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Antioxidant activity and metabolite changes in *Centella asiatica* with different drying methods using FTIR- and quantitative HPLC-based metabolomics

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Abstract

Centella asiatica, known as Indian or Asiatic pennywort, is consumed raw as salad or used as a brain tonic, treatment of Alzheimer's disease, and memory improvement. Differences in the drying method will lead to different levels of phytochemical profile and biological activity. Therefore, the present work aimed to investigate the Fourier transforms infrared (FTIR) spectra fingerprint profiles, HPLC analysis of four bioactive compounds, and antioxidant activity of *C. asiatica* samples exposed to various drying methods, including air-, oven-, and sun-drying. Results showed that all samples had identical FTIR spectra patterns, but there were differences in the absorbance intensities at 1692 and 1634 cm^{-1}, showing the effect of drying methods on the content of extracts' bioactive compounds. These differences were analysed by chemometrics namely principal components analysis (PCA), and groupings were shown for the three samples. Based on the IC_{50} values, oven-drying (OD) had the highest antioxidant activity, followed by sun-drying (SD) and air-drying (AD), with IC_{50} values of 52.25, 94.18, and 99.29 µg/mL, respectively. HPLC analysis showed that OD had a higher percentage for madecassoside and asiaticoside with values of 0.86 and 0.96%, respectively, compared to SD and AD. Meanwhile, AD had the highest content of madecassic and asiatic acids, with values of 0.50 and 0.48%. The absorbance and antioxidant activity data for the three *C. asiatica* extracts were analysed for the correlation using an orthogonal partial least square. Results showed that at 1006 - 989 cm^{-1}, it positively correlated with antioxidant activity, and could be identified as the C–O functional group of alcohol and phenol.

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Introduction

Centella asiatica, also known as Indian or Asiatic pennywort, and locally known as *pegagan* in Indonesia, is a medicinal plant widely used in commercial or traditional medicines. This plant can be consumed raw as a salad, and also used for treating various diseases, including brain tonic, Alzheimer, memory improvement, healthy aging, stomachache, cough, inflammation, muscle soreness, asthma, leprosy, fever, and blood booster (Gray *et al.*, 2017; Sabaragamuwa *et al.*, 2018; Thakurdesai, 2021; Verma *et al.*, 2021; Seong *et al.*, 2023; ShanmugaPriya *et al.*, 2023). Additionally, *C. asiatica* has several biological activities such as wound healing, anti-inflammatory, anticancer, antioxidant, antimicrobial, antidiabetic, anti-amnesic, and anticholinergic activities (Arora *et al.*, 2018; Wong and Ramli, 2021; Tripathy *et al.*, 2022; Tanga *et al.*, 2022; Arribas-López *et al.*, 2022; Yi *et al.*, 2023). These biological activities are due to the presence of bioactive metabolites such as triterpenoid, saponin, alkaloid, phenolic, flavonoid, tannin, steroid, and glycoside (Belwal *et al.*, 2019; Ren *et al.*, 2021; Mohapatra *et al.*, 2021; Kunjumon *et al.*, 2022; Masi *et al.*, 2022; Zheng *et al.*, 2022).

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Antioxidant is one of the biological activities of *C. asiatica* that have been widely reported, with the responsible chemical contents being phenolic and flavonoid compounds, including rutin, quercetin, and catechin (Quyen *et al.*, 2020; Abu Bakar *et al.*, 2022).

The composition of metabolites in plant extracts is generally influenced by post-harvest preservation methods such as drying (Yilmaz and Alibas, 2021; Guadalupe-Moyano *et al.*, 2022). During post-harvest, drying plays a crucial role in preserving the herb quality by reducing the water content, and preventing enzymatic damage due to microbial growth (Benjamin *et al.*, 2022). The process can be carried out with heat such as oven-drying (OD), sun-drying (SD), microwave-drying, or without heat sucah as freeze-drying and air-drying (AD). Air- and oven-drying methods are preferable due to the relatively low cost and easier material handling. However, air drying needs a relatively longer time to dry the samples, particularly for plants with high water content. This will decrease the quality of the samples, including alteration in the taste, colour, and loss or degradation of some bioactive components (Thamkaew *et al.*, 2021). Both changes in the composition and the number of metabolites due to variations in drying methods can be quantitatively and qualitatively monitored. Fourier transform infrared spectroscopy (FTIR) is a qualitative method that can provide information about the spectroscopic fingerprinting and the intensity of the band of a specific functional group in the samples.

