

Quantitative HPLC and FTIR-based Metabolomics for Clustering *Centella asiatica* Cultivation Ages and Evaluation of Their Radical Scavenging Activity

(Kuantitatif HPLC dan Metabolomik berasaskan FTIR untuk Pengelompokan Umur Penanaman dan Penilaian Aktiviti Pemusnahan Radikal *Centella asiatica*)

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ABSTRACT

Centella asiatica is a medicinal plant used to treat stomachache, cough, sore throat, blood booster, and skin diseases. The difference in harvesting time is among the factors that can significantly affect the chemical composition of *C. asiatica*. The purpose of this study was to evaluate the difference of *C. asiatica* cultivation ages through a metabolomics (targeted and untargeted) analysis using FTIR spectra, HPLC analysis of four main components, and evaluation of its radical scavenging activity. The results showed that differences in cultivation ages affected the chemical composition of *C. asiatica*. It was shown by the FTIR spectrum indicating the vibration of several functional groups such as OH, C-H, C=O, C=C, C-O, C-N, C-O phenol, and alkyl halides with differences in their intensities. The results of the HPLC showed that *C. asiatica* harvested after four months post-planting (MPP) had the highest content of madecassoside, asiaticoside, madecassic acid, and asiatic acid, respectively. A principal component analysis (PCA) was carried out to clustering *C. asiatica* based on its cultivation ages. The PCA results showed that each sample could be grouped with a total variance of the first two principal components (PC) using peak area of the four main components analyzed by HPLC and absorbance at wavenumber 400-4000 cm⁻¹ from FTIR spectra were 98% and 95%, respectively. The radical scavenging activity demonstrated that the 4 MPP had the highest inhibition of about 53.81±0.92%. So, *C. asiatica* at 4 MPP gave optimum level four main metabolite content and radical scavenging activity.

Keywords: Antioxidant; *Centella asiatica*; FTIR spectra; HPLC; metabolomics

ABSTRAK

Centella asiatica adalah ubat yang digunakan untuk mengubati sakit perut, batuk, sakit tekak, penggalak darah dan penyakit kulit. Perbezaan masa penuaian adalah antara faktor signifikan yang dapat mempengaruhi komposisi kimia *C. asiatica*. Tujuan kajian ini adalah untuk menilai perbezaan waktu penanaman *C. asiatica* melalui analisis metabolomik (disasarkan dan tidak disasarkan) menggunakan spektrum FTIR, analisis HPLC daripada empat komponen utama, dan penilaian aktiviti penghapusan radikalnya. Hasil kajian menunjukkan bahawa perbezaan tempoh penanaman mempengaruhi komposisi kimia *C. asiatica*. Ia dibuktikan oleh spektrum FTIR dengan getaran beberapa kumpulan berfungsi seperti OH, C-H, C=O, C=C, C-O, C-N, C-O fenol dan alkil halida dengan perbezaan keamatan mereka. Hasil HPLC menunjukkan bahawa *C. asiatica* yang dituai setelah empat bulan selepas penanaman (MPP) mempunyai kandungan madecassoside, asiaticoside, asid madecassic dan asid asiaticoside tertinggi. Analisis komponen utama (PCA) dilakukan untuk pengelompokan *C. asiatica* berdasarkan tempoh penanamannya. Hasil PCA menunjukkan bahawa setiap sampel dapat dikelompokkan dengan total varians daripada dua komponen utama (PC) pertama yang menggunakan puncak adalah daripada empat komponen utama yang dianalisis oleh HPLC dan penyerapan pada bilangan gelombang 400-4000 cm⁻¹ daripada spektrum FTIR masing-masing adalah 98% dan 95%. Aktiviti penghapusan radikal menunjukkan bahawa 4 MPP mempunyai perencatan tertinggi sekitar 53.81±0.92%. Oleh itu, *C. asiatica* pada 4 MPP memberikan tahap optimum bagi empat kandungan metabolit utama dan aktiviti pembersihan radikal.

Kata kunci: Antioksidan; *Centella asiatica*; HPLC; metabolomik; spektrum FTIR

INTRODUCTION

Centella asiatica is a medicinal plant that grows wild in the tropics and subtropics, widely used as herbal medicine and functional food. The biological activities of *C. asiatica* have been reported, such as antioxidant, antibacterial (Rattanakom & Yasurin 2015), antimicrobial (Netala et al. 2015), antimigraine (Bobade et al. 2015), and cytotoxic (Pittella et al. 2009). There are four main components reported in *C. asiatica*, i.e. madecassoside, asiaticoside, madecassic acid, and asiatic acid (Rafi et al. 2018). Other compounds such as sterols, polyacetylene, flavonoids, and phenolic acids have also been present in *C. asiatica* (Atake et al. 2007).

The consistency of this biological activity depends on the composition and concentration of bioactive metabolites contained in a medicinal plant. The composition of chemical components present in medicinal plants can be influenced by growing conditions, including plant origin, different soil types, different cultivation environments, and harvest times (Kunle et al. 2012). The safety, efficacy, and stability of herbal medicinal products are primarily determined by variations in the composition produced from medicinal plants. Changes in chemical composition and biological activity due to different plant ages need to be evaluated on the levels of bioactive metabolites.

