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Research Article

Fingerprint and Multivariate Analysis of *Apium Graveolens* L. From Different Geographic with Spectroscopic ATR-FTIR

Wendy Nora Martian[1], Dini Kesuma[2,*], Rima Via Angraini[1]

ABSTRACT

Background: *Apium graveolens* L. or widely known as celery is a plant that comes from the Apiaceae family. *Apium graveolens* L. is found in the Indonesian Herbal Pharmacopoeia as a nutritious plant. Plants that spread throughout Indonesia, both highlands and lowlands, contain 3-n-butylphthalide, D-limonene, Linalool, Luteolin, Apigenin and Apiin compounds. Differences in geographical location of growth resulted in differences in the content and concentration of *Apium graveolens* L. **Aim and Objectives:** This study was conducted to determine the differences of *Apium graveolens* L. based on its distribution in Indonesia. Indonesia does not yet have a standard for *Apium graveolens* L. **Material and Methods:** this research uses *Apium graveolens* provided by the Center for Research and Development of Traditional Medicinal and Medicinal Plants (B2P2TOOT) Tawangmangu. 10 samples from different regions in Indonesia will be read using the ATR-FTIR instrument in the finger print area as well as PCA (principal principal) multivariate analysis. component analysis) and HCA (Hierarchical Clustering Analysis). **Results:** The PCA results show the highest number of variants on PC 1 91,98% and PC 2 variants at 4,71%. In the quadrant 4 score plot, it was found that there was a closeness between the standard sample and the S2 sample (Materia Medika Batu). **Conclusion:** The result of this study is that there is a mutually supportive relationship from spectrum readings, sample location (temperature, soil moisture content, height (m a.s.l) with multivariate analysis (PCA, HCA results) when compared to the geographic location of each sample.
Key words: *Apium graveolens* L, ATR-FTIR fingerprint area, Multivariate analysis.

Wendy Nora Martian[1], Dini Kesuma[2,*], Rima Via Angraini[1]

[1]Master of Industrial Pharmacy, Faculty of Pharmacy, University of Surabaya, Surabaya, East Java 60293, INDONESIA.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Surabaya, Surabaya, East Java 60293, INDONESIA.

Correspondence

Dini Kesuma

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Surabaya, Surabaya, East Java 60293, INDONESIA.

E-mail: dinikesuma75@gmail.com

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INTRODUCTION

Indonesia is identical with biological wealth which causes many plants to be used as alternative medicine, as well as making herbal medicine products widely circulated. Data from BPOM (food and drug regulatory agency) from 2017-2022, herbal medicine has a higher number than synthetic drugs.[1,2] The data is directly proportional to consumer demand. All herbal medicines are summarized in the Indonesian herbal pharmacopoeia. Starting from organoleptic to testing, everything is written in the Indonesian herbal pharmacopoeia.[3] This is done as a regulation and ensures the safety level of herbal medicine. *Apium graveolens* L. is one of the plants contained in the Indonesian Herbal Pharmacopoeia. This plant has properties as a blood pressure-lowering (anti-hypertensive) with several components in it, including 3-n-butylphthalide, D-limonene, Linalool, Luteolin, Apigenin and Apiin compoundstas.[4-6] Indonesian people very often use this plant as a vegetable in cooking and in the form of herbal medicine. In the Indonesian Herbal Pharmacopoeia mentioned *Apium graveolens* L. has flavonoids as much as 1.98% in the form of Apigenin. Differences in the chemical content of herbal medicines are influenced by several factors including the location of cultivation, harvest time, and differences in varieties of raw materials.[7] BPOM (regulatory agency in Indonesia) has issued many licenses for *Apium graveolens* L products, standardization is needed from the selection of raw materials to finished products.[3] This standardization requires QC (Quality Control) to improve the quality of herbal medicine products. Starting from the evaluation of macroscopic and microscopic observations on specimens as well as chemical, biological and physicochemical analysis.[8] From the row material, assessment of stability, efficacy, safety of the finished product, and the provision of product information to ensure proper use by consumers. The need for an instrument that provides fast, sensitive and accurate results is the ATR-FTIR spectroscopic.[9-11] Attenuated Total Reflectance - Fourier Transform Infrared (ATR - FTIR) is an instrument that helps to read the spectrum, and functional groups of a compound. FTIR spectrum contains multivariate data in the form of absorbance in the range of wave numbers 4000 - 650 cm^{-1}.[12] Each sample has a different spectrum identity, the spectrum has dominant peaks that show the differences in each sample.[8,12,13] ATR-FTIR spectroscopy method combined with multivariate analysis is highly recommended for qualitative or quantitative QC tools for individual or mixed herbal medicine.[14-16] Multivariate analysis used is PCA (Principal component analysis) as could be used as classification and HCA (Herarchical Cluster Analysis) relating the variables on a object to a discrete value of property one of wishes to predict, grouping, the herbal component from different origin.[17,18]