The effects of different drying methods on the metabolite profile (qualitative and quantitative) and the biological activities of *C. asiatica* need to be evaluated to determine the correlation with one another. An effective method for this evaluation is metabolomics which is comprehensively used to analyse the composition and determine the number of metabolites in a cell, tissue, and organism (García *et al.*, 2020). Metabolomics uses FTIR spectra profiles to group drying methods and assess the correlation with antioxidant activity. Due to the extensive number of variables, the FTIR spectra profiles-based grouping requires applying multivariate analysis such as principal component analysis (PCA) and orthogonal partial least square (OPLS). In the present work, PCA has been applied in grouping the samples based on the different drying methods using the absorbance variables in the samples. Meanwhile, OPLS determined the correlation between the functional groups of the chemical compounds found in the extracts and *C. asiatica* antioxidant activity. In addition, the present work also evaluated the level changes of four major bioactive compounds found in *C. asiatica*, namely asiaticoside, madecassoside, asiatic acid, and madecassic acid using HPLC when exposed to different drying methods.

Several investigations explored the correlation between the number of metabolites and various drying methods. Sajak *et al.* (2016) reported the effect of different drying methods and the ratio of solvent to phytochemical components of *Ipomoea aquatica*, while Li *et al.* (2022) showed the influence on *Lonicerae japonicae*. Awin *et al.* (2016) reported phytochemical profiles and biological activities of *Curcuma*, exploring the extraction with different solvents and drying methods. According to Maulidiani *et al.* (2013), 3,5-O-dicaffeoyl-4-O-malonilquinic acid (irbic acid), 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, 5-O-caffeoylquinic acid (chlorogenic acid), quercetin, and kaempferol derivatives are responsible for the antioxidant activity of *C. asiatica*. However, no reports were found exploring the effect of different drying methods on FTIR profiles and the correlation with antioxidant activities, and also for quantitative analysis of four major bioactive compounds in *C. asiatica*. Therefore, we took this opportunity to analyse *C. asiatica* by observing the difference in FTIR spectra patterns combined with chemometrics to determine the effect of various drying methods. The present work would identify the most important functional group of the antioxidant activity of *C. asiatica*. Also, we performed quantitative HPLC analysis on four bioactive compounds in *C. asiatica* with different drying methods.

Materials and methods

Chemicals and materials

The *C. asiatica* leaves were obtained from the Experimental Garden of the Tropical Biopharmaca Research Center (TropBRC), IPB University (-6.54701, 106.71589). These leaves were harvested from a 4-month-old *C. asiatica*, and identified by Mr. Taopik Ridwan, a botanist from TropBRC, and the voucher specimen was stored in TropBRC, IPB University. Other materials included filter paper, ethanol (Merck, Darmstadt, Germany), ascorbic acid/vitamin C (Sigma-Aldrich, St. Louis, United States), Kbr (Sigma-Aldrich, St. Louis, United States), and 2,2-diphenil-1-picrylhydrazyl radical

(Sigma-Aldrich, St. Louis, United States). The chemicals used were madecassoside (87.5%), asiaticoside (88.8%), madecassic acid (88.1%), and asiatic acid (92.6%), obtained from ChromaDex Inc. (Santa Ana, CA, USA). Acetonitrile and water used in HPLC analysis were analytical- or HPLC-grade (Merck, Darmstadt, Germany).

Sample preparation

The samples were divided into three groups, namely AD, OD, and SD, according to Sajak *et al.* (2016). The leaves were separated from the root, washed with tap water, and dried. The samples dried using the AD method were placed under the sun indirectly, with the aid of a roof. The samples dried using the SD method were placed in an open space directly exposed to the sunlight. The samples dried using the OD method were placed in an oven at 40°C. Each sample was treated to reduce the water content to less than 10%, and milled using a mill to a size of 60 mesh.

Sampel extraction

Samples were extracted in ethanol at a ratio of 1:10 for 1 h at room temperature using the sonication method, with five replications. The extract was concentrated using a rotary evaporator for FTIR measurement and antioxidant assay.

