

ISOLATION OF ETHYL TRANS-*p*-METHOXYCINNAMATE FROM *Kaempferia galanga* L. RHIZOMES BY USING *n*-HEXANE

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Abstract

Kaempferia galanga L. rhizomes contain ethyl trans-*p*-methoxycinnamate as a major secondary metabolite compound and biomarker. Various extraction methods with different solvents can isolate ethyl trans-*p*-methoxycinnamate with potential bioactivities. This paper reported the isolation of ethyl trans-*p*-methoxycinnamate from two varieties of *K. galanga* L. rhizomes by maceration in *n*-hexane. The small and large varieties of *K. galanga* samples produced ethyl trans-*p*-methoxycinnamate in 1.43% and 1.84% yields, higher than other methods and polar solvents of previous research. Structural elucidation of the obtained ethyl trans-*p*-methoxycinnamate was performed by FTIR, MS, and ¹H NMR spectroscopies.

Keywords: ethyl trans-*p*-methoxycinnamate, *Kaempferia galanga* L., maceration, *n*-hexane

Introduction

Kaempferia galanga L., which belongs to the Zingiberaceae family, is an annual herbaceous plant that grows well in humid areas and at ground altitudes of up to 1000 meters above sea level. The distinctive aroma of *K. galanga* L. rhizomes is influenced by secondary metabolites, which are dominated by cinnamic acid derivatives such as ethyl trans-*p*-methoxycinnamate (Figure 1) and ethyl

cinnamate (Nurhaslina *et al.*, 2023). Ethyl trans-*p*-methoxycinnamate is a biomarker of *K. galanga* L. Rhizome, which has been reported to have potential as an anti-inflammatory (Sukkasem *et al.*, 2024), anti-larvacide (AlSalhi *et al.*, 2020), anticancer (Lallo *et al.*, 2022), and vasorelaxant agent (Srivastava *et al.*, 2021).

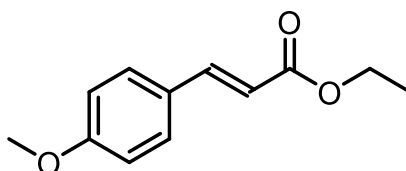


Figure 1. Structure of ethyl trans-*p*-methoxycinnamate

K. galanga L. rhizome extraction can be executed by using various methods such as distillation, percolation, soxhletation, and maceration (Nurhaslina *et al.*, 2023). Literature studies reveal that

water distillation of *K. galanga* L. rhizome from several regions in India for 4-6 hours produces 0.9–1.6% essential oil with 37.35–52.54% ethyl trans-*p*-methoxycinnamate (AlSalhi *et al.*, 2020;

Lal *et al.*, 2023; Srivastava *et al.*, 2021). Muderawan *et al.* (2022) reported that the extraction of two types of *K. galanga* L. rhizomes from Bali, Indonesia, using the steam distillation method for 4 hours produced 2.81% and 3.6% of essential oil, respectively, with ethyl trans-*p*-methoxycinnamate levels of 43.37% and 24.93%. Those studies show that the yield of *K. galanga* L. rhizome oil is not positively correlated with the concentration of ethyl trans-*p*-methoxycinnamate (Sulistyaningsih *et al.*, 2023).

The extraction of *K. galanga* L. rhizome using organic solvent was reported to produce ethyl trans-*p*-methoxycinnamate crystals. Suzana *et al.* (2011) obtained ethyl trans-*p*-methoxycinnamate in 1.25% yield from *K. galanga* L. rhizome by percolation with 96% ethanol for 24 hours. Next, *K. galanga* L. rhizome was extracted by soxhletation in ethanol for 2 hours yielding 0.98% ethyl trans-*p*-methoxycinnamate (Hakim *et al.*, 2018). Furthermore, soxhletation of *K. galanga* L. rhizome for 6 hours using *n*-hexane produced ethyl trans-*p*-methoxycinnamate in 0.33% yield (Rasyid *et al.*, 2022).

