Short Communication:

Data Fusion of UV-Vis and FTIR Spectra Combined with Principal Component Analysis for Distinguishing of *Andrographis paniculata* Extracts Based on Cultivation Ages and Solvent Extraction

Antonio Kautsar[1,2], Wulan Tri Wahyuni[1,2], Utami Dyah Syafitri[3], Syifa Muflihah[1], Nursifa Mawadah[1], Eti Rohaeti[1], Zulhan Arif[1], Bambang Prajogo[4], Muhammad Bachri Amran[5], Abdul Rohman[6], and Mohamad Rafi[1,2*]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Tanjung Kampus IPB Dramaga, Bogor 16680, Indonesia*

[2]*Tropical Biopharmaca Research Center- Institute of Research and Community Services, IPB University, Jl. Taman Kencana No. 3 Kampus IPB Taman Kencana, Bogor 16128, Indonesia*

[3]*Department of Statistics, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Meranti Kampus IPB Dramaga, Bogor 16680, Indonesia*

[4]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Jl. Mulyorejo Kampus C Unair, Surabaya 60286, Indonesia*

[5]*Analytical Chemistry Research Group, Institut Teknologi Bandung, Jl. Ganesha No. 10, Bandung 40132, Indonesia*

[6]*Department of Pharmaceutical Chemistry Faculty of Pharmacy, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*

* ***Corresponding author:***

email: mra@apps.ipb.ac.id

Received: October 3, 2020
Accepted: March 15, 2021

DOI: 10.22146/ijc.60321

Abstract: Andrographis paniculata *is one of the medicinal plants used for the treatment of antidiabetic. Cultivation ages and solvent extraction affected metabolites' composition and concentration that directly cause the plant's efficacies. This research aimed to distinguish* A. paniculata *based on cultivation ages and solvent extraction using data fusion of UV-Vis and FTIR spectra combined with principal component analysis (PCA).* A. paniculata *with 2, 3, and 4 months post-planting were extracted by water, 50% ethanol, 70% ethanol, and ethanol. In each extract, we measured UV-Vis and FTIR spectra. Then, we used the data fusion from both spectra. We used UV-Vis and FTIR absorbance from 200–400 nm and 1800–400 cm^{-1}, respectively. Each extract gives a similar pattern of UV-Vis and FTIR spectra, only differ in their intensities. PCA score plot in two and three-dimensional showed* A. paniculata *extracts could be distinguished based on cultivation ages and solvent extraction with a total variance of 86 and 92%, respectively. Furthermore, this study confirms the data fusion of UV-Vis and FTIR spectra could distinguished* A. paniculata *extracts combined with chemometrics based on cultivation ages and solvent extraction.*

Keywords: A. paniculata*; data fusion; FTIR; UV-Vis; PCA*

■ INTRODUCTION

Andrographis paniculata is a medicinal plant commonly found in South and Southeast Asian countries such as Indonesia, India, Malaysia, Pakistan, and Sri Lanka [1]. In Indonesia, it is known as *sambiloto*. *A. paniculata* is mostly used as a traditional medicine in some countries. It has several biological activities, such as antibacterial, antimalarial, antipyretic, anti-

inflammatory, and antidiabetic [2-4]. It is well known that bioactive compounds from medicinal plants are responsible for their biological activity. The main compounds in *A. paniculata* are included in the diterpene lactone class, such as andrographolide, neoandrographolide, and 14-deoxyandrographolide [5]. Yusuf et al. [6] reported that *A. paniculata* has a different accumulation of active components in the early generative stages. The highest andrographolide content is in the leaves, while the smallest content is in the seeds. Biological activities in plants are influenced by several factors, such as growing conditions, cultivation ages, and post-harvest processes [7]. Furthermore, the type and concentration of extracting solvents can affect the extracted metabolite's composition and concentration [8].

Data fusion is a new branch in chemometrics analysis. It has been widely used in recent years because it can combine several different analytical techniques to improve the quality and quantity of information obtained [9]. The resulting data has a large dimension and matrix of several instruments used, so it requires a combination of multivariate analysis in understanding the data as a whole. In general, data fusion is divided into three levels: low, medium, and high. Low-level data fusion consists of a series of simple data with different origins, while medium-level data fusion is a combination of data extracted from original data with data reduction. High-level data fusion is built from several data fusion models separately in each data, and then the model produces a final fusion of the data. Data fusion has been widely used in food analysis, bioinformatics, soil mapping, business, herbal medicine, etc. [10].