MATERIALS AND METHODS

Material

Standard of *Apium graveolens* L. came from the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Tawangmangu and UPT Laboratory of Herbal

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Figure 1: Fresh *Apium graveolens* L.

Figure 2: Dry *Apium graveolens* L.

Figure 3: Powdered *Apium graveolens* L.

Materia Medika Batu. *Apium graveolens* L. samples were selected from 10 regions in Indonesia that have been determined.

Tools The tools used in this study include a blender, a sieve with a mesh size of 80, as well as checking the moisture content with a Moisturizer Analysis Hb43 Mettler-Toledo GmbH tool, using an Olympus Type x 23 microscope, ATR-FTIR (Attenuated Total Reflectance Fourier) spectrophotometry Transform Infrared) Agillent carry 630, Origin 2018 multivariate analysis software.

Methods sample preparation

Standard sample B2P2TOOT Tawangmangu was dried in an oven at a temperature of +/- 420C, while the other plant samples were air-dried in a room protected from direct sunlight. All plant samples were mashed with a blender and sieved with a mesh size of 80, and the moisture content was checked using Moisture Analysis Hb43 Mettler-Toledo GmbH.

FTIR absorbance spectrum

In this study, a qualitative analysis will be carried out, selecting samples of celery plants taken from several islands in Indonesia with the aim of being able to describe the condition of celery plants scattered in Indonesia. The reading of all samples, both celery and capsules, used the Agillent carry 630 ATR-FTIR (Attenuated Total Reflectance Fourier Transform Infrared) spectrophotometric instrument using the Quality Assessment method in the Lab. Research Faculty of Pharmacy, University of Surabaya, Surabaya, East Java. Readings of all samples, both plant and commercial capsules, produced absorbance spectra, readings were carried out at a wavelength of 4000 cm^{-1} – 650 cm^{-1} using a resolution of 4cm^{-1} which is commonly used for solid samples and 64 scans.[11] Tested on standard samples in accordance with the requirements in FHI (Indonesian Herbal Pharmacopoeia). Followed by qualification using polystyrene to ensure that the absorbance readings must be carried out before readings with the ATR-FTIR instrument. After that, the standard sample of celery was read 3 times for 3 days to see the results of absorption in the hope of having the same pattern. All samples of celery and capsules were read with the ATR-FTIR instrument to get the wave number and absorbance results. Selected finger print area at wave number 1500cm^{-1} - 650 cm^{-1} to get the pattern for each sample.[19,20] Absorbance results are continued for the calculation of %cv (coefficient variation) and SD (standard deviation). The requirements for the sample to be able to do multivariate test are to have %cv.

Multivariate analysis

The multivariate method (MVA) is a statistical technique that allows simultaneous analysis of several variables. This helps to study relationships on a large scale as well as complex data sets. This study uses Origin 2018 Software as a tool for multivariate analysis. The multivariate analysis used is Principal Component Analysis (PCA) to explain the variance-covariance structure of a set of variables through linear combinations as well as a dimension reduction technique and Hierarchical Clustering Analysis (HCA) to classify samples in certain clusters so that they can see the closeness of each sample.

RESULT

Physical characteristics of *Apium graveolens* L. This study used 10 samples of *Apium graveolens* spread across Indonesia. Initial observations in accordance with the provisions in the Indonesian

Figure 4: Physical observations.

