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Analysis for quality of senggugu plant and herbal product (*Rotheca serrata* (L.) with metabolomics approach

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ABSTRACT: Senggugu plant (*Rotheca serrata* (L.) has many benefits and has the potential as a raw material for herbal medicine, so an identify in the form of an FTIR fingerprint profile is needed. The purpose of this study was to ensure the quality of simplisia and herbal product of senggugu roots and leaves by metabolomics approach. Simplisia and herbal products powder of senggugu leaves and roots were extracted with 70% ethanol solvent using ultrasonic method. The dried extracts of simplisia and herbal products were analyzed by FTIR and TLC-Densitometry. Data obtained from FTIR and TLC-Densitometry analyzed with multivariate data analysis techniques, Principal Component Analysis (PCA) to obtain fingerprint profiles. The PCA results obtained a total Principal Component value of 98,7% and showed that leaf simplisia and herbal products of senggugu leaves can cluster well, while root simplisia and herbal products of senggugu roots were at a distance from the plot. The analysis with OPLS-DA showed the same fungtional groups, which are C=H, O-H, C=C. The results of antioxidant activity testing of 70% ethanol extracts of leaves, roots, and herbal products of senggugu obtained strong antioxidant activity ability with IC_{50} values of 80,08; 92,12; 80,53; and 94,03 ppm.

KEYWORDS: Senggugu herbal product (*Rotheca serrata* (L); fingerprint; FTIR, HPTLC; metabolomics.

INTRODUCTION

The use of herbal products has become a trend in Indonesian society, this is driven by the great potential of plants in Indonesia as medicinal plants with their own characteristics. Products from the senggugu plant are available in capsule form containing roots and leaves. The quality and authenticity of the simplicia as raw materials require quality control by conducting authentication tests. Fingerprint profiles can be used as an identity in the quality control of herbal products. Through a metabolomic approach, the composition of bioactive compounds contained in cells, tissues or organisms can be detected comprehensively and quantitatively. [1]

Senggugu plants (*Rotheca serrata* (L.) is a plant belonging to the Lamiaceace family, which is widely distributed in tropocal and subtropical regions [2]. Almost all parts of the senggugu plant are used to treat diseases, especially the roots and leaves. The chemical copounds contained in senggugu include tepenoids or steroids, phenolics, alkaloids, flavonoids, saponins, and tannins. The secondary metabolites with potential antioxidant poperties are phenolic compounds and flavonoids [3]. The presence of abundant active compouds makes the senggugu plant a potential source for herbal medicine. The extracs used in these products are interesting to test, wether the content of root and leaves extracs in products produced by different manufacturers have the same characteristics when compared to the extracs simplisia of senggugu roots and leaves as the positive control [4]. Therefore, identification is needed in order to test the quality in the form of fingerprint profiles obtained using FTIR with a metabolomic approach, and antioxidant activity tests are carried out.

In research by Ayu Febrianti, et al (2021) examining the antioxidant activity of the senggugu leaves using dichloromethane solvent extracted using the maceration method. The average IC_{50} value was 90.3470 µg/mL strong antioxidant activity [5]. Another research by Nasrudin, et al (2015) examining the antioxidant activity of the senggugu root bark using ethyl acetate solvent extracted using the maceration method. The average IC_{50} value was 30.968 ± 0,686 µg/mL very strong antioxidant activity. Based on the two studies that have been carried out, it can be interpreted that senggugu has strong antioxidant activity.[6]

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This research was conducted to determine the antioxidant activity of senggugu plant with leaves and roots extracted using the Ultrasound Assisted Extraction (UAE) method using 70% ethanol as a solvent. İdentifiction was carried out using FTIR instrument the data obtained was analyzed using PCA multivariate technique, and antioxidant activity test was carried out using DPPH (*2,2-diphenyl-1-picryllhydrazil*). Analysis multivariant with SIMCA.

▪ MATERIALS AND METHODS

Materials

The materials used in this research were simplisia and herbal products powder of senggugu plant roots and leaves (Rotheca serrata (L.), 70% ethanol (pro analisis, Merck), methanol (pro analisis, Merck), DPPH, vitamin C(Farmakope standard), quercetin markers (Sigma-Aldrich).

