects that often occur when consumed continuously are loss of vision and hearing, stroke, heart attack, and can cause death [4]. One method used to analyze or detect the presence of acetaminophen and phenylbutazone in herbal medicine is the spectrophotometric method [5]. The spectrophotometric method is one of the common analytical methods developed to determine the levels of drug compounds in pharmaceutical preparations [6].

Spectrophotometry has a lower selectivity compared to other methods such as FTIR spectrophotometry, thin layer chromatography and high performance liquid chromatography. This is because the compounds to be analyzed are not single compounds. So the spectra will overlap. In addition, for the high performance liquid chromatography methods many people have conducted research, on the other hand they are also wasteful and expensive while the spectrophotometric method is cheaper and not many have done [7].

To produce a new, faster and validated package of analysis methods, this study intends to develop a spectrophotometric analysis method combined with multivariate calibration to simultaneously determine the levels of acetaminophen and phenylbutazone in herbal medicine [8]. Multivariate calibration of the chemometrics method that is often used is PLS (partial least square) [9].

This is because PLS is a regression that is calculated using the least squares by connecting two matrices, matrix X spectra data and Y matrix reference value. The combination of this method is able to produce an accurate spectrophotometric analysis method even though it is done without a separation process [10].

Materials and Methods

Materials

Herbal medicine with 30 different brands taken from Malang city, acetaminophen (Merck, Darmstadt, Jerman), phenylbutazone (Merck, Darmstadt, Jerman), ethanol (Merck, Darmstadt,

Jerman), methanol (Merck, Darmstadt, Jerman), distilled water.

Instruments

Agilent Cary 8454 spectrophotometer (Santa Clara, California, USA) equipped with 1.0-cm quartz cells, microlit lab micropipette (Santa Clara, California, USA), millipore (Merck, Darmstadt, Jerman), Laboratory ultrasonic bath PS 10000 (Los Angeles, California, USA).

Calibration and Validation Sample Preparation

The first step is to weigh all the working standards of acetaminophen, phenylbutazone, and complete herbs according to the doses listed in Table 1 for calibration and Table 2 for validation. After that, mix the three substances (acetaminophen, phenylbutazone, and herbal medicine) evenly into the vial bottle.

Then, weigh 50 mg of the mixture and put it into a 50 ml measuring flask. The 50 mg mixture is dissolved with methanol to the existing calibration sign so that the solution is obtained in accordance with the concentration shown in Table 1.

After this step, sonify for 10 minutes with a frequency of 50% (Solution A). Take as much as 0.5 ml of solution a using a measuring pipette which is then put into a 25 ml measuring flask and dissolved with methanol until the calibration mark is there. The solution is filtered first by using a micron millipore filter with a size of 0.2 microns. Then scanning the spectra with wavelengths between 200-350 nm with intervals of absorbance values per 1 nm.

Table 1: Comparison of acetaminophen, phenylbutazone, and herbal medicine for calibration

Number of calibration	Herbal medicine (mg)	Acetaminophen (mg)	Phenylbutazone (mg)	Acetaminophen %	Phenylbutazone %
1	100	0	0	0	0
2	400	50	50	10	10
3	65	50	50	50.10	50.10
4	50	100	100	40	40
5	50	100	50	50	25
6	50	50	100	25	50
7	50	50	150	20	60
8	50	100	50	60	20
9	50	400	50	80	10
10	50	50	400	10	80
11	50	450	0	90	0
12	50	0	450	0	90
13	50	50	50	55.55	55.55
14	50	75	50	62.65	58.57
15	50	50	75	58.57	62.65
16	50	60	50	62.3	51.25
17	50	50	50	58.31	47.30
18	50	30	50	47.30	58.31
19	50	125	50	55.55	52.22
20	50	50	125	52.22	55.55

Table 2: Comparison of acetaminophen, phenylbutazone, and herbal medicine for validation

Number of validation	Herbal medicine (mg)	Acetaminophen (mg)	Phenylbutazone (mg)	Acetaminophen %	Phenylbutazone %
1	50	50	85	27.02	45.94
2	50	85	50	45.94	27.02
3	50	65	50	39.39	30.30
4	50	50	65	30.30	39.39
5	50	70	50	41.17	29.41
6	50	50	70	29.41	41.17
7	50	95	50	48.71	25.64
8	50	50	95	25.64	48.71
9	50	85	65	42.5	32.5
10	50	65	85	32.5	42.5

Herbal Medicine Sample Preparation

In this study, there were 30 types of herbal medicine on the market in the East Java area analyzed. The sampling method used in this study is using purposive sampling. Where sampling with the intention is the selection of samples based on several criteria made by researchers. The sample criteria in question are: herbal medicine in the form of powder or capsules, herbal medicine for rheumatic pain and market samples taken in cities around East Java.

