Inhibition of α-glucosidase activity, metals content, and phytochemical profiling of *Andrographis paniculata* from different geographical origins based on FTIR and UHPLC-Q-Orbitrap HRMS metabolomics

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Abstract. *Rafi M, Septaningsih DA, Karomah AH, Lukman, Prajogo B, Amran MB, Rohman A. 2021. Inhibition of α-glucosidase activity, metals content, and phytochemical profiling of* Andrographis paniculata *from different geographical origins based on FTIR and UHPLC-Q-Orbitrap HRMS metabolomics. Biodiversitas 22: 1535-1542.* Ensuring consistency of quality, safety, and efficacy of herbal medicines from raw materials to finished products is important because of the variability in medicinal plants' active components. One of the plants that have been used as an antidiabetic herbal medicine is *A. paniculata*. This study aims to determine α-glucosidase inhibitory activity, the content of several metals, FTIR spectrum profile, and putative identification of *A. paniculata* metabolites using UHPLC-Q-Orbitrap HRMS from different geographical origin. We found that ethanol extract of *A. paniculata* gave higher inhibition of α-glucosidase activity compared to water extract. Eight metals were determined using FAAS and FAES, and calcium showed the highest content in all *A. paniculata* samples. FTIR spectra of *A. paniculata* showed a similar profile and only differed in the absorbance. We detected the presence of OH, C=O, C=C aromatic, and C-O functional groups in *A. paniculata* extract. About 32 metabolites were putatively identified in *A. paniculata*, mainly from phenolic and diterpene lactones class compounds based on UHPLC-Q-Orbitrap HRMS. Using a combination of FTIR spectra and peak area from 32 identified peaks, we can clustered *A. paniculata* based on its geographical origin. Based on the result obtained, the geographical origin of *A. paniculata* affected the metals and metabolites composition and level, resulting in different levels of α-glucosidase inhibitory activity.

Keywords: *Andrographis paniculata*, geographical origins, α-glucosidase, metabolomics, FTIR, UHPLC-Q-Orbitrap HRMS

INTRODUCTION

The raw material for medicinal plants needs quality testing to ensure consistency of quality, safety, and efficacy of herbal medicines because medicinal plants have large variability in the content of their active components. Genotypic factors, extracting solvents, harvesting time, post-harvest process, and season can affect bioactive compounds' composition and concentration in medicinal plants (Zantar et al. 2015). The growth location is also very influential on the active compounds contained therein (Khattak and Rahman 2015). A species of the same medicinal plant from different growing locations will contain different metabolite compounds due to differences in environmental conditions, such as temperature, humidity, the water content in the soil, and salinity (Sampaio et al. 2016; Borges et al. 2017). Therefore, research on the effect of different growing locations is fundamental to determine the quality of herbal medicinal raw materials.

The evaluation of the quality of herbal medicinal raw materials is currently determined by measuring the concentration of one or a group of chemical components that have certain pharmacological activities in the constituent medicinal plants (Brinckmann 2013). Quality control of medicinal plants can be carried out using a metabolomics approach to obtain information about the chemical components that can be detected to evaluate their raw materials. There is a possibility that a medicinal plant may work synergistically (van der Kooy et al. 2009). Several metabolomic approaches can be used to evaluate the quality of medicinal plants, such as metabolite profiling and fingerprint analysis (Awin et al. 2016; Umar et al. 2021; Aziz et al. 2020). Spectroscopic techniques like FTIR is widely used for fingerprint analysis because easy to use, fast, and cheap (Bunaciu et al. 2011). UHPLC-Q-Orbitrap HRMS is an attractive and efficient instrument in

metabolomics analysis because highly selective and sensitive and able to accurately identify the metabolite contain in plant sample (Xiao et al. 2012)

Andrographis paniculata was chosen as the sample because widely used in herbal medicinal products. *A. paniculata* has several biological activities, one of which is antidiabetic (Aneesh et al. 2018). This biological activity is caused by the bioactive metabolites contained therein. The main metabolite compounds contained in *A. paniculata* are the diterpene lactone and flavonoid groups. Besides that, the metal composition and content in *A. paniculata* is also responsible for its medicinal properties and its toxicity (Mythili et al. 2011). Several previous studies have reported the metal content (Mythili et al. 2011) and metabolite profile of *A. paniculata* (Chua et al. 2013; Song et al. 2013; Yusof et al. 2015; Aneesh et al. 2018; Maria et al. 2018; Tajidin et al. 2019; Rafi et al. 2020a; Rafi et al. 2020b). Those studies only reported the metabolite profile of *A. paniculata* and the differences in the composition and concentration of metabolites because of different plant parts and age, harvest time, mature and young leaves, and solvent extraction. However, no one has reported the influence of growth location especially in Indonesia associated with the metabolite profile and biological activity of *A. paniculata*. Therefore, this study aims to determine the α-glucosidase inhibitory activity, metal content, differences in the FTIR spectra profile, and putative identification of metabolites present in *A. paniculata* extract from several regions in Java island, Indonesia using UHPLC-Q-Orbitrap HRMS.

