# PROCEEDING

## The 2nd International Seminar of Basic Science

## Natural Science For Exploration The Sea-Island Resources

Ambon, May 31st 2016


Organized by

Faculty of Mathematics and Natural Science

Pattimura University

# PROCEEDINGS

The 2nd International Seminar of Basic Science

*"Natural Science for Exploration The Sea-Island Resources"*

Poka-Ambon, 31st May 2016

**Mathematic and Natural Science Faculty**
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2nd edition

# Welcoming Address By The Organizing Committee

Today, We have to thank the The Almighty Allah SWT for the implementation of this international seminar. This is the second seminar about Basic Science in The Faculty of MIPA Pattimura University. The seminar under the title “Natural Sciences for Exploration the Sea-Island Resources” will be carried out on May 31st 2016 at Rectorate Building, Pattimura University. There are 200 participants from lecturers, research institute, students, and also there are 34 papers will be presented.

My special thanks refer to the rector of Pattimura University and the Dean of MIPA Faculty, Prof. Dr. Pieter Kakissina, S.Pd., M.Si. I also would like to express my deepest gratitude to Prof. Amanda Reichelt-Brushett, M.Sc., Ph.D. ; Kazuhiko Ishikawa, Ph.D. ; Nicolas Hubert, Ph.D. ; Prof. Dr. Kirbani Sri Brotopuspito ; Prof. Dr. Marjono, M.Phil. ; Gino V. Limon, M.Sc., Ph.D. as the keynote speakers.

The last, We hope this international seminar usefull for all of us, especially Mollucas People and very sorry if any mistake. Thank you very much.

**Dr. La Eddy, M.Si.**
Chairman of Organizing Committee

# Opening Remarks
# By Dean of Mathematic and Natural Sciences Faculty

I express my deepest gratitude to The Almighty God for every single blessing He provides us especially in the process of holding the seminar until publishing the proceeding of International Seminar in celebrating the 18th anniversary of MIPA Faculty, Pattimura University. The theme of the anniversary is under the title "Natural Sciences for Exploration the Sea-Island Resources". The reason of choosing this theme is that Maluku is one of five areas in Techno Park Marine in Indonesia. Furthermore, it is expected that this development can be means where the process of innovation, it is the conversion of science and technology into economic value can be worthwhile for public welfare especially coastal communities.

Having the second big variety of biological resources in the world, Indonesia is rich of its marine flora and fauna. These potential resources can be treated as high value products that demand by international market. Basic science of MIPA plays important role in developing the management of sustainable marine biological resources.

The scientific articles in this proceeding are the results of research and they are analyzed scientifically. It is expected that this proceeding can be valuable information in terms of developing science and technology for public welfare, especially people in Maluku.

My special thanks refer to all researchers and reviewers for your brilliant ideas in completing and publishing this proceeding. I also would like to express my gratefulness to the dies committee-anniversary of MIPA Faculty for your creativity and hard working in finishing this proceeding, God Bless you all.

**Prof. Dr. Pieter Kakisina, S.Pd., M.Si.**
Dean of Mathematic and Natural Sciences Faculty

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Hotel Mutiara Ambon

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# SPECTRUM ANALYSIS NEAR-INFRARED SPECTROSCOPY (NIRs) OF CAJUPUT OIL

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## ABSTRACT

Cajuput oil is produced by the distillation of leaves and twigs of the *Melaleuca leucadendra* trees which grow wild and are cultivated in Indonesia. It is important to identify the substance of cajuput oil used, and it is conventionally measured using Gas Chromatography Mass Spectroscopy (GC-MS) method. This method is time consuming, leaves chemical residual, relatively expensive, and has not supported the need of quick measurement as well as provided online service in essential oil industry yet. One of potential methods to fulfill those needs is Near Infrared/NIR Spectroscopy. The result of this study shows specific NIRS spectrum pattern of cajuput oil, and the result of the overall identification shows that the cajuput oil consists of two major groups: the hydrocarbon and oxygenated hydrocarbon. The NIRs spectrum result is supported by the result of GC-MS which shows that the main compound of this oil is 1,8 Cineol which reach 72.11% of oxygenated hydrocarbon. Therefore, it can be concluded that the component compound in cajuput oil can be analyzed using NIRs method in a short period, accurate, and can be made as finger print in detecting the substance in cajuput oil products.