First, weigh as much as 50 mg for each herbal sample and then put it in a 50 ml measuring flask. The sample is dissolved using methanol until the calibration mark is available. After that, sonification is done for 10 minutes with a frequency of 50% (Solution A). Then take a solution of 0.5 ml using a measuring pipette and put it into a 25 ml measuring flask.

Then, dissolve with methanol until the calibration mark is there. The solution is filtered first by using a micron millipore filter with a size of 0.2 microns. After that, scanning spectra is carried out with wavelengths between 200 - 350 nm at intervals of 1 nm.

Statistical Analysis

In data collection, this study used the chemometrics method. The chemometrics method first prepared a calibration sample of 20 samples with several series of different concentrations between acetaminophen and phenylbutazone. Second, prepare a validation sample of 10 samples with different concentration series between acetaminophen and phenylbutazone.

Scan using wavelengths spectrophotometer with between 200 - 350 nm. The results of the analysis data are compared with data from samples of herbal medicine on the

market. The obtained spectrum data processing is processed using R studio software. The data obtained is changed and incorporated in one Excel 2010 software paper with wavelengths as columns and samples as rows. One excel software working paper is then input into the R studio software and calculated using formulas in R studio. Spectrum on the Excel worksheet is then inputted into the R studio software. Furthermore, the data were analyzed by multivariate regression using the PLS method to see the relationship between absorbance (variable X) and concentration (variable Y).

Before the regression process is selected data or variables using the GA-PLS method? Data that has been selected is validated Leave One out (LOO) cross validation. This LOO validation will produce the value of Root Mean Square Error of Prediction (RMSEP) which is shown in the form of a plot. The number of components used for data processing is indicated by a decrease in steep plots and forming angles.

The next step is to look at the selected absorbance and the significance value of each absorbance. After doing the regression process, the PCA method was used to find out the groups of each sample analyzed, which were included in the group of without drugs and herbal groups with drugs.

Results and Discussions

Chemometrics Model of Acetaminophen and Phenylbutazone

Spectrum data is changed using excels software. A total of 60 data absorbances of the sample were put together into one worksheet. In the worksheet, there are columns that represent wavelengths from 200 nm to 350 nm, while in rows represent numbers or labels on the sample.

Furthermore, the data from one excel worksheet was submitted to the R studio software, which then performed a statistical processing of absorbance data. Processing statistics was performed using multivariate calibration methods with Partial Least

Square (PLS) techniques [11]. In PLS techniques, variables that have a high correlation with response variables will be given additional weight to produce more effective predictions [12].

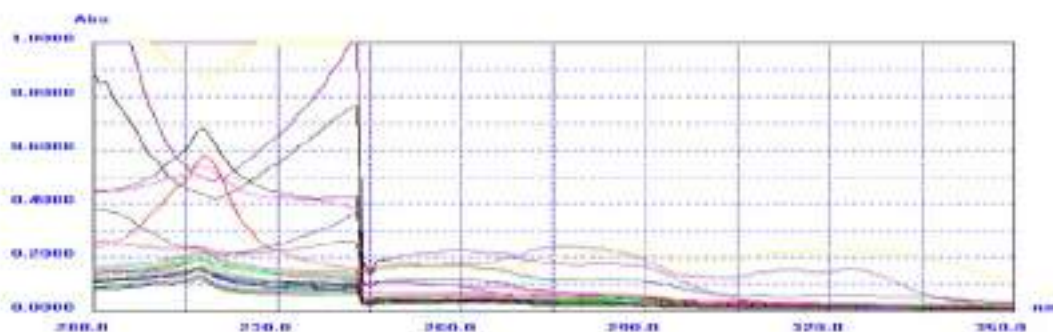


Fig. 1: Spectra from the UV-Vis Spectrophotometer

The absorbance data obtained from calibration and validation sample readings were used to make a calibration model using the Partial Least Square method using R studio software [13]. The selection of data or variables carried out using genetic algorithm

method combined with PLS regression. This method is able to select and sort variables to produce a significant model. Then a regression process is performed from the PLS method (Fig.2).