MATERIALS AND METHODS

Sampling location of *Andrographis paniculata*

The leaves and stems *of A. paniculata* were collected in April 2019 from several locations in Java island, Indonesia, as shown in Table 1. The plants used in this research were 3 months old. The sample was identified by an expert from Tropical Biopharmaca Research Center (TropBRC), IPB University, Bogor, Indonesia. A voucher specimen from all samples was stored at TropBRC, IPB University, Bogor, Indonesia.

Table 1. Location of collected sample

Code of sample	Location of sample
AP1	Dramaga, Bogor, West Java
AP2	Nagrak, Sukabumi, West Java
AP3	Bayumanik, Semarang, Central Java
AP4	Karangpandan, Karanganyar, Central Java
AP5	Kejayan, Pasuruan, East Java
AP6	Batu, East Java
AP7	Poncol, Magetan, East Java

Procedures

Preparation and extraction of Andrographis paniculata

A. paniculata samples are sorted from damaged samples, washed with running water to remove impurities, and then drained. After that, we chopped the samples into thin slices and dried them at temperature 45 °C for 36 hours, protected from direct sunlight. Furthermore, dry sorting is carried out to remove particles during drying, then weighing the dried sample. A total of 10 g powder of dried *A. paniculata* was macerated with ethanol p.a. three times in 24 hours. After that, the filtrate was concentrated using a rotary evaporator. The water extract was made by decoction. As much as 50 g of dried samples added into a decoction container. A decoction was carried out for 30 minutes at 90 °C. The water extract was then dried with a freeze-dryer until the dry extract was obtained.

Determination of α-glucosidase inhibitory activity

Determining the α-glucosidase enzyme's inhibitory activity, we followed the procedure described by (Subramanian et al. 2008). Preparation of 0.1 U/mL α-glucosidase enzyme solution was made by dissolving the enzyme using a phosphate buffer pH 7.0 containing bovine serum albumin 2000 mg/mL. For the extract solution of *A. paniculata*, as much as 50 mg of each extract is dissolved to 10 mL. Subsequently, dilution was carried out with a phosphate buffer pH 7.0 to obtain a concentration of 6.25-25.00 mg/mL. As a positive comparison for the inhibitory activity, we used andrographolide and acarbose with a concentration of 1.25-20.00 mg/mL.

A total of 10 μL of each sample concentration were incubated together with 50 μL of 0.5 U/mL enzymes for 5 minutes and 25 μL of 0.55 mM p-nitrophenyl α-D-glucopyranoside. The reaction mixture was then incubated at 37 °C for 30 minutes. After that, the reaction was stopped by adding 100 μL Na_2CO_3 0.2 M. The substrate's enzymatic hydrolysis was monitored at 405 nm by the amount of p-nitrophenol released using a microplate reader. The same treatment was applied to the andrographolide and acarbose as the positive control. Meanwhile, blank control only contains enzymes and substrates. The percentage of inhibition is calculated using the formula:

$$\% \text{ inhibition} = \frac{\text{Abs}_{\text{negative control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{negative control}}} \times 100\%$$

The IC_{50} of the sample extract was calculated by regression analysis based on the percentage of inhibition from several concentrations used in this study.

Determination of metals concentration in Andrographis paniculata

Before measuring metals concentration, all of the samples were digested first to make sample solutions using a microwave-assisted digestion procedure. About 0.25 g each sample was mixed with 10 mL HNO_3 65% and 2 mL H_2O_2 30% and digested using microwave digestion method. We determined Na and K using flame atomic emission spectrophotometer, while Ca, Mg, Zn, Fe, Cd, and Pb using flame atomic absorption spectrophotometer (GBC

Avanta AAS, GBC Scientific Company, Victoria, Australia) in *A. paniculata* sample.

Measurement of FTIR spectra

All of the *A. paniculata* samples were measured with a Thermo Scientific Nicolet iS10 FTIR spectrophotometer equipped with OMNIC and TQ Analyst software (Thermo Fisher Scientific. Inc, Madison, USA). The sample measurement technique using attenuated total reflectance (ATR) with the extract was placed on the ATR crystal. FTIR spectra were recorded at wavenumbers 4000 to 650 cm^{-1}, with scans 32 and resolution about 4 cm^{-1}. Measurements were made 2 times and recorded as absorbance data. After scanning, the ATR crystals were cleaned with ethanol and acetone two times. At each sample measurement, a background measurement is carried out to avoid spectrum variations between times. Background measurements are carried out by measuring the IR absorption without the presence of a sample.