Differences in the chemical composition of medicinal plants can be evaluated using a metabolomics approach to determine their quality. Using metabolomics,

we analyzed small molecules in a particular cell, tissue, or organism qualitatively and quantitatively to interpret and compare metabolite profiles from several analytical instruments. We could use many analytical instruments such as GC-MS (Intararuchikul 2019; Rattanakom & Yasurin 2015), HPLC or LC-MS (Syarifah et al. 2017; Umar et al. 2021), NMR (Songvut et al. 2021), and FTIR (Rohaeti et al. 2021). There are several main approaches in metabolomics, namely targeted metabolite analysis, metabolite profiling, and fingerprinting.

Profiling the composition of metabolites and biological activities of medicinal plants is very necessary because it provides an overview of the biochemical state of a plant to monitor the quality of herbal medicinal raw materials. In a previous study conducted by our group, a metabolite profiling of *C. asiatica* has been performed for differentiation in cultivation age (3-, 4-, and 5-months post-planting) (Rafi et al. 2018). In this work, we expanded the cultivation age from 2 until 6 months post-planting (MPP) and evaluated the metabolite profile and radical scavenging activities of *C. asiatica*. In connection with the differences in the planting period of *C. asiatica*, standardization of *C. asiatica* extract will be very helpful so that for its application, uniformity of active ingredients, safety, and efficacy can be guaranteed.

Therefore, profiling was carried out in this study using a metabolomics approach, namely, metabolite fingerprinting using the FTIR spectrum and targeted analysis of madecassoside, asiaticoside, madecassic acid, and asiatic acid (Figure 1) in *C. asiatica* using HPLC at 2

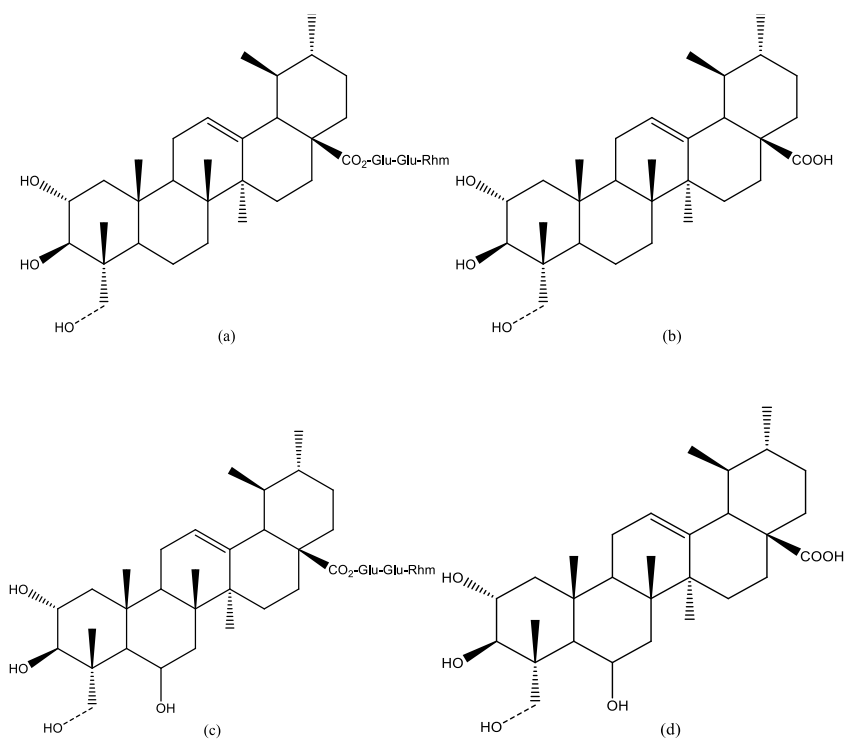


FIGURE 1. Chemical structure of (a) asiaticoside, (b) asiatic acid, (c) madecassoside, and (d) madecassic acid

to 6 MPP. The HPLC chromatogram and FTIR spectrum obtained will have a large amount of data, so that it requires the help of multivariate analysis to facilitate the interpretation of the grouping of the planting period. Measurement of radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was also conducted to determine the potency of antioxidants in of *C. asiatica* extract for each month post-planting.

MATERIALS AND METHODS

SAMPLE PREPARATION

Samples of *C. asiatica* were obtained from the Experimental Garden of the Tropical Biopharmaca Research Center (TropBRC), IPB University, Bogor, Indonesia. The voucher specimen (BMK0105082016) was stored in TropBRC, IPB University. *C. asiatica* was planted in 15 plots in TropBRC medicinal plant garden (141 msal), IPB University in three replications with a 2 - 6 MPP. Each sample was randomly harvested and then washed to remove impurities attached to the sample and then quenched using liquid nitrogen so that the metabolites contained in the sample did not change. About 250 mg of the sample was weighed, extracted with 10 mL ethanol (Merck, St. Louis, USA), and sonicated for 30 min. The filtrate was filtered with a Whatman filter membrane (0.22 µm pore size; PTFE; P/N E252, Buckinghamshire, England).

