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Correlation between FTIR fingerprint spectra and inhibition of α-glucosidase activity of Andrographis paniculata using partial least squares regression analysis

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Abstract*:* *Andrographis paniculata* has been used empirically by people as traditional medicine to treat diabetes. In this study, we correlated the FTIR spectra and α-glucosidase inhibition by using chemometrics analysis to obtain a functional group from the metabolite present in *A. paniculata* herb exhibited α-glucosidase inhibitory activity. *A. paniculata* herb powder was extracted using water, ethanol, ethyl acetate, and n-hexane using ultrasonication method. The dried extract obtained was then measured for its FTIR spectra and determined for α-glucosidase inhibitory activity. Absorbance data from the FTIR spectra of A. paniculata herb extracts were used as a principal component analysis (PCA) variable for grouping extract based on the solvent extractor used. In addition, partial least square regression analysis (PLSR) is also used to predict functional groups from metabolites that cause inhibition of α-glucosidase. In the PLSR, we correlated using the FTIR spectrum's absorbance values and IC_{50} of α-glucosidase inhibitory activity. Before being subjected to PCA and PLSR, the FTIR spectrum was preprocessed using standard normal variate. PCA using the absorbance data from 1800–1000 cm^{-1} and 2800–3400 cm^{-1} could group the extract based on extracting solvent used with a cumulative percentage of the two principal components (PC), namely PC-1 and PC-2 are 86%. The PLSR analysis showed that OH, C=O, C=C, and C-O are the predicted functional groups related to the inhibition of α-glucosidase. So, those functional groups are present in the metabolites in *A. paniculata* herb extracts that contributed to the inhibition of α-glucosidase.

Keywords: *Andrographis paniculata*, α-glucosidase, chemometrics, FTIR spectra

Abstrak: *Andrographis paniculata telah digunakan secara empiris oleh masyarakat sebagai obat tradisional untuk mengatasi diabetes. Dalam penelitian ini, kami mengorelasikan spektrum FTIR dan aktivitas penghambatan α-glukosidase menggunakan analisis kemometrik untuk mengidentifikasi gugus fungsi yang terdapat pada metabolit herba A. paniculata yang menunjukkan aktivitas penghambatan α-glukosidase. Serbuk herba A. paniculata diekstraksi menggunakan air, etanol, etil asetat, dan n-heksana dengan metode ultrasonikasi. Ekstrak yang telah dikeringkan kemudian dianalisis menggunakan spektroskopi FTIR dan dievaluasi aktivitas penghambatan α-glukosidasenya. Data absorbansi dari spektrum FTIR ekstrak herba A. paniculata digunakan sebagai variabel dalam analisis principal component analysis (PCA) untuk mengelompokkan ekstrak berdasarkan pelarut yang digunakan pada proses ekstraksi. Selain itu, analisis regresi partial least squares (PLSR) digunakan untuk memprediksi gugus fungsi dari metabolit yang menghambat α-glukosidase. Pada PLSR, kami mengorelasikan nilai absorbansi spektrum FTIR dengan nilai IC_{50} aktivitas penghambatan α-glukosidase. Sebelum dianalisis dengan PCA dan PLSR, dilakukan prapemrosesan spektrum FTIR menggunakan metode standard normal variate. PCA menggunakan data absorbansi pada rentang 1800–1000 cm^{-1} dan 2800–3400 cm^{-1} berhasil mengelompokkan ekstrak berdasarkan pelarut ekstraksi, dengan persentase kumulatif dua komponen utama (PC), yaitu PC-1 dan PC-2, sebesar 86%. Analisis PLSR menunjukkan bahwa gugus fungsi –OH, C=O, C=C, dan C–O merupakan gugus fungsi yang paling kuat berkaitan dengan penghambatan α-glukosidase. Dengan demikian, gugus-gugus fungsi tersebut terdapatpada metabolit ekstrak herba A. paniculata dan berkontribusi terhadap penghambatan α-glukosidase.*

Kata kunci: *Andrographis paniculata, α-glukosidase, kemometrik, spektrum FTIR*

INTRODUCTION

Diabetes mellitus is an endocrine system disease characterized by blood glucose levels above the normal range. Now, diabetes mellitus is a common disease that has been rapidly developing worldwide. It is estimated that by 2045, there will be 783.2 million people with diabetes, an increase of 46% from 2021 (Sun *et al*. 2022). Treatment for diabetes mellitus generally uses synthetic drugs such as metformin, which, although effective, often causes side effects such as digestive disorders and hypoglycemia. (Li *et al*. 2012). Therefore, it highlights the need for research to develop safer and more effective natural-based treatment alternatives.