Separation and putative identification of metabolites using UHPLC-Q-Orbitrap HRMS

A. paniculata powder was extracted using methanol with a ratio between sample and solvent of about 1:10 for 30 minutes at room temperature. Separation of *A. paniculata* metabolites was performed according to the procedure described by Rafi et al. (2021) using Vanquish Flex UHPLC-Q Exactive Plus Orbitrap-High Resolution Mass Spectrometer with *Accucore^{TM} phenyl hexyl* (100 x 2.1 mm, 2.6 μm) as separation column. We used mobile phase 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with gradient elution as follow: 0.00-1.73 min (30-40%B); 1.73-6.93 min (40-75 %B); 6.93-7.80 min (75-100 %B); 7.80-8.23 (100-30 %B), 8.23-13.00 min (30 %B). The flow rate was maintained at 0.2 mL/min and an injection volume of about 2 μL. Other conditions for UHPLC-Q-Orbitrap HRMS analysis were as followed: the source of MS ionization used was ESI (+) with Q-Orbitrap mass analyzer with m/z range from 133-2000 m/z. The collision energy used for fragmentation was 18, 35, and 53 eV. Spray voltage used about 3.8 kV, the capillary temperature is 320°C, sheath gas, and auxiliary gas flow rates are 15 and 3 mL/min, respectively. We used scan type full MS/dd MS^2 for positive ion mode.

Putative identification of metabolites detected in *A. paniculata* extracts was performed using mass spectrum obtained from UHPLC-Q-Orbitrap HRMS and processed by Compound Discoverer version 2.2. We employed an in-house database collected from various scientific articles related to *A. paniculata* to identify the metabolites. In the software, we performed select spectra, align retention time, detect unknown compounds, group of unknown compounds, predict compositions, search mass list, fill gaps, normalized areas, and marked background compounds for putative identification of *A. paniculata* metabolites.

Data analysis

Distinguishing of *A. paniculata*, according to geographical origin, was performed using principal component analysis (PCA). We used the Unscrambler X version 10.1 software (CAMO, Oslo, Norway) for running PCA. Two PCA models were made with absorbance data from the IR spectra of the sample in the region 4000-650 cm^{-1} and relative peak area of 32 as the variables from UHPLC-Q-Orbitrap HRMS chromatogram.. Before subjected to PCA, we preprocessed FTIR spectra using baseline correction and standard normal variate (SNV).

RESULTS AND DISCUSSION

Extraction yield and inhibition of α-glucosidase of *Andrographis paniculata* extracts

In this study, sample extraction was carried out using maceration with ethanol p.a. solvent and decoction with distilled water. The yield extract and inhibition of α-glucosidase is shown in Table 2. In general, the highest yield was shown by the ethanolic extract compared to the water extract of *A. paniculata* from different locations of growth. The yield of extracts in each location was different because of the environmental condition. The highest yields of ethanol and water extracts were obtained from *A. paniculata* obtained from Dramaga, Bogor (AP1), and *A. paniculata* collected from Batu, Malang (AP6) showed the lowest yield.

Each extract's inhibitory strength was expressed by a concentration value capable of inhibiting enzyme activity as much as 50% (IC$_{50}$). The lower the IC$_{50}$ value, the more active the extract is. The results showed that the ethanol extract was more active than the water extract. AP1 ethanol extract had the strongest activity. The solubility of andrographolide, which can inhibit α-glucosidase, is thought to be the leading cause of this difference. This is because andrographolide is easily dissolved in ethanol.

Metals concentration in *Andrographis paniculata*

Eight metals were analyzed in *A. paniculata* samples, namely Na, K, Ca, Mg, Zn, Fe, Cd, and Pb. The analysis results of several alkali metals, alkaline earth, transition metals, and precious metals can be seen in Figure 1. The analysis results show that all metals are contained in each sample. However, each sample contains metals with different concentrations. Ca was detected with the highest content in all regions compared to other metals. The Ca and Fe content of AP3 and AP5 are quite different compared to the other 5 regions. The metal Na, K, Mg, and Zn content is relatively close to its value whereas Cu and Cd were not found to be present in all regions. This indicates the influence of the growing location on the metal concentration contained in *A. paniculata*. Based on these data, calcium is the metal with the highest concentration contained in all samples.

FTIR spectra of *Andrographis paniculata*

The FTIR spectrum is used to identify functional groups present in the metabolites present in a sample. The FTIR spectrum pattern of *A. paniculata* from various sampling locations will show very similar patterns because the metabolites' composition in one species will undoubtedly be relatively the same, as shown in Figure 2.

The difference in absorption bands seen from each sample of *A. paniculata* from various growth locations can be observed at the wave number 1500-1200 cm^{-1}, the fingerprint area. This difference indicates the diversity of metabolites extracted from each sample. Also, different absorbance intensities in several absorption bands in each extract indicated a difference in the metabolites' levels. The difference in the number of these metabolites affects biological activities such as inhibition of the enzyme α-glucosidase, which also has different values, as shown in Table 2.

