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Rapid and Simultan Determination of Phenylbutazone in Herbal Remedies Using Infrared Spectroscopy Combined With Chemometric

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ABSTRAK

Fenilbutason merupakan salah satu obat yang sering ditambahkan kedalam jamu. Menurut peraturan perundang-undangan di Indonesia, hal tersebut merupakan hal yang dilarang. Oleh karena itu, merupakan kebutuhan mendesak untuk mengembangkan metode yang cepat, dan dapat diandalkan untuk mendeteksi dan menentukan bahan kimia obat (BKO) dalam jamu. Penelitian ini bertujuan untuk mendeteksi dan menghitung fenilbutason dalam obat herbal menggunakan spektroskopi ATR-FTIR yang dikombinasikan dengan kemometrika. *Stepwise Multiple Linear Regression* (SMLR) digunakan untuk mengidentifikasi hubungan antara konsentrasi dan bilangan gelombang. Semua sampel diklasifikasikan dan didiskriminasikan masing-masing berdasarkan BKO yang ditambahkan dengan akurasi dan presisi yang sempurna. Hasil penelitian menunjukkan bahwa keberadaan BKO berhasil ditentukan pada konsentrasi 1 hingga 85 mg dalam 100 mg obat herbal dengan RMSECV 0,01134874 dan R^2 0,9984 dengan menggunakan teknik ATR-FTIR yang dikombinasikan dengan kemometrika.

Kata Kunci:
Fenilbutason; BKO; ATR-FTIR; Kemometrika

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ABSTRACT

Phenylbutazone is one of common adulterated drug in herbal remedies. According to legal regulations in indonesia, it is prohibited to adulterate those ingredients in herbal remedies. So, it is an urgent need to develop rapid, and reliable method for detection and determination adulterants in herbal medicine. This study aims for the detection and determination of Phenylbutazone in herbal remedies using ATR-FTIR spectroscopic combined with chemometrics. Stepwise Multiple Linear Regression (SMLR) was used identify the relationship between concentrations and wave numbers. All samples (Unadulterated and adulterated) were classified and discriminated respectively to their added contents with perfect accuracy and precision. The results suggest that existence of the active substance could be successfully determined at the levels in the range of 0.01 to 85 mg in totally 100 mg of herbal remedies with RMSECV 0.016054643 and R^2 0.9984 respectively by using ATR-FTIR technique combined with chemometrics.

Keywords:
Phenylbutazone, adulteration, ATR-FTIR, chemometrics

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1. Introduction

Herbal medicine is an Indonesian traditional medicine in the form of ingredients or hereditary herbs based on experience that has been used for treatment. As many as 60 percents of the people in Indonesia still consumed herbal medicine and 95 percent of the consumer feel that herbal medicine to improve health and cure diseases. During pandemic period in 2020, the turnover of herbal medicine reached Rp. 20 trillion with exports of Rp. 16 trillion. Even in 2026, global market revenue for herbal medicine is estimated to reach US$ 218.9 billion [1].

However, in the last 20 years there have been many adulterated drugs were found in herbal medicine. Many manufacturers intentionally adulterate their product to improve therapeutic effect and give more benefits to the herbal manufacturers [2]. Based on the results of sampling and testing conducted on July 2020 to September 2021, National Agency of Drug and Food Control found as many as 53 (fifty three) items of traditional medicinal products, 1 (one) item of health supplements containing adulterated drugs. Even, total findings of illegal herbal medicines and health supplements containing adulterated drugs found in 3,382 production and distribution facilities have an economic value of 21.5 billion. One of common adulterant was phenylbutazone that found in herbal medicine for gout and rheumatism therapy [3].