MEASUREMENT OF FTIR SPECTRUM

The FTIR spectrum was made for three replications. The potassium bromide (KBr) pellet of the extract was prepared by mixing 2 mg of sample with 200 mg of KBr. Pellets are made using a hand press. FTIR spectra were recorded using an FTIR Tensor 37 spectrophotometer (Bruker Optik GmbH, Karlsruhe, Germany) with a deuterated triglycine sulfate detector in the mid-infrared region (400-4000 cm⁻¹) at a resolution of 4 cm⁻¹ with a scan count of 32 operated with OPUS software version 4.2 (Bruker Optik GmbH, Karlsruhe, Germany). The FTIR spectrum was stored in data point table (DPT) format. The original FTIR spectrum was pretreated by Savitzky-Golay smoothing and maximum normalization on Unscrambler® X software version 10.1 (CAMO, Oslo, Norway).

DETERMINATION OF FOUR ACTIVE COMPOUNDS USING HPLC

Quantitative analysis of madecassoside, asiaticoside, madecassic acid, and asiaticoside acid was performed

by HPLC with the procedure described by Rafi et al. (2018). HPLC LaChrome Elite L-2000 equipped with UV-Vis detector L-2420 and oven L-2300 (Hitachi, Tokyo, Japan) was used for separation and detection of the four compounds. The column used is Shim-pack VP-ODS C18 (150 × 4.6 mm) (Shimadzu, Kyoto, Japan). The mobile phase used a combination of acetonitrile (A) and water (B) with a gradient elution system of 20-45% A for 20 min and 45-65% at 20-40 min. Other controlled conditions were a flow rate of 1 mL/min, a column temperature of 40 °C, and a detection wavelength of 206 nm. Next, 20 µL of the sample was injected with the HPLC system. Determination of the four main components in *C. asiatica* was performed in triplicates for each sample.

DETERMINATION OF RADICAL SCAVENGING ACTIVITY

Measurement of radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method followed the procedure described by Sugunabai and Karpagam (2015). *C. asiatica* extract was dissolved in ethanol with a concentration of 100 µg/mL. A total of 100 µL of sample extract and 100 µL of DPPH 30 g/mL were added to each well, then incubated for 30 min in a dark room. After that, the absorbance (A) was measured at a wavelength of 517 nm. Each sample was measured in triplicates. The following formula determines free radical scavenging activity.

$$\% \text{ inhibition} = \left[1 - \frac{A_{\text{sample}} - A_{\text{control}(+)}}{A_{\text{blank}} - A_{\text{control}(+)}} \right] \times 100\%$$

DATA ANALYSIS

Statistical comparison for four major compounds content and radical scavenging activity was carried out using one-way analysis of variance (ANOVA) followed by the Duncan test. We used a significant difference at the 95% confidence level (p<0.05) was used. Principal component analysis (PCA) was used for clustering *C. asiatica* based on cultivation age using FTIR spectra and four major components level with Unscrambler® X software version 10.1 (CAMO, Oslo, Norway).

RESULTS AND DISCUSSION

QUANTITATIVE ANALYSIS OF FOUR MAJOR COMPOUNDS IN *C. asiatica*

Different cultivation ages can be a factor that affects the bioactive component level contained in a medicinal plant (Rafi et al. 2018). We performed quenching to collect the *C. asiatica* sample to stop all the sample's

metabolic processes and no enzymatic reactions. This study quantified four main components (madecassoside, asiaticoside, madecassic acid, and asiatic acid) in the *C. asiatica* samples from 2 to 6 MPP.

Madecassoside, asiaticoside, madecassic acid, and asiatic acid were detected at retention times of 8.9, 10.8, 20.2, and 24.8 min, respectively, gave a strong signal on the HPLC chromatogram (Figure 2). The content of each compound varies significantly. Madecassoside and asiaticoside were the most dominant components, while

madecassic acid and asiatic acid were the lowest in most analyzed samples. We also found that each sample showed the levels of four analytes in 4 MPP were the highest than other samples (Table 1). Madecassoside, asiaticoside, and madecassic acid content increased in samples from 2-4 MPP and decreased from 4-6 MPP samples. Different patterns were shown in asiatic acid wherein 4 and 5 MPP showed no significant difference. These results suggest that differences in levels of chemical components can be affected by growing environmental conditions and cultivation age.