The extraction of *K. galanga* L. rhizomes using the maceration method was carried out in methanol, ethanol, and ethyl acetate. Hudha *et al.* (2015) reported the extraction of *K. galanga* L. rhizomes by maceration in ethanol at 99% and obtained ethyl trans-*p*-methoxycinnamate in 0.138% yield. The extraction with a similar method in ethyl acetate gives the ethyl trans-*p*-methoxycinnamate a 0.55% yield (Komala *et al.*, 2017). Moreover, Winingsih *et al.* (2021) obtained the ethyl trans-*p*-methoxycinnamate in 0.02% yield by macerating *K. galanga* L. rhizomes in methanol. Maceration of *K. galanga* L. rhizomes has also been carried out in *n*-hexane and gave the essential oil (7.93–8.76% yield) with 57.17–60.62% of ethyl

trans-*p*-methoxycinnamate (Muderawan *et al.*, 2022).

Although the yield of ethyl trans-*p*-methoxycinnamate from *K. galanga* L. rhizomes is affected by the type of ecospecies or variety of *K. galanga* L., the choice of the appropriate extraction method and the type of solvent are also very essential. On the other hand, studies on the isolation of ethyl trans-*p*-methoxycinnamate from *K. galanga* L. rhizomes by maceration in *n*-hexane are rarely reported. Therefore, this study aims to evaluate the use of maceration method in *n*-hexane to obtain ethyl trans-*p*-methoxycinnamate from *K. galanga* L. rhizomes. The structure of the isolated ethyl trans-*p*-methoxycinnamate was then established by FTIR, MS, and ¹H NMR spectroscopy techniques.

Research Methods

Materials

K. galanga L. rhizomes were purchased from the local market in Pasuruan, East Java (A1), and Rembang, Central Java (K1), Indonesia. *n*-Hexane, ethanol, and ethyl acetate were in analytical grade and obtained from a commercial supplier (Fulltime, China).

Instrumentation

The purity of the compound was checked by analytical thin layer chromatography (TLC) on pre-coated silica gel 60 F₂₅₄ plates (Merck, Germany), and the spots were visualized by using UV lights at 254 nm (Desaga, Germany). Melting points were tested using the Fisher-Johns Melting Point Apparatus (Fisher Scientific, USA). ¹H NMR spectra were recorded on a Bruker Avance Neo 500 MHz (Bruker, Switzerland) in CDCl₃, with TMS as an internal standard and the chemical shifts being represented in parts per million (ppm, δ values). FT-IR spectra were recorded on the Shimadzu 8400S. LCMS mass spectra were recorded on an Xevo G2-XS Qtof (Waters, USA).

Sample preparation

K. galanga L. rhizomes were sorted and washed under running water. The clean rhizomes were drained, chopped, and air-dried for 5 days. Dried rhizomes were coarsely powdered and saved for the next step.

Extraction and isolation of ethyl *trans-p*-methoxycinnamate

Powdered *K. galanga* L. rhizomes (150 g) were subjected to maceration with *n*-hexane (600mL) for 2×24 hours. The mixture was separated by filtration and the light-yellow filtrate was evaporated from the solvent using rotary vacuum evaporator to obtain brownish yellow extract. The concentrated extract was washed with cold *n*-hexane and stored in refrigerator for initiating the crystals formation. The crystals were then filtered

and washed with cold *n*-hexane, dried at room temperature, and purified by recrystallization from ethanol:*n*-hexane (1:9). The purity of the obtained crystals was confirmed by TLC and melting point. The pure crystals were weighed and analyzed using ¹H-NMR, FTIR, and mass spectroscopies.

Results and Discussion

In this study, the rhizomes of *K. galanga* L. were purchased from two different regions. Sample **A1** was obtained from Pasuruan, East Java with an average altitude of 100–500 meters above sea level, and sample **K1** was collected from Rembang, Central Java (500–750 meters above sea level). The typical **A1** *K. galanga* L. rhizomes are small with a light brown epidermis, while sample **K1** is large *K. galanga* L. rhizomes with a dark brown epidermis (Figure 2).