FTIR and UV-Vis spectroscopies have been widely used for quality control in food and herbal medicinal products because of their advantages, such as fast, cheap, and easy. FTIR provides spectrum information of functional groups with different and complex fingerprint patterns. UV-Vis produces a spectrum with visual parameters from the variation of UV-Vis light absorption in the sample but has limitations for characterization and authentication [11]. The combination of FTIR and UV-Vis can produce information that is more accurate and useful in interpreting data than it is used separately in multivariate analysis.

Application of data fusion has been extensively used in improving the quality of herbal medicines with a lot of data fusion research that leads to the discrimination of origin and types of medicinal plants. There are several papers that have reported using data fusion of UV-Vis and FTIR spectra, such as discrimination of porcini mushrooms [12], traceability of Boletaceae mushrooms [13], differentiation and comparison of *Wolfiporia cocos* raw materials [14], discrimination of wild *Paris Polyphylla* Smith var. *yunnanensis* [15], discrimination of species in Ganodermataceae mushrooms [16], and geographical traceability of *Eucommia ulmoides* leaves [17]. However, improving herbal medicines' quality at the specific age of harvest and solvent extraction for *A. paniculata* is still limited. This study aims to distinguish *A. paniculata* with differences in harvest age and solvent extraction using UV-Vis and FTIR spectra fusion data combined with multivariate analysis, such as principal component analysis.

■ EXPERIMENTAL SECTION

Materials

A. paniculata was collected as 2, 3, 4-month post-planting (MPP) from *Pusat Studi Biofarmaka Tropika* medicinal plant garden (IPB University, Bogor, Indonesia) in 2019. Ethanol p.a. (Merck, Darmstadt Germany), potassium bromide spectroscopy grade (Sigma Aldrich, Missouri, USA), Whatman filter paper No.40 (Merck, Darmstadt, Germany) and distilled water (Hydro, Jakarta, Indonesia) was used in this work.

Instrumentation

Rotary evaporator-114 (Buchi, Flawil, Switzerland) was used for the evaporation of solvent extraction, and freeze-dryer ALPHA 1-2 LDPlus (Christ, Osterodeam Harz, Germany) was used for drying the extract. UV-Vis spectra were performed in UV-Vis spectrophotometer U-2000 (Hitachi, Tokyo, Japan). FTIR spectra were measured in Bruker Tensor 37 FTIR spectrophotometer (Bruker Optik GmbH, Karlsruhe, Germany).

Procedure

Sample preparation and extraction

Samples were sorted then cleaned by washing with water. Afterward, samples were dried and pulverized into a powder. About 2.5 g of powder was added to 25 mL of extraction solvent then sonicated for 30 min at room temperature. The solvents used for extraction were water, and 50% ethanol, 70% ethanol, and ethanol p.a. with four replication. The filtrate was collected, then concentrated by rotary evaporator R-100 (Buchi, Flawil, Switzerland), then dried using a freeze-dryer ALPHA 1-2 LDPlus (Christ, Osterodeam Harz, Germany).

UV-Vis spectra measurement

About 7.5 mg of extract was added into 100 mL of each solvent that was used for extraction. Each solution was analyzed by a UV-Vis spectrophotometer U-2000 (Hitachi, Tokyo, Japan) in a range of 200–800 nm with an interval of 0.5 nm. Afterward, all of the spectra were recorded and converted in MS. Excel.

FTIR spectra measurement

About 2.0 mg of extract was mixed with potassium bromide into a pellet. Furthermore, the pellet was analyzed by FTIR spectrophotometer Tensor 37 (Bruker, Ettlingen, Germany) with deuterated triglycine sulfate (DTGS) detector. FTIR spectra were recorded in the region of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans/min controlled by OPUS 4.2 software (Bruker, Ettlingen, Germany). All of the spectra were collected and converted into MS. Excel.

Chemometrics analysis

Significant differences in the extraction yield results were determined using analysis of variance (ANOVA) followed by the Duncan test. PCA was used to discriminate *A. paniculata* extracts based on cultivation ages and solvents extraction. UV-Vis and FTIR spectra data (absorbance) were used as variables for data fusion. PCA was performed with the Unscrambler X version 10.4 (CAMO, Oslo, Norwegia).