FTIR spectra measurement

A 2 mg extract sample was mixed with 200 mg KBr to prepare pellets, which were created using manual compression equipment (Shimadzu, Tokyo, Japan). Subsequently, FTIR spectra were recorded using FTIR Tensor 37 Spectrophotometer (Bruker Optik GmBH, Karlsruhe, Germany), equipped with DTGS (deuterated triglycine sulphate) detector in the mid-infrared (4000 - 400 cm^{-1}) at 4 cm^{-1} resolution, with 32 numbers of scanning, operated with OPUS version 4.2 software (Bruker Optik GmBH, Karlsruhe, Germany). FTIR spectra in the OPUS format were stored in a data point table (DPT), and the original spectra were pre-treated. The data were normalised, ensuring that the least absorbance was set to 0, and the highest at 2, followed by FTIR measurements with five replications.

Antioxidant activity

Antioxidant activity was assessed using the DPPH method by referring to a procedure used by Arora *et al.* (2018). *C. asiatica* extracts were prepared in concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL using serial dilution with ethanol as the solvent. As the positive control, vitamin C solution was prepared in concentrations of 100, 50, 25, 12.5, and 6.25 µg/mL. The extract of each sample (100 µL) and DPPH solution (50 µg in 1 mL ethanol) were added to a 96-well plate, and incubated at room temperature for 30 min in a dark room. Subsequently, the absorbances were measured at 517 nm, with the same treatment being applied to the positive control. The absorbance of each sample with various concentrations was measured in triplicate. Free radical scavenging activity was calculated using Eq. 1:

$$\text{Inhibition (\%)} = \left[1 - \left(\frac{\text{Asampel - Acontrol}}{\text{Ablank - Acontrol}}\right)\right] \times 100\% \quad \text{(Eq. 1)}$$

where, A = Absorbance.

The result was reported based on the IC_{50} value, which is the concentration where the samples remove 50% of DPPH free radicals.

HPLC analysis

Determination of madecassoside, asiaticoside, madecassic acid, and asiatic acid contents in *C. asiatica* was carried out according to Rafi *et al.* (2018) using a HPLC system (LC-20A series, Shimadzu, Kyoto, Japan) equipped with a diode-array UV-vis detector and shim-pack VP-ODS C18 column (150 × 4.6 mm i.d., 4.6 µm particle size) (Shimadzu, Kyoto, Japan). The mobile phase consisted of acetonitrile (A) and water (B) flowing at a gradient elution program for 40 min with a flow rate of 1 mL/min. Column temperature was kept at 40°C, and a detection wavelength of 206 nm was used for analysis.

Data analysis

FTIR spectral data were converted to Excel files, and multivariate analysis, including PCA and OPLS, was performed with SIMCA-P version 14.0 software (Umetrics, Umea, Sweden). PCA analysis was carried out using the absorbance data of functional groups, pre-processed as smoothing with a scale of 11 to eliminate the noise. For the OPLS analysis, FTIR absorbance data was used as the X matrix (estimator), while the IC_{50} values from antioxidant analysis were used as the Y matrix (response). The quality of the model was described with model accuracy (R_2Y) and prediction accuracy

(Q_2Y) criteria. Furthermore, several OPLS outputs were used in interpreting the data, including Score, Y-related Coefficient, and VIP (variable influence of projection) plots.

Results and discussion

Drying methods and water content of C. asiatica

C. asiatica samples were divided into three groups, and subjected to different drying methods, namely AD, SD, and OD, carried out in five replicates. These different drying methods affected the compositions and the number of chemical compounds in samples. AD was performed by placing the samples under indirect sunlight, with the aid of a roof, for 7 h/d for 14 d. SD was performed by placing the samples under direct sunlight for 7 h/d for 9 d, while OD was performed by placing the samples in an oven with a lamp at 40°C for 10 h/d for 9 d. These treatments were performed to bring down the water content to less than 10%. Based on the results, it was discovered that higher temperatures affected the time needed for drying. The goal of drying to a water content of less than 10% was to enable storage for an extended period, and avoid environmental influences such as moulds.