Phenylbutazone is a drug class of non-steroidal anti-inflammatory drugs (NSAIDs) derived from pyrazole. Uncontrolled consumption of phenylbutazone, could damage liver and kidneys. Even in regular consumption, phenylbutazone could cause gastric bleeding, gastric pain, nausea, vomiting, edema, anemia, agranulocytosis and aplastic anemia [4]–[6]. Because of these dangerous side effects, phenylbutazone usage is very limited and considered. Phenylbutazone drugs can still be considered if there are patients who have bechterew syndrome attacks and acute gout attacks [7]. However, adulteration of phenylbutazone was strictly forbidden according to PERMENKES No. 007 of 2012.

Analysis of phenylbutazone content is usually conducted using UV spectrophotometry, TLC and HPLC [7], [8]. However, the results of the spectrophotometry method are less sensitive and has selectivity problem and analysis using TLC and HPLC require complicated preparations, time-consuming and high cost. Therefore, this study uses an infrared spectrophotometer (FTIR) instrument. Because infrared spectrophotometry is rapid, cost-effective, and undestructive to the sample. This study utilized fingerprint patterns and wavenumbers to characterize adulterated and non-adulterated samples combined with Stepwise Multiple Linear Regression.

2. Method

Materials

Herbal medicines, KBr (Merck, US), phenylbutazone standard obtained from Sigma Aldrich (US).

Preparation of standard

Phenilbutazone standard was mixed homogenously with activated KBr (50: 950) using mortar and stamper. The standard is stored in dark and airtight bottle.

Adulterated sample preparations.

In this study, total of 7 different herbal medicines were used as matrixes. Into these blank herbal medicines (table 1), the phenylbutazone standard is added with varied levels of 1%; 5%; 10%; 15%; 20%; 25%; 40%; 45%; 80; and 85% then mixed homogenously using mortar and stamper.

Table 1. Sample name and Composition

Name	Composition
Jaya Asli	*Zingiberis rhizoma, Curcumae rhizoma, Andrographis folium, Carryophilly flos, Gendarusae folium*
Tawon	*Royal jelly, Foeniculi fructus, Liqusti racic, Conidii radic*
Pro Urat	*Bupleuri radix, Curcuma domestica rhizoma, Polyanthi folium, Plantago mayor folium, Cyperia rhizoma, Phaleria fructus, Piperis nigri fructus, Zingiberis rhizome*
Uratan	*Nigellae sativae semen, Cinanmomi burhani cortex, Zingiber officinalis rhizoma, Curcuma domesticae rhizoma, Andrograpis paniculata herba, Elaeocarpus grandiflora fructus, Feniculum vulgare fructus*
Basmurat	*Polyanthi folium, Sojae semen, Alii saviti bulbus*
Nosrat	*Zingiber rhizoma, Piperis nigri fructus, Orthosiphonis rhizoma, Retrofracti fructus*
Wangton	*Zingiberis rhizoma, Chobati rhizoma, Asari herba, Epimedii herba.*

Scanning and Data Analysis

Adulterated samples are placed on ATR (Attenuated Total Reflectance) crystals at controlled temperatures. Measurements were made at 32 scans, and at separating power (resolution) 1 cm^{-1}. After scanning, the ATR crystal plate was cleaned with alcohol and dried with a tissue. To avoid spectral variations between times, the base spectrum is measured each time a sample measurement is made. All spectra were recorded at 4000 to 400 cm^{-1} and replicated 3 times. Obtained data were processed and analized using Stepwise Multiple Linear Regression based on the coefficient of determination (R2) and RMSECV values with Minitab 18.

3. Results and Discussion

Quantitative analysis using infrared spectroscopy is based on the ability to form fingerprint pattern. Each compound will generate different infrared spectrum like shown in figure 1.

