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Penentuan Kadar Sitronelal Total dan Pengembangan Model Klasifikasi FTIR-Kemometrik Minyak Jowitt *Cymbopogon winterianus* dari Berbagai Ketinggian Daerah Tanam

Determination of Total Citronellal Levels and Development of FTIR-Chemometric Classification Model of Cymbopogon winterianus Jowitt Oil from Different Altitudes of Planting Areas

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ABSTRACT

Cymbopogon winterianus Jowitt oil (CWJ oil) is a vegetable oil with very wide uses in food sector, cosmetics, medicines and chemical industry. Citronellal compound is one of the quality requirements that determine the quality of CWJ oil. This study aims to developed a FTIR-chemometric classification model and to determine the total citronellal content of C. winterianus Jowitt oil grown in low and medium lands. The sample used was citronellal oil from Jember and Banyuwangi regions. The cleaned CWJ herbal samples were withered, chopped and distilled to produce CWJ oil. CWJ oil was scanned using FTIR spectroscopy and then the FTIR spectras were analyzed using LDA, SVM and SIMCA to form a CWJ oil classification model. The selected classification model was then validated using leave one out cross validation and external validation. The LDA classification model showed the best results with 100% accuracy of model recognition and validation. The results of the determination of total citronellal levels of CWJ oil using the hydroxylamine method showed that samples of CWJ oil from Banyuwangi had higher levels of total citronellal than samples from Jember. The average levels of total citronellal CWJ oil from Banyuwangi and Jember regions were 32.84%, and 26.06%, respectively . This method can be applied to sample discrimination in order to guarantee the quality of raw materials, especially CWJ.

INTRODUCTION

Indonesia is one of the largest essential oil producing countries in the world. There are twenty types of essential oil-producing plants that have high economic value, including Cymbopogon oil. *C. winterianus* Jowitt oil (CWJ oil) is a vegetable oil with very wide uses in the food sector, cosmetics, medicines, and chemical industry. One of the chemicals compounds of Cymbopogon oil is citronellal, it is widely used as a raw material for mosquito repellent gels and lotions, soaps, disinfectants, and vegetable pesticides. It also has bactericidal activity on several bacterial species (Timung 2016). Citronellal also has anti-inflammatory activity and can protect cells from oxidative reactions (Melo 2011).

The phytochemical content of plants is influenced by various factors, including environmental factors. Differences in geographic location can produce different compositions of secondary metabolites in plants (Kardono 2003). In essential oil-producing plants, growing environmental temperature, irradiation intensity, and altitude can affect the composition of essential oils produced (Murningsih 2009). Essential oils produced by citronellal plants, in addition to being influenced by treatment Post-harvest, several growth environmental factors also affect the yield, composition, and quality of citronellal oil (Blank 2007).

Determination of the classification model of sample can be formed using the FTIR (Fourier Transformed Infrared) spectroscopy method. FTIR spectroscopy is an effective analytical technique because it is simple and fast to process, non-destructive to samples, and has high sensitivity, data resolution, and data acquisition speed. FTIR is commonly used with chemometric statistical analysis methods to facilitate the interpretation of data as needed (Gad 2012). The combination of FTIR and chemometrics is proven to be able to classify and identify medicinal plants (Cao 2017) and red ginger (Purwakusumah 2014)

This study aims to develop a chemometric-FTIR classification model and to determine the total citronellal content of *C. winterianus* Jowitt oil from different altitudes.

METHODOLOGY

The materials used in this study were samples of citronellal (*C. winterianus* Jowitt) variety G2 taken from Jember and Banyuwangi districts and were determine by Materia Medika, Purwodadi, East Java, Indonesia. The reagent used were 97% ethanol, distilled water, hydrochloric acid (HCl) 37% (Sigma-Aldrich, St. Louis, Amerika), potassium hydroxide (KOH), and bromphenol blue indicator (Merck, Darmstadt, Germany).

The tools used in this research were ALPHA II Compact FTIR Spectrometer (Bruker, Billerica, Massachusetts, Amerika), OPUS software, The Unscrambler X ver. 10.2 (Camo, Oslo, Norwegia) software, SPSS 20.0 (IBM, Armonk, New York, Amerika) software, system distillation tool water, digital analytical balance, and ultrasonicator, and glassware.