***Keywords:*** Cajuput oil, GC-MS, NIRS, 1,8 Cineol

## INTRODUCTION

Cajuput oil is one of essential oil which is produced by the distillation of leaves and twigs of *Melaleuca leucadendra* trees which has particular and nutritious scent, and it also one of forestry products that has widely known by societies. The main compound of cajuput oil is 1,8 Cineole ($C_{10}H_{18}O$), as well as the alcohol's occurrence from terpineol ($C_{10}H_{17}O$), and several kinds of terpenes such as 1-pinene, valerate and aldehyde benzoate [1]. In Indonesia, the oil that its 1.8 cineole concentrate is above 55% is considered as the first quality, and under this concentrate is considered as the standard quality [2]. It is also supported by Department of Standard National Indonesia that requires cajuput oil containing 50-65% 1,8 Cineole (*SNI*-Indonesian National Standard 06-3954-2006). The benefits of Cineole are can reduce headache, toothache, rheumatics, convulsions, anti-mosquito [3], cockroach (*Periplaneta americana*) repellent [4], as fumigant [5], stimulate blood circulation without irritating air circulation on the skin [6], anti-microbe [7;8], analgesic and anti-inflammation [9; 10], as cytotoxic and anti-tumor [6], relief coughs in acute bronchitis patients in their four-day treatment [11].

The quality and the compound of cajuput oil are highly significant to the benefit of cajuput oil used by the consumers. Therefore, it is important to analyze the compound of the

cajuput oil used. The most commonly used test to determine the chemical composition in cajuput oil using Gas Chromatography mass Spectroscopy (GC-MS). It is the conventional method used to detect the components of essential oil. This method is time consuming, leaves chemical residual, relatively expensive, and has not supported the need of quick measurement as well as provided online service in essential oil industry yet. Therefore, one of the potential methods to fulfill those needs is Near Infrared Spectroscopy (NIRs). The NIR spectra read both the organic and inorganic compounds which have specific absorption bands which is different from one another every time infra red wavelength is given to it. Thus, it offers non-destructive testing (NDT) method to evaluate its physical, mechanical and chemicals characteristics. The NIR spectroscopy principle relies on the harmonic and an-harmonic absorptions from the molecular motions which caused molecular vibrations in wavelength transitions in the infrared region where the overtone and combination vibrations are in near infrared intervals [12]. Bunning-Pfaue & Kehraus [13] are also stated that NIRs method is reliable, relatively not expensive, and fast and can give distinctive mark on the compounds' products. Therefore, the contents of cajuput oil expected can be analyzed using NIR spectroscopy to ease the future testing method and to save the cost. On the other hand, the contents of cajuput oil will also be measured using Gas Chromatography Mass Spectroscopy (GC-MS)

## MATERIALS AND METHODS

### Material

The cajuput oil, branded name Eriwakang, distillated from *Melaleuca leucadendra* leaves which was acquired from citizens' distillation in Suli Village, Salahutu Subdistrict, Central Maluku Regency was provided as the sample.

### The Refining of Cajuput Oil

The cajuput oil was refined using chemical method with anhydrous sodium sulfate ($Na_2SO_4$) and if the color of essential oil is brighter than before, it shows that the distillation process is done.

### NIR Spectroscopy Analysis

Near Infrared Spectrometer NIRFLEX solid N-500 made by BUCHI was used to measure the component contents of pure cajuput oil's compound. The transreflectance measurement was used to measure, where 2 ml samples were put on a petri dish and covered by a reflector. The sample under the reflector has 0.3 mm thickness. This sample was scanned for 21 times in wavelength interval 4000-6300 $cm^{-1}$ then it was averaged to get the good signal/noise ratio (s/n ratio). The spectra reflection in this measurement was 4 $cm^{-1}$. The baseline was corrected using the first derivative while the acceleration of power split using the second derivative. Every derivative number is started with spectrum smoothing using Savitzky-Golay polynomial order 3 windows 11.