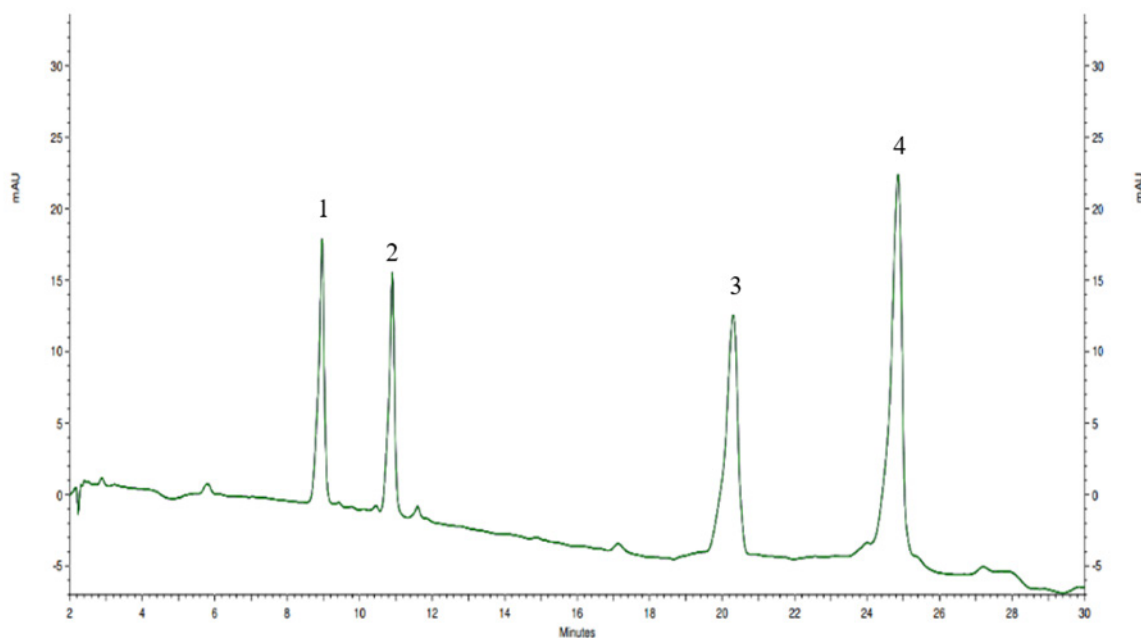


FIGURE 2. HPLC chromatogram of four major components in *C. asiatica* (4 MPP), madecassoside (1), asiaticoside (2), madecassic acid (3), and asiatic acid (4)

TABLE 1. Four major bioactive components content in *C. asiatica*

| Chemical components | Content (mg/g) | | | | |
|---------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | 2 MPP | 3 MPP | 4 MPP | 5 MPP | 6 MPP |
| Madecassoside | 0.38 ± 0.09 ^a | 1.18 ± 0.11 ^b | 2.92 ± 0.18 ^c | 1.94 ± 0.13 ^d | 1.59 ± 0.04 ^c |
| Asiaticoside | 0.59 ± 0.13 ^a | 1.83 ± 0.12 ^b | 3.33 ± 0.15 ^d | 2.40 ± 0.23 ^c | 1.74 ± 0.12 ^b |
| Madecassic acid | 0.16 ± 0.03 ^a | 1.09 ± 0.04 ^c | 1.49 ± 0.23 ^d | 1.17 ± 0.11 ^c | 0.63 ± 0.04 ^b |
| Asiatic acid | 0.57 ± 0.86 ^a | 0.86 ± 0.04 ^a | 1.34 ± 0.22 ^b | 1.29 ± 0.03 ^b | 0.50 ± 0.01 ^a |

FTIR SPECTRUM OF *C. asiatica*

FTIR spectrum can provide very useful information to describe a sample's characteristics and see changes

in concentration and metabolite profiles of a plant (Purwakusumah et al. 2014). The FTIR spectrum of *C. asiatica* extract with different cultivation ages gave

identical patterns that only differ in the intensities of each peak. This indicates that the chemical compounds contained in each sample are almost similar (Figure 3). The interpretation of the FTIR spectrum of *C. asiatica* extract at various harvest periods showed vibrations from several functional groups. The functional groups presence were OH, C-H (-CH₂ and -CH₃), C=O aromatic, C=C benzene, C-O, C-N (aromatic amine), C-O phenol, C-N aliphatic amine, and alkyl halide (Table 2). All FTIR

spectra of *C. asiatica* give a similar pattern but differ in the peak intensities. These differences showed that each group of the sample has different metabolite levels. Based on the resulting FTIR spectra, it is known that *C. asiatica* extracts with 4 MPP had the highest absorbance intensity, then followed by sample extracts of 5, 3, 2, and 6 MPP. These results correlate with the highest madecassoside, asiaticoside, madecassic acid, and asiatic acid AA level at 4 MPP.