Results showed that AD, SD, and OD samples fulfilled the requirements, with average water contents of 8.05, 7.98, and 6.80%, respectively, as shown in Table 1. The water content of each sample was significantly different ($p < 0.05$). AD had the highest value, followed by SD and OD, due to the higher drying temperature which led to a faster transpiration process. Furthermore, it was discovered that OD with the highest drying temperature had the lowest water content, followed by SD and AD. Humidity is another distinguishing factor among the three drying methods. Drying with OD yielded a constant temperature and humidity, as the weather did not affect the method. Meanwhile, the weather affects AD and SD methods, causing fluctuation in temperature and humidity, making consistent application of the same treatment every day difficult.

Table 1. Water, madecassoside (M), asiaticoside (A), madecassic acid (MA), and asiatic acid (AA) contents, and antioxidant activity of *C. asiatica* extracts from different drying methods.

Drying method	Water content (%)	Content (%)				IC_{50} (µg/mL)
		M	A	MA	AA	
AD	8.05^b	0.75 ± 0.03^a	0.86 ± 0.02^a	0.50 ± 0.01^c	0.48 ± 0.01^c	99.29^c
SD	7.98^b	0.73 ± 0.03^a	0.81 ± 0.03^a	0.29 ± 0.01^b	0.27 ± 0.01^b	94.18^b
OD	6.80^a	0.86 ± 0.04^b	0.96 ± 0.04^b	0.18 ± 0.01^a	0.19 ± 0.01^a	52.25^a

Mean values followed by different lowercase superscripts are significantly different by Duncan's *post hoc* test at $p < 0.05$.

The drying results using AD, SD, and OD methods yielded different colours. OD produced a yield with a greener colour compared to AD and SD yields. This colour difference was attributed to the exposure of samples to sunlight during the drying process. OD samples were heated only in the oven with infrared radiation from the infrared lamp, and heated at 40°C, with relatively constant humidity. Meanwhile, AD and SD methods were treated with different temperatures, and highly depending on the weather, presenting challenges in maintaining consistent conditions. AD and SD methods gave yield with browner colour compared to OD due to the exposure of the samples to sunlight and ultraviolet radiation, both directly and indirectly. This colour change was related to the chlorophyll degradation into pheophytin, which was brown, during the drying process. One of the most important properties of chlorophyll is its ability to light, heat, oxygen, and chemical degradations (Rydzyński *et al.,* 2019). Although this phenomenon also occurred in AD, the samples were not directly exposed to the sunlight, and the colour change was insignificant.

FTIR spectra of samples

FTIR spectra obtained from the ethanolic extract of *C. asiatica* using three different drying methods gave identical patterns. These patterns showed the metabolites present in the extract through a functional group interpretation. The extract is known to contain a variety of metabolites, including phenolic and triterpenoid (Kunjumon *et al.,* 2022). Phenolic compounds are characterised by aromatic or aliphatic structures with at least one aromatic ring

connected to more hydroxyl (-OH) groups. The presence of an aromatic ring is shown by the vibration of the aromatic CH- functional group in the wavenumber range above 3000 cm^{-1} (3050 - 3010 cm^{-1}), which is also observed at 900 - 690 cm^{-1} due to a strong coupling with the adjacent hydrogen atom. This vibration can be used in the substituent position determination in the aromatic ring. Furthermore, the vibration of C=C in the aromatic ring is observed at the wavenumber 1600 and 1475 cm^{-1}.

The presence of a weak overtone peak at the area of between 2000 and 1667 cm^{-1} is a character that can be used in the substituent position determination in the aromatic ring. Generally, the -OH functional group vibrates at about 3600 cm^{-1}, with an -OH phenolic commonly found at 3610 cm^{-1}. The carbonyl ortho- in the intra-molecular hydrogen bond in the phenolic compound may shift the -OH band to a lower frequency. Alcohol and phenolic compounds can also show a C-O vibration absorbance band at the wavenumber 1260 - 1000 cm^{-1} (Pavia *et al.*, 2001).

The drying process is responsible for the oxidation of the organic components. For example, the hydroxyl group can transform into an aldehyde, ketone, and carboxylic acid, increasing the absorbance value. The aldehyde functional group is characterised by C=O vibration at 1740 - 1725 cm^{-1} for aliphatic aldehyde. Furthermore, there is C=O conjugation with α, β-C=C with C=O at 1700 - 1680 cm^{-1} and C=C at 1640 cm^{-1}, and C=O conjugation (1700 - 1660 cm^{-1}) with phenyl (1600 - 1450 cm^{-1}) for phenyl ring. The presence of aldehyde is also characterised by CH- strain, consisting of weak peaks at 2860 - 2800 and 2760 - 2700 cm^{-1} (Pavia *et al.*, 2001).