### Gas Chromatography Mass Spectroscopy (GC-MS)

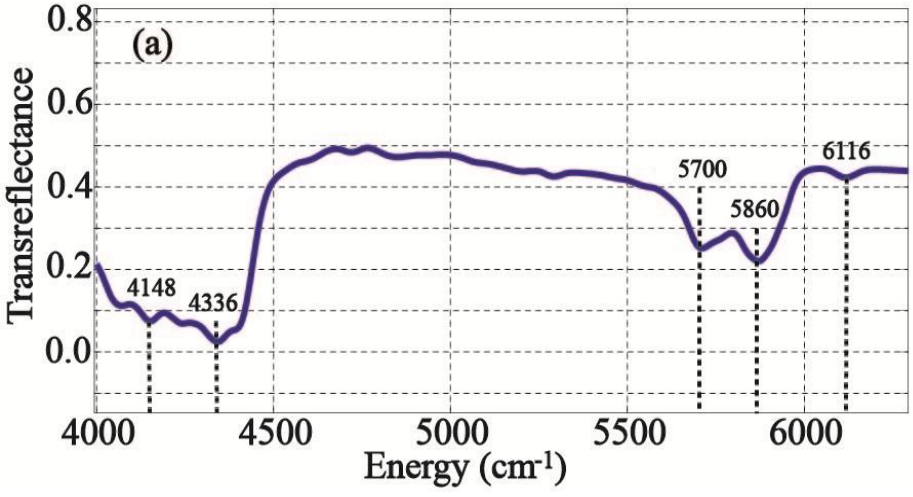
The component structures of pure cajuput oil were identified using GC-MS SHIMADZU QP-5000. The column used in this study was Rastek RXi-5MS, which length is 30 m, ID 0.25mm. Helium was used as the carrier gas (0.3 ml/min) and the column pressure is 13.7 kPa were used to operate the machine. The heating temperature in the column is 70 $^0C$ in injection split model. The total flow is 80 ml/min, column flow is 0.50 ml/min, and linier speed is 25.9 cm/s.

## RESULTS AND DISCUSSION

### Cajuput Oil Analysis using NIR Method

Before analyzing the cajuput oil using NIR Method, it was refined using chemical method with anhydrous sodium sulfate (Na2SO4) to turn its color into brighter color. Na2SO4 was added in order refining and remove the dispersed water in the oil since the new distillated essential oil usually contains little amount of distillated water which can caused slow reaction between the water and the essential oil [1]. The water contained in the cajuput oil can caused hydrolysis process to create sodium which turns the cajuput oil color into darker color. If it happens, the distillation process has to be performed to improve the quality of the cajuput oil.

NIR spectrum was acquired using transreflectance measurement technique which the samples' geometric wavelength was twice of the samples' thickness. Since the sample thickness was 0.3 mm, therefore the geometric wavelength was 0.6 mm. transreflectance spectrum of the pure cajuput oil was shown in Figure 1(a). In this spectrum, absorption is shown by the valley. Where the lesser the value, the stronger the absorption is. From the measurement, it is shown that the lowest transreflectance is 0.02455, which means that this spectrum has not reached the saturation condition, so the samples' thickness is considered adequate. Just like the NIR spectrum in general, the absorption of overtone and combination vibrations which appear in NIR area always have wide absorption features as those vibrations are very close. Wide absorption which appears in the region around 4050-4400 $cm^{-1}$ and 5650-6000 $cm^{-1}$ were the characteristic of hydrocarbon chain in general. Apparently, there are several prominent typical absorptions, such as in 4148 $cm^{-1}$, 4336 $cm^{-1}$, 5700 $cm^{-1}$, 5860 $cm^{-1}$, and 6116 $cm^{-1}$. Another structure form that spectrum is more difficult to be identified therefore two times of derivative process are needed. The second derivative spectrum of pure cajuput oil is shown in Figure 1(b). It is important to be noted that the second derivative spectrum will indicate the absorptions' position which have not been determined accurately (because it looks like a peak, not a valley) in transreflectance spectrum. In the second derivative spectrum, the valley position in transreflectance spectrum will be indicated by the peak position on the second derivative spectrum. The valley on the second derivative spectrum does not have any physical meaning.