■ RESULTS AND DISCUSSION

Extraction Yield of *A. paniculata*

A. paniculata metabolites were extracted using sonication at room temperature with different solvent extraction. We found that the percentage yield of extraction using various solvents and cultivation ages have slight differences (Table 1). According to Cui et al. [18], the composition and concentration of chemicals present in the plant are affected by solvent extraction used and cultivation ages. In this study, we got that cultivation ages and solvent extraction give a different level of metabolites extracted in *A. paniculata*.

The highest percentage of yield was obtained using 70% ethanol extract from 4 MPP, and the lowest is water extract from 2 MPP of *A. paniculata*. The trend in the percentage of yield showed that the pattern was increased except on 50% ethanol and ethanol. When extracted using different solvent extraction, *A. paniculata* from different cultivation ages showed that 50% ethanol extract has the highest yield in 2 and 3 MPP of *A. paniculata*. Whereas for the 4 MPP of *A. paniculata*, 70% ethanol extract gave the highest yield value. From the results obtained, at the age of 4 MPP, the more polar metabolites are extracted more in *A. paniculata*.

UV-Vis Spectra of *A. paniculata*

Fig. 1 shows the UV-Vis spectra of all sample extract solutions. As shown in Fig. 1, a similar pattern was

Table 1. Yield of *A. paniculata* extracts

Extraction solvents	Yield (%)		
	2 MPP	3 MPP	4 MPP
Water	16.24 ± 1.83[a]	16.99 ± 0.96[a]	21.90 ± 0.83[b]
50 % ethanol	22.97 ± 2.35[b]	23.80 ± 1.57[b]	18.76 ± 1.16[a]
70 % ethanol	22.06 ± 0.41[b]	22.24 ± 1.58[b]	23.37 ± 2.80[b]
Ethanol	18.58 ± 2.90[a]	18.31 ± 0.49[a]	16.62 ± 1.46[a]

The reported values are mean ± SD of the quadruplicate for each sample. The mean ± SD within each extract in the same column followed with different superscript letters represent significant differences at $p < 0.05$

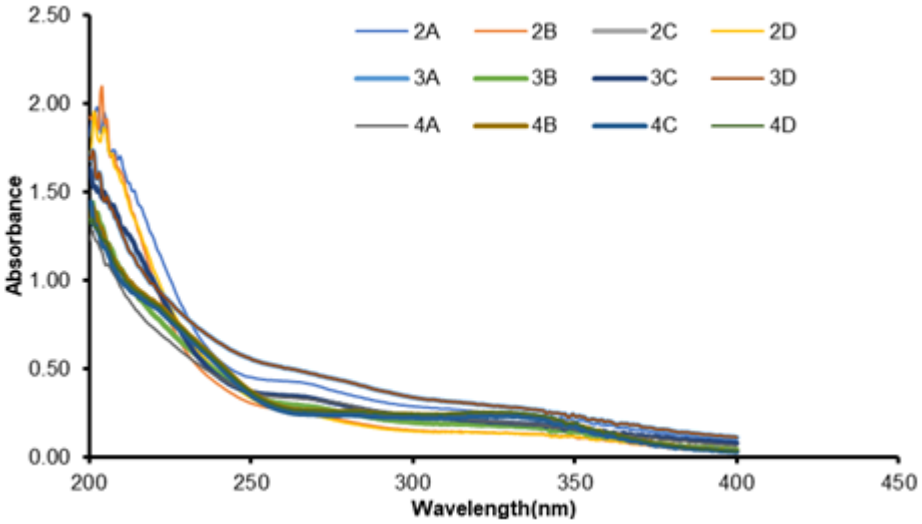

Fig 1. UV-Vis spectra of *A. paniculata* extracts: 2 MPP: water (2A), 50% ethanol (2B), 70% ethanol (2C), ethanol (2D); 3 MPP: water (3A), 50% ethanol (3B), 70% ethanol (3C), ethanol (3D); 4 MPP: water (4A), 50% ethanol (4B), 70% ethanol (4C), ethanol (4D)

obtained from all UV-Vis spectra of the samples. Differences were only found for their intensity. It means no differences in the composition of metabolites extracted only differ for their level of concentration. About two regions with a maximum absorption appeared in the range of 270–290 nm and 310–330 nm. Both of the peaks resulted from the absorption of carbonyl chromophore with an electron transition from n→π*.