As presented in Figure 1, FTIR spectra interpretation of *C. asiatica* extracts from various drying methods showed typical peaks for the three FTIR spectra. However, there were differences at the wavenumber of 1800 - 1600 cm^{-1} for the three FTIR spectra of *C. asiatica* extracts. FTIR spectra of OD extract had a pair of peaks, with the peak at the wavenumber 1692 cm^{-1} being stronger than at 1634 cm^{-1}, including AD and SD extracts. FTIR spectra of AD extract had a pair of peaks weaker than OD and SD, with the peak at 1692 cm^{-1} being weaker than 1634 cm^{-1}. FTIR spectra of SD extract had a pair of weak peaks at the wavenumber 1692 and 1634 cm^{-1}, where the higher peak was stronger than AD spectra but weaker than OD spectra. For SD, the peak at 1634 cm^{-1} was stronger than AD but weaker than OD. Additionally, differences were observed in the peaks at wavenumber 1413 - 1058 cm^{-1} among the three FTIR spectra of *C. asiatica* extracts. FTIR spectra of OD extract showed the most peaks, while AD extract had the least and the weakest peaks compared to OD and SD.

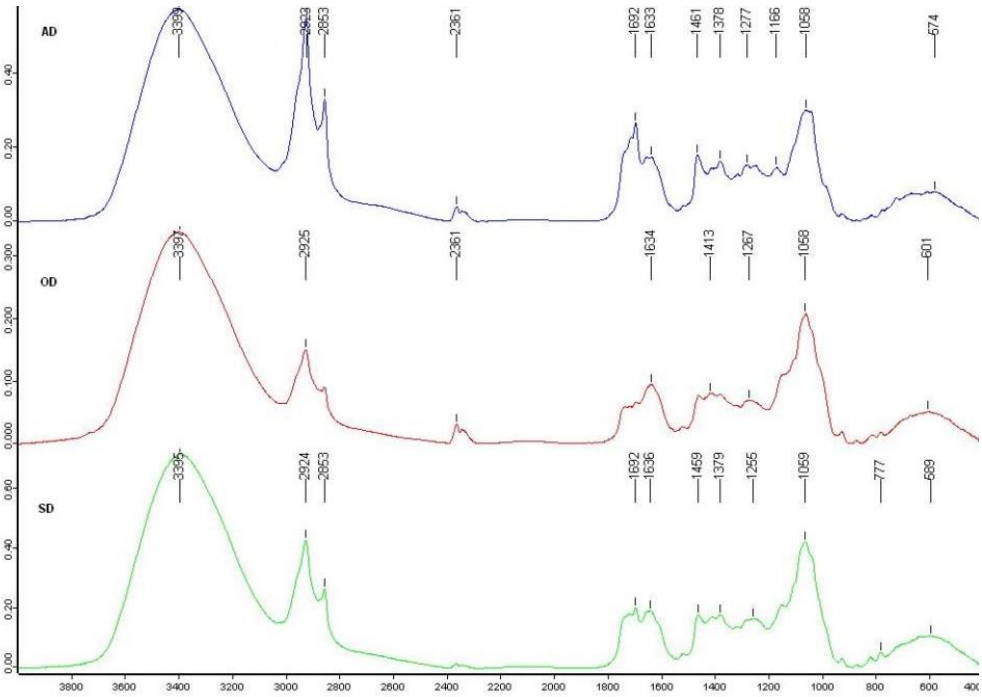

Figure 1. FTIR spectra of OD, AD, and SD samples.

Based on FTIR spectra, it was evident that the three FTIR spectra of *C. asiatica* extract with different drying methods showed identical patterns but had varying intensities at a pair of peaks at the wavenumber 1692 and 1634 cm^{-1}. This showed that different drying methods can lead to variation in FTIR spectra of extracts, resulting in various active compounds in *C. asiatica* extracts. These differences may be further identified using chemometrics such as PCA.