Senggugu plant extraction

The Ultrasound-Assisted Extraction (UAE) method using 70% ethanol was used to produce senggugu leaf and root extract. 20 grams of senggugu leaves and root with 200 mL of solvent (1:10 ratio) was extracted at 25°C for 30 minutes using an ultrasonic bath with a frequency of 50 kHz. The filtrate was collected and concentrated using a rotary vacuum evaporator (pressure 175 mbar, temperature 40°C, speed 70 rpm) to obtain a crude extract of senggugu leaves and roots. The extract was then dried with freeze drying. The concentrated extract was weighed, and the yield value was calculated. The crude were analyzed by FTIR spectroscopy the data obtained was analyzed using PCA multivariate technique, and the antioxidant activity test assesed by 2,2-diphenyl-1-picrylhydrazyl (DPPH).

FTIR Analysis

Secondary metabolites fingerprinting was performed with an FTIR spectrophotometer (SHIMADZU-japan). The sample was provided by mixing the crude extract with potassium bromide and made into a pellet. FTIR spectrum was recorded by scanning the sample in the regions from 4000 to 400 cm-1 with a resolution od 4 cm-1. The spectra were saved in IRSolution then converted to Microsoft excel.

Data analysis

The multivaiate data analyses, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA), were performed with SIMCA which represented the FTIR measurement results and the ordinate data.

DPPH radical scavening activity

The DPPH stock solution was prepared by weighing 4 mg DPPH then dissolved in 25 mL methanol, and the stock solution of the extract were made at a concentration of 1000 µg/mL. Ascorbic acid and methanol were used as a positive and negative control, repectively. DPPH solution was dispensed into a test microplate reader of the tested extracts with the concentrations of 250, 375, 500, 625, 750 µg/mL were immediately introduced. The reaction mixtured was incubated at ambient temperature for 30 minutes inthe dark, and then the absorbance was measured at 517 nm. DPPH radical scavening activity of the extracts was expressed as IC_{50}.

▪ RESULTS AND DISCUSSION

Characteristics of ethanol extract

The sample used in this study was fresh Senggugu plant leaves and roots obtained from Balai Penelitian Tanaman Obat dan Aromatik (BALITRO), Bogor, which has previously been determined at the Biodata Collection Room, Department of biology, Univesity of Indonesia, which showed that the sample of this study was *Rotheca serrata*. Senggugu plant leaves simplicia is dried simplicia, fresh green, and has a characteristic aroma, and senggugu roots simplicia is dried simplicia, fresh brown, and has a characteristic aroma.

Table 1. Extract characteristics.

No.	Parameter	Senggugu leaves ethanol extract	Senggugu roots ethanol extract	Herbal product of leaves ethanol extract	Herbal product of roots ethanol extract
1	Colour	Green	Brown	Green	Brown
2	Aroma	Aromatic	Aromatic	Aromatic	Aromatic
3	Taste	-	-	-	-

FTIR analysis

Evaluation of metabolite composition extracted from different parts of Rotheca serrata, plant (leaves and roots) was carried out using FTIR spectroscopy in the range 4000 to 400 cm-1 with the main purpose to the overall functional groups of metabolites found in the samples [7]. FTIR spectra for each sample were recorded andf from the average FTIR absorption spectra of all extracts (Figure 1), se could see a similar pattern among the four different extracts.

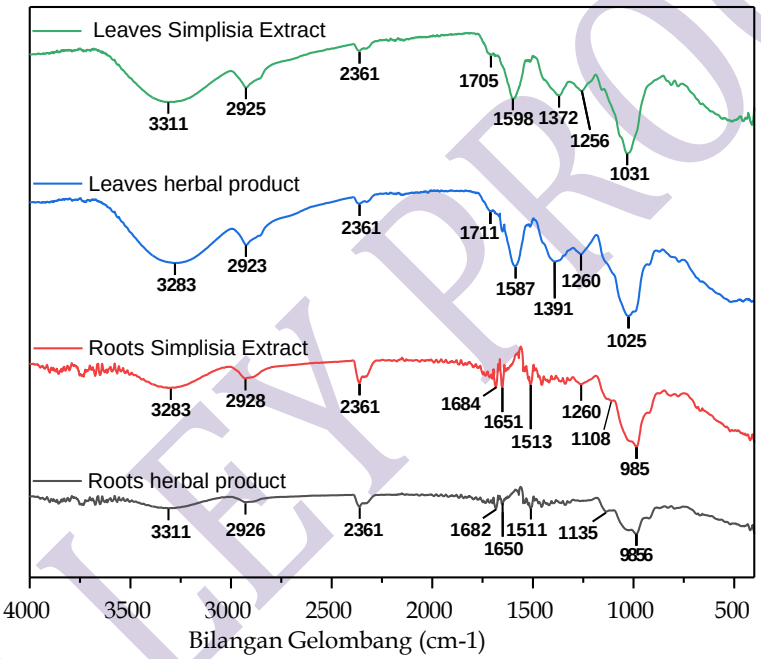


Figure 1. FTIR spectra of leaves simplicia, roots simplicia, senggugu herbal product.

