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Table of contents

Volume 819

2021

◀ Previous issue Next issue ▶

2nd International Conference Earth Science And Energy 11 November 2020, Kuala Lumpur, Malaysia

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Open all abstracts

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Ganoderma boninense control in palm oil plantations using *Trichoderma harzianum* in various Media

Aries Sukariawan, Eka Bobby Febrianto, Sakiah, Muhammad Ridho and Doli Karnando

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Preparation and characterization of cellulose microcrystalline made from palm oil midrib

Pada Mulia Raja, Ika Ucha P. Rangkuti, M. Hendra Ginting, Giyanto and Wahyu F. Siregar

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OPEN ACCESS 012003

The study of soil biological and chemical properties on palm oil plant rhizosphere with and without palm oil mill effluent applications

Megawati Sidiyasa, Sabah, Aprilia S. Sutanto, A Lawary and R Mahmuda

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Evaluation of the Value of the Defective and Taste of Arabica Coffee (*Coffea Arabica L*) West Sumatera

Rince Alfia Fadri, K Kesuma Sayuti, Novizar Nazir and Irfan Suliansyah

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Utilization of alternative ingredients as combination feed on the growth of catfish (Clarias sp.)

RC Mukti, M Amin and Yulisman

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Assessment on shallot farming development in North Padang Lawas Regency, North Sumatra

Moral Abadi Girsang, Palmarum Nainggolan, Shabil Hidayat, Suheri Sitepu and Khadijah El Ramija

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Application of Trichoderma as an Alternative to the use of Sulfuric Acid Pesticides in the Control of Diplodia Disease on Pomelo Citrus

Mei Silvia and Sutarman

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The Effect of Trichoderma and Onion Extract on the Success of Grafting in Mango Seedlings

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Isolation and Performance Testing of *Bacillus subtilis* As Biological Agents to Control the Diplodia Disease on Siam Citrus

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K-Nearest Neighbor Algorithm to Identify Cucumber Maturity with Extraction of One-Order Statistical Features and Gray-Level Co-Occurrence

Syamsudduha Syahrerini, Denny Syamsudin, Dwi Hadidjaja Rasjid Saputra and Akhmad Ahfas

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Determination of Volatile Compounds on Traditional Fermentation of *Chao Teri*

Syahriati, UA Nur Fitriani and Muhammad Yusuf

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Physicochemical, nutritional, and color characteristic of Nori Snack from Snakehead Fish (*Ophiocephalus striatus*), *Eucheuma cottonii*, and *Gracilaria sp*

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Reproductive characters of hampala fish (*hampala macrolepidota kuhl & Van Hasselt, 1823*), correlation with body length

Suhestri Suryaningsih, Sri Sukmaningrum, Adi Amurwanto and Aswi Andriasari Rofiqoh

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Efficacy of application time of *Penicillium sp.* suspension on White Root Fungus (*Rigidoporus lignosus*) in Nutmeg (*Myristica fragrans*)

Chairudin, Agustinur and J Permadi

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The correlation between mangrove ecosystem with shoreline change in Indramayu coast

Sofyan Hasanuddin Nur and Endang Hilmi

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Geothermal Well Targeting in Consideration To Geological Structures of Mataloko Field, Flores

Untung Sumotarto, Yudistian Yunis, Fajar Hendrasto, Kris Pudyastuti, Evan R. Sammuel, Dhany Rizky and Arthur G.P. Nayoan

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New Insight of Surface and Subsurface Sedimentology of Salodik Group, Banggai Basin

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Petrophysical Analysis to Determine the Initial Oil Reserves in the AHP Field

Apolonius Harda Putranta Sudi, Ratnayu Sitaresmi and Prayang Sunny Yulia

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Compatibility Test for Screening Surfactant Flooding

M Dhafa, R Setiati, M T Malinda and A Anugrahadi

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The Opportunities and Constraints of Wind Energy

A A S Siahaya, R Setiati and M T Malinda

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Effect of Improvement of Temperature and Gas Amount on Nitrogen Flooding

M T Malinda, R Setiati, M A Azizi and Y F Simbolon

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Laboratory Study: The Effect of Clay Mineral in Sandstone on Resistivity of Rock

