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**RESEARCH INNOVATION ON MODELING, SIMULATION,
AND ITS APPLICATIONS**

Editors

Dr. Wuryansari Muharini Kusumawinahyu
Danny Prasetyo Hartanto
Rizka Firdausi
Mutya Fani Atsomya

Mathematics Department on Behalf of Faculty of Sciences
Brawijaya University



GC-MS and NMR Analysis of ethyl-p-methoxycinnamate Isolated From *Kaempferia galanga* L Using Distillation Method

Lusiani Dewi Assaat^{1*}, Tun Tedja Irawadi², Irmanida Batubara³

¹ Faculty of Mathematics and Natural Sciences Education, Sultan Ageng Tirtayasa University, Serang, Indonesia, assaat@yahoo.com

² Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agriculture University, Indonesia, tun_tedja@yahoo.com

³ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Bogor Agriculture University, Indonesia, imebatubara@gmail.com

Abstract

The purposes of this research were to isolate and analyze ethyl-p-methoxycinnamate which is the main component of *Kaempferia galanga* L. Volatile oil of *K. galanga* L obtained by distilled fresh rhizome using simple distillation method. The main components of *K. galanga* L volatile oil were ethyl-p-methoxycinnamate, ethyl cinnamate, and δ -3-carene. The ethyl-p-methoxycinnamate compound analyzed using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). The result of GC-MS analysis showed that ethyl-p-methoxycinnamate is the main component in the volatile oil (26.4%). The structure then confirmed using ¹H-NMR, COSY, HMQC, and HMBC analysis in NMR.

Keywords: ethyl-p-methoxycinnamate, GC-MS, NMR

I. INTRODUCTION

Kencur (*Kaempferia galanga* L) is one of the rhizomes commonly used by people of Indonesia to treat swelling, rheumatism, cough, abdominal pain, expectorant, bacterial infection, and used as ingredients for preparing 'Jamu', a local health tonic [1]. The main components of kencur volatile oils are pinene (1.28%), camphene (2.47%), carvone (11.13%), benzene (1.33%), eucalyptol (9.59%), borneol (2.87%), methyl cinnamate (23.23%), pentadecane (6.41%) and ethyl-p-methoxycinnamate (31.77%) [2].

Recently a lot of research established to led the utilization of the main components of *K. galanga* L, such as ethyl-p-methoxycinnamate. Ethyl p-methoxycinnamate could inhibit the proliferation of HepG2 cells in a dose-dependent manner by inducing cells to enter into apoptosis. Therefore, it is important to choose the method how to isolate ethyl-p-methoxycinnamate from *K. galanga* L. Supercritical CO₂ extraction method was used to isolate ethyl p-methoxycinnamate with yield about 2.50% [3].

In this study, the distillation method used to isolate ethyl p-methoxycinnamate. By using this method on *K. galanga* L rhizome, essential oil from *K. galanga* L will be obtained which is expected to contain ethyl p-methoxycinnamate with the highest yield. On other hand, distillation method is a simple method because it is only use water as a solvent, instead of the other chemical solvents. By using the water solvent is expected that isolated compound

have a high level of safety when it will be used as a medicine.

II. MATERIAL AND METHODS

Plant materials

The 1.0-1.5 years old *K. galanga* L rhizome were collected from pasar induk Kramat Djati. The rhizome determined by Herbarium Bogoriense, Biology research center LIPI, Bogor, Indonesia.

Preparation of *K. galanga* L distillate

About 500 g *K. galanga* L rhizome washed and cut into small pieces. The rhizomes were distilled for 4 hours. During the process, solvent temperature is set at 95-105 °C.

Water vapor will carry the components of volatile oil, then the essential oil is collected. From the essential oil and residue, we can collect a white crystals after left over night. This white crystals collected in the bottles for further analysis.

Identification of compound

The chemical components of white crystals was determined by Agilent Technologies 6890 Gas Chromatograph with Auto Sampler and 5973 Mass Selective Detector and Chemstation data system. The operating parameters were as follows: Column: HP 5 WAX. Ionization mode EI; electron energy 70 eV; Capillary Column 30 m x 0.25 mm x 0.25 μ m Film Thickness; interface temperature 280°C; ion source temperature 280°C; inject volume 1 μ l; column

temperature 60°C-240°C. The spectra were recorded and compared with the terpene library.

Compound were identified by $^1\text{H-NMR}$, COSY, HMQC, and HMBC. Methanol- d_3 was used as the NMR solvent. These NMR measurements was performed by using JEOL EC600NMR.

