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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 derivates 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 compound of ethanolic extract of kencur and its crystal were obtained during the cold extraction process using (GC-MS). The

precent 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, Indoenesia. The cleaned of kencur fresh rhizomes then dried for a few days and were made into powder.

Extraction

The dried powder of kencur rhizomes (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 amylum. 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 L/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	*trans*-Ethyl cinnamate	5.05	176	176, 148, 131, 103, 77
	22.114	99	Ethyl-*cis*-*p*-methoxycinnamate	2.85	206	206, 161, 134, 77
	22.866	98	Methyl 4-methoxycinnamate	4.02	192	192, 161, 133
	25.976	99	Ethyl-*trans*-*p*-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-*trans*-*p*-methoxycinnamate	96.20	206	206, 161, 134, 77

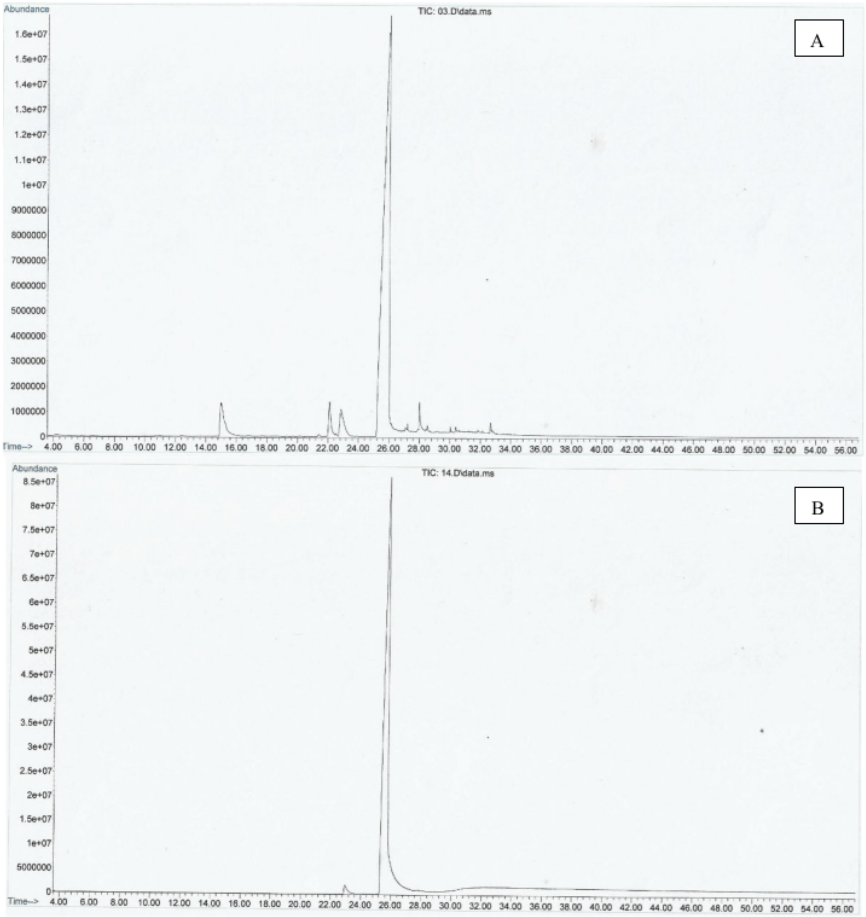

Figure 1. The chromatogram of ethanolic extract (A) and crystal (B) of kencur

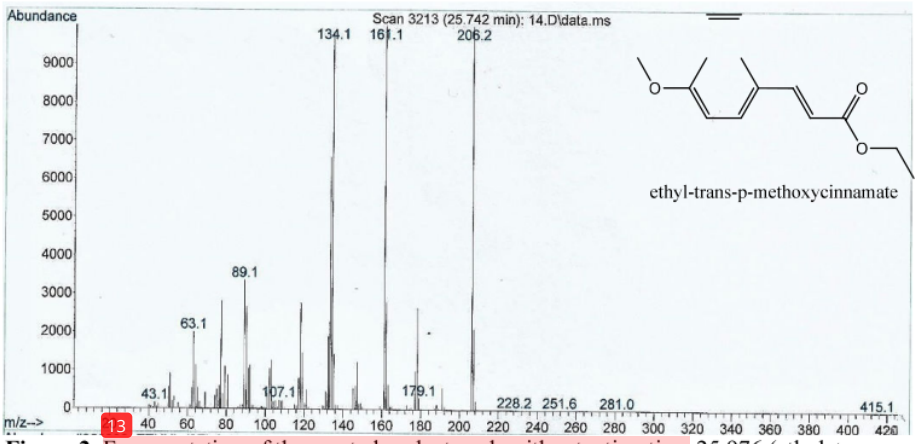

Figure 2. Fragmentation of the most abundant peak with retention time 25.976 (ethyl-*trans-p*-methoxycinnamate) in ethanolic extract and 25.742 in crystals of kencur with an estimated molecular weight of 206.

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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