Ratnayu Sitaresmi, Rosidelly, Lia Mega and M. Taufiq Fathaddin

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Drill Holes Spacing Analysis for Estimation and Classification of Coal Resources Based on Variogram and Kriging

M D Ramadhan, I Marwanza, C Nas, M A Azizi, W Dahani and R Kurniawati

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The Palu Earthquake Formed a New Geological Structure

A. Anugrahadi, U. Sumarto and T. T. Purwiyono

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Solar Energy Hybrid Control System Design Photovoltaic Using Fuzzy Logic Controller

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Analysis of Overcurrent Safety in Miniature Circuit Breaker *AC (Alternating Current)* and *DC (Direct Current)* in Solar Power Generation Systems

Jamaaluddin Jamaaluddin, Indah Sulistiyowati, BWA. Reynanda and Izza Anshory

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The Application of Geostatistical Analysis on Radiometric Mapping Data to Recognized the Uranium and Thorium Anomaly in West Sulawesi, Indonesia.

I Gde Sukadana, I Wayan Warmada, Agung Harijoko, Frederikus Dian Indrastomo and Heri Syaeful

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Causes, Dynamics, and Environmental Conflict Resolution Models in Coal-Fired Steam Power Plant Development Locations
Ria Ishardanti, Herdis Herdiansyah and Francisia Saveria Sika Ery Seda
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Illegal artisanal and small-scale mining practices: re-thinking the harm
Putu P. S. Agustina, Herdis Herdiansyah and Anggi A. Harahap
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Good Governance Role for a Sustainable Solid Waste Management in Rural Community
Ari Naldi, Herdis Herdiansyah and L G Saraswati Putri
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Climate Village Program for Climate Change Adaptation and Mitigation for Green Villages
Herdis Herdiansyah
[+ Open abstract](#) [View article](#) [PDF](#)
-
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Sustainable Development on Indonesian Environmental Risk Assessment
Agastyo Djanardono Basoeki, Herdis Herdiansyah and Yuki M. Adhitya Wardhana
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Soybean production response: A study of Jambi's acreage response under policy program
Edison and Dharia Renate
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Contribution of Oil Palm Plantation to Household in Kolaka District of Southeast Sulawesi
Haji Saediman, Rahmayana Rahmayana, Yusna Indarsyih, Nuryamin Budi and Lukman Yunus
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Protecting Biodiversity: The Importance of Understanding the Role of Government and Society in Human-Urban Wildlife Interaction in Indonesia

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The effect of maggots lentera flies (*hermetia illucens*) growing media as the solution of using organic waste

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012048

Habitat characteristics and population of *leptophryne javanica* in Curug Cisurian, Kopi Bojong, and Ciinjuk in Gunung Ciremai National Park

Ika Karyaningsih, Nurdin, W Indra, Nina Herlina and Ai Nurlaila

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012049

Structure and similarities of Understorey Vegetation Communities in the mining area of PT. Indocement Tungal Prakarsa Tbk. Palimanan Unit, Cirebon

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Dynamics of dengue hemorrhagic fever incidence and climate as potential factors in Palembang 2013 – 2019

Krisna Delita, Nurhayati Damiri, Rico Januar Sitorus, Poeji Loekitowati Hariani, Fika Minata Wathan and H Annisa Tassia

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012052

Quality of Fermented Purple Sweet Potato (*Ipomea batatas* var. *Ayamurasaki*) (Tapai) in Various Yeast Concentration and Steaming Time

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012053

Characteristics of Dry Carrot Noodle (*Daucus carota* L) with Proportion of Wheat Flour and White Oyster Mushroom Flour (*Pleurotus ostreatus*)

L Hudi, I A Saidi, R Azara and T R Pratiwi

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012054

Determination of Level of Lead (Pb) and Cadmium (Cd) Migration on Bowl and Clear Plastic Cup Using Atomic Absorption Spectrophotometer

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Optimization and Validation Methods for Assay of Acyclovir Cream Determination by High Performance Liquid Chromatography

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Total Flavonoids Content of Polar Extracts of *Cayratia trifolia* Leaves

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012057

Chemical Component of Kencur (*Kaemferia galanga* L.) Ethanolic Extract Using Gas Chromatography-Mass Spectrometry

