

The Influence of Ginger Rhizome Extraction on the content of the active ingredients [6]-Gingerol Produced

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Abstract. One of the products derived from ginger rhizomes is oleoresin which contains a high concentration of active compounds and has the potential to be used not only as a medicine but also as a beneficial natural ingredient material to human body. Ginger oleoresin is a valuable product that contains several compounds and provides functional health benefits as well as immunity booster in large groups of people. Gingerol is an important component of ginger rhizome oleoresin extract. Gingerol (C₁₇H₂₆O₄) is one of the compounds found in ginger oleoresin that decomposes easily into Shogaols (C₁₇H₂₄O₃). The ethanolic extract of zingiber rhizomes powder was processed under the best yield of oleoresin was 38.313% with concentration of [6]-gingerol was 0.39 mg/g extract, which was produced with conditions of 60 °C extraction temperature in 120 minutes. While the best content of [6]-gingerol in oleoresin was 1.33 mg/g extract with ginger oleoresin yield of 21.821%, which was produced with conditions of 50 °C extraction temperature in 60 minutes.

Key words: Ginger, ginger oleoresin, [6]-gingerol, solid-liquid extraction, yield

1. Introduction

Zingiber officinale is commonly known as ginger, which is a monocotyledon belonging to family Zingiberaceae[1]. Ginger is a plant that originated from Asia and has been harvested from various parts of the world including Jamaica, and the West Indies. Ginger has a slightly spicy flavor and rhizome can be used as the ingredients of traditional medicine [2]. Ginger is a popular base ingredient in traditional beverages in Indonesia[3], [4]. This is due to the presence of 6-gingerol, which plays a role in pharmaceutical and disease prevention due to the presence of carbon chains C1 to C5, which play an important role in exhibiting different pharmacological properties [5], [6]. Gingerol is one of the precious active compounds in ginger root in addition to zingiberene, shogaol, β -bisabolene, α -farnesene, β -sesquiphellandrene, α -curcumene, ginger oil, and resin [7], [8]. Gingerol can be extracted using the liquid-solid extraction method with a specific solvent and temperature.

Extraction is one of the methods used to extract and separate an active compound contained in a material using a specific method based on the active compound to be extracted. The extraction and processing methodologies used are critical for the overall quality of the extract as well as the quantitative measurement of temperature-sensitive major actives present in the extract.

The current development of extraction technology has developed very advanced uses such as extraction with supercritical fluid pressure or better known as Supercritical Fluid Extraction (SFE), extraction using hydrofluorocarbon or refrigerant as the fluid or known as Phytonic Extraction, and the use of enzymes or more known as Enzymatic Assisted Extraction (EAE) [9].

The purpose of this study was to investigate the sonication of oleoresin and the concentration of [6]-gingerol produced.

2. Materials and methods

2.1. Preparation of dry ginger samples

Ginger rhizomes were purchased and harvested from local farmers, Banten province, with the age of harvest 6-7 months, The ginger samples were cleaned, washed, and sliced to thin pieces (2-3 mm), then dried using an oven drier, with the temperature of the drier was 60°C. After being dried for 4-5 days, the samples were ground using a mini grinder and meshed (80 mesh), so that fine ginger powder was obtained.

2.2. Chemical and reagents

Food grade ethanol 96% from a local wholeseller, 6-gingerol standard from Sigma-Aldrich, Methanol and HPLC grade acetonitrile from Sigma-Aldrich, Milli-Q water, Helium Gas for GC-MS.

2.3. Yield of oleoresin obtained

Dried samples of the ginger (20 g) were extracted using ethanol as the solvent, with a solid to the solvent ratio (S/L, % w/v) was 1:7, extraction time of 60-480 minutes. The ethanolic extraction process was carried out using a water bath equipped with a Sonicator (Selecta Ultrasonics, 360 Watt). The received extracts were filtered through Whatman No.41 filter paper to remove powder and then concentrated using a rotary evaporator (Heidolph) with vacuum condition of 7 millibar and a temperature of 22°C. Oleoresin yield was calculated on weight on a percent basis, with the following equation Eq- 1:

$$\text{Yield of oleoresin (\%)} = \frac{m_a}{m_b} \times 100\%$$

Eq- 1

Where, m_a = Weight of dried ginger, g
 m_b = Weight of oleoresin obtained, g

2.4. Ginger oleoresin component analysis

GC-MS analysis was used for determining extract components. The GC-MS analysis was performed on Agilent 7890B GC/MS system with 5977A MSD using a DB-5MS-UI column with dimensions of 30 m x 0.25 μ m x 0.25 μ m. Helium was used as the carrier gas. Temperatures process at 300 °C, with an initial temperature of 40°C and a holding time of 1 minute. The extract's components were identified by comparing their mass spectra to those in the NIST/NBS libraries.