Madecassoside, asiaticoside, madecassic acid, and asiatic acid contents of C. asiatica

Madecassoside (M), asiaticoside (A), madecassic acid (MA), and asiatic acid (AA) were the main compounds found in *C. asiatica,* and their contents are shown in Table 1. Based on the results obtained, OD had a higher percentage for madecassoside and asiaticoside, with a value of 0.86 and 0.96%, respectively, compared to SD and AD. Meanwhile, AD had the highest content of madecassic and asiatic acids, with a value of 0.50 and 0.48%, respectively. The content of these four compounds in each sample was significantly different ($p < 0.005$), showing that the variation in drying methods affected the content of the main metabolite in *C. asiatica*. This phenomenon occurred due to differences in humidity and drying temperature of each method, which had the greatest effect on the metabolite content.

Antioxidant activity

Based on Table 1, the IC_{50} value of each sample was significantly different ($p < 0.05$). OD samples had lower IC_{50} values compared to SD and AD, showing greater antioxidant activity. This phenomenon occurred because of the time spent from drying to antioxidant activity, where the longer drying time reduced antioxidant activity. This may be affected by the enzymatic oxidation process, causing polyphenols to oxidise, and decrease the number of polyphenols available (Miao *et al.,* 2022). OD treatment resulted in the highest antioxidant activity compared to the others due to decreased levels of phenolic compounds that have antioxidant activity in plants. Specifically, phenolic acid is a compound that plays an active role in antioxidant activity. The high antioxidant activity in OD samples can be attributed to the drying process occurring at the optimum temperature of approximately 40 - 60°C (Roslan *et al.*, 2020).

Based on the data presented in Table 1, AD and SD extracts showed IC_{50} values of 99.29 and 94.18 µg/mL, respectively. This differed from that of OD extract at 52.25 µg/mL, showing superior antioxidant activity with the least IC_{50} value among the other extracts. The variation in results was attributed to the drying process occurring at an optimum and constant temperature.

SD and AD samples were exposed to inconsistent drying temperatures, contributing to the potential to decompose the antioxidant compounds. This was evident in the colour of SD and AD samples, which shifted from a pale green to a brownish colour, showing a decrease in quality. These results showed that drying treatment significantly influenced antioxidant activity, with SD and AD treatments causing a decrease in the antioxidant activity of *C. asiatica* extracts.

FTIR spectra grouping with PCA

The complex pattern of FTIR spectra is difficult to directly interpret, requiring the application of multivariate analysis for interpretation. One common method used in multivariate analysis is PCA, a chemometric method used to simplify and extract relevant information from complex chemical data. This method can reduce big-sized data to its principal component (PC), representing the structure and variance in the data (Granato *et al.*, 2018). In PCA, FTIR spectra is pre-treated with an objection to avoid any problems raised by the baseline shift, and increase the resolution of overlapping spectra (Tsagkaris *et al.,* 2023).

In the present work, PCA analysis of FTIR spectra resulting from *C. asiatica* extracts with AD, SD, and OD methods yielded a relatively great value of 99.30%, as presented in Figure 2. This showed that the absorbance variable of *C. asiatica* extracts can explain approximately 99.30% of data variance. According to Granato *et al.* (2018), the closer the spectra of two samples are, the greater the similarities.

Figure 2 shows that *C. asiatica* samples treated with different drying methods are separated into their respective groups. The plot distribution of every *C. asiatica* sample based on AD, SD, and OD methods, with three replications for each analysis. PCA also showed that the distributions of OD and SD samples were more centred, showing smaller data variances compared to AD samples.

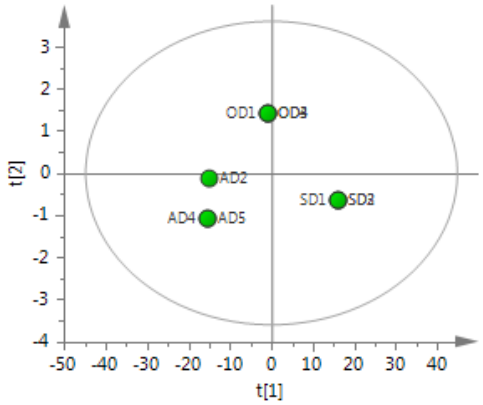

Figure 2. PCA score plot of FTIR spectra of AD, OD, and SD samples.