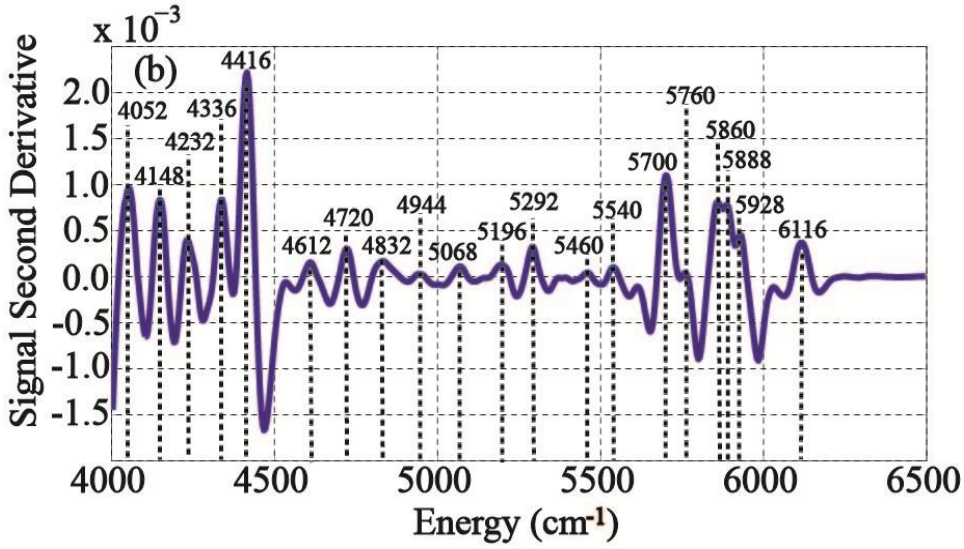



Figure 1. (a) The transreflectance spectrum of the pure cajuput oil, (b)The second derivative spectrum of the cajuput oil

Figure 1(b) the second derivative spectrum of the pure cajuput oil shows the variations in peaks which has specific absorption area on wave number 4052, 4148, 4612, 4832, 5068, 5928 and 6116 (aromatic compound C-H aryl); 4232, 4336 (aliphatic hydrocarbon compound); 4416, 5460, 5540, 5700 (volatile combination compound C, H, O); 4720 (methanol compound O-H); 4944 (ester compound); 5860, 5888 (ether compound), and 5196, 5192 (polyvinyl alcohol compound O-H).

The strongest signal in the second derivative of the cajuput oil was shown in wavenumber 4416 $cm^{-1}$ which is identified as chemical group combination of C-H-O. Therefore, it can be concluded that the major compositions of the cajuput oil is a chemical group combination of the aromatic compound C-H-O. This data is also supported by the result of data analysis using GC-MS in Table 2, which shows the highest composition in the cajuput oil is Cineole 72.11% with chemical compound $C_{10}H_{18}O$.

The previous study, carried out by Schimleck, *et al*. [14], showed the Cineole spectrum from the sample leaves producing cajuput oil (*Melaleuca cajuputi*) has absorption around wavenumber 1650-1800 and 2200-2400 nm (6060-5600 and 4545-4167 $cm^{-1}$), and it is also in line with the result of a study by Ebbers, *et al*. [15] that confirm the NIR spectrum data from pure 1,8 Cineole has the absorption wave number range of 1700-1800 and 2200-2300 nm (5882-5600 and 4545-4348 $cm^{-1}$). The absorptions shown on the wave number from the studies conducted by Schimleck, *et al*. [14] and Ebbers, *et al*. [15] were also shown in spectrum data of the distillated cajuput oil that it shows absorption in those wave number ranges too. In wave number range 1650-1800 (6060-5600 $cm^{-1}$) NIRs spectrum data of the cajuput oil shows the absorption in wave number 5700, 5760, 5860, 5888 and 5928 $cm^{-1}$. Then, in the range wave number 2200-2400 nm (4545-4167 $cm^{-1}$) the NIRs spectrum data of the cajuput oil shows the absorption in wave number 4232, 4336, and 4416 $cm^{-1}$.

The data tabulation from the absorption peaks identification in the second derivative spectrum of cajuput oil is provided in Table 1.

Table 1. The absorption peaks identification in the second derivative spectrum of cajuput oil [16]

| No. | Energy ($cm^{-1}$) | Functional Group | Type of Material |
|---|---|---|---|
| 1. | 4052, 4148 | C-H Aromatic C-H (aryl) | Aromatic compound |
| 2. | 4232, 4336 | C-H Methylene C-H | Aliphatic compound C-H, hydrocarbon |
| 3. | 4416 | O-H/C-H/C-H-O combination | Aromatic compound |
| 4. | 4612 | C-H Aromatic C-H (aryl) | Aromatic compound |
| 5. | 4720 | O-H/C-O combination from | Methanol compound O-H |
| 6. | 4832 | methanol | Aromatic compound |
| 7. | 4944 | C-H Aromatic C-H (aryl) | Esther compound |
| 8. | 5068 | C=O esther & acid (C=OOR) | Aromatic compound |
| 9. | 5196, 5292 | C-H Aromatic C-H (aryl) | Polyvinyl alcohol compound |
| 10. | 5460, 5540, | O-H hydrogen | OH |
| 11. | 5700 | O-H/C-H/C-H-O/C-H=O | Aromatic compound |
| 12. | 5760 | combination | Aromatic compound |
| 13. | 5860, 5888 | C-H methyl, Aromatic ($ArCH_3$) | Ether compound |
| | 5928, 6116 | C-H methyl C-H, ether ($R\text{-}O\text{-}CH_3$) | Aromatic compound |
| | | C-H Aromatic C-H (aryl) | |

The data on table 1 describes that cajuput oil consists of 2 big groups, the hydrocarbon group which is formed from Hydrogen (H) and Carbon (C), and the oxygenated hydrocarbon group which is formed from Carbon (C), Hydrogen (H) and Oxygen (O).