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Effect of Glycerin as Plasticizer in Formulation of Grape Seed Oil (*Vitis vinifera* L.) Emulsion Peel-Off Mask

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The use of coconut water (*cocos nucifera l.*) as alternative media to substitute Sabouraud Dextrose Agar (SDA) for the growth of *aspergillus flavus*

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Diversity of insect pollinator on farmland near to mount Ciremai National Park

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Vegetation structure and species composition of habitat types *Goniothalamus macrophyllus* (Blume) Hook.f. and Thomson in Lowland Forest, Kuningan Regency, West Java

Ilham Adhya, Yayan Hendrayana, Toto Supartono, Agus Yadi Ismail and Nurdin

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Activity of valerian catalase in Ultisol growing media by adding humic acid

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Siam Madu citrus seeding in supporting the development of Sumatra citrus zone

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012068

Characterization of GABA (gamma-aminobutyric acid) levels some fermented food in Indonesia

Heny Herawati, Diana Nur Afifah, Eni Kusumanigtyas, Sri Usmiati, Agus S. Soemantri, Miskiyah, Elmi Kamsiati and Muchamad Bachtiar

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Differences in the effect of online nutrition education through lectures without and with online games on increasing knowledge of Nutrition, Iron, and vitamin C intake of young women

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Diversity and abundance of insect pollinator on *dimocarpus longan l.* in Sawitsari research station, Sleman, Yogyakarta

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Iron supplement for anemia developed from home industry waste of snake fruit Pondoh (Snake fruit *edulis Reinw.*)

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Innovation development information system for sales of traditional food processed product web based in the Cibiruang Village

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ET Asmorowati and D Sarasanty

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Chemical Component of Kencur (*Kaemferia galanga* L.) Ethanolic Extract Using Gas Chromatography-Mass Spectrometry

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Abstract. *Kaemferia galanga* L. or kencur is a traditional plant that known has potential antioxidant and anti-inflammatory activities because of its chemical compound such as ethyl *p*-methoxycinnamate. This study aimed to analyze the chemical compound of ethanolic extract of kencur using gas chromatography-mass spectrometry. Kencur was extracted by cold maceration using ethanol-water (70:30) at room temperature. From this procedure, the filtrate (extract of kencur) and crystal are produced. The crystal then was crystallization using ethanol. The ethanolic extract and crystal were analyzed qualitatively using gas chromatography-mass spectrometry. The results show that in the ethanol extract identified there were 5 compounds, namely ethyl-*trans-p*-methoxycinnamate (84.55%); ethyl cinnamate trans (5.05%), methyl 4-methoxycinnamate (4.02%), ethyl-*cis-p*-methoxycinnamate (2.85%) and benz[A]azulene-1,4-dione, 2,3-dimethyl- (1.49%). Whereas the crystals are ethyl-*trans-p*-methoxycinnamate (96.20%) and methyl 4-methoxycinnamate (1.62%). It was concluded that ethyl-*trans-p*-methoxycinnamate was the main compound of kencur which was easily obtained by cold extraction.

1. Introduction

Ethyl *p*-methoxycinnamate is one of the compound from derivatives cinnamate from non-flavonoids phenolic groups [1] that have exhibited anti-inflammatory properties. This compound also plays a role in pharmacological activities such as anticancer, antimicrobial, angiogenesis inhibitors, etc. [2]. Ethyl *p*-methoxycinnamate can be found in high levels *Kaemferia galanga* L. (or kencur) rhizome. Ethanol is a solvent suitable for extraction of ethyl *p*-methoxycinnamate than chloroform, dichloromethane and hexane [3]. Gas Chromatography-Mass Spectrometry (GC-MS) is the reproducible, accurate and suitable for analysis and identification the ethyl *p*-methoxycinnamate from extracts of kencur [4]. This study aimed to analyze the ethyl *p*-methoxycinnamate and other chemical compounds of ethanolic extract of kencur and its crystal were obtained during the cold extraction process using (GC-MS). The



percent of ethyl *p*-methoxycinnamate could be used as a biomarker compound for standardization of kencur rhizome as a natural source of pharmaceutical drugs[5].