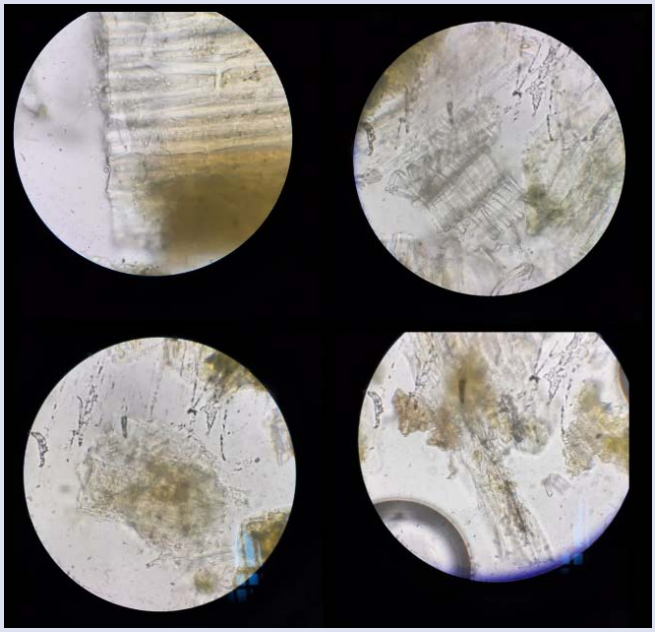

Figure 5: Microscopic observations.