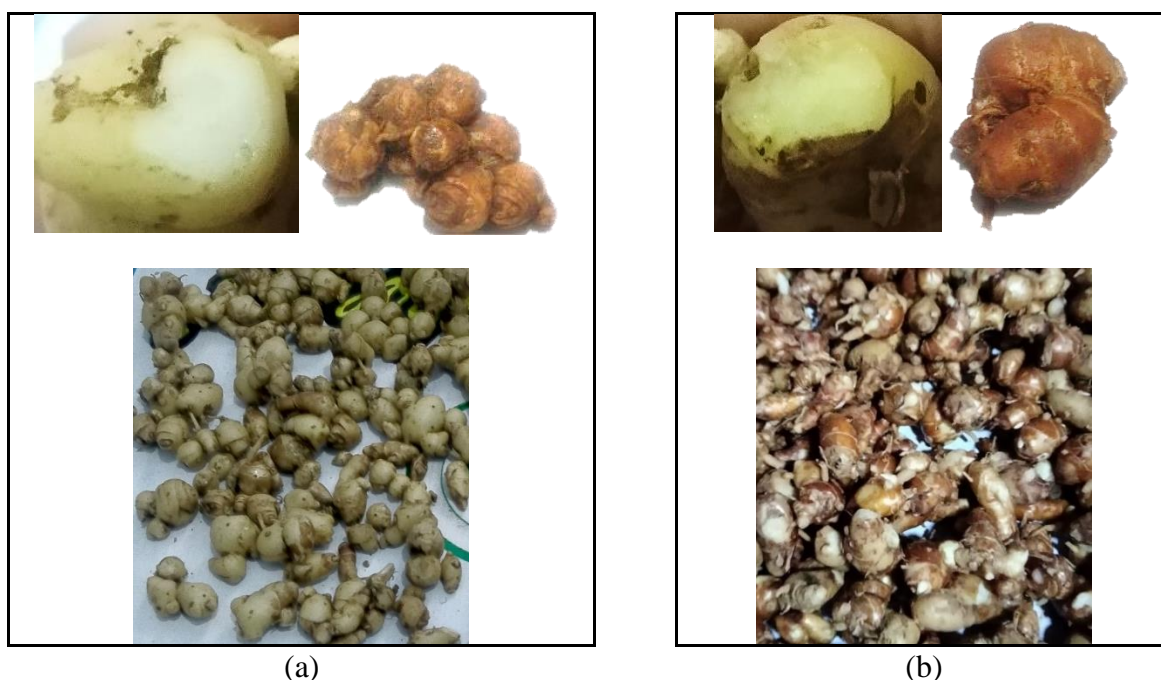


Figure 2. *K. galanga* L. rhizomes. (a) small **A1** and (b) large **K1** *K. galanga* L. rhizomes.

Ethyl *trans-p*-methoxycinnamate was isolated through a simple and economical maceration method in *n*-hexane (Hidayat and Wulandari, 2021; Nuzula *et al.*, 2023; Panjaitan and Yuliana, 2022). Even though it has disadvantages in terms of time, maceration is a suitable method for

extracting thermolabile compounds like ethyl *trans-p*-methoxycinnamate (Winingsih *et al.*, 2021). A non-polar ethyl *trans-p*-methoxycinnamate was reported as completely soluble in *n*-hexane which is also nonpolar. The yield of ethyl *trans-p*-methoxycinnamate may

therefore be higher (Hidayat and Wulandari, 2021).

Briefly, *K. galanga* L. rhizomes powder was soaked in *n*-hexane for 2×24 hours at room temperature. The light-yellow extract was then separated from the solvent until a concentrated brownish-yellow extract was obtained. The concentrated extract was washed with cold *n*-hexane and stored in the refrigerator until the crystals formed,

which were then filtered and washed again with cold *n*-hexane. The crystals were dried at room temperature and purified by recrystallization from ethanol:*n*-hexane (1:9) (Figure 3).