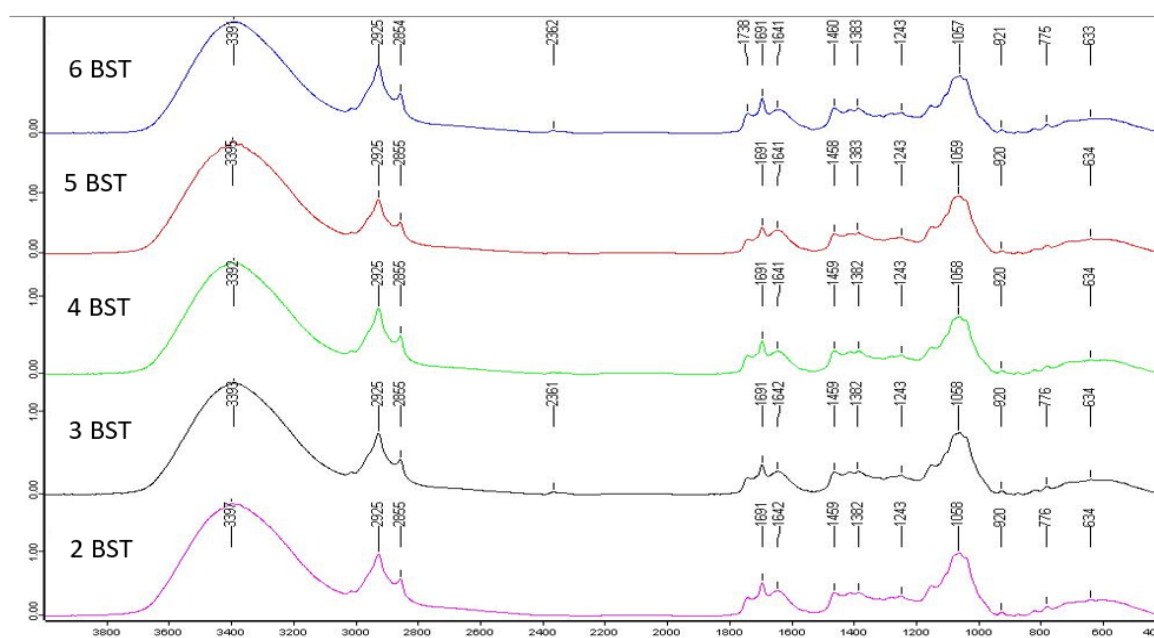


FIGURE 3. FTIR spectra of *C. asiatica* based on cultivation ages

TABLE 2. Identification of functional group from the FTIR spectra of *C. asiatica*

| Wavenumber (cm ⁻¹) | Identified functional groups | Intensities |
|--------------------------------|--|-------------|
| 3399-3391 | OH | Strong |
| 2854-2925 | C-H (-CH ₂ dan -CH ₃) | Medium |
| 1691 | C=O aromatic | Medium |
| 1641-1642 | C=C benzene | Medium |
| 1461-1058 | C-O | Medium |
| 1382-1383 | C-N (aromatic amine) | Weak |
| 1243 | C-O (phenol) | Strong |
| 1056-1059 | C-N aliphatic amine | Medium |
| 600-800 | Akyl halide | Weak |

RADICAL SCAVENGING ACTIVITY OF *C. asiatica*

Measurement of radical scavenging activity using the DPPH method was based on the reaction between a stable free radical from DPPH (1,1-diphenyl-2-picrylhydrazil) with antioxidant components to form diphenylpicrylhydrazine. This reaction occurs through binding one electron of hydrogen by the nitrogen atom in DPPH, whose electrons are unpaired, resulting in a color change. A UV-VIS spectrophotometer measured the intensity of the purple color lost at a wavelength of 517 nm. Therefore, the higher the radical scavenging activity, the lower the absorption.

The level of % inhibition indicates free radical activity. The results obtained from *C. asiatica* extract at various cultivation ages showed the five extracts' different free radical scavenging activities (Figure 4). The highest percent inhibition value of *C. asiatica*

extract was found at 4 MPP (53.81%) and the lowest at 2 MPP (39.13%) with concentration of 100 µg/mL. Based on this finding, it proved that the higher the content of madecassoside, asiaticoside, madecassic acid, and asiatic acid, the higher the radical scavenging activity.

Also from the ANOVA resulted in 4 and 5 MPP showed no significant difference ($p < 0.05$) and the content of asiatic acid supports this ANOVA result. These results suggested an effect of the planting period on the content of active compounds, causing differences in the biological activity level of *C. asiatica*. Other influences can come from differences in the geographical location of where the plant grows, the part used, and the type of solvent used (Liu et al. 2016). Based on the content of active compounds, FTIR spectrum, and radical scavenging activity, it can be concluded that the *C. asiatica* at four MPP is the best condition to harvest according to its four major components and radical scavenging activity.

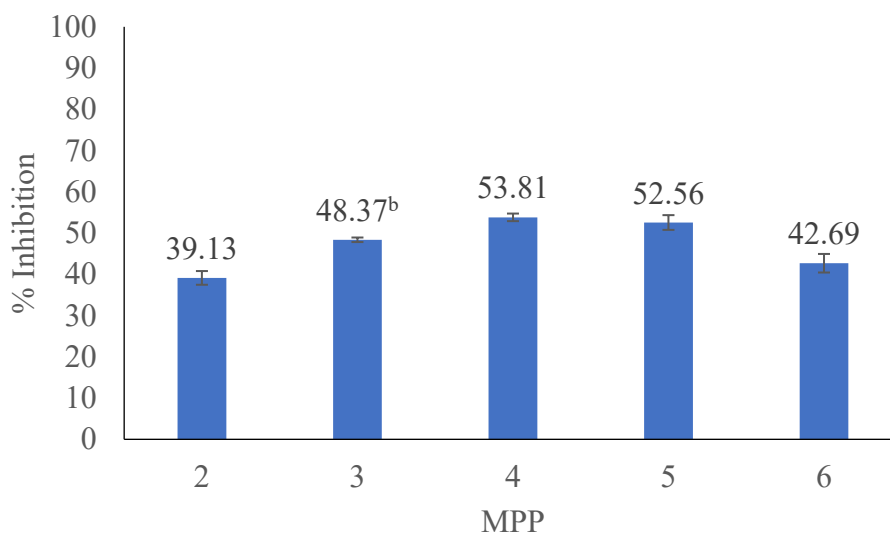


FIGURE 4. Inhibition antioxidant activity of *C. asiatica* extract from 2 until 6 months post-planting