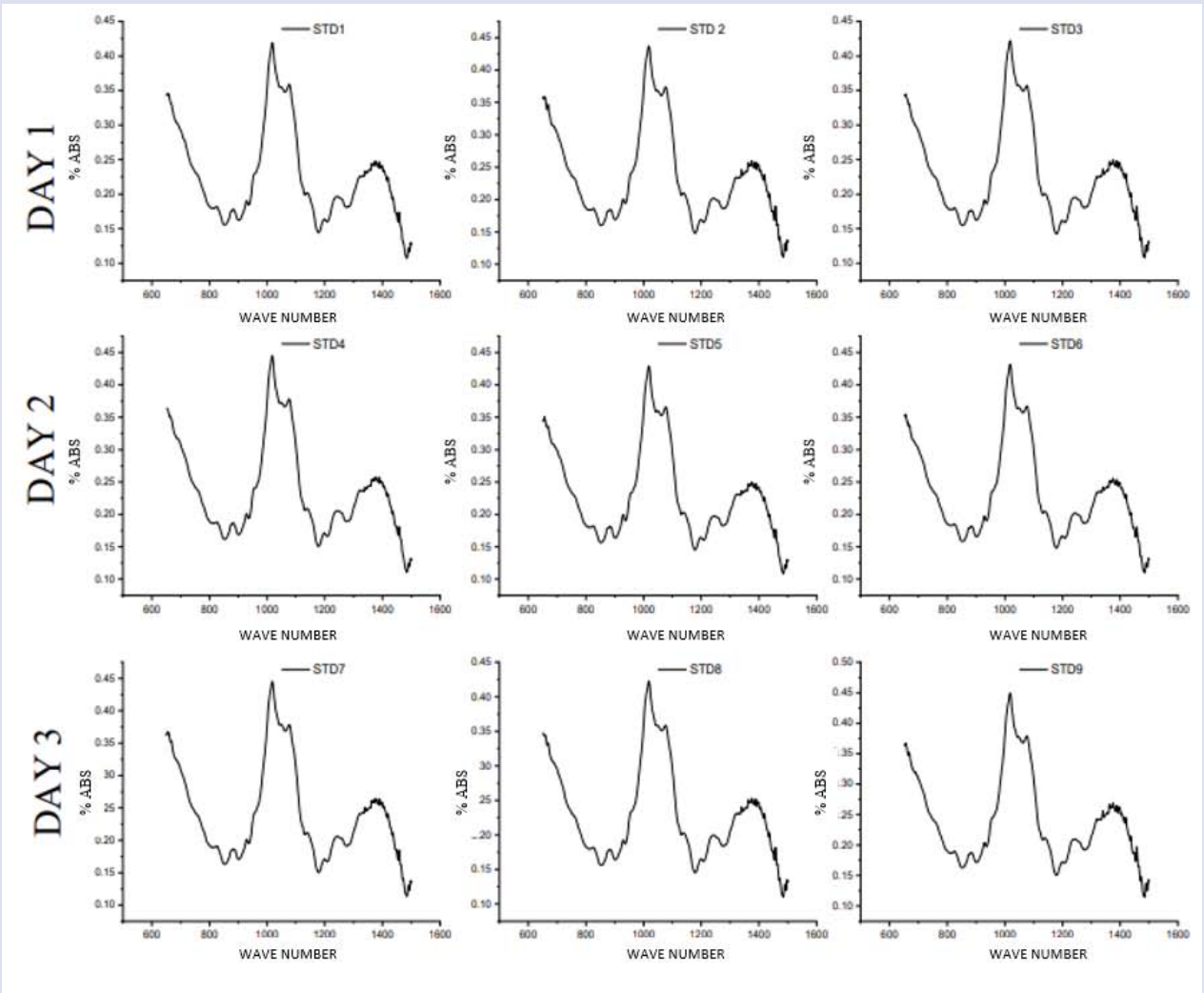

Figure 6: Absorbance spectrum (Finger Print) - standard replication in 3 days.

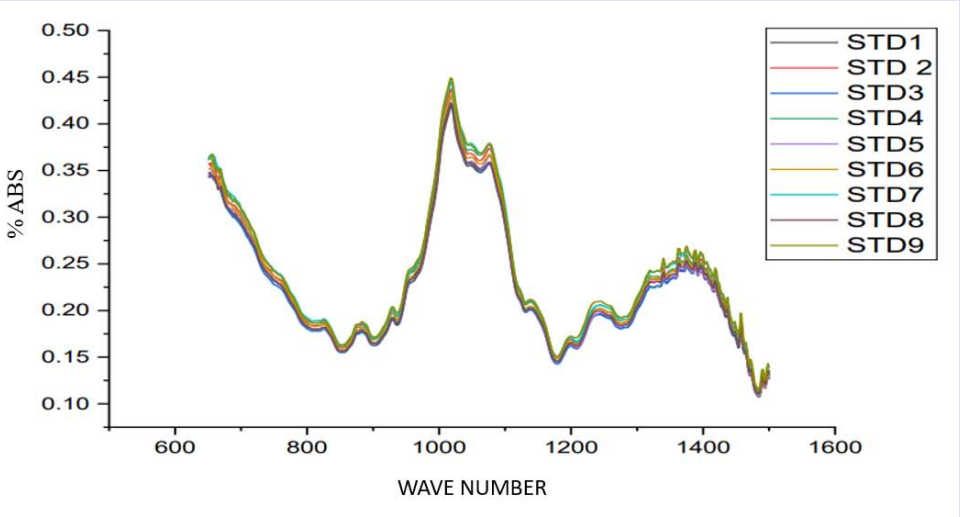

Figure 7: Absorbance spectrum standard overlay.

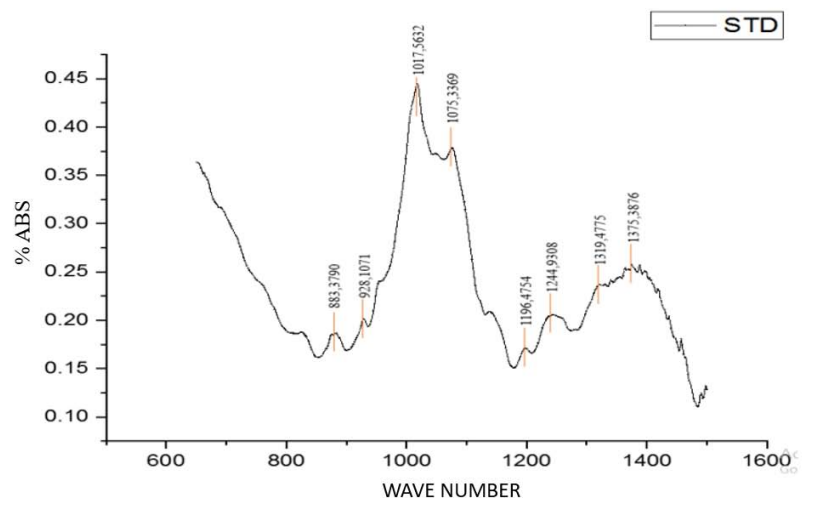

Figure 8: Absorbance spectrum standard.