FTIR Spectra of *A. paniculata*

FTIR spectra of *A. paniculata* extracts (Fig. 2) also showed a similar pattern and differences only in each peak's absorbance intensities. This confirms the UV-Vis spectra measurement results, which also produce the same thing, which is different for the extracted metabolites' concentration level. The difference can be seen on the peak at wavenumber 3400, 2930, 2852, 1728, 1620, 1386, and 1076 cm^{-1}. Each peak is representing a functional group from metabolites present in the *A. paniculata*. The peak at 3400 cm^{-1} is from the stretching vibration of hydroxyl (-OH). Meanwhile, peaks at 2930, 2852, and 1386 cm^{-1} are from the stretching vibration of methyl (C–H). Other peaks on 1728 and 1620 correspond to the stretching vibration of the carbonyl (C=O) functional group, and the peak at 1076 cm^{-1} is from the stretching vibration of aliphatic amine (C–N).

Absorbance intensity showed a variation value on each sample. A peak at 1728 cm^{-1} in water extract has low intensity than other extracts used in this study. However, a peak at 1620 cm^{-1} showed that the absorbance intensity is higher than the other extracts. Pandey and Mandal [19] reported that the type of solvents could affect the distribution and concentration of composition extracted, while part of the plants can also impact the extraction.

Discrimination of *A. paniculata* Extracts

We used a combination of data fusion from absorbance data of UV-Vis and FTIR spectra in the range of 200–400 nm and 1800–400 cm^{-1}, respectively. Preprocessing of spectra was performed before we used it for the PCA. Preprocessing aims to enhance the quality of data, for example, reducing noise to give better result output of the grouping sample [20]. We used multiple preprocessing for UV-Vis, such as baseline, normalize, and smoothing, while for FTIR spectra, we used baseline, standard normal variate, and smoothing.

PCA works by simplifying variables by reducing their dimension into principal components. Two and three-dimensional (2D and 3D) PCA score plots of *A. paniculata* (Fig. 3) showed that all extracts were grouped according to the cultivation ages and solvent extraction