Based on FTIR analysis of leaves root, and herbal product of herbal senggugu, several distinctive absorbance peaks were obtained. İn the four extracts, there is an O-H froup in the range of 3590-3650 cm-1, then there is a C-H group in the range of 2850-2970 cm-1, there is a C=O group in the range of 1690-1760 cm-1, there is a C=C alkene group in the wave number range of 1610-1680 cm-1, there is an aromatic C=C group in the wave number range of 1500-1600 cm-1, there is a C-H group at wave nuber 1340-1470 cm-1, and there is a C=H group in the range of 676-995 cm-1 [8]. The same functional groups in leaves and roots extract indicate the possibility of the same compound content.

Data Analysis

Principal component analysis (PCA)

A series of multivariate analyses were applied to clustes the leaves and roots extract based on the FTIR spectra. The R solution version 4-3.0 was applied to asses the overall variation of the metabolite levels. This methode is useful to explain the data set variance and grouping the samples based on their similarity. [9]

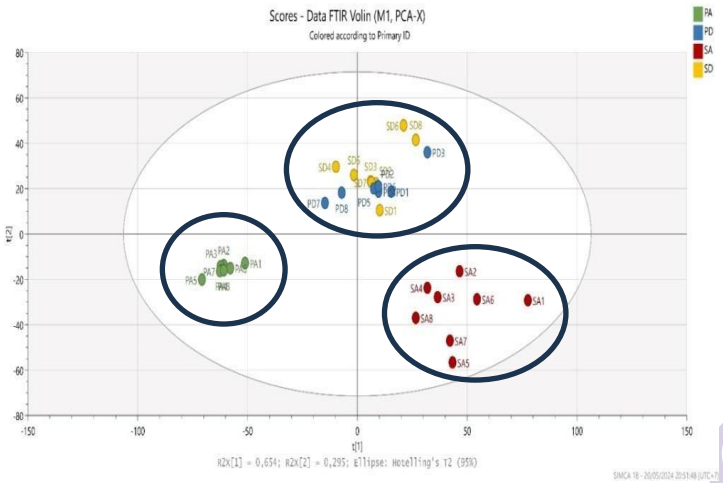


Figure 2. Score plots shows clustering of the samples

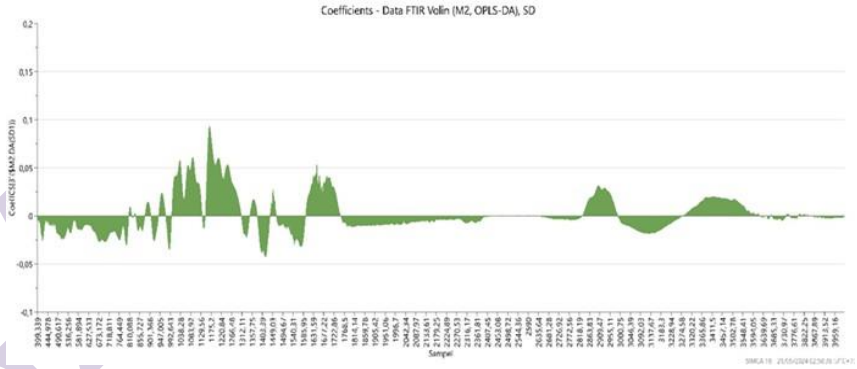
SD	= Leave simplicia	PD	= Leaves product
SA	= Root simplicia	PA	= Root product

Based on this figure, it can be seen that the leaf simplicia group and leaf product are located in the same quadrant, indicating that leaf products taken from the online shop are appropriate of correctly derived from leaf simplicia. Whereas in root simplicia and root products, it can be seen that the grouping is located in the different quadrants with the dictance of the score plots far apart so that it can be said that what is written on the etiquette of root products does not match the results

Separate patterns in root product and root simplicia can occur due to there are other compositions in the product that are not included in the etiquette.

Orthogonal Partial Least Squares – Discriminant Analysis (OPLS-DA)

The functional group absorption data from the sample was analyzed by PCA, further analysis was carried out using OPLS-DA, which is useful for knowing the metabolite functional groups in leaf, root, and herbal products of senggugu. The result of OPLS-DA is a coefficient plot [10] (Figure 4).