2.5. [6]-gingerol content analysis

Ginger extract was collected from all experiment was analysed using high-performance liquid chromatography (HPLC) for quantifying the content of [6]-gingerol. Prior to analysis, samples and standard solutions were prepared and filtered through a syringe filter membrane (0.45 μ m nylon-66) from All-pure Inc. The [6]-gingerol analysis method using HPLC was performed under the following conditions: The HPLC systems (ShimadSIL-20AHT-UFLC) connected to a photodiode array detector (SPD-20A), equipped with a C18 HPLC column (4.6 x 150 mm) with a 5 μ m particle size from YMC.

Mobile phase A: Water and B: Acetonitrile were used in gradient mode as follows: A/B (45/50) for 0 min, A / B (39/61) for 3 min, and A / B (45/55) for 10 min, and finally hold for 5 min. The total run time analysis was performed for 15 mins. At a 1.0 ml/min flow rate, the chromatogram was recorded at 280 nm.

HPLC conditions and preparation of the [6]-gingerol standard: The [6]-gingerol standard (5 mg) was added to HPLC grade methanol to produce a stock solution of 100 µg/ml. A serial dilution of 6.25; 12.5; 25; 50, and 100 µg/ml working standards were made from the stock solution. All this solution was stored at 4 ° C until used [10].

The oleoresin extracts were analyzed using Waters-HPLC systems, photodiode-array detector (PDA), and empower three chromatography data software. The quantification of [6]-gingerol was obtained after comparing the standard calibration curve of [6]-gingerol. All measurements of the extracted experiment sample were injected and compared to the standard calibration of [6]-gingerol.

2.6. Gingerol Extraction Kinetics by Mathematical Modelling

[6]-gingerol extraction kinetics was assumed to follow the second-order kinetic model, which usually used in the process of solid-liquid extraction[11]–[13]. The mathematical expression of the model is shown in **Error! Reference source not found.**:

$$\frac{dC_t}{dt} = k(C_s - C_t)^2 \quad \text{Eq- 2}$$

Where k is the extraction rate constant of second-order kinetic, C_s is the capacity of extraction or extract at saturation concentration, C_t is extract concentration at any time. If $t = 0$, the value of C_t is 0, a new equation, **Error! Reference source not found.**, obtained from Eq- 2 integration, as follows:

$$C_t = \frac{C_s^2 kt}{1 + C_s kt} \quad \text{Eq- 3}$$

Eq- 3 Linearization is shown from **Error! Reference source not found.**, as follows:

$$\frac{t}{C_t} = \frac{t}{kC_s^2} + \frac{t}{kC_s} \quad \text{Eq- 4}$$

Since the initial rate of extraction (h) as C_t/t , when $t = 0$, so the h can be determined with Eq- 5, as follows:

$$h = kC_s^2 \quad \text{Eq- 5}$$

All experimental data were fitted in to the equation models, while coefficient of determination (R^2) was used as a criterion for adequacy of fit. Estimation of activation energy (E_a) in the extraction process is crucial. The activation energy of gingerol extraction was obtained by relating the kinetic model shown in Eq- 4 with the Arrhenius equation, shown in **Error! Reference source not found.** and **Error! Reference source not found.**, as follows

$$h = C^{-E_a/(RT)} \quad \text{Eq- 6}$$

$$\ln k = -\frac{E_a}{R} \left(\frac{1}{T} \right) - \ln C \quad \text{Eq- 7}$$

Where C is the concentration of gingerol, k is the constant of kinetic rate, E_a is the activation energy, R is the constant of ideal gas, and T is the temperature of extraction. The slope obtained from data fitting was E_a/R , and E_a was determined by multiplying the slope with R (8.314 J/mol K).