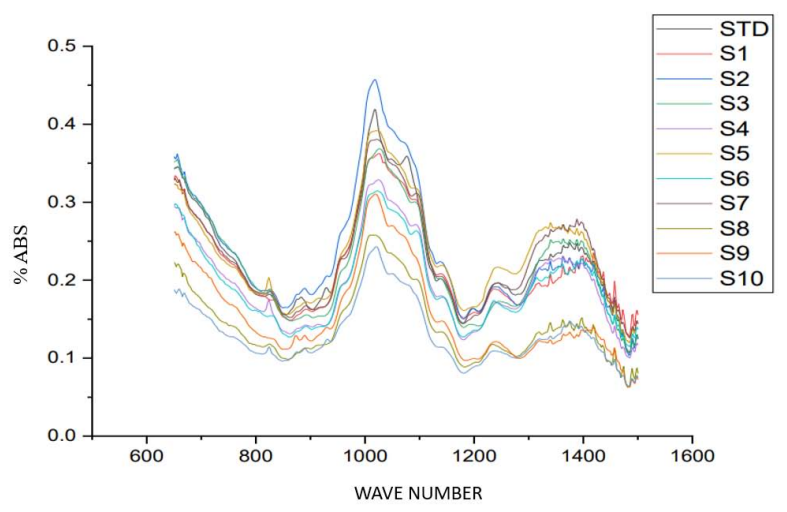

Figure 9: Absorbance spectrum standard and 10 sample overlay.

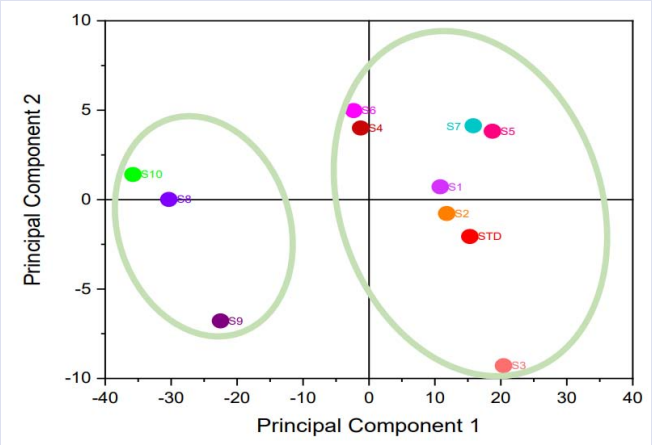

Figure 10: PCA Plot Score (*Apium graveolens* L.).

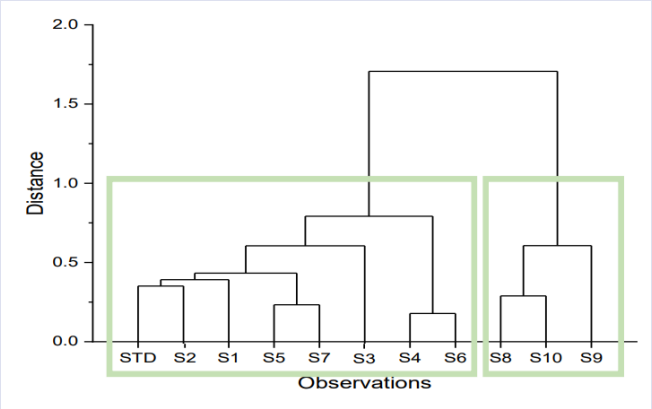

Figure 11: HCA (*Apium graveolens* L.).

Table 1: Geographical location and climatic conditions of *Apium graveolens* L.