Table 3 showed the identified functional groups present in *A. paniculata* extracts. The absorption band of stretching vibration around 3300 cm^{-1} was identified as OH groups and detected in all *A. paniculata* samples. Other functional groups detected in all samples were C=O (stretching vibration), C=C aromatic (stretching vibration), and C-O for C-OH (bending vibration) at wavenumber 1730 cm^{-1}, 1600 cm^{-1}, and 1029 cm^{-1}, respectively.

Putative identification of metabolites in *Andrographis paniculata*

The putative identification of metabolites in *A. paniculata* extract using UHPLC-Q-Orbitrap-HRMS was detected as many as 28-32 metabolites from seven growth sites (Table 4). The identified metabolites belong to the phenolic acid group, diterpene lactones, and flavonoids. Three phenolic acid compounds were identified in *A. paniculata* extract, namely cinnamic acid, caffeic acid, and 3-O-caffeoylquinic acid (Table 4). Cinnamic acid and caffeic acid are phenolic acids that produce fragmentation by releasing H_2O and COOH molecules, namely at m / z $149 \rightarrow 131 \rightarrow 105$ and $181 \rightarrow 163 \rightarrow 136$.

Diterpene lactones are the main class of compounds present in *A. paniculata*. In this study, we managed to identify as many as 15 terpene compounds of the lactone group, as shown in Table 4. Of the several metabolites belonging to the terpene lactone class, andrographolide is the main characteristic compound in *A. paniculata*. Andrographolide with m/z 351 (Rafi et al. 2021) was identified through detection of fragments with m/z 333 [M + H-H_2O]$^+$, 315 [M + H-2H_2O]$^+$, 297 [M + H-3H_2O]$^+$, 285 [M + H-2H_2O-12]$^+$, and 257 [M + H-2H_2O-12-28]$^+$. This compound is the most dominant in the diterpene lactone group and has a high abundance, especially in the leaves (Komalasari and Harimurti 2015). Andrographolide is also a compound that plays an important role in biological activity as an antidiabetic (Nugroho et al. 2012).

Apart from phenolic acid and diterpene lactones, *A. paniculata* also has several flavonoid compounds (Gan et al. 2019; Rafi et al. 2021). A total of 14 flavonoid compounds were identified in the samples used, such as apigenin, luteolin, methoxy flavone derivatives, and andrographidin A. Quantitatively, the relative content of 32 metabolites from seven regions is shown in Figure 3. The andrographolide content (18) of A. paniculata samples in each area is relatively the same, while the 14-Deoxy-andrographolide content is higher in the AP3 and AP5 regions.

Tabel 2. Yield of extracts, and IC_{50} of α-glucosidase inhibitory activity of *Andrographis paniculata* extracts

Code of sample	Yield (%)		IC_{50} (mg/mL) (n = 3)	
	EtOH	Water	EtOH	Water
AP1	14.97	12.20	6.56±0.22[a]	13.86±0.23[b]
AP2	13.84	11.47	8.58±0.22[a]	12.65 ± 0.46[b]
AP3	14.21	11.58	8.24±0.44[a]	11.82 ± 0.72[b]
AP4	14.63	12.15	8.77±0.23[a]	11.55 ± 0.17[b]
AP5	14.02	12.01	9.11±0.34[a]	25.39 ± 3.03[b]
AP6	13.77	11.23	9.21±0.27[a]	12.09 ± 0.07[b]
AP7	13.91	11.68	6.56±0.24[a]	13.17 ± 0.64[b]
Acarbose			3.89 ± 0.20[a]	
Andrographolide			7.22 ± 0.33[a]	

Note: The mean ± SD within each measurement in the same column, followed with different lowercase letters, represents significant differences at $p < 0.05$.

Tabel 3. Functional group detected in *Andrographis paniculata* extracts

Wavenumber (cm^{-1})	Functional group
3320-3300	O-H (stretching vibration)
2919-2910	C-H sp^3 (stretching vibration)
2851-2849	C-H aldehyde (stretching vibration)
1733	C=O (stretching vibration)
1611-1600	aromatic C=C (stretching vibration)
1415	C-H sp^3 (bending vibration)
1232-1220	C-O ether (stretching vibration)
1029	C-OH alcohol ((stretching vibration)
900-888	C-H alkene (bending vibration)

Clustering of *Andrographis paniculata*

Andrographis paniculata taken from several locations on the island of Java, Indonesia, has an FTIR spectrum fingerprint profile and a UHPLC-Q-Orbitrap HRMS chromatogram similar to one another. The only difference lies in the level of metabolite concentration detected. Therefore, in grouping samples based on their growth locations, chemometric analysis is needed, such as principal component analysis (PCA). PCA itself can simplify variables by reducing dimensions and providing an overview of sample grouping through the main component (PC) (Theodoridis et al. 2012). In this study, the two models of PCA was created using the absorption value of functional groups at a wavelength of 4000-650 cm^{-1} and the compounds' peak area values identified as variables.