3. Results and Discussion

3.1. Yield of oleoresin

Table 1 and Figure 1 summarize the findings found in studies conducted on the effect of the sonicator on the oleoresin and [6]-gingerol. Table 1 shows the effect of temperature and reaction time on the oleoresin yield obtained, with the highest oleoresin yield obtained at temperature of 60 °C and extraction time of 120 minutes, with the acquisition of oleoresin yield reaching 38.313%.

Table- 1. The yield of ginger oleoresin (%)

The temperature of Extraction, (° C)	Time of Extraction, (minutes)	The yield of Ginger Oleoresin (%)
40	60	17.036
	120	21.847
	180	23.498
	240	19.563
50	60	21.821
	120	25.969
	180	23.348
	240	30.010
60	60	20.395
	120	38.313
	180	22.631
	240	30.335

Figure 1 shows the yield extraction comparison between extraction temperature at certain extraction time. While 60 minutes extraction time was conducted, the highest temperature for yield extraction is at 50 °C. When extraction time was at 120 minutes, the best temperature for yield extraction is at 60 °C. At 180 minutes extraction time, the chart shows that 40 °C has the highest yield of extraction. Extraction temperature of 60 °C has the highest yield of extraction at 240 minutes extraction time.

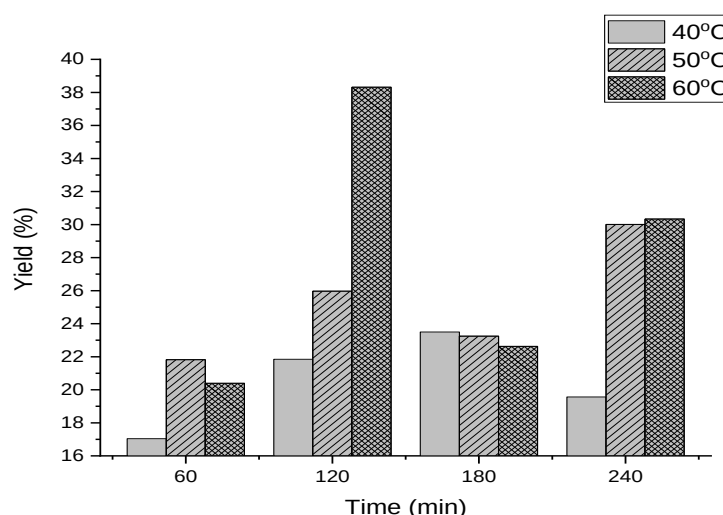


Figure 1. The yield of extraction bar chart

3.2. The component analysis of ginger oleoresin

The components of ginger oleoresin are determined using GC-MS. The extract's composition was quite complicated, as expected, with many components resolved using GC-MS.

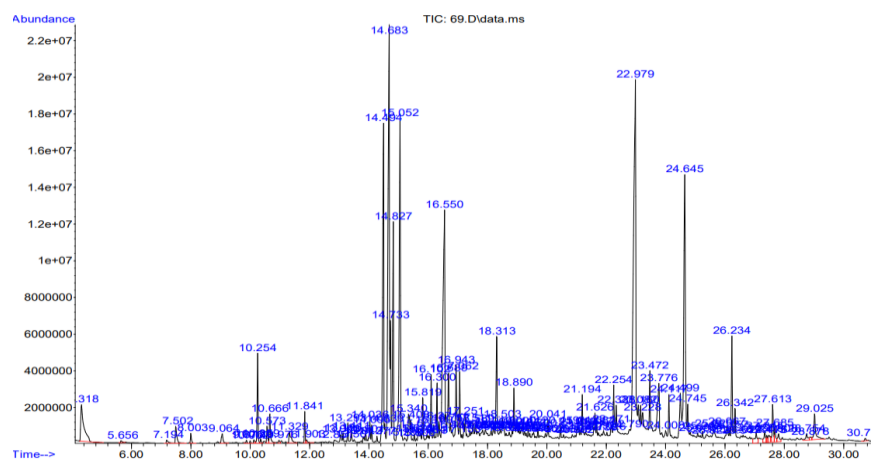


Figure 2. GC-MS Chromatogram of ginger oleoresin extract

GC-MS analysis commenced, resulting 136 peaks identification of the extract, which are shown in Figure 2. The retention time of [6]-gingerol is shown in chromatograms ranging from 6.00-30.00 minutes. All the data obtained from the experiment analysis then were listed into table and complex compounds with high quality value were selected. The compounds' nomenclatures were in IUPAC format. Therefore, NIST/NBS libraries was used to translate the nomenclatures into trivial format. Table-2 represents the component of ginger oleoresin extract.