The TLC analysis of the isolated crystals was performed with the *n*-hexane:ethyl acetate (1:1). The results showed the presence of a single spot with a retardation factor (R_f) value of 0.76 for both samples (Figure 4).

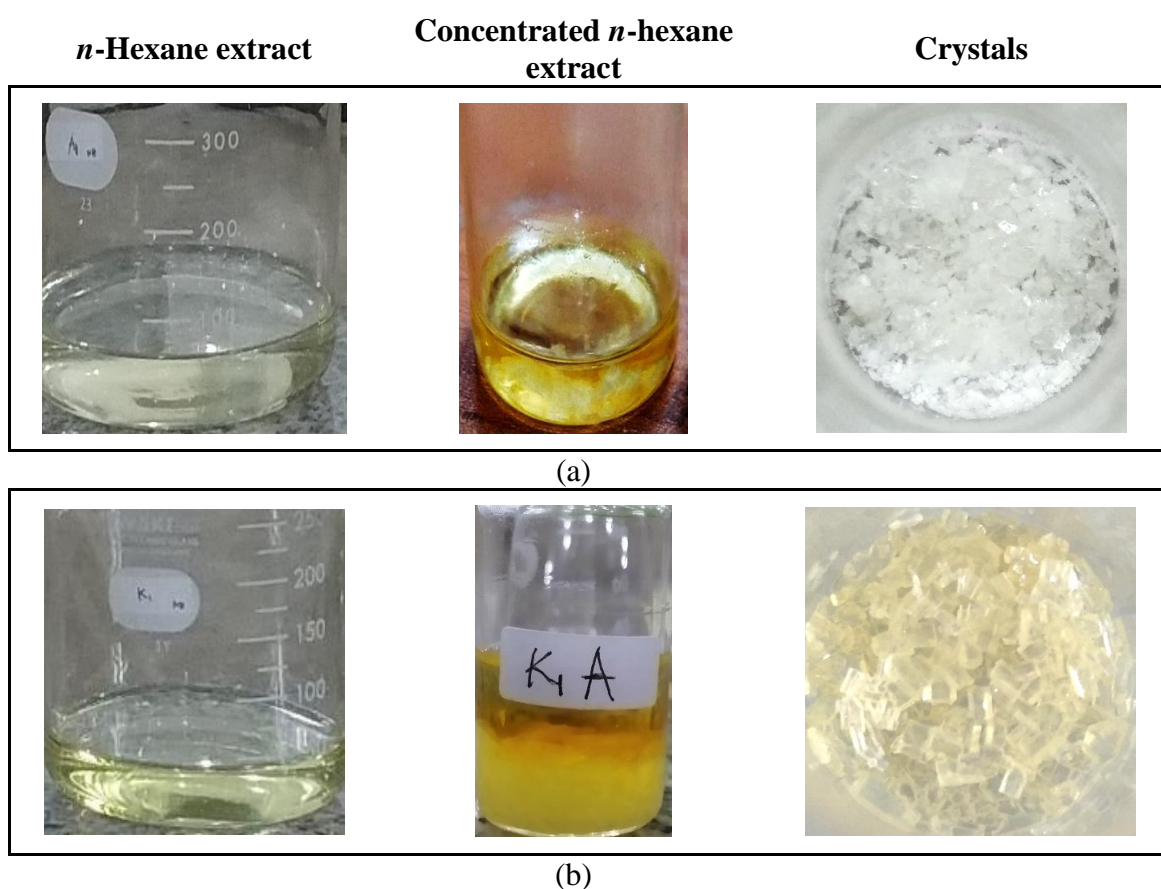


Figure 3. The *n*-hexane extract, concentrated *n*-hexane extract, and crystals obtained from (a) A1 and (b) K1



Figure 4. TLC profile of isolated crystal of (a) A1 and (b) K1

The purity test of the crystals using the TLC with 3 different eluents and two-dimensional (2D) TLC (Figure 5) also gave a single spot indicating that the isolated crystals were pure. This result is supported by the melting point analysis which gives a value of 49–50 °C for **A1**