Plot scores were generated from PCA analysis of *A. paniculata* extract with FTIR spectrum data (Figure 4A) and metabolite peak area (Figure 4B) as variables. The two score plots show the extract group based on the location of the growth. In the PCA score plot using FTIR spectra, we used three PC resulted in a three-dimensional score plot with the cumulative percentage of the three PCs used were 83%. While for the PCA score plot using UHPLC chromatogram, we used two PCs that accounted for 93% of the cumulative percentage of the two PCs. Using FTIR spectrum data does not produce a good enough grouping than metabolite compound data, which means that each sample from each region cannot be appropriately distinguished only by FTIR spectrum fingerprint data.

Tabel 4. Putative identification of metabolites in *Andrographis paniculata* from several locations of growth

| Senyawa	Mw	Error (ppm)	MS & MS/MS	Formula	AP1	AP2	AP3	AP4	AP5	AP6	AP7
3-O-Caffeoylquinic acid	354.09502	-0.17	355, 163	$C_{16}H_{18}O_9$	✓	✓	✓	✓	✓	✓	✓
Cinnamic acid	148.05241	-0.13	149, 131, 105	$C_9H_8O_2$	✓	✓	✓	✓	✓	✓	✓
Caffeic acid	180.04243	0.95	181, 163, 136	$C_9H_8O_4$	✓	✓	✓	✓	✓	✓	✓
3-O-β-D-Glucopyranosyl andrographolide	512.26133	-1.60	513, 351, 333, 315, 297, 285, 257	$C_{26}H_{40}O_{10}$	✓	✓	✓	✓	✓	✓	✓
Apigenin	270.05214	-2.53	271, 153, 119	$C_{15}H_{10}O_5$	✓	✓	✓	✓	✓	✓	✓