Table- 2. Component of ginger oleoresin extract [14]

No	Component	Retention Time	No	Component	Retention Time
1	δ -Curcumene	14,686	15	Xanthorrhizol	17,585
2	α -Farnesene	14,737; 16,01	16	Aminosalicyclic Acid	18,997
3	β -Bisabolene	14,825	17	Camphorsulfonic acid	19,513
4	β -Sesquiphellandrene	15,052	18	Humulane-1,6-dien-3-ol	19,551
5	α -Amorphene	15,342	19	Gerany-p-cymene	19,702
6	Khusimone	16,186	20	Geranyl octadecaic	26,344
7	(1R,4R,5S)- α -Acoradiene	16,299	21	Geranyl linoleate	27,617
8	ι -Gurjunene	16,375	22	Citronellyl linoleate	27,34
9	Zingerone	16,552; 17,698; 17,837; 18,127; 18,429; 19,249; 19,299; 20,559; 20,862; 20,95; 21,744; 21,908; 22,021; 22,79; 23,08	23	Palmitic acid	20,043

No	Component	Retention Time	No	Component	Retention Time
10	Natural β -Eudesmol	16,69	24	Shogaol	23,156; 23,231; 24,012; 25,386; 25,525; 25,626; 26,231
11	cis-Thujopsene	16,753	25	Gingerol	22,336; 24,504
12	γ -Curcumene	16,942	26	Hydrocinnamic acid	24,113
13	Schyobunol	17,131	27	[6]-Gingerdiol 3,5-diacetate	24,643; 29,029
14	β -Curcumen-12-ol	17,245			

3.3. [6]-Gingerol content analysis

The content of [6]-gingerol in ginger oleoresin is measured using HPLC. Ginger oleoresin were diluted in acetonitrile until 100 ppm (w/v) concentration. Diluted ginger oleoresin filtered, then 2 mL of filtered samples were injected into HPLC compartment vials. The raw data obtained from HPLC were area values, area values data were processed with standard curve that was prepared before the analysis resulting [6]-gingerol content in ppm inside the vials. The calculation to determine the [6]-gingerol content in extract [6]-gingerol content in mg/g extract follows Eq- 8:

$$[C]_{Extract} = C_{vials} \times V \quad Eq- 8$$

Where $[C]_{Extract}$ is [6]-gingerol content in extract (mg/g), C_{vials} is [6]-gingerol content in ppm inside the vials, and V is solvent volume used for dilution in litre. The result of content analysis is as follows:

Table- 3. [6]-gingerol content analysis result

The temperature of Extraction, ($^{\circ}$ C)	Time of Extraction, (minutes)	[6]-Gingerol Content (ppm)	[6]-Gingerol Content in extract [mg/g]
40	60	5.945	0.32
	120	7.056	0.44
	180	6.713	0.28
	240	2.775	0.14
50	60	17.481	1.33
	120	6.350	0.53
	180	6.061	0.28
	240	5.735	0.30
60	60	9.464	0.62
	120	5.013	0.39
	180	5.901	0.26
	240	7.127	0.61

3.4. Gingerol Extraction Kinetics by Mathematical Modelling

Data obtained from HPLC analysis then fitted into the kinetics modelling equations. Figure 3 to Figure 5 represents resulting kinetics curve from fitted concentration data in modelling equations. Figure 3 shown fitted data into 0th order kinetics model. The linear equation of the 0th order kinetics is $y = 7E-05x + 0.0081$, and R^2 value of 0.967 was obtained.