Another attribute of PCA is biplot, which shows a relationship among variables, a relative similarity among the objects of observation, as well as the position among the object of observation and variables. In Figure 3, the samples were well grouped into the type of drying methods, namely AD, OD, and SD. The results showed that SD samples had a higher correlation to the functional groups than AD and OD samples. This phenomenon was attributed to the absorbance intensities observed from SD spectra, which were higher than AD and OD spectra, as shown in Figure 1. Based on the spectra, it was known that the spectra with the highest absorbance intensity value showed a higher correlation to the functional groups. This showed that a higher absorbance intensity of an extract would result in greater metabolite compound content.

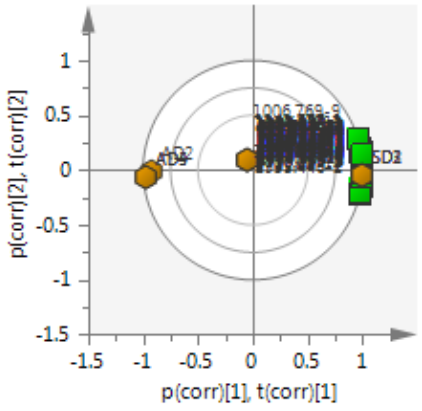

Figure 3. Biplot of FTIR spectra of AD, OD, and SD samples.

Correlation of FTIR spectra and antioxidant activity using OPLS

OPLS analysis has been used widely to identify the main variable influencing the samples grouping based on the characteristics of the Y data matrix (antioxidant activity). The principle of OPLS is to correct two kinds of data matrices, namely metabolite chemical profile (X component) and bioactivity (Y component). The result of OPLS shows output in the form of plots, including score, Y-related coefficient, and X variance. The score plot will show the grouping of plots of various *C. asiatica* extracts with different drying methods-based antioxidant activities. Meanwhile, the correlation of two matrices, the functional group and antioxidant activity data, will be observed using a Y-related coefficient plot, where the highest value shows a significant correlation. Functional group data correlated to antioxidant activity is expressed in the negative area, while the uncorrelated data is positive (Xu *et al.*, 2021).

FTIR analysis was used in the present work to identify the distribution of wavenumbers with relatively significant activity in various extracts using an X variance plot. The area with the least peak value was further identified for the functional group. In identifying the active compound, a plot of variable influence on projection (VIP) was used as a parameter of the X signal important to the Y data. Meanwhile, the Y-related coefficient plot was used to distinguish the signal positively and negatively correlated to the Y data matrix (antioxidant activity). VIP plot gave a positive correlation value for all signals, while a coefficient plot provided both positive and negative correlation values. The signal with a VIP value of more than 1 would be selected with an error bar diagram not touching the X-axis (Feng *et al.*, 2022).

The result of the score plot presented in Figure 4 shows good separation, effectively distinguishing the active (OD) from the less active extracts (SD and AD). The separation between samples with low and high activity facilitated identifying the functional groups in *C. asiatica* extracts. The accuracy of the OPLS model can be obtained through R_2Y and Q_2Y values. R_2Y value is the number of Y variables that may be explained by the model and the model fit review. Meanwhile, Q_2Y values, resulting from cross-validation, quantitatively measure the correlation between the prediction and the actual data (Blasco *et al.*, 2015). From the OPLS modelling of the relation between functional group and antioxidant activity, R_2Y and Q_2Y values of 0.93 and 0.88 were obtained, respectively. Based on standard requirements, acceptable OPLS models should have R_2Y and Q_2Y values ranging from 0.5 to 1. The results showed that the model was good because the two values met the standard requirements.

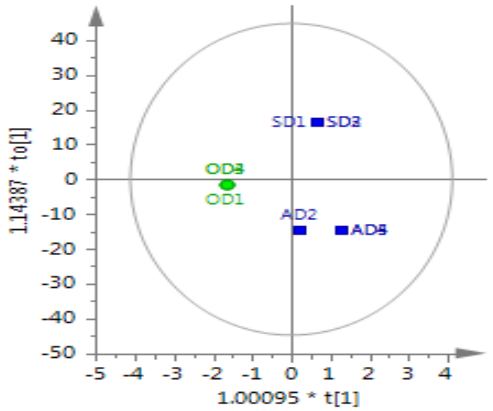

Figure 4. OPLS score plot of *C. asiatica* antioxidant activity from AD, OD, and SD samples.