Andrographis paniculata is a medicinal plant that has been used in traditional medicine systems for a long time. This is due to the content of various secondary metabolites from the diterpenoid, flavonoid, and polyphenol compound classes, some of which are the main bioactive compounds, such as andrographolide (Dwivedi *et al*. 2021; Rafi *et al*. 2022). These compounds exhibit various pharmacological activities, including antidiabetic activity. However, the quality and quantity of the bioactive compounds produced are greatly influenced by several factors, such as the part of the plant (Bai *et al*. 2020; Maslahat *et al*. 2025), planting age (Tajidin *et al*. 2019), extraction solvent (Lezoul *et al*. 2020; Rafi *et al*. 2023; Akhter *et al*. 2024), geographical origin (Hayati *et al*. 2024, etc. The type of solvent used in the extraction process affects the extraction of compounds from the plant sample, which in turn influences the pharmacological activity of the extract.

Evaluation of the effects of different solvents on metabolite composition and their correlation with pharmacological activity is currently being widely developed using the metabolomics approach. Metabolomics comprehensively, quantitatively, and qualitatively analyzes metabolites found in organisms, utilizing multivariate analysis (Nalbantoglu 2019). This approach enables the identification of metabolite profiles and changes in metabolites under various conditions, linking pharmacological activity with metabolite signals generated from the spectrum or chromatogram. One method often used in metabolomics is the combination of FTIR spectra with chemometrics, as it offers several advantages, including speed, accuracy, and reliability in evaluating the effects of solvent variations. FTIR spectrum fingerprints also have high stability values (Måge *et al*. 2021).

Previous studies using a combination of FTIR spectra with chemometric analysis such as PCA have succeeded in grouping samples based on differences in extraction solvents such as in Sudanese honey (Tahir *et al.* 2017), *Sonchus arvensis* (Rafi *et al*. 2021), *Syzygium polyanthum* (Rohaeti *et al.* 2021). Additionaly, partial least square regression (PLS-R) has been employed to investigate the correlation between the functional groups of compounds and the biological activities of the three samples. However, this is the first attempt at grouping A. paniculata extracts based on differences in solvent polarity and their correlation with α-glucosidase inhibitory activity. Therefore, this study aims to classify A. paniculata extracts based on differences in solvent types (water, ethanol, ethyl acetate, and n-hexane) and predict the functional groups of metabolites that contribute to α-glucosidase inhibitory activity using FTIR spectra combined with PCA and PLS-R.

MATERIALS AND METHOD

Materials, Chemicals, and Instruments

A. paniculata herb was obtained from *Balai Penelitian Tanaman Obat dan Rempah* in the 2020 and identified at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Ethanol, ethyl acetate, *n*-hexane, and distilled water were purchased from Merck (Darmstadt, Germany). Potassium bromide (spectroscopy grade), α-glucosidase enzyme from *Saccharomyces cerevisiae*, p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate, acarbose, bovine serum albumin (BSA), sodium carbonate, dimethyl sulfoxide (DMSO), monobasic potassium phosphate KH_2PO_4, and dibasic potassium phosphate K_2HPO_4 were obtained from Sigma Aldrich (St. Louis, USA). The instruments used are FTIR Tensor 37 spectrophotometer (Bruker Optik GmbH, Ettlingen, Germany), microplate reader ELX 800 (Epoch BioTek, Winooski, USA), ultrasonicator LC 30 H (Elma Schmidbauer GmbH, Singen, Germany), and rotary evaporator (Heidolph, Germany).