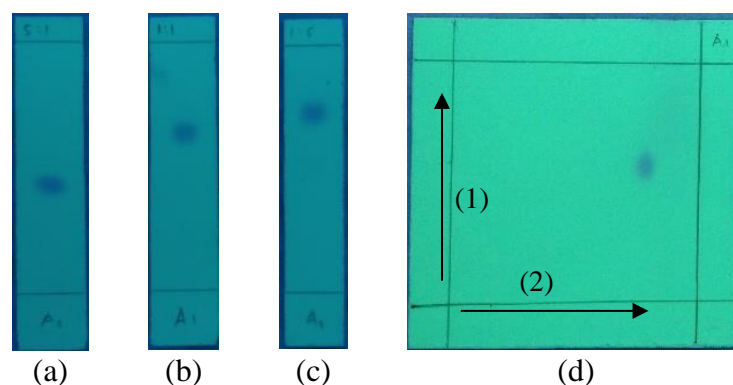


Figure 5. TLC purity check by three different eluents and 2D of isolated crystals. (a) *n*-hexane:ethyl acetate = 5:1, (b) *n*-hexane:ethyl acetate = 1:1, (c) *n*-hexane:ethyl acetate = 1:5, (d) (1) *n*-hexane:ethyl acetate = 3:1, d (2) *n*-hexane:ethyl acetate = 1:1.

Structural identification of the crystal using an NMR spectrometer (CDCl₃, 500 MHz) yields a ¹H NMR spectra as shown in Figure 6. The ¹H NMR spectra show four types of protons: two groups of methyl protons (CH₃), one group of methylene protons (CH₂), two methine (CH) proton groups, and two aromatic proton groups which correspond to the structure of ethyl *trans-p*-methoxycinnamate. The triplets signal with integration 3 at chemical shift (δ) of 1.32 ppm (*J* = 7 Hz) is the CH₃ proton signal, while the singlet signal with integration 3 at δ 3.82 ppm corresponds to the OCH₃ proton. The two integrated quartet signal at δ 4.25 ppm (*J* = 7 Hz, 7 Hz) is the signal for the CH₂ proton. Two CH protons appear as a doublet signal at δ 6.30 ppm (*J* = 16 Hz) and δ 7.63 ppm (*J* = 16 Hz), each with an integration 1. The doublet signals with two integrations each

and 49–51 °C for **K1**. This value corresponds to the melting point of the standard ethyl *trans-p*-methoxycinnamate (National Center for Biotechnology Information, 2024). A narrow range (1–2 °C) of melting point indicates a relatively pure compound (Mohrig *et al.*, 2014).

at δ 6.89 ppm (*J* = 8.5 Hz) and δ 7.46 ppm (*J* = 9 Hz) are aromatic proton signals.

Identification of ethyl *trans-p*-methoxycinnamate structure by NMR was strengthened by infrared (Figure 7) and mass spectra data (Figure 8). The infrared spectra of ethyl *trans-p*-methoxycinnamate show absorption at wave number (ν) 1701 cm⁻¹ for the carbonyl ester group (C=O). The absorption at ν 2981 cm⁻¹ and ν 2937 cm⁻¹ is suitable for C-H sp³. Moreover, the absorption at ν 1628 cm⁻¹ for C=C and at ν 1602 cm⁻¹ for aromatic C-C in the structure of ethyl *trans-p*-methoxycinnamate. The mass spectrum of ethyl *trans-p*-methoxycinnamate shows the molecular ion peak [M+H]⁺ at *m/z* 207.1021 for C₁₂H₁₅O₃, which corresponds to the calculated *m/z* for ethyl *trans-p*-methoxycinnamate of 207.1021.