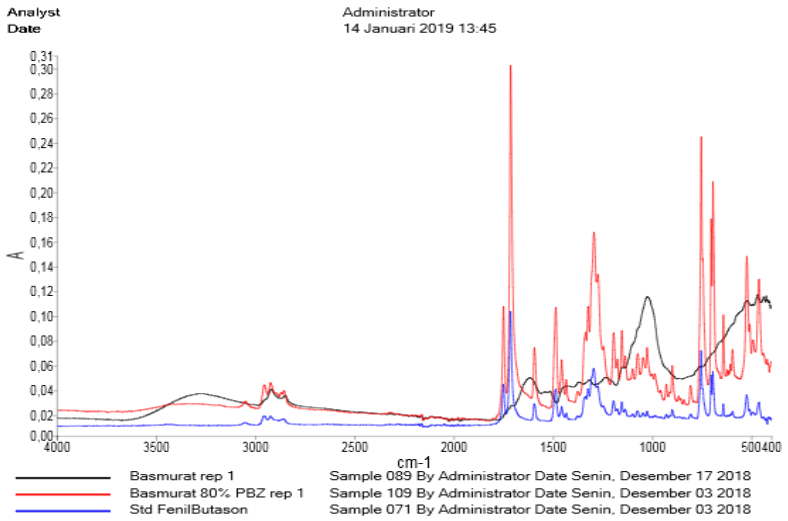

Figure 1. Overlay spectra of Bamurat herb, Basmurat herb spiked with phenylbutazone and phenylbutazone standard

The aim of overlaying is to compare three types of samples to see similarities and differences in each spectrum. The sample (basmurat) which was adulterated by phenylbutazone has nearly similar spectra to phenylbutazone standard and it was easy to pick several peaks that match with concentration of adulterated phenylbutazone. However, it was different when all samples were overlayed like shown in figure 2.

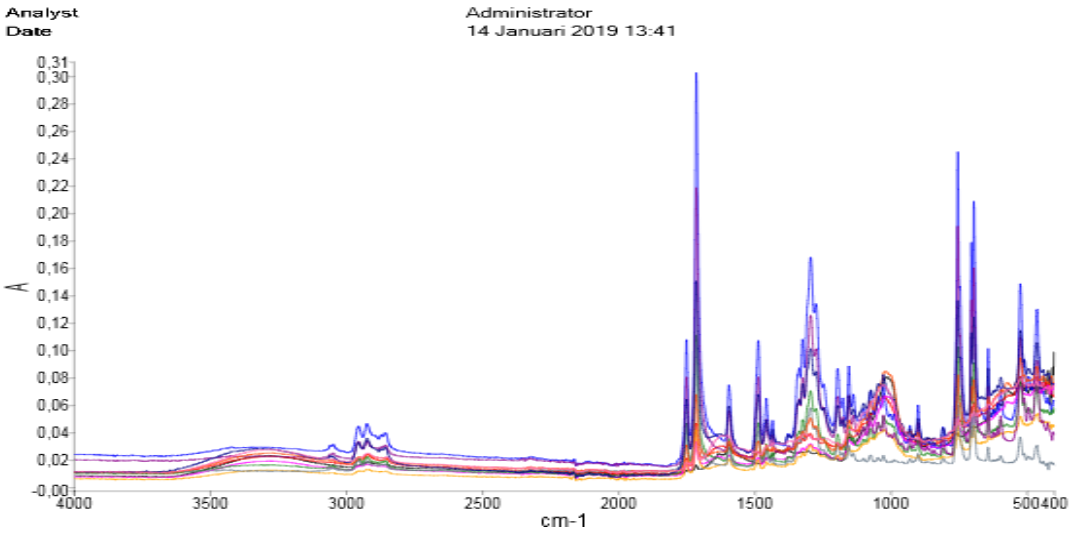

Figure 2. Overlay spectra of phenylbutazone standard with adulterated samples in many concentration