Sample Preparation

The samples were cleaned of other foreign materials with running water and then air-dried. The dried samples were ground using a blender and then sieved to obtain a powder with a particle size of 4/18. A total of 20 g of sample powder was added to 100 mL of solvent (ethanol, ethyl acetate, n-hexane, and water) and extracted using ultrasonication at 40 kHz for 30 minutes. Extraction was performed thrice, first with 50 mL, then for the second and third times, each time using as much as 25 mL of extracting solvent. The solution was filtered and the filtrate obtained was concentrated using a rotary evaporator. We performed the extraction in five replicates. The yield percentage of the extract was calculated using the Equation (1).

$$\text{Yield (\%)} = \frac{\text{berat ekstrak (g)}}{\text{berat sampel bubuk (g)}} \text{x}100\% \quad ...(1)$$

Inhibition of α-Glucosidase Activity Assay

A total of 50.0 µL of solution extract was added to 450 µL of phosphate buffer, pH 7.0, and 250 µL of 10 mM PNPG substrate solution. Furthermore, the solution was preincubated for 5 minutes at 37°C, then 250 µL of the enzyme solution was incubated at 37 °C. After incubation, 1000 µL of 0.2 M sodium carbonate was added. A solution of up to 200 µL was added to the microplate reader, and the absorbance was measured at λ = 405 nm. Acarbose was used as a positive control. The percentage of inhibitory activity was calculated using the following Equation (2).

$$\%\text{Inhibition}=\frac{Absorbansi\ blanko - Absorbansi\ sampel}{Absorbansi\ blanko} \text{ x } 100\% \quad ...(2)$$

The IC_{50} value was calculated by plotting the concentration versus the enzyme's degree of inhibition (%).

FTIR Spectrum Measurement

Samples were prepared as pellets by mixing 2 mg of each extract with 200 mg of KBr. KBr pellet analysis was performed using a Tensor 37 FTIR spectrophotometer equipped with a deuterated triglycine sulfate detector. FTIR spectra were recorded in the 4000-400 cm^{-1} range with a resolution of 4 cm^{-1} using OPUS 4.2 software (Bruker, Ettlingen, Germany). The absorbance data from each extract stored in a data point table format.

Data analysis

Experimental data are reported as the' mean ± standard deviation of five independent experiments. To determine significant differences, data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test if $p < 0.05$. Significant differences were set at the 5% level ($p < 0.05$). Chemometric analysis of PCA and PLS-R was performed using The Unscrambler X software version 10.1 (Camo, Oslo, Norway). Before analysis, the FTIR spectrum was preprocessed using the standard normal variate (SNV) method. PCA was used to differentiate each extract based on its extraction solvent using absorbance data variables in the 4000-400 cm^{-1}, 1800-1000 cm^{-1}, and 2800-3400 cm^{-1} regions. PLS-R aims to predict functional groups with antidiabetic activity from *A. paniculata* extract, carried out by correlating the FTIR spectrum (x-axis) with the IC50 value from the enzyme activity test of α-glucosidase (y-axis).

RESULT AND DISSCUSION

Yield percentage of the extract

The yield of extracts from *A. paniculata* herbs obtained using four different solvent extraction methods (water, ethanol, ethyl acetate, and *n*-hexane) is shown in Table 1. Based on the results obtained, water extract produces the highest yield (9.64%), compared to the other extracts. Analysis of variance revealed that the type of solvent had a significant effect on the percentage of extract yield at a 5% significance level (p-value < 0.05). The ethyl acetate and *n*-hexane extracts did not show significant differences, whereas the water and ethanol extracts exhibited significant differences. This indicates that the type and concentration of compounds extracted depend on the solvent used. In addition, the metabolites contained in *A. paniculata* herbs are polar to semipolar based on the yield of the extract obtained, with the majority being polar.