The cleaned CWJ herbal samples were withered, chopped and distilled to produce CWJ oil. 2.5 kg of CWJ herbal enter a distillation apparatus and add 10-11 liters of water until the citronella herb is submerged. Heat the distiller to 100°C. The steam from boiling the citronella herb in the kettle will flow through the condenser pipe. In the liquid storage tube, the water layer at the bottom can be removed periodically during the distillation process by opening the drain valve so that only citronella oil is accommodated. The total time used during the distillation process of citronella oil was 4 hours. CWJ oil was scanned using FTIR spectroscopy at 4000–650 cm^{-1} and then the FTIR spectras were analyzed using linear discriminant analysis (LDA), support vector machine (SVM) and soft independent modelling of class analogy (SIMCA) to form a CWJ oil classification model. The classification model was carried out by taking FTIR spectras data from CWJ oil from the training set sample, then classifying the model using chemometric techniques. The FTIR spectrum of the training set samples at all wavelengths was used to form the LDA, SVM and SIMCA models using 2 categories, namely "Jember" and "Banyuwangi". The labeling is intended to categorize the sample based on its area, "Jember" with a low altitude category and "Banyuwangi" with a medium altitude category. The selected classification model was then validated using leave one out cross validation (LOOCV) as internal validation and external validation. LOOCV was validation that performed by leaved one data and the remaining data used to form new model. External validation used independent sample to evaluated the accuracy of prediction of the classification model (Wulandari 2022)

Determination of citronellal content using hydroxylamine method. A sample of 0.8 grams of oil was weighed then added 20 mL of 5% ethanolic hydroxylamine solution, 10 mL of 0.5 N KOH solution, and 5 drops of bromphenol blue. This solution was then titrated using 0.5 N HCl solution until the blue color of the solution changed to yellowish green. This titration was replicated three times and also carried out on blanks solution, which was titrant mixtures without containing samples.

Tabel 1. Result of Citronellal Content and Yield Of CWJ Oils

Code (Location of sample)		Altitude (amsl)*	Yield (%w/w)	Citronellal level (%±SD, n=3)*
Jember region	D (Demangan)	22	0.48	26.97 ± 0.242
	CS (cangkring siang)	198	0.52	27.91 ± 0.165
	M1 (Mencek 1)	83	0.44	23.33 ± 0.242
	M2 (Mencek 2)	111	0.62	28.05 ± 0.035
	B (Baratan)	133	0.46	24.06 ± 0.139
	U (Undangan)	300	0.48	42.04 ± 0.163
Banyuwangi region	S (Sukabumi)	345	0.44	40.89 ± 0.011
	T (Tegalpakis)	410	0.50	25.81 ± 0.253
	GN (Glen Nevis)	458	0.44	24.61 ± 0.113
	CL (Curah Leduk)	593	0.41	30.86 ± 0.002

*amsl : above mean sea level
*SD : standard deviation

RESULT AND DISCUSSION

In this study, the yield of oil produced was 0.41 up to 0.62 %w/w. The yield is the percentage of results obtained from the weight of *Cymbopogon* herbal divided by the weight of the oil produced. The citronellal level obtained from the Jember area was 23.33% up to 28.05%, while for the Banyuwangi area was 24.61% up to 42.04% (**Table 1**). In the unpaired t-test, results showed sig. value of 0.002 (2-tailed) < 0.01, indicating that there is a significant difference in average total citronellal content between the two categories of citronella oil samples from the Jember and Banyuwangi regions. According to SNI (1995), citronellal oil is considered to meet the quality requirements if it has a citronellal content of at least 35%. Citronella oil samples that meet these requirements are samples from the Banyuwangi region with location codes Undangan and Sukabumi with a total citronellal content of 42.04% and 40.89%, respectively. This shows that the content of citronella in CWJ oil is most likely influenced by several factors other than the altitude of the planting area, *i.e.*, environmental temperature, intensity of radiation, and nutrient levels or soil fertility (Octriana 2019).