12S-Hydroxy andrographolide	368.2197	-0.51	369, 351, 333, 315, 297, 285, 257	$C_{20}H_{32}O_6$	✓	✓	✓	✓	✓	✓	✓
Paniculide B	280.13095	-0.44	281, 263, 245, 217	$C_{15}H_{20}O_5$	✓	✓	✓	✓	✓	✓	✓
5,7-Dihydroxy-8-methoxyflavone	284.06819	-0.99	285, 119	$C_{16}H_{12}O_5$	✓	✓	✓	✓	✓	✓	✓
Apigenin-7-O-beta-D-glucuronide	446.12082	80.50	447, 271	$C_{22}H_{22}O_{10}$	✓	✓	✓	✓	✓	✓	✓
5,4'-Dihidroxy-7,8,2',3'-tetramethoxyflavone	374.09998	-0.50	375, 360, 342	$C_{19}H_{18}O_8$	-	✓	✓	✓	✓	✓	✓
Luteolin	286.04656	-4.12	287	$C_{15}H_{10}O_6$	✓	✓	✓	✓	✓	✓	✓
14-Deoxy-17-hydroxy-andrographolide	352.22466	-0.89	353, 335, 317, 299, 287, 259	$C_{20}H_{32}O_5$	✓	✓	✓	✓	✓	✓	✓
5-Hydroxy-7,8,2'-trimethoxyflavone 5-glucoside	490.14757	0.12	491, 329	$C_{24}H_{26}O_{11}$	✓	✓	✓	✓	✓	✓	✓
Paniculide C	278.11549	0.24	279, 261	$C_{15}H_{18}O_5$	-	✓	✓	✓	-	✓	✓
5,4'-dihydroxy-7,8-dimethoxyflavone	314.07876	-0.88	315, 197, 119	$C_{17}H_{14}O_6$	✓	✓	✓	✓	✓	✓	✓
Andrographic acid	364.18871	0.33	365	$C_{20}H_{28}O_6$	-	✓	✓	✓	✓	✓	-
14-deoxy-andrographiside	496.26592	-2.62	497, 317, 299, 287, 259	$C_{26}H_{40}O_9$	✓	✓	✓	✓	✓	✓	✓
Andrographolide	350.20816	-3.32	351, 297, 285, 257	$C_{20}H_{30}O_5$	✓	✓	✓	✓	✓	✓	✓
Apigenin 7,4'-dimethyl ether	298.08378	-1.21	299, 133	$C_{17}H_{14}O_5$	✓	✓	✓	✓	✓	✓	✓
5-Hydroxy-7,8-dimethoxyflavanone	300.09918	-2.07	301, 197, 105	$C_{17}H_{16}O_5$	✓	✓	✓	✓	✓	✓	✓