α-glucosidase Enzyme Inhibitory Activity

Inhibition of α-glucosidase by *A. paniculata* herb extracts was performed *in vitro* with acarbose as the positive control. The results were expressed as IC_{50}, the concentration of extract that inhibits 50% of α-glucosidase activity. Based on the results in Table 1, the ethanol extract showed the highest inhibition of α-glucosidase activity, with an IC50 of approximately 50.76 µg/mL, followed by the water, ethyl acetate, and *n*-hexane extracts. These results indicated that semipolar to polar metabolites exhibit inhibition of α-glucosidase. As we know, andrographolide, a marker metabolite in A. paniculata, is known to possess this activity and can be extracted using polar solvents such as ethanol and water (Rao & Rathod 2015; Astuti *et al*. 2022). Significant differences in the IC_{50} values of the four solvent extractions indicated that each extract contained α-glucosidase inhibitory compounds at varying concentrations. So, selecting the solvent extraction will affect the level of α-glucosidase inhibitory activity.

FTIR Spectral Fingerprint Analysis

Fingerprint analysis using FTIR aims to identify the sample's characteristics and determine the functional groups present in its chemical components.

Table 1. Yield percentage and IC50 of α-glucosidase inhibitory activity of A. paniculata herb extract

Solvent	Yield (%)	IC_{50} (µg/mL)
Water	9.64 ± 0.95 a	61.04 ± 0.89a
Ethanol 96%	5.56 ± 0.61 b	50.76 ± 1.42b
Ethyl acetate	1.19 ± 0.09 c	73.13 ± 1.25c
n-Hexane	0.44 ± 0.04 c	89.97 ± 0.77d
		Acarbose = 53.62

Note: Mean of 5 replications. Numbers in the same column followed by different letters indicate significantly different results at $p < 0.05$ (Tukey).

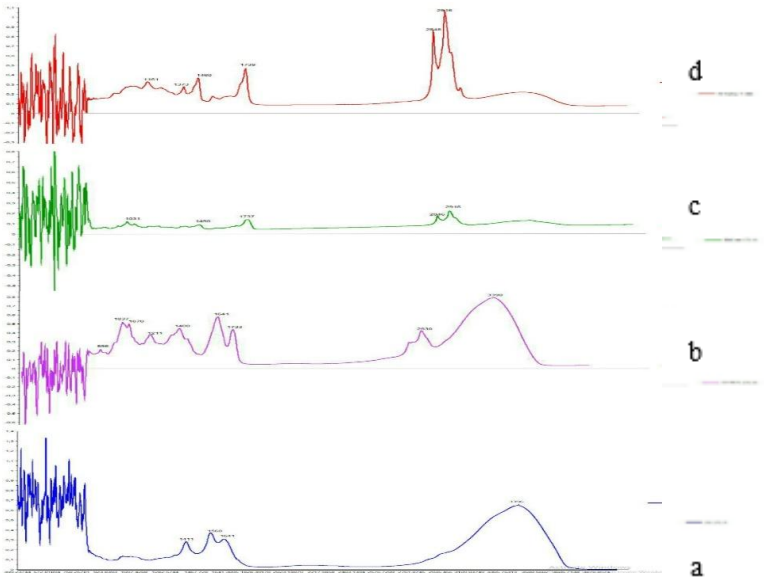


Figure 1. FTIR spectrum of water (a), ethanol (b), ethyl acetate (c), and *n*-hexane (d) extract of *A. paniculata* herb

Figure 1 shows the FTIR spectra profile of four extracts of *A. paniculata* herb. We observed a distinct FTIR spectrum profile for each extract, as each extract gave a different absorption band. *A. paniculata* herb extract shows the presence of metabolites with several types of functional groups based on the FTIR spectra profile (Table 2).

At a wavenumber of approximately 3400 cm^{-1}, we observed a broad absorption band, particularly in the ethanol and water extracts of A. paniculata herb, indicating the presence of a hydroxyl group (-OH). An absorption band occurred at 2810–3000 and 3000-3100 cm^{-1}, indicating the presence of -CH and =CH stretching vibrations, respectively. Meanwhile, absorption in the range of 1700–1780 cm^{-1} suggests the presence of a carbonyl (C=O) group from an ester, and at wavenumbers 1580–1670 cm^{-1}, absorption is related to the presence of alkenes (C=C) through stretching vibrations. In addition, absorption in the range of 980–1390 cm^{-1} indicates the presence of C-O and C-C groups through stretching vibrations (Yusof *et al*. 2015). However, a disturbing noise was detected at wavenumbers 400–800 cm^{-1}, so it could not be analyzed further.