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The condition of soil fertility which was not a controlled in this study was a factor that caused the total citronellal levels of the samples obtained did not meet the quality requirements. The soil fertility effected the secondary metabolites produced, including citronellal (Silalahi 2020). Climate also affects the composition of essential oil components produced by CWJ. In this study, the samples used by the author were harvested during the rainy season. The citronellal content produced during the dry season is greater than the rainy season (Dacosta 2019). Land conditions suitable for growth are 100-600 m above sea level (DPL), but citronella can still grow up to 1,200 masl. The required light intensity is 100%, and 2,000-3,000 mm of rainfall is suitable; soil elements such as soil chemistry also determine growth (Rosman, 2012).

Based on previous studies, differences in location, topography and altitude can affect the total citronellal content of citronellal oil, where the total citronellal content of citronellal oil grown in higher plains areas will be greater than the total citronellal content of citronellal oil. planted in the lower plains (Suryani & Nurmansyah 2013; Sulaswaty 2019). Differences in geographical location, topography, and altitude cause different chemical compositions and different soil conditions so that the metabolic processes carried out by plants will also be different and produce different compositions of secondary metabolites (Danh 2009).

The method of developing a classification model was carried out by taking FTIR spectras data from CWJ oil from the training set sample, then classifying the model using chemometric techniques. The FTIR spectrum of the training set samples at all wavelengths was used to form the LDA, SVM and SIMCA models using 2 categories, namely "Jember" and "Banyuwangi". The labeling is intended to categorize the sample based on its area, "Jember" with a low altitude category and "Banyuwangi" with a medium altitude category. The results of the classification model of CWJ oil were listed in **Table 2** and **Figure 1**.

Figure 1 shows that the model can classify samples into their respective categories, namely the "Jember" category, which is in the red circle, and the "Banyuwangi" category, which is in the blue circle.

Table 2. Result of LOOCV classification models

Code of Sample Leaved	Accuracy of Prediction		
	LDA	SVM	SIMCA
D (Demangan)	100%	47,06%	100%
M 1 (Mencek 1)	100%	47,06%	100%
M 2 (Mencek 2)	100%	47,06%	100%
GN (Glen Nevis)	100%	47,06%	100%
CL (Curah Leduk)	100%	47,06%	100%
U (Undangan)	100%	47,06%	100%
average	**100%**	**47,06%**	**100%**

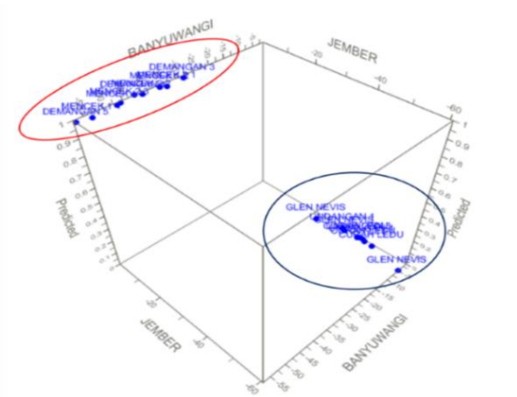

Figure 1. LDA classification model of CWJ oil. "Jember" category, which is in the red circle, and the "Banyuwangi" category, which is in the blue circle

The ability to recognize of classification models can be showed from the value of % accuracy of pediction. The accuracy value of the three models, LDA, SVM and SIMCA, were 100%, which indicates that the three models are able to predict all samples into correct category. (**Table 3**)

The LDA classification model shows the best results with 100% accuracy of pediction of classification model and validation. External validation of LDA results showed 100% accuracy because LDA was easily understood with a confusion matrix and figure description. External validation of SVM and SIMCA results have an accuracy of less than 100% because SVM is an easy algorithm and nonlinear mapping. Also, SVM is only fitted for a small number of sample. SIMCA may be categorized a sample into one, two, or three classes and not classify at all, so the external validation result was uncategorized or had 0% of prediction accuracy. (Wulandari 2022). (**Table 4**)

Tabel 3. The Accuracy of Prediction of Classification Models

Model	Accuracy of Prediction
LDA	100%
SVM	100%
SIMCA	100%

Table 4. Result of External Validation of Classification Model

Classification Model	% Accuracy of Prediction
LDA	100%
SVM	50%
SIMCA	0%

CONCLUSION

The LDA classification model of FTIR spectra can classify citronella oil from different altitude planting area. The average levels of total citronellal CWJ oil from Banyuwangi and Jember regions were 32.84%, and 26.06%, respectively. This method can be applied to sample discrimination in order to guarantee the quality of raw materials, especially CWJ.