(a)

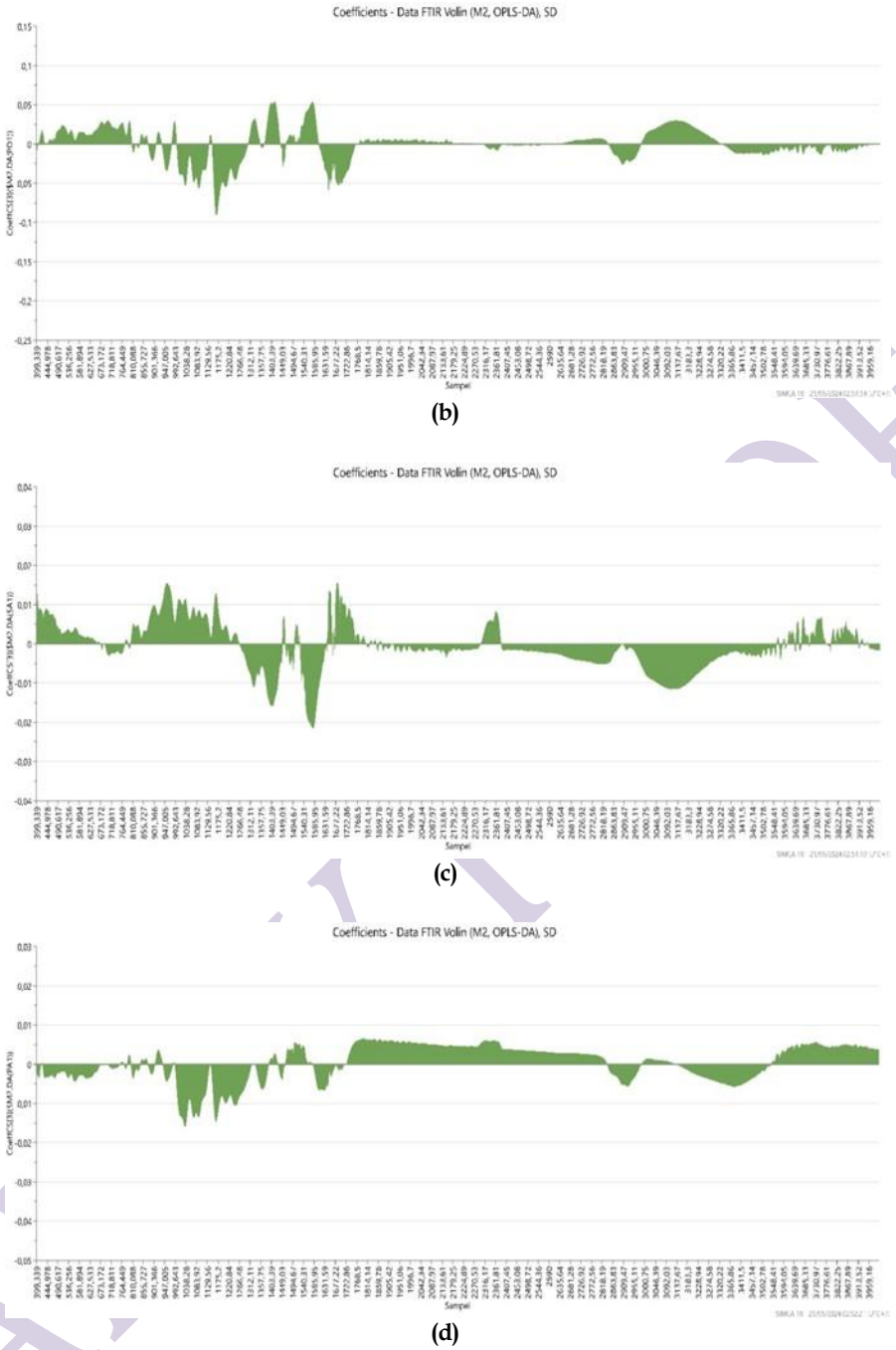


Figure 4. This is a figure of coefficient plot of OPLS-DA such as; **(a)** Coefficient plot OPLS-DA of leaves simplicia extract; **(b)** Coefficient plot OPLS-DA of leaves product extract; **(c)** Coefficient plot OPLS-DA of leaves simplicia extract; **(d)** Coefficient plot OPLD-DA of roots product extract.