Code	Location	Geography position	Heigh	Rain fall	State of Water in Soil	Temperature (°C)
S1	Sumatera, Lampung Sedayu, semaka, Tanggamus, No. Determination : 074/497/102.7-A/2021	5°31'55"S 104°27'52"E	513 m a.s.l	200 - 300 mm	80 - 100%	27.563
S2	Jawa, Jawa Timur UPT lab. Materia Medika, Jl. Lahor no.87, Batu, No. Determination : 074/466/102.7-A/2021	7°55'04"S 112°31'10"E	895 m a.s.l	100 - 150 mm	80 - 100%	22.016
S3	Jawa, Jawa Timur Kebun Inspirasi Sultan Hidroponik, Sumber Pinang, Kecamatan Silo, Jember, No. Determination: 074/524/102.7-A/2021	8°14'42"S 113°50'38"E	241 m a.s.l	100 - 150 mm	-	27.44
S4	Kalimantan, Kalimantan Selatan Tariwin, Batu Mandi, Balangan, No. Determination : 074/520/102.7-A/2021	2°28'04"S 115°31'43"E	50 m a.s.l	200 - 300 mm	80 - 100%	26.75
S5	Sulawesi, Sulawesi Selatan Jalan Poros Loka, Bonto Marranu, Uluere, kab. Bantaeng, No. Determination : 074/521/102.7-A/2021	5°26'53"S 119°54'27"E	1.130 m a.s.l	100 - 150 mm	80 - 100%	26.77
S6	Bali, The Blooms garden, Cadikuning, Baturiti, Tabanan No. Determination : 074/522/102.7-A/2021	8°18'12"S 115°09'38"E	1.224 m a.s.l	100 - 150 mm	60 - 80%	27.86
S7	Lombok, NTB Mantang, Batukliang, lombok tengah No. Determination : 074/523/102.7-A/2021	8°36'37"S 116°19'21"E	366 m a.s.l	100 – 150 mm	80 - 100%	27.36
S8	Surabaya, Jawa timur Kampung Simolorejo, kel. Simomulyo, kec. Sukomanunggal, Surabaya No. Determination : 074/663/102.20-A/2022	7°15'24"S 112°42'22"E	4 m a.s.l	20 - 50 mm	20 – 40%	31
S9	Sampang, Madura Jl. Purba Gg: 2 No:11, Kec.Pamekasan, Kab. Pamekasan, Madura No. Determination : 074/664/102.20-A/2022	7°09'52"S 113°28'58"E	14 m a.s.l	20 - 50 mm	0 - 20%	32
S10	Nabire, Papua Tengah Asrama Denzipur 12, Jl. R.E. Martadinata, Sanoba, Distrik Nabire, Kabupaten Nabire, Papua tengah	3°20'22"S 135°32'26"E	24 m a.s.l	150 - 200 mm	60 - 80%	32
STD	Jawa, Jawa Tengah B2P2TOOT Jl. Raya lawu, tawangmangu, karanganyar No. Determinasi : YK.01.03/2/1175/2021	7°37'03"S 111°03'06"E	1.200 m a.s.l	100 - 150 mm	80 - 100%	22.81

Table 2: Moisture content Apium graveolens L.

No.	Sample	%MC
1	S1	2.7
2	S2	3.0
3	S3	2.7
4	S4	2.8
5	S5	2.7
6	S6	2.5
7	S7	2.5
8	S8	2.8
9	S9	2.6
10	S10	2.8
11	STD	2.8

Herbal Pharmacopoeia include physical observations, microscopic tests and testing of water content. From the shape of a single leaf, thin, brittle, jagged leaf, pinnate leaf bone, dark green in Figure 4 in accordance with the provisions in FHI. In addition to looking at the physical form of *Apium graveolens*, it was found macroscopically using an Olympus Type X 23 microscope with a magnification of 40x/0.6 in the form of petiole epidermis, upper epidermis with stomata, transport bundle with ladder type thickening and idioblasts (lower epidermis oil cells with stomata) in accordance with the provisions FHI in Figure 5. This shows the conformity of the sample with the provisions of FHI.

Moisture content (MC) can be measured with a %MC less than 10% as shown in Table 2. Sample S2 results. The reading is carried out at a wavelength of 1500 – 650 cm^{-1} or what is often called the fingerprint area due to the large number of dominant peaks. From samples. The reading of the absorbance spectrum of the sample is carried out based on Lambert-beer's law which states that there is a linear relationship between absorbance and concentration.[14] From Figure 8. Shows (Materia medika Batu) has a high MC compared to the other samples, but all samples are included in the FHI provisions which state that the water content for celery plants is less than 10%.[3]

ATR-FTIR absorbance spectrum

From *Apium graveolens* L. The reading of a standard sample of *Apium graveolens* (Tawangmangu) using the ATR-FTIR Agilent 630 Carry is shown in Figure 6. performed 3 times a day and for 3 consecutive days, in order to provide accurate, the dominant peak of *Apium graveolens* at wave numbers 883.3790 cm^{-1} and 928.1071 cm-1 which indicates a strong absorption of the functional group = C-H, at wave numbers 1017.3369 cm^{-1}, 1075.3369 cm $^{-1}$, 1196.4754 cm^{-1}, 1244.9308 cm^{-1}, 1319.4775 cm^{-1} and 1375.3876 cm^{-1} which indicate a strong absorption of the C – O functional group.