The FTIR spectrophotometer effectively identifies the functional groups of a compound in a sample, such as plants. Its use is still limited because the complexity of the sample matrix and the resulting spectrum influence it. This weakness has been overcome based on the results of various research papers using chemometrics. Chemometrics is an alternative method for analyzing plant components using multivariate statistical analysis based on specific characteristics. It has to produce more accurate data and information. Thus, applying chemometrics can expand the potential use of the FTIR spectrophotometer in analyzing plant chemical composition

Principal Component Analysis for Clustering *A. paniculata* Extracts

PCA was performed to visualize and explore patterns, clusters, or outliers by simplifying variance into several new variables called principal components (PC). PCA results are visualized in the form of a score plot, which is a projection of several objects into two principal components that facilitate the identification of similarities or differences between samples (Pinto 2017). In this study, the classification of *A. paniculata* herb extracts was performed using the FTIR spectra absorbance data. Before being subjected to the PCA, we preprocessed the data using the standard normal variate (SNV) method. With SNV, spectral data is standardized to correct for spectrum shifts due to differences in concentration or instrument variations, increasing the accuracy and robustness of the model (Cheng *et al.* 2024).

In this work, we used absorbance from each FTIR spectrum of all extracts at wavenumber 4000–400 cm^{-1} and absorbance from segmentation wavenumber at 1800–1000 cm^{-1} and 2800–3400 cm^{-1} for PCA. The PCA score plot for all extracts is shown in Figure 2. The PCA score plot, using absorbance from 4000–400 cm^{-1} (Figure 2), shows a total PC value of 64%. This indicates a diverse sample that can be explained by the model, with a variance of up to 64%, comprising PC1 at 39% and PC2 at 25%. However, the grouping of *A. paniculata* herb extracts based on solvent extraction is not clearly separated, especially for the ethyl acetate and *n*-hexane extracts. This could be due to the presence of noise at wavenumbers 800–400 cm^{-1}, which may interfere with the classification results obtained by PCA. When we used the absorbance data from

Table 2. Functional groups identified in *A. paniculata* herb extract

Wavenumber (cm -1)	Functional group
3400	-OH
3000-3100	=CH
2810–3000	-CH
1700–1780	C=O or ester (RCOOR)
1580–1670	C=C
980-1390	C-O and C-C

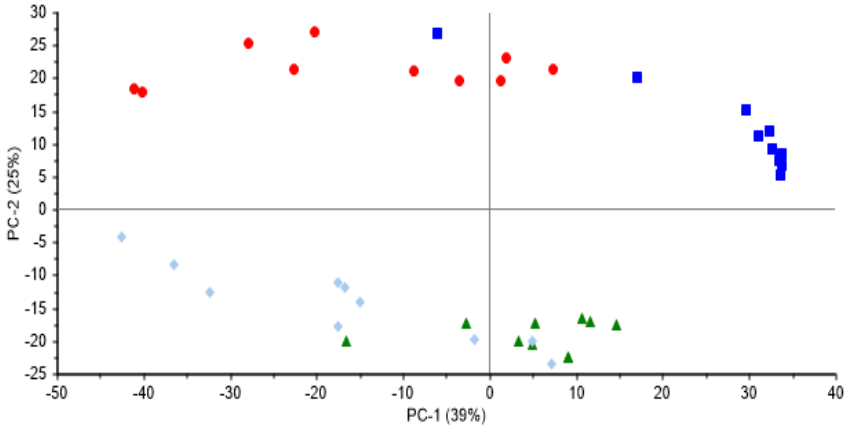


Figure 2. PCA plot score with absorbance data from 4000-400 cm^{-1} of *A. paniculata* herb extract with water (■), ethanol (●), ethyl acetate (▲), and *n*-hexane (◆).