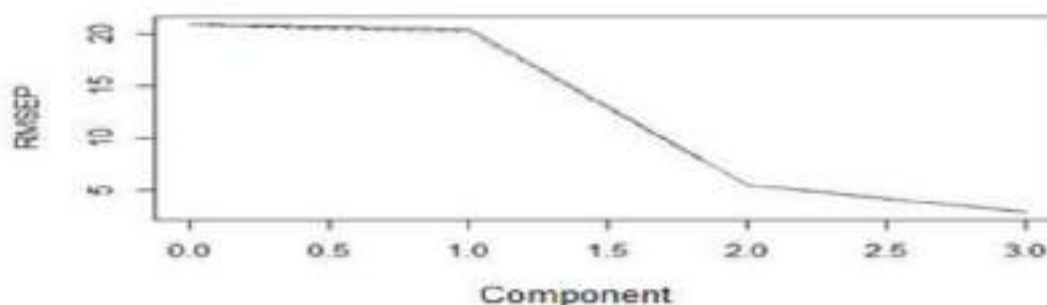


Fig. 2: RMSEP plot of acetaminophen

The value of RMSEP for acetaminophen compounds was produced at 2.981. This process is used to find the number of

components used (fig.2). The intercept and correlation coefficients generated were - 0.0784 and 0.9889 (Fig.3).

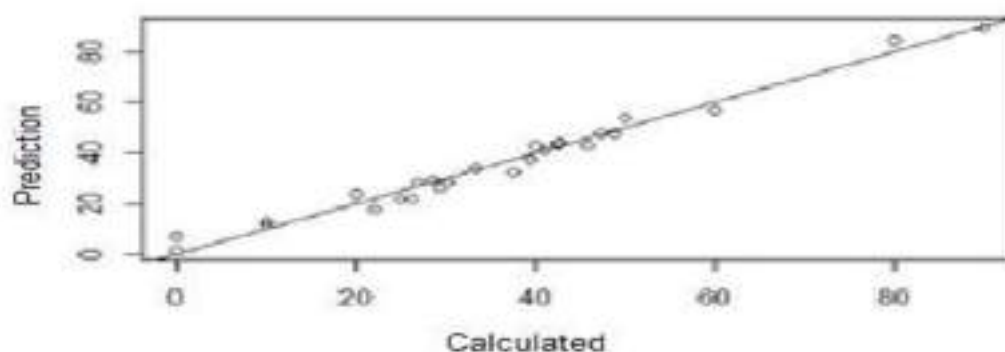


Fig. 3: Acetaminophen calibration curve

The R value that has been obtained is used to test the validity of a model or equation that has been produced [14]. After that the R value obtained is compared with the value of R that has been used as a reference for research based on the number of samples used [15]. If the R value obtained is close to the required value, it can be concluded that the relationship between the sample value and the absorbance value can be said to be proportional. In this study, a reference to the

standard R value used is standard R [16]. The correlation coefficient value from statistical data analysis is expected to reach the standard $R > 0.99$. The resulting correlation coefficient is 0.9889. In this case it can be concluded that the modeling or equation obtained has fulfilled the minimum R value requirements and shows the correlation between absorbance (Variable X) and concentration (variable Y) [17].