In figure 2, it was very complicated to pick several peaks that have correlation with concentration of adulterated phenylbutazone. In such condition, SMLR was used to remove uncorrelated variables (peaks) while regressing multiple variables simultaneously [9]. This method was used to identify and measure relationship between concentration values of phenylbutazone in the samples and the absorbance values of wave numbers. This SMLR is a development of a forward selection method in which the variable entered is a variable that has a high-value correlation then is continued for a

variable whose correlation value is higher after being combined with the previous variable. But in practice, it is combined with a backward selection method that served to eliminate insignificant variables [10]. The results is 0.2374 + 18.66 (1736) - 5,187 (1713) + 53.44 (1517) - 193.1 (1508) + 118.1 (1507) + 2.47 (1075) + 12,017 (755) - 23,59 (464) + 16,53 (460). From the equation, specific wave numbers whis have significant correlation with adulterated phenylbutazone were described. According to SMLR, 1736, 1713, 1517, 1508, 1507, 1075, 755, 464, and 460 cm^{-1} were chosen as significant wave numbers. The functional groups responsible for phenylbutazone were compiled in Table 2.

Table 2. Functional groups of phenylbutazone in Adulterated Herbal Medicine

Wave numbers	Functional Groups
1736 and 1713	Carbonil (C=O)
1517	C-C ring and C-N
1508 and 1507	C = C aromatic
1075	C - C

Source: Auer, et.al, 2003 [11]

Phenylbutazone wave numbers in standard, have significant distinctive differences and uniqueness compared to phenylbutazone wavenumbers in the herbal medicine matrixes. This occurs because the matrixes consist of multiple plant extract with multi-component which are very possible to have functional group characteristic similar to phenylbutazone. Then the wave number selected from the SMLR modeling is the wavenumber that identifies phenylbutazone in the typical herbal medicine matrixes [12].

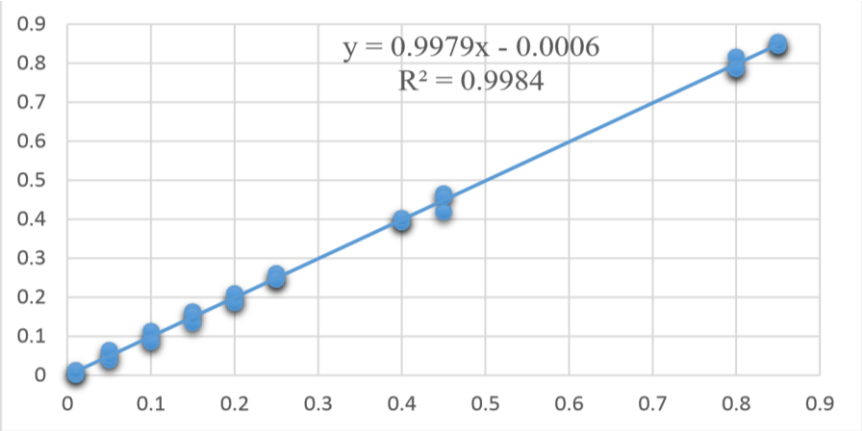

Figure 3. Correlation between prediction and actual value of adulterated phenylbutazone in samples

The results of the data processed from 30 samples using the SMLR method generated P <0.05. The results are said to have the best values that can be seen from the parameters of the value of determination coefficient (R2) and the value of RMSECV [9]. RMSECV is a parameter representing capability of the model to predict total concentration including adulterated concentration of phenylbutazone in samples within the validation range as determined by the concentration range of the adulterated analytes. The RMSECV results is 0.01134874. These results are already very good because the value obtained is very small and approaches the value of 0 which indicates that the value has a very small error rate or is said to have excellent precision while the value of R2 is 0.9984. These results are already very good because the value of the coefficient of

determination is close to the value of 1 which describes a very high level of accuracy like shown in figure 3 [13]–[15].

4. Conclusion

The Fourier-transform infrared spectroscopy (FTIR) method combined with the Stepwise Multiple Linear Regeneration (SMLR) could carry out a quantitative analysis of phenylbutazone in herbal medicine in various concentration variations. The application of this method is very simple, fast, inexpensive, and non-destructive.

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