2. Material and Methods

Collection of plant

The kencur rhizome were collected from the Unit Konservasi Budidaya Biofarmaka (UKBB), Pusat Studi Biofarmaka Tropika LPPM IPB, Bogor, West Java, Indonesia. Plant was identified in Herbarium Bogoriense, Biology Research Centre, Indonesian Institute of Sciences/Lembaga Ilmu Pengetahuan Indonesia (LIPI), Cibinong, Indonesia. The cleaned of kencur fresh rhizomes then dried for a few days and were made into powder.

Extraction

The dried powder of kencur rhizomes (1 kg) was extracted in ethanol:water (70:30, v/v) using cold maceration method in macerator for 3 x 24 h. The extract then was decanted and filtered through a Whatman filter paper. The filtrate was evaporated using a vacuum rotary-evaporator N-1200 BS series EYELA (Shanghai, China) at 50 °C[6]. The residue from filtration procedure contains the crystals and amyllum. The residue was collected, washed using ethanol, decanted, and recollected then crystallization using ethanol solvent. The ethanolic extract and crystals then were analyzed using gas chromatography-mass spectrometry.

Phytochemical Screening of Ethanolic Extract

The phytochemical compounds of kencur ethanolic extract, such as phenolics, flavonoids, alkaloids, tannins, and saponins were qualitatively identified following standard procedures describes in the Harborne[7] and Indonesian Herb Pharmacopoeia[6].

Analysis of Chemical Compounds

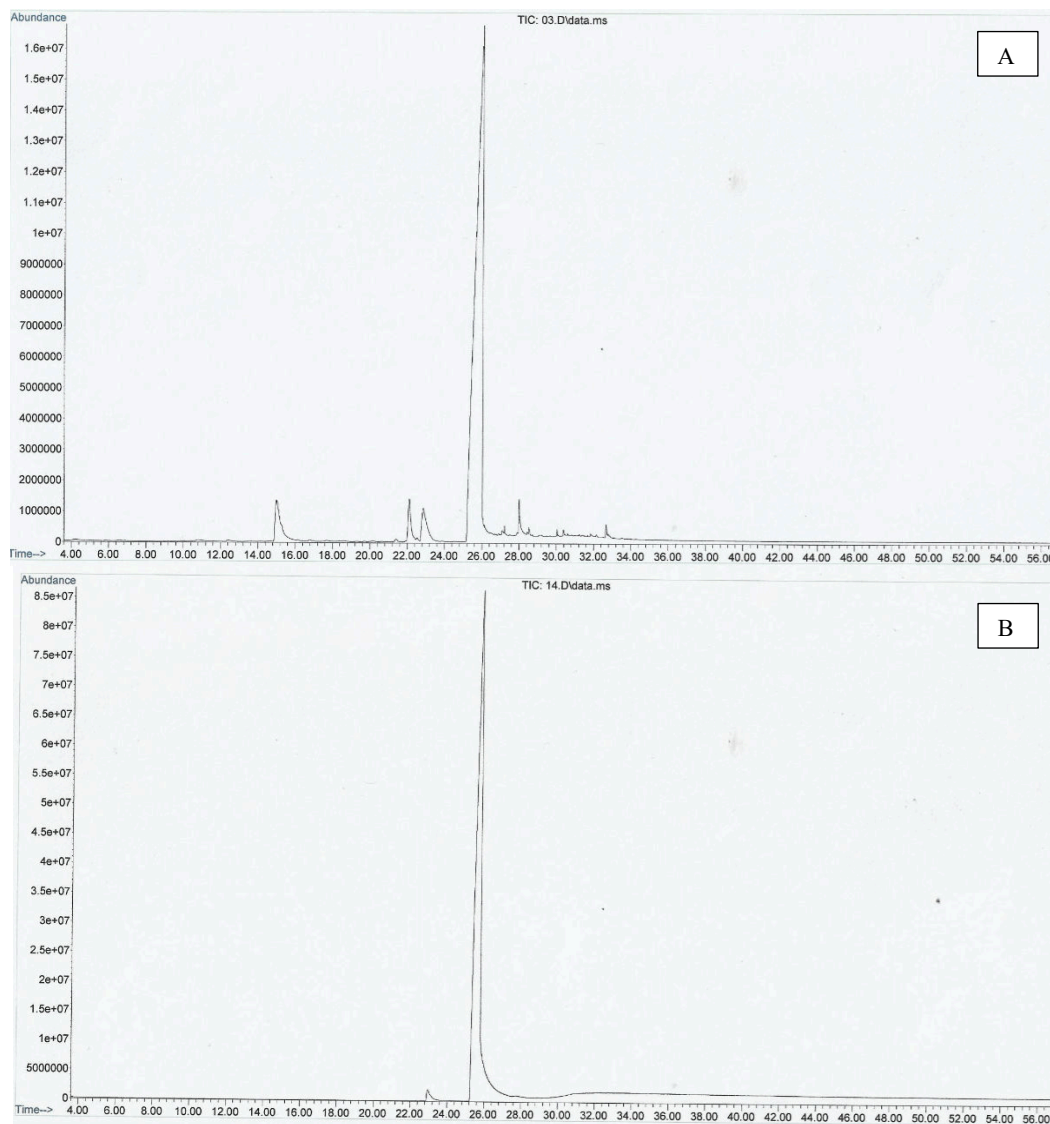
Analysis of chemical compounds of the extract and crystal was performed in the Laboratorium Kesehatan Daerah, Dinas Kesehatan, Pemerintah Provinsi DKI Jakarta, Indonesia. The ethanolic extract and crystal were dissolved in methanol. The solution then derived by bis-(trimethylsilyl) acetamide reagent and 20 µL of pyridine. The obtained solution was incubated for 60 min at 80°C. Five µL of each sample solution was analyzed with the Gas Chromatography with AutoSampler (Agilent Technologies 7890), 5975 Mass Selective Detector and Chemstation data system. The separation was carried out using the capillary column (HP Ultra 2) with a length of 30 m × 0.20 mm I.D × 0.11 µm of film thickness. The carrier gas was helium at a constant flow rate of 1.2 mL/min. The injection port temperature was set at 250 °C. The initial temperature of the oven was set at 80 °C then hold for 0 min, rising at 3 °C/min to 150 °C then hold for 1 min and finally rising at 20 °C/min to 280 °C then hold for 26 min. The components were identified based on compatibility with an authentic mass spectrum in Wiley electronic library. The criteria for the major compounds in the GC-MS chromatogram if the compound has a percent area >5% (Herebian *et al.* (2009). The similarity of mass spectra of the sample with a library was determined at a qualifier value of at least 80%.