Andrographidin A	462.15198	-1.33	463, 301, 197	$C_{23}H_{26}O_{10}$	✓	✓	✓	✓	✓	✓	✓
5-Hydroxy-7,2',6'-trimethoxyflavone	328.09435	-1.03	329, 314, 299	$C_{18}H_{16}O_6$	✓	✓	✓	✓	✓	✓	✓
5-Hydroxy-3,7,8,2'-tetramethoxyflavone	358.10486	-1.09	359, 329, 197	$C_{19}H_{18}O_7$	✓	✓	✓	-	-	✓	✓
Neoandrographolide	480.27237	0.11	481	$C_{26}H_{40}O_8$	✓	✓	✓	-	-	-	-
14-Acetyl-andrographolide	392.22	-0.28	393, 315, 297, 285, 257	$C_{22}H_{32}O_6$	✓	✓	✓	✓	✓	✓	✓
Andrograpanin	318.2187	-2.50	319, 301, 289, 261	$C_{20}H_{30}O_3$	✓	✓	✓	✓	✓	✓	✓
14-Deoxy-11-oxoandrographolide	348.1936	-0.21	349, 331, 313, 285	$C_{20}H_{28}O_5$	-	-	✓	-	-	-	-
14-Deoxy-andrographolide	334.2134	-3.02	335, 317, 299, 287	$C_{20}H_{30}O_4$	✓	✓	✓	✓	✓	✓	✓
Andrographolactone	296.1769	-2.46	297, 269	$C_{20}H_{24}O_2$	✓	✓	✓	✓	✓	✓	✓
Dehydro-andrographolide	332.1977	-3.19	333, 315, 297, 285, 257	$C_{20}H_{28}O_4$	✓	✓	✓	✓	✓	✓	✓
5,7,2',3'-Tetramethoxy-flavanone	344.1259	-0.29	345	$C_{19}H_{20}O_6$	✓	✓	✓	✓	✓	✓	✓
Dihydroskullcap-flavone I	316.0945	-0.59	317, 299, 197, 121	$C_{17}H_{16}O_6$	✓	✓	✓	✓	✓	✓	✓
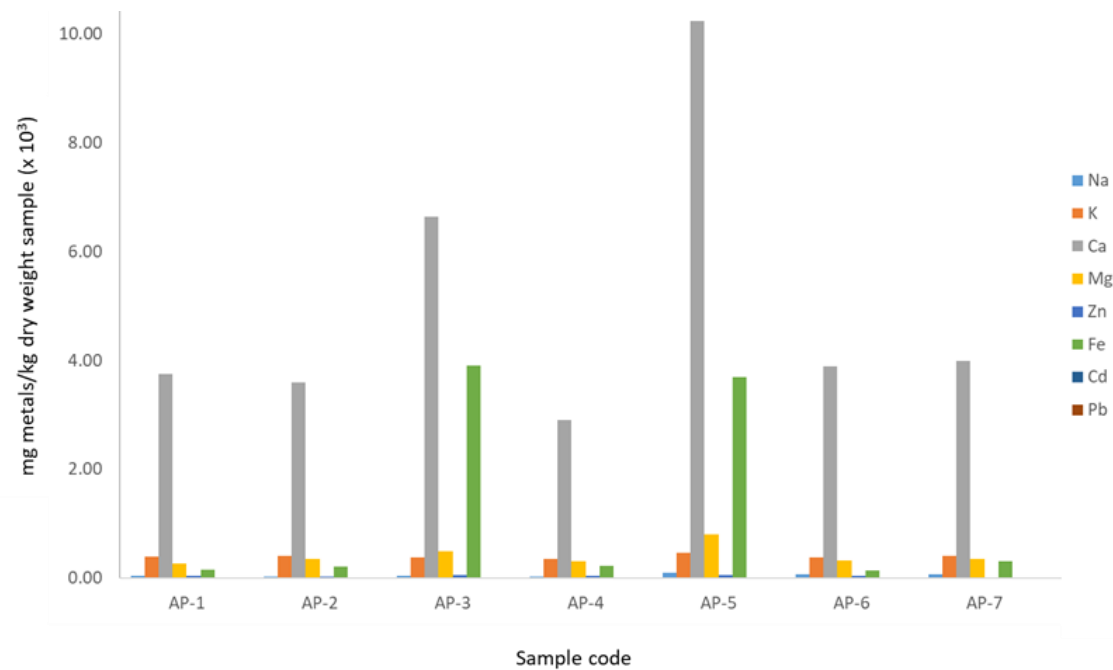
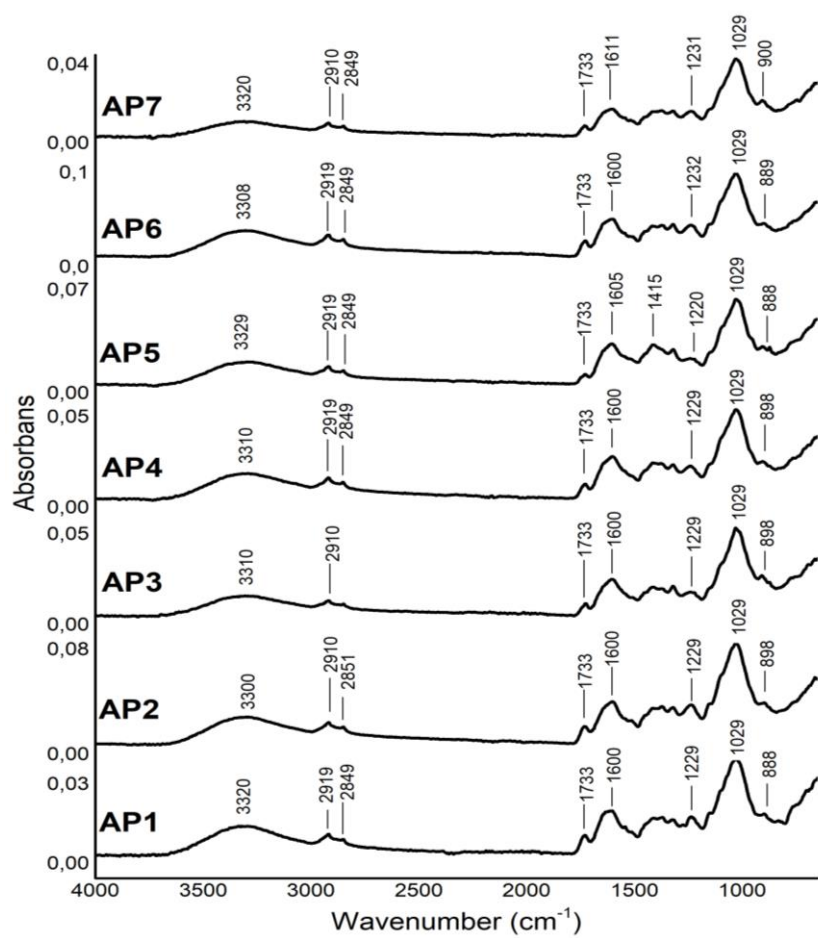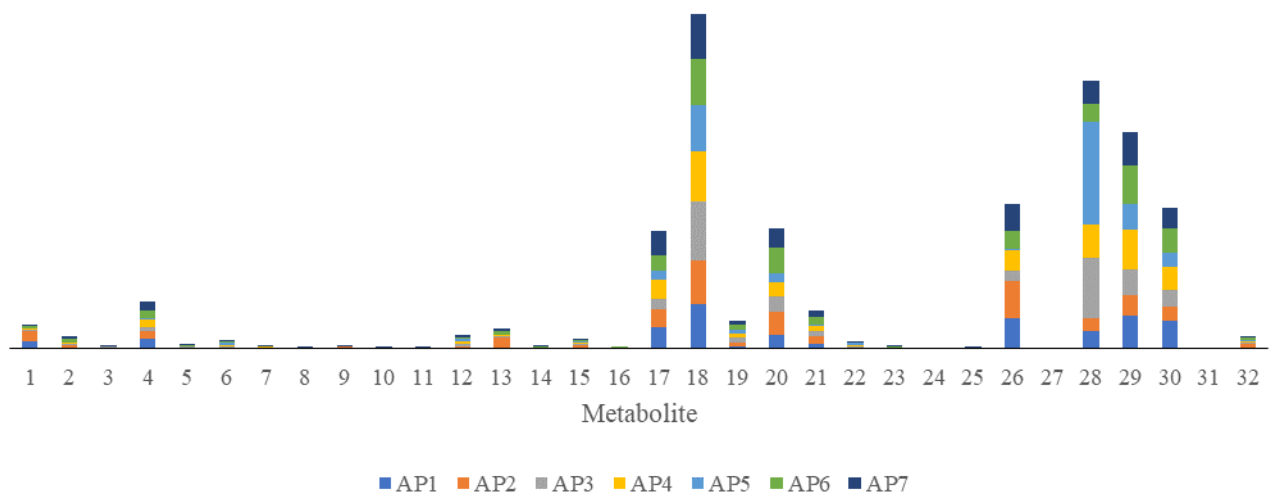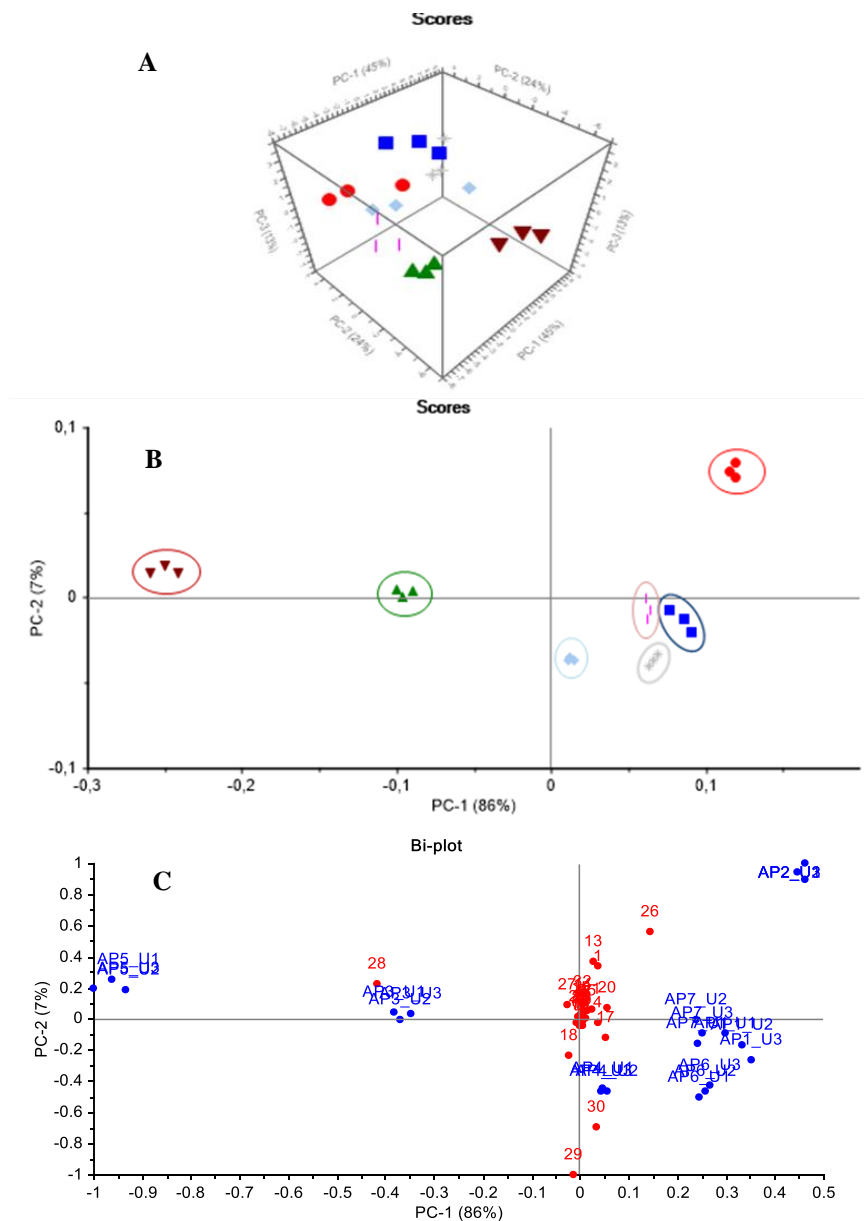**Figure 1.** Metals composition and level in *Andrographis paniculata*