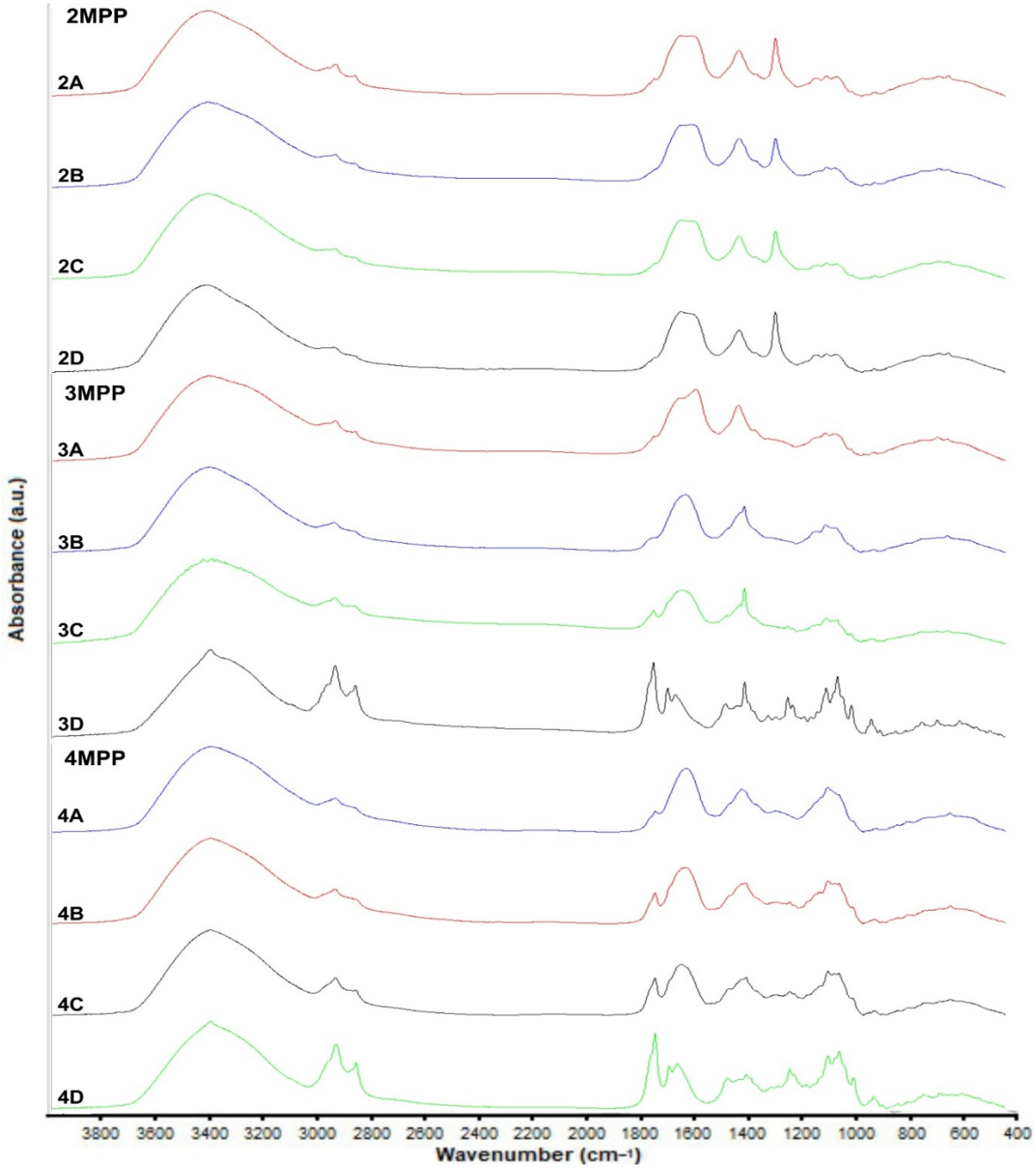

Fig 2. FTIR spectra *A. paniculata* extracts: 2 MPP: water (2A), 50% ethanol (2B), 70% ethanol (2C), ethanol (2D); 3 MPP: water (3A), 50% ethanol (3B), 70% ethanol (3C), ethanol (3D); 4 MPP: water (4A), 50% ethanol (4B), 70% ethanol (4C), ethanol (4D)

with a good separation of group. Only water extract of 2 MPP and 3 MPP is very close to each other in the two PCA score plots because the level concentration of extracted metabolites is not different in the two samples. According to Table 1, it can be seen that water extract of 2 MPP and 3 MPP are not significantly different from the Duncan test. The cumulative percentage of principal components is 86% and 92% using 2D and 3D PCA score plots, respectively. The cumulative percentage of all PC used in the 2D and 3D score plot is higher than 70%, giving good 2D and 3D visualization.