III. RESULT AND DISCUSSION

Distillation process of *K. galangal* rhizome resulted *K. galanga* L essential oil. In cold conditions, a white crystal formed in the essential oil. Its yield of white crystal is about 0.28%. the white crystals were collected in a vial for further analysis. GC-MS spectra showed that white crystal is a pure compound (Fig 1).

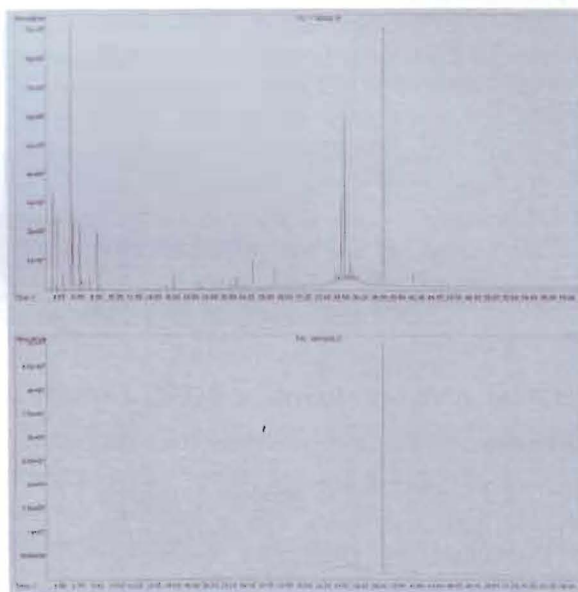


Fig.1. The spectra for *K. galanga* L essential oil (upper) and white crystal (down).

GC-MS spectra showed that main compound essential oil were ethyl-*p*-methoxycinnamate, ethyl cinnamate, and δ -carene (upper). Chromatogram peak from white crystals spectra showed at retention time 38,44 min. GC-MS:EI;m/z :206 (M-1) (down). Result of mass spectrum analysis compared with library index mass spectrum. Therefore, the analysis was continued using *Nuclear Magnetic Resonance* (NMR).

White crystals analyzed using $^1\text{H-NMR}$, COSY, HMQC, and HMBC. White crystals: $^1\text{H-NMR}$ δ :6.3176 (1H, d, J=16.5Hz H-2), δ : (1H, d, J=16.5Hz H-3), δ :7.4908 (2H, d, J=6.9Hz H-5, H-9), δ :6.9045 (2H, d, J=6.9Hz H-6, H-8), δ :3.7861 (3H, s, H-10), δ :4.1898 (2H, k, H-11), δ :1.2807 (3H, t, H-12). $^{13}\text{C-NMR}$ δ :167.74 (C-1), δ :114.97 (C-2), δ :144.56 (C-3), δ :127.01 (C-4), δ :129.59 (C-5), δ :114.08 (C-6), δ :161.79 (C-7), δ :114.08 (C-8), δ :129.59 (C-9), δ :54.52 (C-10), δ :60.12 (C-11), δ :13.30 (C-12).

Overall analysis is summarized in the following table:

Table. 1. The result of $^1\text{H-NMR}$ spectrum analysis

No Carbon	Chemical shifts (δ , ppm)	Integration	Multiplicity	Coupling constant (J, Hz)
1	-	-	-	-
2	6.3176	1	Duplet	16.5
3	7.5871	1	Duplet	16.5
4	-	-	-	-
5,9	7.4908	2	Duplet	6.9
6,8	6.9045	2	Duplet	6.9
7	-	-	-	-
10	3.7861	3	Singlet	-
11	4.1898	2	Quartet	-
12	1.2807	3	Triplet	-

Below is a figure of a complete analytical result analysis of ethyl-*p*-methoxycinnamate.

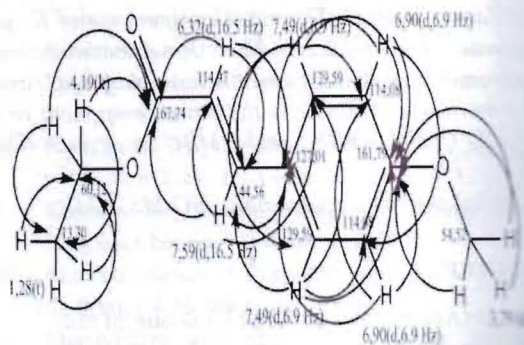


Fig. 2. The result of $^1\text{H-NMR}$, COSY, HMQC, and HMBC

IV. CONCLUSION

Ethyl-*p*-methoxycinnamate compound could be isolated from *K. galanga* L (26.4%) using distillation method. The result of GC-MS and NMR showed that the white crystals obtained from distillation is ethyl-*p*-methoxycinnamate compound.

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