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REFERENCES

Blank AF, Costa AG, Arrigoni M, Cavalcanti SCH, Alves PB, Inneco R, Ehlert PAD, Sousa IF. 2007. Influence of season, harvest time, and drying on java citronellal (*Cymbopogon winterianus*) volatile oil. *Brazilian Journal of Pharmacognosy*. 17(4): 557–564.

Cao, Zheng, Wang, Z. Shang dan J. Zhao. 2017. Classification and Identification of Rhodobryum roseum Limpr. and Its Adulterants Based on Fourier Transform Infrared Spectroscopy (FTIR) and Chemometrics. PLOS One: 1- 14.

Dacosta M, Sudirga SK, Muksin IK. 2019. Perbandingan Minyak Atsiri Tanaman Sereh Wangi (*Cymbopogon*): Jurnal Simbiosis. 1: 25-31.

Danh LT, Mammucari R, Truong P, Foster N. 2009. Response surface method applied to supercritical carbon dioxide extraction of *Vetiveria zizanioides* essential oil. Chemical Engineering Journal. 155(3): 617-626

Gad, HA., SH. El-Ahmady, MI. Abou-Shoer, dan MM. Al-Azizi. 2012. Application of chemometrics in authentication of herbal medicines: a review. Phytochemical Analysis. 24:1–24.

Kardono LBS, Artanti N, Dewiyanti ID, Basuki T, dan Padmawinata K. 2003. Selected Indonesian Medical Plants: Monographs and Description. Volume I. Jakarta: Gramedia.

Melo MS, Guimaraes AG, Santana MF, Siqueira RS, Lima ADBD, Dias AS, Santos MRV, Onofre ASC, Quintans JSS, DP et al. 2011. Anti-inflammatory and redox-protective activities of citronellal. *Biologi Research.* 44: 363– 368.

Murningsih T. 2009. Studi fitokimia baeckea frutences l: pengaruh faktor lingkungan terhadap komposisi minyak atsiri. Berita Biologi. 9(5):66825.

Octriana L, Istianto M. 2019. Efektivitas Minyak Sereh Wangi dalam Mengendalikan Kutu Putih Pepaya Paracoccus. Jurnal Budidaya Pertanian. 17: 15-22.

Purwakusumah, ED., M. Rafi, UD. Syafitri, W. Nurcholis, MAZ Adzkiya. 2014. Identifikasi dan Autentifikasi Jahe Merah menggunakan Kombinasi Spektroskopi FTIR dan Kemometrik. Agritech. 34(1): 82-87

Rosman. 2012. Kesesuaian Lahan Dan Iklim Tanaman Sereh. Jakarta: Litbang Pertanian, Kementerian Pertanian.

Silalahi, M. 2020. Essential Oil pada Tanaman Sereh. Jurnal lmiah Multi Sciences. 12: 7-13.

Sulaswaty A, Rusli M.S, Abimayu H, Tursiloadi S. 2019. Minyak Serai Wangi dan Produk Turunannya. Jakarta : LIPI.

Suryani, E & Nurmansyah. 2013. Penampilan Beberapa Klon Unggul Serai Wangi pada Dua Agroekologi Berbeda di Sumatera Barat. Buletin Penelitian Tanaman Rempah dan Obat. 24: 2.

Timung R, Barik CR, Purohit S, Goud VV. 2016. Composition and anti-bacterial activity analysis of citronella oil obtained by hydrodistillation: Process optimization study. *Industrial Crops and Products.* 94: 178–188.

Wulandari L, Idroes R, Noviandy TR & Indrayanto R. 2022. *Profiles of Drug Substances, Excipients, and Related Methodologi. 47(1)* Elsevier Inc.