Based on the results described above, the following is the equation of the concentration of acetaminophen:

$$y = -0,0784 + 150,5811 X_1 - 171,7370 X_2 + 84,0094 X_3$$

X1 is absorbance at wavelength 263, X2 is absorbance at wavelength 277 and X3 is absorbance at wavelength 310. Variables from equations that have been produced can be said that these variables are significant because they have a value of $P < 0.05$ which means the null hypothesis is rejected and an alternative hypothesis is accepted. The value

of RMSEP for phenylbutazone compounds was 4,559. This process is also used to find the number of components used which can be seen in Figure 4. The intercept values and correlation coefficients generated are respectively -0.0727 and 0.9728 (Figure 5). The following is the calibration curve obtained as follows:

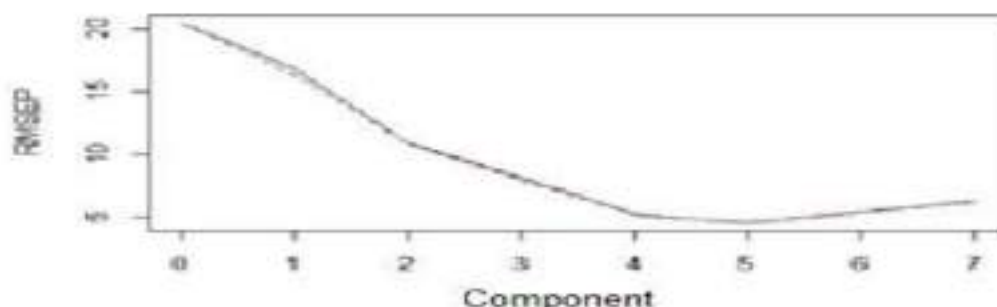


Fig. 4: RMSEP plot of phenylbutazone

The R value that has been obtained is used to test the validity of a model or equation that has been produced [18]. After that the value of R obtained is compared with the value of R that has been used as a reference for

research. If the R value obtained is greater than the required value, it can be concluded that the relationship between the sample value and the absorbance value can be said to be proportional [19].

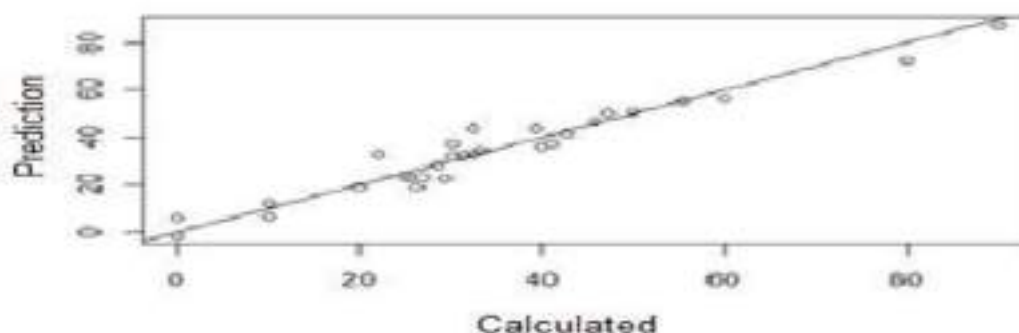


Fig. 5: Phenylbutazone calibration curve

The correlation coefficient obtained in this study fulfills the two standard R values obtained which are equal to 0.9728. It can be concluded that the equation or modeling

produced meets the requirements. Based on the results obtained, the following is obtained phenylbutazone concentration equation:

$$y = -0,0727 + 77,271 X_1 - 134,954 X_2 - 90,848 X_3 - 22,129 X_4 - 103,610 X_5 - 119,013 X_6 - 82,823 X_7$$

X1 is absorbance at wavelength 202, X2 is absorbance at wavelength 205, X3 is absorbance at wavelength 247, X4 is absorbance at wavelength 285, X5 is absorbance at wavelength 294, X6 is absorbance at wavelength 296, and X7 is absorbance at wavelength 342. The variables of the equation produced can be said to be significant.

This can be seen from the p-value obtained, namely $P < 0.05$, which means the null hypothesis is rejected and the alternative hypothesis is accepted. But there are 2 variables that are not significant with a value of $P > 0.05$, which is a variable with absorbance at wavelength 285 and absorbance at wavelength 342.

Principal Component Analysis

Processing the first data produces a diagram called a hierarchical diagram. A hierarchical diagram is a grouping of initial data by

equating data from each existing data group [20]. Hierarchical diagrams are also used to search areas of clusters which are then created or used as data for PC 1 and PC 2 or Dim 1 and Dim 2 [21]. PCA is an axis playback technique in such a way that PC 1 lies in maximum variation and PC 2 is located at playback in the direction that gives the next maximum variation So that the data can be described in two dimensions instead of a number of n origin data (Fig.6).