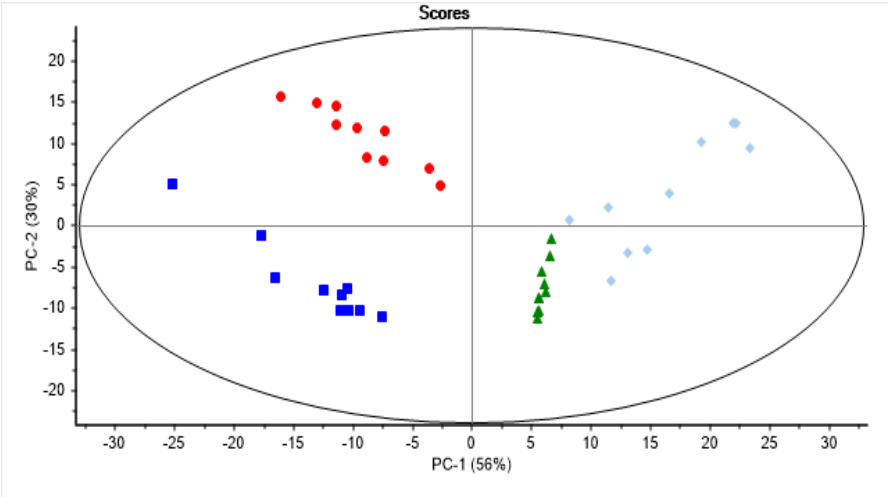
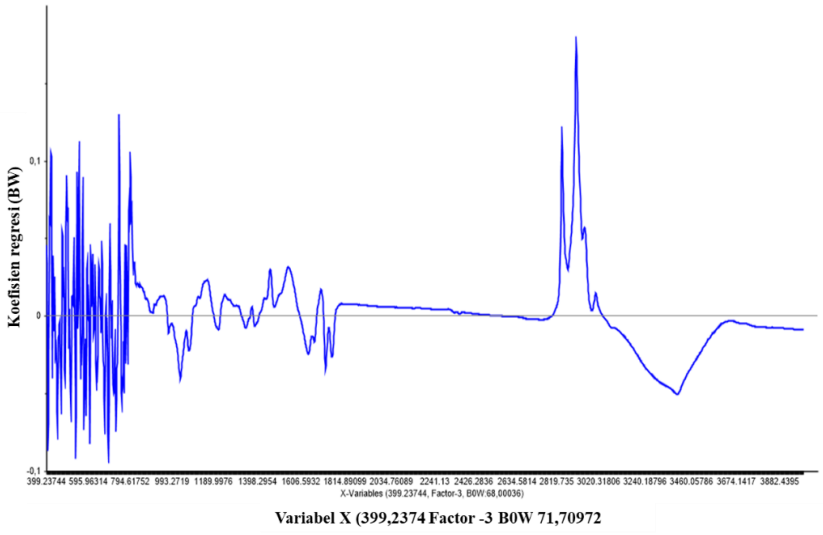


Figure 3. PCA plot scores with absorbance data from 1800-1000 cm $^{-1}$ and 2800-3400 cm $^{-1}$ of *A. paniculata* herb extract with: water (■), ethanol (●), ethyl acetate (▲), and *n*-hexane (◆).

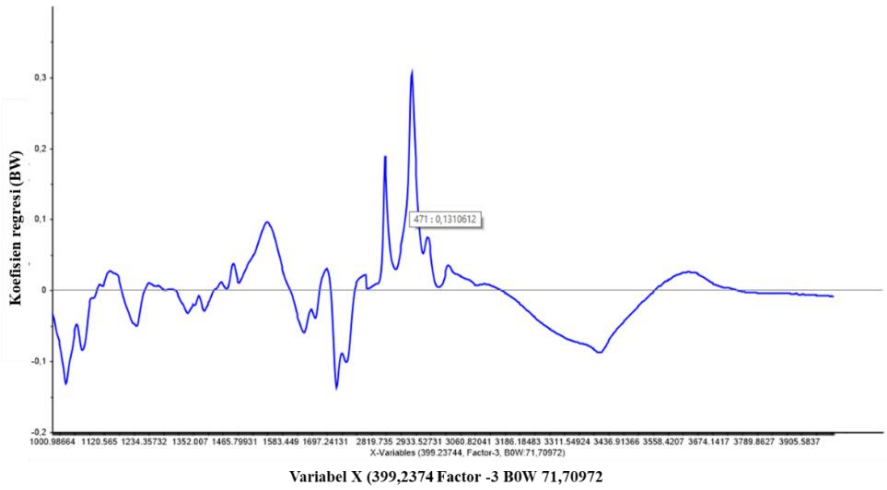
segmentation, wavenumbers 1800–1000 cm^{-1} and 2800–3400 cm^{-1} improved the grouping of the four A. paniculata herb extracts, as shown in Figure 4. A PCA score plot using absorbance data from wavenumbers 1800–1000 cm^{-1} and 2800–3400 cm^{-1} produced a higher PC value than one without segmentation, which was 86%. The grouping pattern between extracts was also more clearly visible. This increase was due to the characteristics of the infrared region in the wavenumber range, which was more specific intra-molecularly, clarifying the grouping of *A. paniculata* herb extracts based on differences in metabolite composition.