CLUSTERING OF *C. asiatica* BASED ON CULTIVATION AGE

Clustering of *C. asiatica* with different cultivation ages could be performed using the level of four major components present in *C. asiatica* determined by HPLC and FTIR spectra. Comparison of HPLC chromatogram profiles of *C. asiatica* were very similar and differed only by the peak area of each analyte. The retention time value is relatively consistent, but the intensity of the peak area

of the sample varies, which means that the concentration of chemical components in each sample is different. In addition, the FTIR spectrum of all *C. asiatica* samples has a very complex pattern that is difficult to interpret directly in classifying them based on planting age. This makes it difficult to visually distinguish the *C. asiatica* based on the time of planting. Therefore, a combination of fingerprints and multivariate analysis for clustering *C. asiatica* were used in this work.

One method that is often used in multivariate analysis is principal component analysis (PCA). PCA simplifies the variables in the analysis results using HPLC or FTIR spectra into only a few main variables, namely principal component (PC). Using the first two PC, we could get sample grouping based on the similarity or dissimilarity of the variables in the group. The variables used to cluster *C. asiatica* based on cultivation age were the levels of four main components and absorbance values from the FTIR spectrum.

Before being subjected to PCA, the peak area of the four main components in *C. asiatica* was carried out

data for pretreatment. This process was performed to get good results because the data quality greatly affected the clustering result. One of the common data pretreatments is transformation using the scaling method. This method is generally applied using the standard deviation as a factor. In this study, scaling was done by dividing the value of each variable by the standard deviation value minus 1 (Hendriks et al. 2005). Based on the PCA score plot using the peak area of four major components, each sample of *C. asiatica* was grouped according to its cultivation ages with a total percentage of PC-1 and PC-2 about 98% (Figure 5). The results of PCA are good

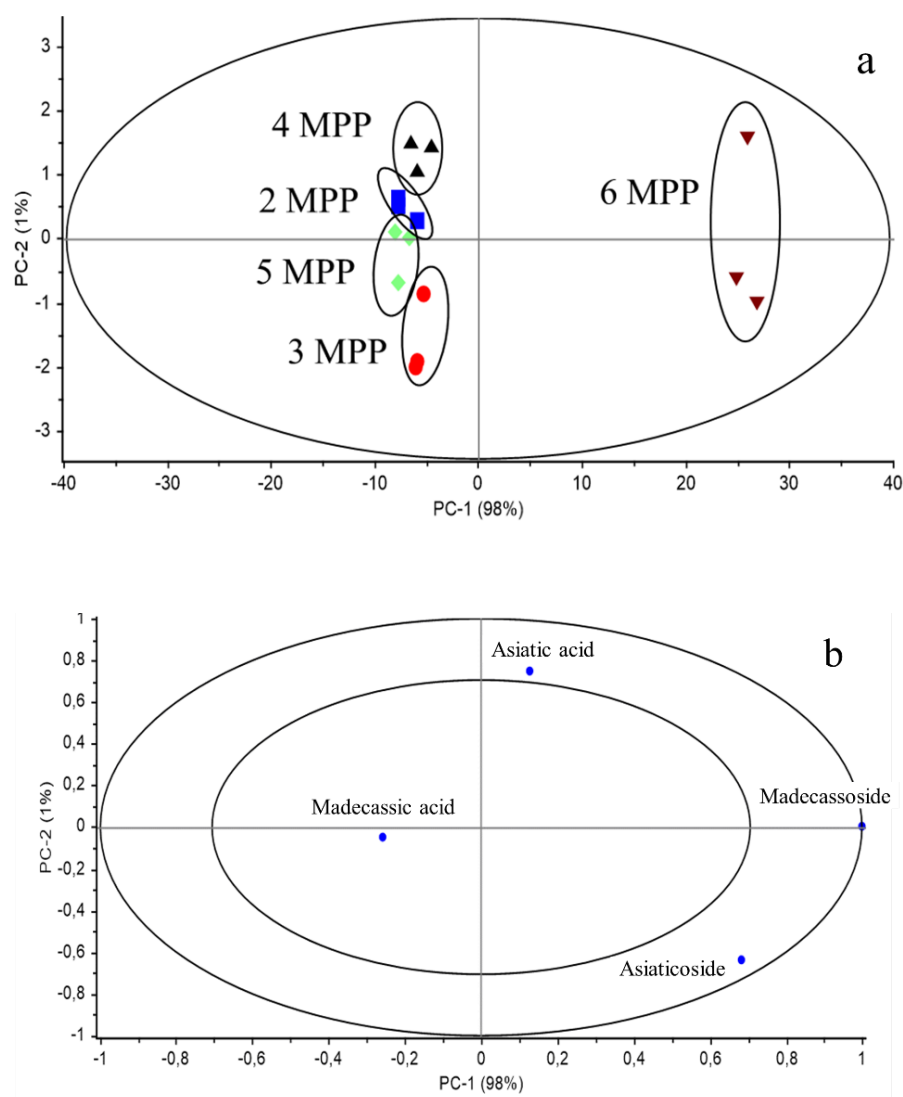


FIGURE 5. PCA score plot (a) dan loading plot (b) using peak area of four major components in *C. asiatica*