Figure 2. FTIR spectrum pattern of *Andrographis paniculata* from various sampling locations

Figure 3. Relative amount of metabolites present in *Andrographis paniculata* from seven locations of growth

Figure 4. PCA score plot of FTIR spectra in 3D (A), PCA socre plot (B) and and biplot (C) of 32 identified metabolites in 2D. AP1 (■), AP2 (●), AP3 (▲), AP4 (◆), AP5 (▼), AP6 (✚), dan AP7 (❙)

Groups close together to show a high similarity of metabolite profiles, while samples with different metabolite profiles will form distant groups. Figure 4C is a combination of the score plot and loading plot (biplot) to show the metabolite that contributes to the separation of A. paniculata according to its geographical origin. Separation of AP5 and AP3 from other groups was influenced by 14-deoxy-andrographolide, while for AP2 because of andrograpanin compound. Dehydroandrographolide is responsible for the separation of AP1, AP4, AP6, and AP7.

In conclusion, *Andrographis paniculata* extract from seven locations of growth has different α-glucosidase inhibitory activity and metals composition. Ethanolic extract of *A. paniculata* showed more significant inhibition of α-glucosidase activity compared to water extract. Calcium was found more abundant in *A. paniculata* than other metals determined in this study. FTIR spectrum and UHPLC-Q-Orbitrap HRMS chromatogram of *A. paniculata* showed a similar profile and only differed in their metabolites intensity. We found *A. paniculata* extract have OH, C=O, C=C aromatic, and C-O functional group from the FTIR spectra obtained. About 32 metabolites were putatively identified in *A. paniculata*, mainly come from phenolic and diterpene lactone class compounds. Clustering *A. paniculata* based on geographical origin was achieved using FTIR spectra and peak area from 32 identified peaks. Based on the result obtained, we found that the growth location affected the metals and metabolite's composition and level, resulting in different levels of α-glucosidase inhibitory activity.

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