3. Results and Discussion

In this study, the percentage of extraction yield from ethanolic extract of kencur was 9.56%. Organoleptic characteristics of kencur extract show a distinctive odour, light brown colour, thick shape, and a spicy taste on the tongue. According to Indonesian Herb Pharmacopoeia (2008)[6], ethanolic extract of kencur rhizome with good quality is not less than 8.3%. The phytochemical screening shows that ethanolic extract contains phenolics, flavonoids, tannins, saponins, and alkaloids. Chromatogram of kencur ethanolic extract and the crystals are shown in Figure 1 dan Figure 2. The ethanolic extract had five chemical compounds with the major compounds is ethyl *p*-methoxycinnamate (84.55%). Whereas the crystals had two compounds with the major compounds is ethyl *p*-methoxycinnamate (96.20%) (Tabel 1).

Table 1. Chemical compounds of ethanolic extract and crystal of kencur rhizome

Samples	RT	Quality	Compounds	Percentage (%)	Molecular weight	Characteristic MS fragments
Ethanolic extract of kencur	14.998	98	<i>trans</i> -Ethyl cinnamate	5.05	176	176, 148, 131, 103, 77
	22.114	99	Ethyl- <i>cis-p</i> -methoxycinnamate	2.85	206	206, 161, 134, 77
	22.866	98	Methyl 4-methoxycinnamate	4.02	192	192, 161, 133
	25.976	99	Ethyl- <i>trans-p</i> -methoxycinnamate	84.55	206	206, 161, 134, 77
	28.017	83	Benz [A] azulene-1,4-dione, 2,3-dimethyl-	1.49	236	236, 208, 193
Crystal of kencur	22.956	98	Methyl 4-methoxycinnamate	1.62	192	192, 161, 133
	25.742	94	Ethyl- <i>trans-p</i> -methoxycinnamate	96.20	206	206, 161, 134, 77