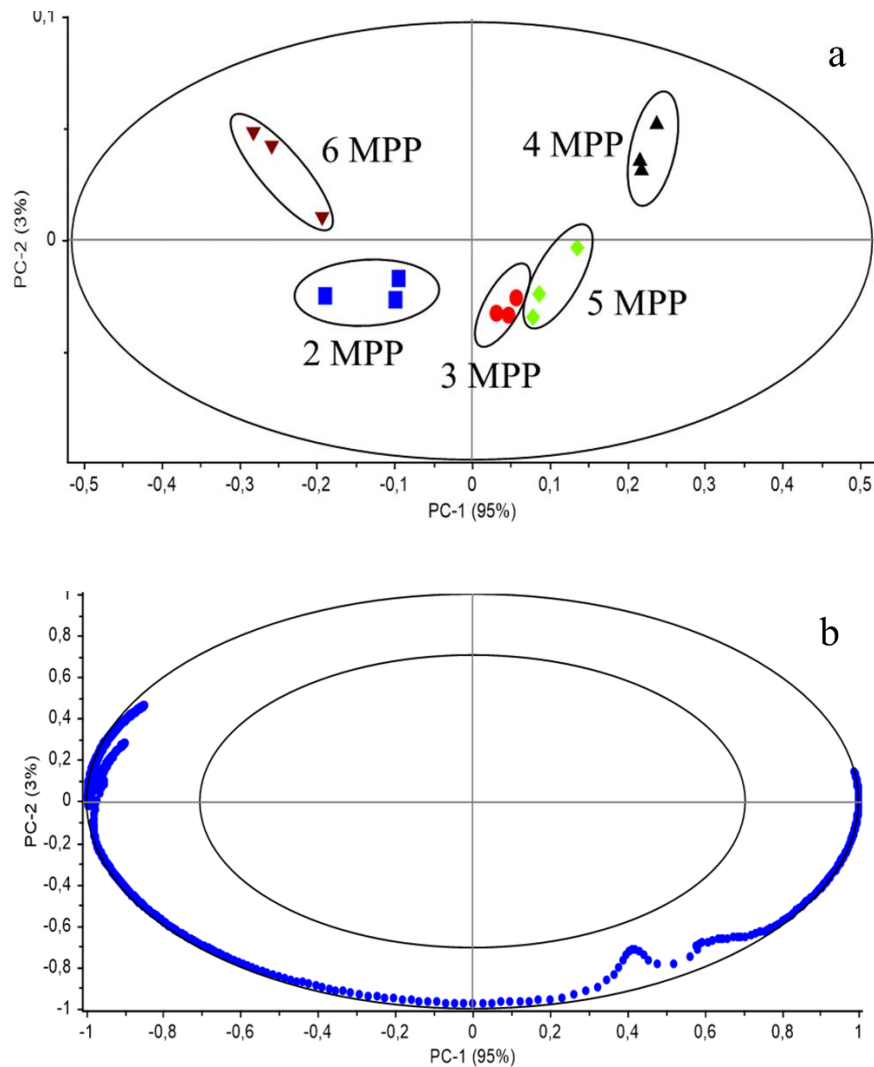


FIGURE 6. PCA score plot (a) dan loading plot (b) using absorbance from the *C. asiatica* FTIR spectra

if the first two PC can describe a large total variation. The 6 MPP was grouped separately compared to the other sample group 2-5 MPP. The closer one sample is to another, the greater the similarity between the samples (Purwakusumah et al. 2014). Meanwhile, the loading plot (Figure 5(b)) illustrates the contribution of the four peaks to the resulting PCA. The circle on the correlation loading plot can show the percent contribution of each variable. The three peaks (madecassoside, asiaticoside, and asiatic acid) showed above 50% contribution, while the madecassic acid peak contributed below 50%. This is because the peak area of madecassic acid is relatively small than other peaks in the analyzed samples.

In addition, we also used the variable absorbance spectrum FTIR for clustering *C. asiatica* with different cultivation ages. PCA results are displayed in the form of a score plot (Figure 6(a)) which shows the grouping of *C. asiatica* samples using absorbance value from their FTIR spectrum. PCA score plot by using the FTIR spectra as the variable showed *C. asiatica* sample at each cultivation age could be grouped with a total variation of PC-1 and PC-2 about 95%. Using the absorbance of FTIR spectra can explain about 95% variations of the data from the *C. asiatica* sample. *C. asiatica* with different cultivation ages could cluster well on the score plot. Groups 3 and 5 MPP had similar metabolite concentration because it

was closer to each other. The same thing occurred to 2 with 6 MPP, while 4 MPP grouped separately. Based on Figure 6(b), the FTIR spectrum shows a contribution of 50% in the grouping.