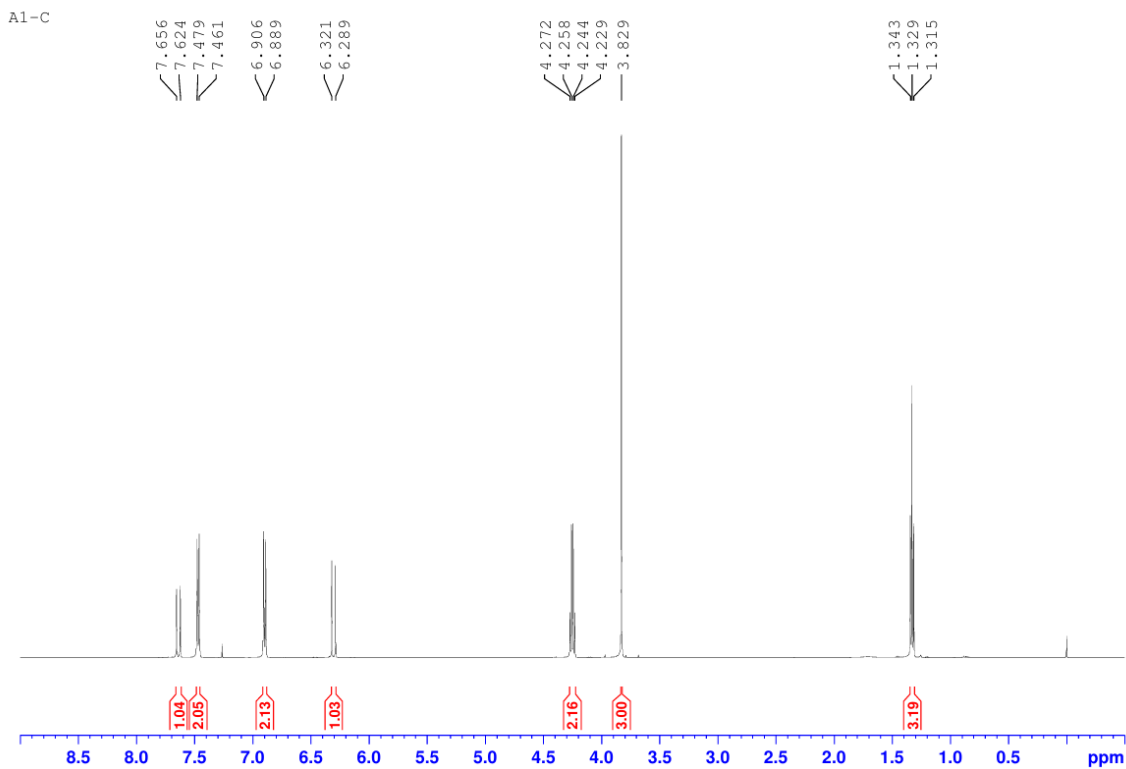


Figure 6. ¹H-NMR spectra of ethyl trans-*p*-methoxycinnamate

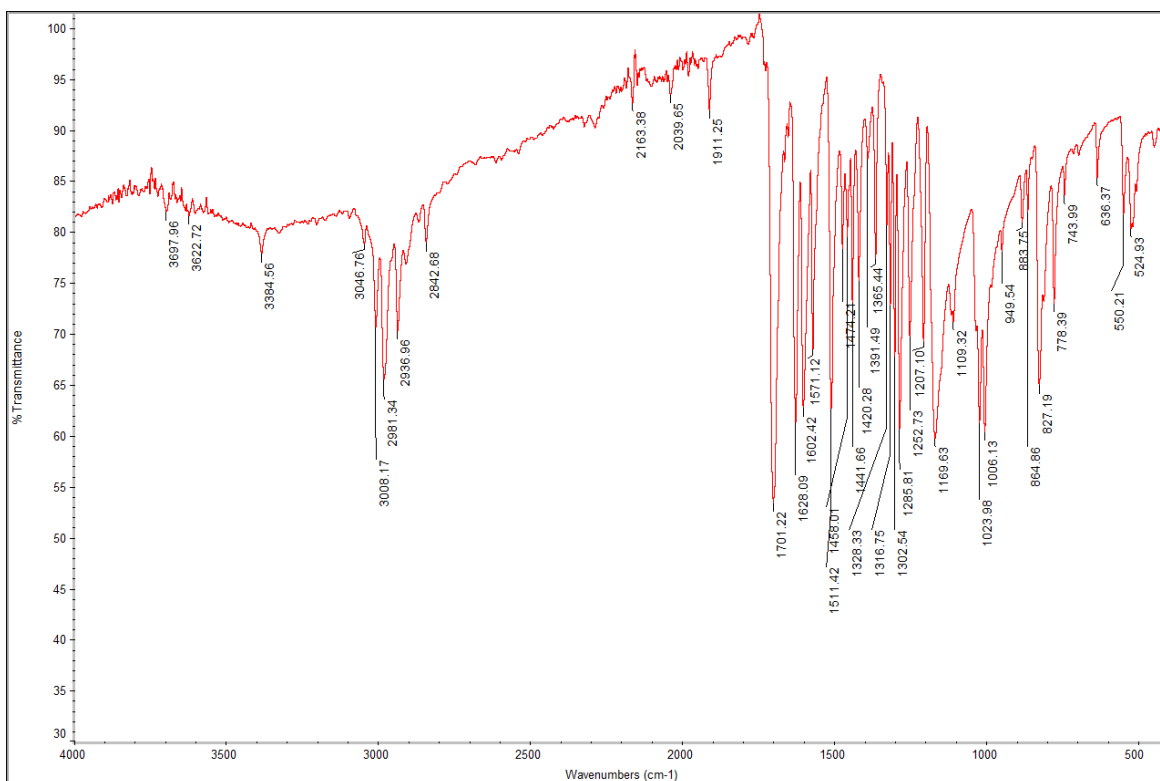


Figure 7. FTIR spectra of ethyl trans-*p*-methoxycinnamate



Figure 8. Mass spectra of ethyl trans-*p*-methoxycinnamate

The isolation of ethyl trans-*p*-methoxycinnamate from rhizomes of *K. galanga* L. using maceration in *n*-hexane has been successfully established. Table 1 tabulating the yields for each sample indicates sample **K1** produced ethyl trans-*p*-methoxycinnamate in a higher yield

compared to sample **A1** by using the same method and solid/liquid ratio. However, the yield for both samples is still within the range of the ethyl trans-*p*-methoxycinnamate in *K. galanga* L. rhizomes reported (0.7–2.5%) (Srivastava *et al.*, 2021).

Table 1. Isolated ethyl trans-*p*-methoxycinnamate from **A1** and **K1**

Parameters	Samples	
	A1	K1
Crystal Mass (g)	2.1435	2.7545
Yield (%)	1.43	1.84
Form	Crystalline Solid	Crystalline Solid
Color	White	Yellowish-white

The ethyl trans-*p*-methoxycinnamate obtained from this study has a higher yield than other reports using methanol (24 hours), ethanol (24 hours), and ethyl acetate (5 days). Those three solvents gave ethyl trans-*p*-methoxycinnamate of 0.35%, 0.14%, and 0.55% yields, respectively (Ahn *et al.*, 2008; Hudha *et al.*, 2015; Komala *et al.*, 2017). *n*-Hexane has also been used in the isolation of ethyl trans-*p*-methoxycinnamate from rhizomes of *K. galanga* L. from Bengal, India, using the ultrasonic-assisted extraction method. The ethyl trans-*p*-methoxycinnamate was successfully obtained in 1.34% yield (Srivastava *et al.*, 2021). The ethyl trans-*p*-methoxycinnamate with 1.25% yield was also produced by the percolation method in ethanol (Suzana *et al.*, 2011). This study demonstrates that maceration in *n*-hexane is a favorable method for extracting ethyl trans-*p*-methoxycinnamate from *K. galanga* L. rhizomes.

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Conclusions

Ethyl trans-*p*-methoxycinnamate was successfully recovered by the maceration using *n*-hexane. Ethyl trans-*p*-methoxycinnamate was obtained as white to yellowish-white crystals in yields of 1.43% (A1) and 1.84% (K1). The structure of ethyl trans-*p*-methoxycinnamate was successfully determined using ¹H NMR, FTIR, and mass spectroscopy.

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