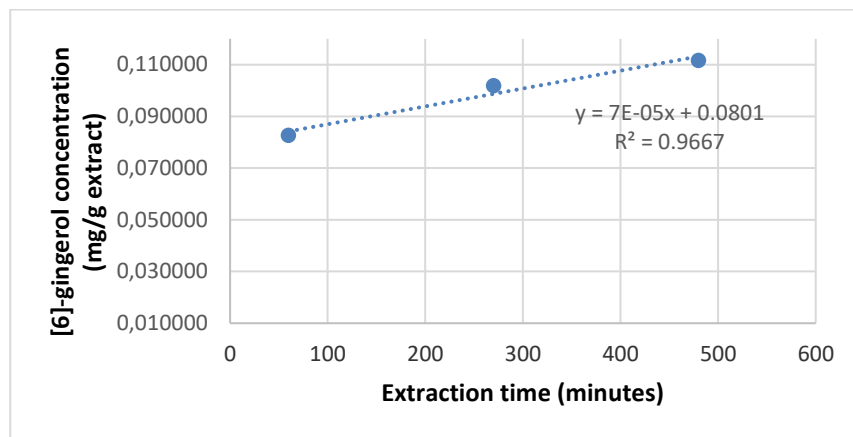


Figure 3. 0th order kinetics model curve

Figure 4 represents fitted data into 0th order kinetics model. The linear equation of the 1th order kinetics is $y = 7E-04x - 2.5166$, and R^2 value of 0.9526 was obtained.

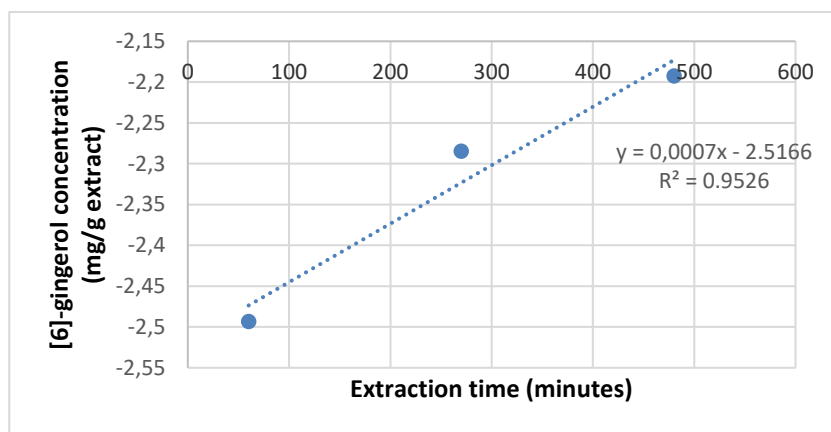


Figure 4. 1st order kinetics model curve

Figure 5 represents fitted data into 2nd order kinetics model. The linear equation of the 2nd order kinetics is $y = 7.5E-04x - 12.312$, and R^2 value of 0.937 was obtained.

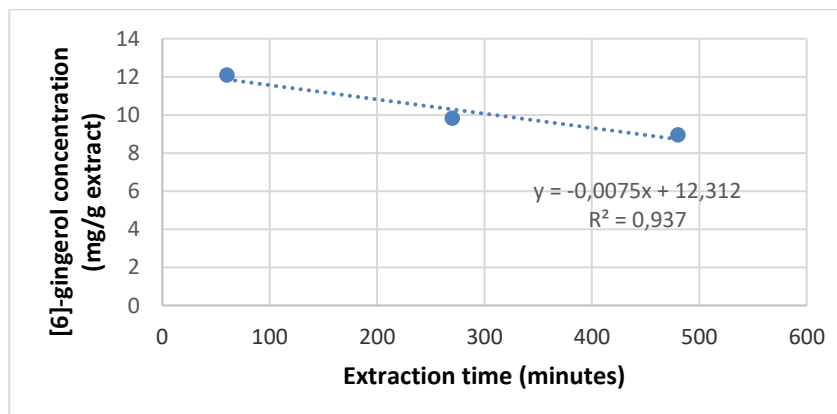


Figure 5. 2nd order kinetics model curve

From the R^2 values which were obtained from the kinetics order curve linear equation, the extraction process in this study follows 0th order kinetics

4. Conclusions

Immersion extraction using ethanolic solvent was performed with sonication. From the results of experiments that have been carried out the experimental extraction, the best yield of oleoresin was 38.313% with concentration of [6]-gingerol was 0.39 mg/g extract, which was produced with conditions of 60 °C extraction temperature in 120 minutes. While the best content of [6]-gingerol in oleoresin was 1.33 mg/g extract with ginger oleoresin yield of 21.821%, which was produced with conditions of 50 °C extraction temperature in 60 minutes. Also, extraction process follows the 0th order kinetics model.

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