CONCLUSION

This study has performed metabolite profiling of *C. asiatica* with different cultivation ages using FTIR spectra and HPLC-based metabolomics and evaluated its radical scavenging activity. Differences in cultivation ages and variation in the location growth factor (soil, water) of *C. asiatica* showed quite different in the peak intensities of its FTIR spectra and HPLC chromatogram obtained. The metabolite profile of *C. asiatica* at 4 MPP showed the highest madecassoside, asiaticoside, madecassic acid, and asiatic acid levels, among other cultivation ages. Clustering of *C. asiatica* based on cultivation ages showed that each sample could be grouped using absorbance from the FTIR and peak area of 4 main components determined by HPLC with a total variation of the first two PC about 95% and 98%, respectively. The content of the four main compounds in the *C. asiatica* correlated with radical scavenging activity, with the 4 MPP sample showing the highest scavenging activity. This shows that *C. asiatica* at 4 MPP gave optimum results for four main metabolite content and radical scavenging activity.

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REFERENCES

- Bobade, V., Bodhankar, S.L., Aswar, U., Vishwaraman, M. & Thakurdesai, P. 2015. Prophylactic effects of asiaticoside-based standardized extract of *Centella asiatica* (L.) urban leaves on experimental migraine: Involvement of 5HT_{1A}/1B receptors. *Chinese Journal of Natural Medicines* 13(4): 274-282.
- Hendriks, M.M.W.B., Cruz-Juarez, L., Bont, D.D. & Hall, R.D. 2005. Preprocessing and exploratory analysis of chromatographic profiles of plant extracts. *Analytica Chimica Acta* 545(1): 53-64.
- Intararuchikul, T., Teerapattarakon, N., Rodsiri, R., Tantisira, M., Wohlgemuth, G., Fiehn, O. & Tansawat, R. 2019. Effects of *Centella asiatica* extract on antioxidant status and liver metabolome of rotenone-treated rats using GC-MS. *Biomedical Chromatography* 33(2): e4395.
- Kunle, O.F., Egharevba, H.O. & Ahmadu, P.O. 2012. Standardization of herbal medicines - A review. *International Journal of Biodiversity and Conservatio* 4(3): 101-112.
- Liu, W., Yin, D., Li, N., Hou, X., Wang, D., Li, D. & Liu, J. 2016. Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Scientific Reports* 6(1): 1-18.
- Netala, V.R., Kotakadi, V.S., Nagam, V., Bobbu, P., Ghosh, S.B. & Tartte, V. 2015. First report of biomimetic synthesis of silver nanoparticles using aqueous callus extract of *Centella asiatica* and their antimicrobial activity. *Applied Nanoscience* 5(7): 801-807.
- Pittella, F., Dutra, R.C., Junior, D.D., Lopes, M.T.P. & Barbosa, N.R. 2009. Antioxidant and cytotoxic activities of *Centella asiatica* (L) Urb. *International Journal of Molecular Sciences* 10(9): 3713-3721.
- Purwakusumah, E.D., Rafi, M., Syafitri, U.D., Nurcholis, W., Agung, M. & Adzkiya, Z. 2014. Identification and authentication of Jahe Merah using combination of FTIR spectroscopy and chemometrics. *Agritech* 34(1): 82-87.
- Rafi, M., Handayani, F., Darusman, L.K., Rohaeti, E., Wahyu, Y., Honda, K. & Putri, S.P. 2018. A combination of simultaneous quantification of four triterpenes and fingerprint analysis using HPLC for rapid identification of *Centella asiatica* from its related plants and classification based on cultivation ages. *Industrial Crops and Products* 122: 93-97.
- Rattanakom, S. & Yasurin, P. 2015. Chemical profiling of *Centella asiatica* under different extraction solvents and its antibacterial activity, antioxidant activity. *Oriental Journal of Chemistry* 31(4): 2453-2459.
- Rohaeti, E., Karunina, F. & Rafi, M. 2021. FTIR-based fingerprinting and chemometrics for rapid investigation of antioxidant activity from *Syzygium polyanthum* extracts. *Indonesian Journal of Chemistry* 21(1): 128-136.
- Songvut, P., Chariyavilaskul, P., Khemawoot, P. & Tansawat, R. 2021. Pharmacokinetics and metabolomics investigation of an orally modified formula of standardized *Centella asiatica* extract in healthy volunteers. *Scientific Reports* 11(1): 1-13.
- Sugunabai, J. & Karpagam, T. 2015. Analysis of functional compounds and antioxidant activity of *Centella Asiatica*. *World Journal of Pharmacy and Pharmaceutical Sciences* 4: 1982-1993.
- Syarifah, V.B., Rafi, M. & Wahyuni, W.T. 2017. High performance liquid chromatography fingerprint analysis for quality control of brotowali (*Tinospora crispa*). *Journal of Physics: Conference Series*. IOP Publishing. 835(1): 012016.
- Umar, A.H., Ratnadewi, D., Rafi, M. & Sulistyarningsih, Y.C. 2021. Untargeted metabolomics analysis using FTIR and UHPLC-Q-Orbitrap HRMS of two *Curculigo* species and evaluation of their antioxidant and α -glucosidase inhibitory activities. *Metabolites* 11(1): 42.

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