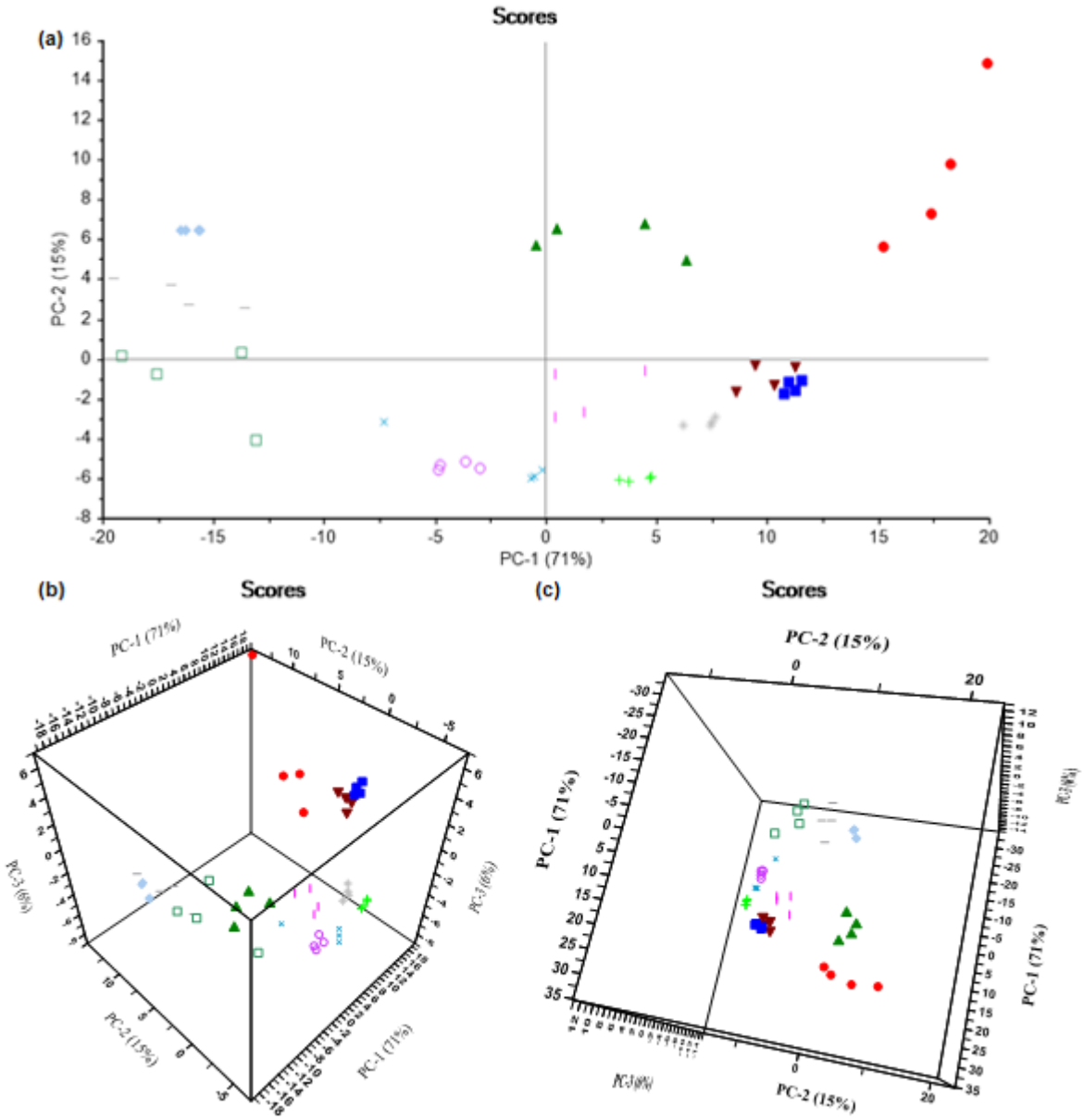

Fig 3. (a) 2 dimension PCA score plot, (b) 3 dimension PCA score plot, and (c) 3 dimension PCA score plot rotated 270 degrees to Z axis. 2 MPP: water (▪), 50% ethanol (●), 70% ethanol (▲), and pure ethanol (♦); 3 MPP: water (▼), 50% ethanol (*), 70% ethanol (|), and pure ethanol (—); 4 MPP: water (+), 50% ethanol (×), 70% ethanol (○), and pure ethanol (▫)

■ CONCLUSION

UV-Vis and FTIR spectra of *A. paniculata* extracts with different cultivation ages and solvent extraction have similar profiles. The differences only in their absorbance intensity. The four months post-planting of *A. paniculata* extracted with 70% ethanol provide a higher yield percentage than other extracts. A combination of UV-Vis and FTIR data with PCA enabled clustering *A. paniculata* extracts according to their cultivation ages and extraction solvents.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledged the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through the World Class

University Program of IPB University to support this research by *Riset Kolaborasi Indonesia* Grant 2019 (No: 0847/IT3.L1/PN/2019).

■ REFERENCES

[1] Valdiani, A., Kadir, M.A., Tan, S.G., Talei, D., Abdullah, M.P., and Nikzad S., 2012, Nain-e Havandi *Andrographis paniculata* present yesterday, absent today: A plenary review on underutilized herb of Iran pharmaceutical plants, *Mol. Biol. Rep.*, 39 (5), 5409–5424.

[2] Tandon, C., Mathur, P., and Sen, M., 2015, *Andrographis paniculat*a Nees (Kalmegh): A review on its antibacterial activities and phytocompounds, *Eur. J. Med. Plants*, 8 (1), 1–10.

[3] Elumalai, S., Banupriya, R., Sangeetha, T., and Madhumathi, S., 2016, Review on phytopharmacological activities of *Andrographis paniculata* (Burm. f.) Nees, *Int. J. Pharm. Bio Sci.*, 7 (4), 183–200.

[4] Aneesh, A., George, A.J., Kariyil, B.J., Khrisna, D., and Sajitha, I.S., 2018, Phytochemical evaluation of *Andrographis paniculata* (L.), *J. Pharmacogn. Phytochem.*, 7 (4), 1949–1953.