This table shows that the functional groups are simillar, so its is likely that the compounds contained in the roots, leaves, and herbal products of senggugu are similar, the possibly of the similarities compound describes the etiquette listed in the product accordingly.

Table 2. Similarities types of bond of the four extracts.

Extract	Types of bond	Similarities types of bond
Leaves simplicia	C-H, C=H, C=C, C-O, C=O	C=H, O-H, C=C
Roots simplicia	C=H, O-H, C=C, C-O, C=O	
Leaves product	C-H, C=H, O-H, C=C	
Roots product	C-H, C=H, O-H, C=O	

DPPH Scavening activity

Vitamin C antioxidant activity test

The positive control used in this study was vitamin C. Vitamin C is a water-soluble antioxidant. The use of positive control in antioxidant activity testing is to determine the antioxidant potential in the leaves, roots, and herbal product of senggugu[11]. Vitamin C was made with five concentration of 1, 2, 3, 4, 5 ppm incubated for 30 minutes in the dark, and then the absorbance was measured 517 nm [12]. Based on the table above, the measurement of antioxidant activity of vitamin C as a positive control obtained IC50 value of 3.20 ppm, it is included in the category of very strong activity [13].

Leaves, roots, senggugu herbal product antioxidant activity test

Antioxidant activity testing on leaves, roots, and herbal product were made with concentration of 50, 75, 100, 125, 150 ppm incubated for 30 minutes in the dark, and then the absorbance was measured 517 nm[12]. Based on the antioxidant activity test results, the antioxidant activity of leaf, roots, and senggugu herbal product obtained IC50 values of 80.80; 92.12; 80.53; 94,08 ppm, it is included in the category of fairly strong antioxidant activity [13]. Then in the results of this study it can also be seen that the antioxidant activity of simplicia leaf extract and leave herbal product is stronger than the root, it is possible because senggugu leaves have a higher content of bioactive compounds and are more effective in producing antioxidant activity than senggugu roots.

Table 2. The results of antioxidant activity testing.

No.	Extract	IC_{50} (ppm)
1	Vitamin C	3.42 ± 0.05
2	Leaves simplicia	80.08 ± 6.14
3	Roots simplicia	92.12 ± 1.09
4	Leaves product	80.53 ± 2.58
5	Roots product	94.03 ± 2.02

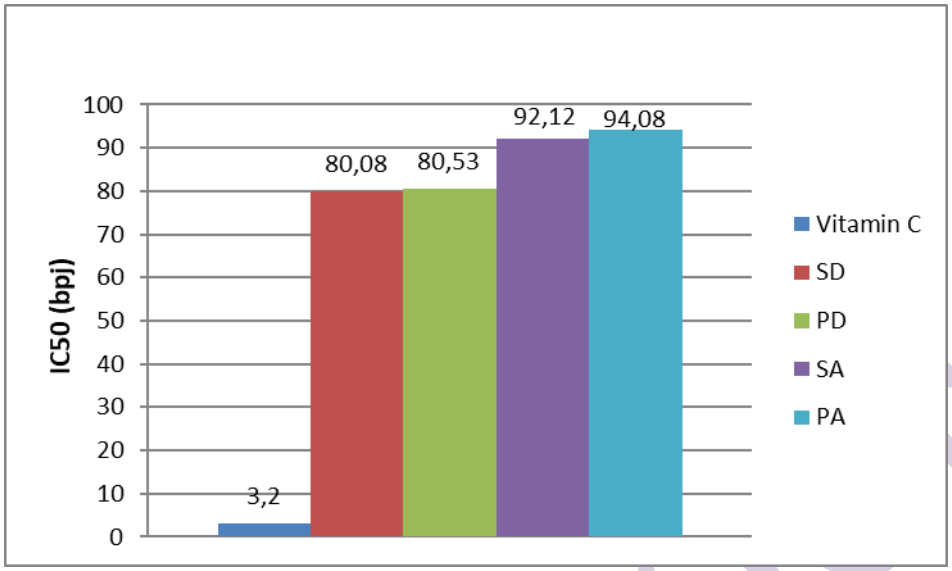


Figure 5. The results of antioxidant activity testing.

- ## CONCLUSION

İn this research, the results of analysis with PCA it can be concluded that hebal products marked or claimed as senggugu leaves are appropriate, while for products claimed as senggugu roots are inappropriate. Based on the results of the antioxidant activity test, leaf, root, senggugu herbal product have a fairly stong stong antioxidant activity.

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