**Cajuput Oil Analysis Using GC-MS**

The data tabulation of cajuput oil component measurement is shown in Table 2.

Table 2. The GC-MS data tabulation of cajuput oil component measurement

| Peak | Compound | Molecule Chemical | Base Peak (%) |
|---|---|---|---|
| 1 | α-Pinene | $C_{10}H_{16}$ | 2.56 |
| 2 | β-Pinene | $C_{10}H_{16}$ | 1.41 |
| 3 | β-Myrcene | $C_{10}H_{16}$ | 1.15 |
| 4 | γ-Terpinene | $C_{10}H_{16}$ | 0.38 |
| 5 | Benzene | $C_{10}H_{14}$ | 0.39 |
| 6 | 1,8-Cineole | $C_{10}H_{18}O$ | 72.11 |
| 7 | γ-Terpinene | $C_{10}H_{16}$ | 2.04 |
| 8 | δ-4-carene | $C_{10}H_{16}$ | 1.03 |
| 9 | 3-Cyclohexen-1- | $C_{10}H_{18}O$ | 0.76 |
| 10 | Methanol | $C_{10}H_{18}O$ | 7.66 |
| 11 | β-fenchyl alcohol | $C_{12}H_{20}O_2$ | 2.24 |
| 12 | β-Terpinyl acetate | $C_{15}H_{24}$ | 5.06 |
| 13 | trans-Caryophyllene | $C_{15}H_{24}$ | 1.95 |
| 14 | α-Humulene | $C_{15}H_{24}$ | 0.68 |
| 15 | δ-Guaiene | $C_{15}H_{24}$ | 0.59 |
| | δ-Guaiene | | |

From the GC-MS measurement it is found that cajuput oil consists of 15 compound contents and the highest content is 1,8 Cineole 72,11% which has met the Indonesia National Standard requirement. Therefore, the quality of the cajuput oil used in this study has the highest quality. The measurement result of the Cajuput Oil using GC-MS can be grouped into big 4 groups: Limonene ($C_{10}H_{16}$) 26.23%, Benzene ($C_{10}H_{14}$) 0.39%, Cineole ($C_{10}H_{18}O$) 72.87%, β-Terpinyl Acetate ($C_{12}H_{20}O_2$) 2,24%, and Sesquiterpene ($C_{15}H_{24}$) 7.28%.

The previous study by Astuti & Aphari [17], showing gcms results cajuput leaves extracted using ethanol by maceration method produces 45 components and 10 components having the highest content are α-selinene (9,07%), Guaiol (7,47%), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phytol (5,24%), Cyclopentanetrione (4,90%), trans-Caryophyllene (4,78%), 10-epi-gamma-eudesmol (4,69%), 1,8-cineole (4,66%), hexadecanoic acid (CAS) Palmitic acid (4,23%), 4-isopropyl-5-methyl-hexa-2,4-dien-1-ol (3,79%), dan 2-propen-1-on3, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-,(E)-(CAS) Pinostrobin (3,71%). Results of they study demonstrate the component with the highest concentration of α-selinene 9,07% and 1,8 cineole is 4,66%. So, it can be said that the method of extraction can affect the outcome of ingredients extracted. Compared the results of research conducted by Astuti & Aphari [17], this research distillation methods produces 1,8- cineole content is 72.11 % higher than the results of research by Astuti & Aphari [17] which uses solvent ethanol by maceration extraction methods.

## CONCLUSION

The spectrum identification result from Near Infrared Spectroscopy (NIRs) shows that the strongest second derivative signal in cajuput oil is explained in wave number 4416 $cm^{-1}$ which is identified as the group combination of C-H-O aromaticity compound. The final result of the cajuput oil compounds in NIRs spectrum, which is also supported by GC-MS spectrum data, is divided into two groups of big compounds. Those are hydrocarbon and oxygenated hydrocarbon. Therefore, Near Infrared Spectroscopy (NIRs) can be used to analyze or find out the component compound of cajuput oil accurately in a short period and it also can be applied online and does not spend expensive cost. From the analysis of the structural component compound of the cajuput oil using GC-MS finds that there are 15 structural compounds, and the content of the major compound of this oil is 1,8 Cineole 72.11 % which produces particular scent of the cajuput oil. Extraction methods may affect the results of eucalyptus oil content extracted.

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