**Figure 1.** The chromatogram of ethanolic extract (A) and crystal (B) of kencur

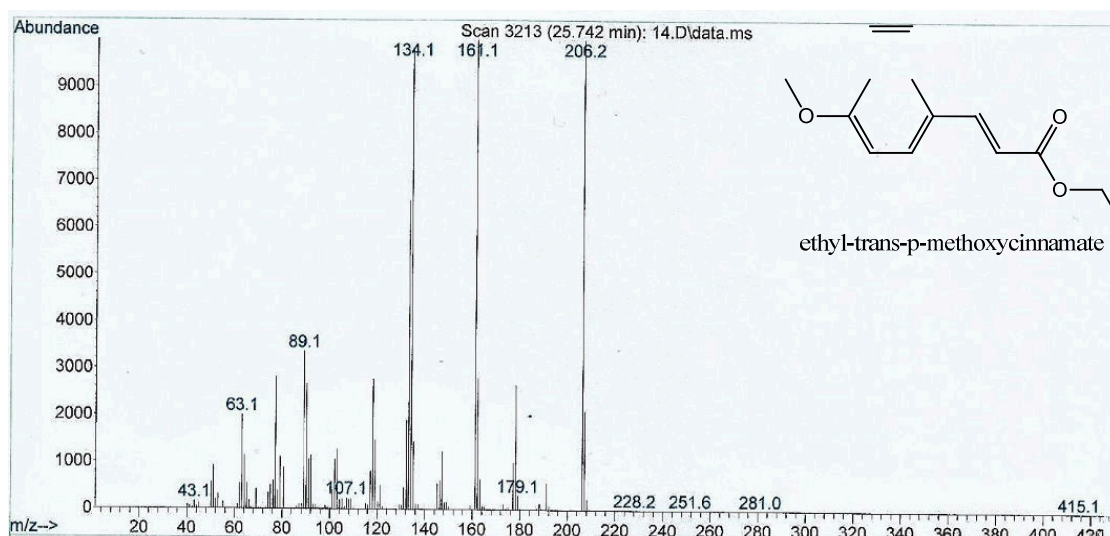


Figure 2. Fragmentation of the most abundant peak with retention time 25.976 (ethyl-*trans-p*-methoxycinnamate) in ethanolic extract and 25.742 in crystals of kencur with an estimated molecular weight of 206.

Based on **FIGURE 1.**, it shows that the peaks in retention times of 25.976 (in ethanol extract of kencur, A) and 25.742 (crystals of kencur, B) dominate the chromatogram pattern. The compound has a molecular weight of 206 with characteristic fragmentation patterns at 134, 161 and 206 m/z as in the mass spectrum of ethanolic extract (A) and crystal of kencur (B). With these data, it shows that the ethanol extract and crystals of kencur are dominated by ethyl-*trans-p*-methoxycinnamate compounds.

Ethyl-*p*-methoxycinnamate is an ester containing a benzene ring and a methoxy group which is nonpolar and contains a carbonyl group that binds to ethyl which is somewhat polar. The crystals were obtained from this extraction (0.71%). According to Indonesian Herb Pharmacopeia (2008)[6], the ethyl-*trans-p*-methoxycinnamate content in 70% ethanol extract of kencur is not less than 4.30%. There is a difference in the yield obtained from crystals with theory because these crystals are produced from a series of extraction procedures with ethanol as a solvent. Ethanol is one of the best solvents to obtain the polyphenol group compound [8]. Thus, it is better if when extracting kencur with a cold maceration process using ethanol it should be more careful so that no ethyl-*trans-p*-methoxycinnamate crystals are left in the residue.

4. Conclusions

In this study, ethyl-*trans-p*-methoxycinnamate was the main compound of kencur ethanolic extract. The crystals produced during the extraction process also show ethyl-*trans-p*-methoxycinnamate purity with one dominant peak. It means that it is effortless to extract this compound using the cold maceration method.

Acknowledgments

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