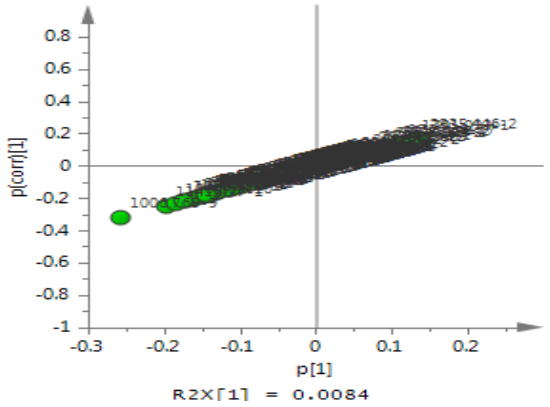

Figure 5. S-plot of OPLS curve of *C. asiatica* antioxidant activity from AD, OD, and SD samples.

The S-plot curve presented in Figure 5 explains the score plot to obtain information about the extract's dominant, active, and non-active functional groups. The position of the functional group is based on the samples in the score plot. Based on the obtained S-plot, it was discovered that the most dominant functional group in OD samples was found at the wavenumber of 1006 - 989 cm^{-1}. The Y-related coefficient plot presented in Figure 6 shows that substitution in the aromatic ring affected the antioxidant activity of *C. asiatica* extracts. Approximately, all functional groups were correlated, including those at 3400 - 3080 cm^{-1}, obtained from the O-H functional group. Furthermore, a significant correlation was found at 1600 - 1475 cm^{-1} for the C=C of the aromatic ring and at 1200 - 980 cm^{-1}, which was suspected to be C-O vibration from alcohol and phenolic.

Another analysis of OPLS is the X variance plot used to identify the dominant peak area in the extract. Based on the data obtained, the functional group at the wavenumber of 1006 - 989 cm^{-1} was the most dominant, which was identified using the VIP (variable influence on projection) plot. VIP plot value shows the wavenumber associated with antioxidant activity, with a value exceeding 0.5 considered significant. Based on the VIP plot, it was discovered that the highest value was found at the area of 1006 - 500 cm^{-1}. VIP value of C-H bending in the aromatic ring was the most correlated to antioxidant activity. In the present work, the analysis results using score plot, S-plot, Y-related coefficient, and X-plot were correlated with one another. This showed that the C-O functional group was mostly correlated to antioxidant activity originating from the phenolic compounds.

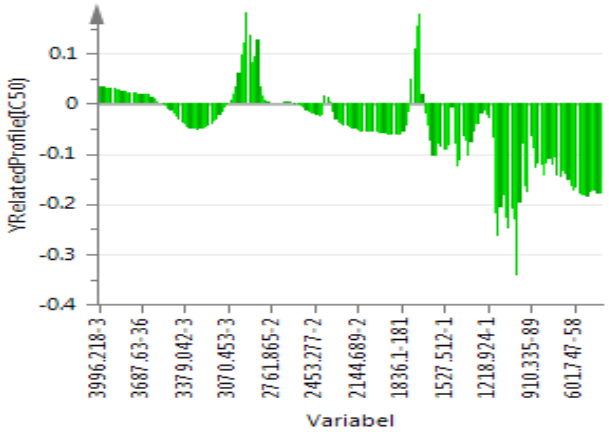

Figure 6. Y-related coefficient plot of correlation of functional group and antioxidant activity of *C. asiatica* extracts from AD, OD, and SD samples.

Conclusion

The present work demonstrated that various drying methods for *C. asiatica* produced relatively different FTIR spectra. These drying methods caused a difference in a pair of peaks at 1692 and 1634 cm^{-1} of FTIR spectra of *C. asiatica* extracts. Based on the results of FTIR spectra combined with PCA, the three extracts were effectively separated into groups, with a total PC of 99.30%. Antioxidant activity test showed that the order of IC_{50}, from the most active, was OD, SD, and AD methods, respectively. This showed that drying methods influenced antioxidant activity, with OD showing stronger FTIR peaks than AD and SD methods at 1692 and 1634 cm^{-1}. Based on OPLS analysis, the C-O functional group of phenolic compounds was the most influential in the antioxidant activity of *C. asiatica* extracts from various drying methods.

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