The results obtained from the data normalization process, in hierarchical diagrams appear a grouping that looks more structured or neatly arranged on each divided cluster [22]. Dim 1 and Dim 2 or can be called PC 1 and PC 2 can already be said to represent some of the data variance up to 90%. The Factor Map diagram results in the distribution of sample groups that are more clearly marked by the interrelation of each sample into the formed clusters [23].

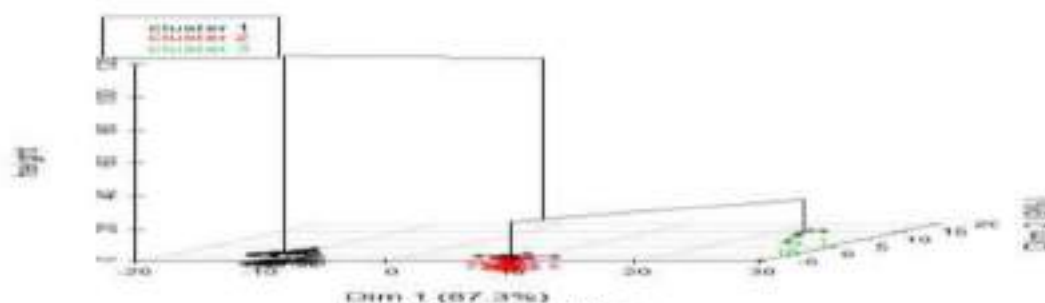


Fig.6: Hierarchical Diagram

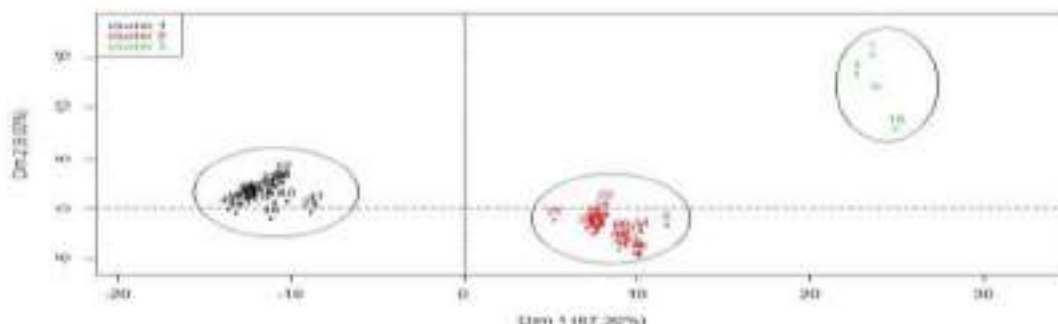


Fig. 7: Factor map diagram

The first cluster that is black is a group of herbs that do not contain drugs. Cluster of the two groups of herbs containing drugs. The second cluster is represented in red. The third cluster represented in green also shows medicinal-free herbs (Fig.7). According to the Republic of Indonesia Drug and Food Supervisory Agency Law No. 23 of 1992 article 1 paragraph 10, traditional medicines are ingredients or ingredients in the form of plant materials, animal materials, mineral materials, galenic preparations or mixtures of these materials which traditionally have been used for treatment based on experience [24].

However, this legal reference does not provide a full guarantee that all herbal medicines on the market are in accordance with the rules. This is in line with the findings of this study as evidenced by the results of the research obtained. Not all herbs in the market are free of drugs. Based on the results of the grouping that has been produced, from 30 market samples analyzed 5 traditional drugs were grouped in the second cluster. So that it can be concluded that the five market samples contain drugs namely acetaminophen and phenylbutazone.

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Conclusion

In reading the sample using the Principal Component Analysis method in 30 traditional medicines found 5 pieces of market samples containing acetaminophen and phenylbutazone. Samples containing drugs with codes J, K, S, U, V.

Author Contributions

Giovanny Cristy Hendarto participated in developing the research protocol. Eva Monica participated in field work supervision. Rollando Rollando participated in developing the research protocol, field work supervision, data analysis and drafting this manuscript.

Conflicts of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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