[5] Sajeeb, B.K., Kumar, U., Halder, S., and Bachar, S.C., 2015, Identification and quantification of andrographolide from *Andrographis paniculata* (Burm. f.) Wall. ex Nees by RP-HPLC method and standardization of its market preparations, *Dhaka Univ. J. Pharm. Sci.*, 14 (1), 71–78.

[6] Yusof, N.A., Isha, A., Ismail, I.S., Khatib, A., Shaari, K., Abas, F., and Rukayadi, Y., 2015, Infrared-metabolomics approach in detecting changes in *Andrographis paniculata* metabolites due to different harvesting ages and times, *J. Sci. Food Agric.*, 95 (12), 2533–2543.

[7] Brinckman J.A., 2013, Emerging importance of geographical indications and designations of origin - authenticating geo-authentic botanicals and implications for phytotherapy, *Phytother. Res.*, 27 (11), 1581–1587.

[8] Kunle, O.F., Egharevba, H.O., and Ahmadu, P.O., 2012, Standardization of herbal medicines - A review, *Int. J. Biodivers. Conserv.*, 4 (3), 101–112.

[9] Silvestri, M., Bertacchini, L., Durante, C., Marchetti, A., Salvatore, E., and Cocchi, M., 2013, Application of data fusion techniques to direct geographical traceability indicators, *Anal. Chim. Acta*, 769, 1–9.

[10] Lahat, D., Adali, T., and Jutten, C., 2015, Multimodal data fusion: An overview of methods, challenges, and prospects, *Proc. IEEE*, 103 (9), 1449–1477.

[11] Borràs, E., Ferré, J., Boqué, R., Mestres, M., Aceña, L., and Busto, O., 2015, Data fusion methodologies for food and beverage authentication and quality assessment - A review, *Anal. Chim. Acta*, 891, 1–14.

[12] Qi, L.M., Li, J.Q., Liu, H.G., Li, T., and Wang, Y.Z., 2018, An additional data fusion strategy for the discrimination of porcini mushrooms from different species and origins in combination with four mathematical algorithms, *Food Funct.*, 9 (11), 5903–5911.

[13] Yao, S., Li, T., Liu, H., Li, J., and Wang, Y., 2017, Traceability of Boletaceae mushrooms using data fusion o UV-Vis and FTIR combined with chemometrics methods, *J. Sci. Food Agric.*, 98 (6), 2215–2222.

[14] Li, Y., and Wang Y., 2018, Differentiation and comparison of *Wolfiporia cocos* raw materials based on multi-spectral information fusion and chemometric methods, *Sci. Rep.*, 8 (1), 13043.

[15] Wu, X.M., Zuo, Z.T., Zhang, Q.Z., and Wang, Y.Z., 2018, FT-MIR and UV–vis data fusion strategy for origins discrimination of wild *Paris Polyphylla* Smith var. *yunnanensis*, *Vib. Spectrosc.*, 96, 125–136.

[16] Li, X.P., Li, J., Liu, H., and Wang, Y.Z., 2020, A new analytical method for discrimination of species in Ganodermataceae mushrooms, *Int. J. Food Prop.*, 23 (1), 227–240.

[17] Wang, C.Y., Tang, L., Jiang, T., Zhou, Q., Li, J., Wang, Y.Z., and Kong, C.H., 2021, Geographical traceability of *Eucommia ulmoides* leaves using attenuated total reflection Fourier transform infrared and ultraviolet-visible spectroscopy combined with chemometrics and data fusion, *Ind. Crops Prod.*, 160, 113090.

[18] Cui, Y., Wang, Y., Ouyang, X., Han, Y., Zhu, H., and Chen, Q., 2009, Fingerprint profile of active component for *Andrographis paniculata* Nees by HPLC-DAD, *Sens. Instrum. Food Qual. Saf.*, 3 (3), 165–179.

[19] Pandey, A.K., and Mandal, A.K., 2010, Variation in morphological characteristics and andrographolide content in *Andrographis paniculata* (Burm. f) Nees of Central India, *Iran. J. Energy Environ.*, 1 (2), 165–169.

[20] Purwakusumah, E.D., Rafi, M., Safitri, U.D., Nurcholis, W., and Adzkiya, M.A.Z., 2014, Identifikasi dan autentikasi jahe merah menggunakan kombinasi spektroskopi FTIR dan kemometrik, *Agritech*, 34 (1), 82–87.