The results of standard readings with other samples were made in overlay view, to find out the similarities or differences. (Figure 9) shows the similarity between the standard and the S2 sample (Materia medika).

PCA multivariate analysis (PCA)

The first multivariate analysis is principal component analysis (PCA) which reduces all data without eliminating existing data but is made in more representative results. In Figure 7. are the results of 10 samples and 1 standard sample? The PCA results show the highest number of variants on PC 1 91,98% and PC 2 variants at 4,71%. In the quadrant 4 score plot, it was found that there was a closeness between the standard sample and the S2 sample (Materia, Medika, Batu). It can be interpreted that the standard and S2 have the same chemical properties, as well as samples S8 and S9, S6 and S4, S7 and S5. This is supported by Table 1. Regarding the temperature and altitude of the location.

HCA multivariate analysis (HCA)

(HCA) is a continuation of PCA, HCA is a dendogram that describes groups in research, as well as to compare PCA results. In Figure 8 there are 2 large clusters namely STD, S2, S1, S5, S7, S3, S4, S6 and S8, S10, S9. HCA gives the same results as PCA, which means that HCA and PCA are chemometric analysis tests that support each other in differentiating samples qualitatively.

DISCUSSION

The results of this study are that the ATR-FTIR instrument can provide precise qualitative testing to make it easier for researchers to differentiate each sample by reading the absorbance spectrum. The resulting spectrum results give dominant peaks which are signs on the test sample. each peak shows a wave number which can determine its functional group.

This replication is very important to provide accurate results in research. Indonesia does not have herbal plant standards, but the B2P2TOOT Tawangmangu government agency has celery plants (*Apium graveolens* L) and issued a letter of determination. *Apium graveolens* L from B2P2TOOT Tawangmangu is used as a standard, but replication testing is needed to ensure the correctness of the ATR-FTIR standard and instrument. On the first day, the standard *Apium graveolens* L was read 3 times, followed by the second and third days. the results of this replication reading are overlaid so that the similarities of each spectrum can be seen, if there are differences it is necessary to re-test to ensure the accuracy of the instrument.

replication of all samples was also read, after all samples were confirmed to be the same as the replication, then proceed to data analysis with Origin 2018 software. PCA and HCA multivarite data analysis was carried out. for qualitative testing, PCA and HCA analysis is enough, but in the future PLS-DA and SIMCA tests are needed.

In the PCA test, it was found that the standard score plot display has the same quadrant as the S2 sample which proves that the S2 sample from the government agency Materia Medika Batu has the same quality as the standard. The PCA results can be related to the geographic location in table 1. The PCA plot score results will be processed by HCA analysis. HCA makes it easier for researchers to differentiate and classify.

CONCLUSION

The use of the ATR-FTIR instrument with a combination of multivariate analysis gives good results for qualitative research. It is necessary to optimize the standard (Tawangmangu) to ensure the accuracy of the instrument and the authenticity of the standard, bearing in mind that Indonesia does not issue herbal medicine plant standards. Spectrum readings based on the fingerprint area (1500 - 650cm^{-1}) can determine the functional group of each peak. PCA results, HCA has a mutually supportive relationship to the geographic location of each sample related to temperature, rainfall, High, water content in the soil. Provide initial screening results to determine the quality and authenticity of *Apium graveolens* L.

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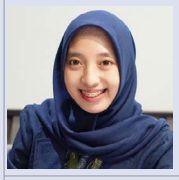
ABOUT AUTHORS

	DR. DINI KESUMA Is an Associate Professor in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Surabaya, Indonesia. She has experience in medicinal chemistry and analytical chemistry. The main focus of her research and publication is cancer drug development in silico and in vitro.
	WENDY NORA MARTIAN is currently a Master of Industrial Pharmacy student, Faculty of Pharmacy, University of Surabaya, Indonesia.
	RIMA VIA ANGRAINI is currently a Master of Industrial Pharmacy student, Faculty of Pharmacy, University of Surabaya, Indonesia.

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