Correlation of FTIR Spectra and α-Glucosidase Inhibitory Activity

PLS-R analysis aims to identify variables with a significant effect. Therefore, we can predict and identify the functional groups in a metabolite that contribute to the inhibition of α-glucosidase activity in *A. paniculata* herb extracts. This method connects the x variable (predictor) with the y variable (response). This study used the absorbance value of



(a)



(b)

Figure 4. PLS-R plot using absorbance data from 4000-400 cm^{-1} (a) and 1800-1000 cm^{-1} and 2800-3400 cm^{-1} (b) of *A. paniculata* herb extract

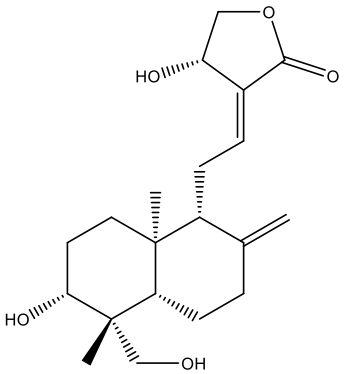


Figure 5. Structure of andrographolide

the FTIR spectrum of *A. paniculata* herb extract as the x variable, while the IC_{50} value from the α-glucosidase inhibitory activity was used as the y variable. Data will be processed before use using the preprocessing method, as in PCA. The results of the PLS regression analysis are presented in the form of a

regression coefficient plot, which provides information on the metabolite functional groups that significantly contribute to the inhibitory activity of α-glucosidase.

Based on the results of the regression coefficient in Figure 4, the correlation between the x and y variables obtained shows peaks that point upward and downward to the x-axis. The downward peaks indicate a positive correlation with the inhibitory activity of the α-glucosidase enzyme, whereas the upward peaks do not contribute to this activity. Functional groups that are positively correlated at wavenumbers 4000–400 cm^{-1} (Figure 4a) are located at 3500–3100 cm^{-1}, 1700–1500 cm^{-1}, 1250–1190 cm^{-1}, and 1100–900 cm^{-1}. In the range 3500–3100 cm^{-1}, the presence of the OH functional group was detected, while in the range 1700–1500 cm^{-1}, the C=O and C=C functional groups were found. The C-C and C-O bonds were identified in 1250–1190 and 1100–900 cm^{-1} (Yusof *et al*. 2015). he presence of OH, C=O, and C=C functional groups can be directly associated with andrographolide compounds (Figure 5). Andrographolide is one of the compounds that is widely studied as a component of the *A. paniculata* herb.

CONCLUSION

This study found that the ethanol extract of *A. paniculata* herb has the highest α-glucosidase inhibitory activity compared to water, ethyl acetate, and *n*-hexane extract. FTIR fingerprinting spectra of all *A. paniculata* extracts showed different profiles, so the extracted metabolites' composition and concentration differed. Classification of four *A. paniculata* herb extracts successfully obtained by PCA using the absorbance data from 1800-1000 cm^{-1} and 2800-3400 cm^{-1}. Correlation between FTIR spectra of *A. paniculata* herb extracts with the IC_{50} of α-glucosidase inhibitory activity by the PLS-R method showed that OH, C=O, C=C, and C-O functional groups are predicted and present in the metabolite contained in *A. paniculata* that has inhibition activity for α-glucosidase.

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