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Gingerol and shogaol on red ginger rhizome (*Zingiber officinale* var. Rubrum) using high-performance liquid chromatography

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ABSTRACT

Red ginger (*Zingiber officinale* var. Rubrum) is one of the traditional medicines of the Zingiberaceae family, which contains phenolic ketone compounds, including gingerol and shogaol. At high temperatures, gingerol compounds become unstable and will change into shogaol. This study optimized conditions for the simultaneous separation of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol from the red ginger extract using the High-Performance Liquid Chromatography method. The analytical conditions consisted of five different methods by modifying the composition of the mobile phase, the elution system, the flow rate of the mobile phase and the optimum UV wavelength. The best conditions for the simultaneous separation using a ratio of water: acetonitrile mobile phase in method 4 with a gradient elution system including 0 min (65:35); 1.5 min (40:60); 5-6.5 min (10:90); 7.5–9 min (0:100); 9.5–12 min (65:35); the mobile phase flow rate was 1.1 mL/min at an optimum wavelength of 230 nm with a retention time of 6-gingerol compound, 6-shogaol, 8-gingerol and 10-gingerol respectively were 4.947; 6.168; 6.554; and 7.412 min and its resolution were 2.267; 1.575; 1.315; and 2.215. Then the tailings factor and asymmetry values were obtained with an average value of ± 1.

Keywords: gingerol, HPLC, red ginger, shogaol

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INTRODUCTION

Red ginger *(Zingiber officinale* var. Rubrum*)* is one of the traditional medicines of the Family Zingiberaceae, which has been very commonly used as an herbal ingredient since ancient times because it contains many properties such as anti-inflammatory, antiplatelet, antitumor, antihyperglycemic, antidiabetic and many more (Fadaki et al., 2017). The main compounds contained in ginger are phenolic compounds (gingerol, shogaol, gingerdiol and gingerdione) and other compounds such as Fe, Mg, Ca, Vitamin C, sesquiterpenes, flavonoids and parasols (Pradhita et al., 2012). Phytochemical tests have been carried out on the rhizomes, stems, and leaves of red ginger; the highest levels of flavonoids were found in the rhizomes using a mixture of 96% ethanol and 12 N HCl with a ratio of 98: 2, is 0.0068% (Herawati & Saptarini, 2020).

Gingerol and shogaol are the main compounds of ginger oil, with the amount of gingerol ranging from 23-25% and shogaol ranging from 18-25%. Gingerols are a series of phenolic ketone homologs consisting of 6-gingerol, 8-gingerol and 10-gingerol (Srikandi et al., 2020). At high temperatures, gingerol compounds become unstable and will turn into shogaol. Several types of shogaol compounds exist, including 6-shogaol, 8-shogaol and 10-shogaol. A study previously mentioned that during the extraction and drying process, it was seen that the content of 6-gingerol decreased at 60°C and when the temperature was increased to 80°C, it resulted in the lowest content of 6-gingerol but on the contrary, increasing 6-shogaol content (Ok & Jeong, 2012).

Several analytical methods are used to identify compounds in medicinal plants, including chromatography consisting of TLC, HPLC and GC or spectroscopy including UV-Vis, NMR, FTIR and mass (Purwakusumah et al., 2014). Several compound analysis methods can be used with instruments, one of which is High-Performance Liquid Chromatography (HPLC). The HPLC is a technique for separating organic and inorganic compounds and analysis of impurities, especially for the non-volatile of compounds (Gandjar & Rohman, 2007). This method is very suitable for identifying non-volatile compounds because it can quantitatively identify the bioactive content in ginger extract, especially the content of gingerol and shogaol (Salmon et al., 2012). There are many known methods for separating a compound, among which the most common is Thin Layer Chromatography (TLC). The TLC method separates an organic compound with a low molecular weight according to its polarity (Usama et al., 2022). TLC analysis is still conventionally compared to HPLC, which has a more time-efficient analysis level in recognizing or separating a chemical compound (Navni et al., 2020).

Using the HPLC method, this research simultaneously optimized analytical conditions to separate the 6-, 8-, 10-gingerol and 6-shogaol compounds. The analytical conditions to be studied include the mobile phase composition, the elution system, the mobile phase flow rate, and the maximum wavelength of UV light for detecting compounds.

MATERIALS AND METHOD

Materials

High-Performance Liquid Chromatography (HPLC) Agilent technologies 1260 Infinity II with wavelength PDA/DAD (Diode-array) detector (190-400 nm), Inertial ODS Column (4,6 mm, 150 mm x 5µm), Mixed standards of 6-gingerol (100%), 8-gingerol (99.1%), 10-gingerol (99.0 %) and 6-shogaol (98.1%) (Sigma), acetonitrile HPLC grade (Merck), methanol HPLC-grade (Merck), phosphoric acid buffer solution 80%. The plant materials were collected from Kalbe Ubaya Hanbang-Bio Laboratory and authenticated by the Center for Traditional Medicine Information and Development (Pusat Informasi dan Pengembangan Obat Tradisional), Faculty of Pharmacy, University of Surabaya.

Methods

Preparation of red ginger extract

Fresh red ginger rhizome was washed, sliced ± 3-4 mm, then dried by aerating and oven at 55°C for ± 3 hours. The next step was to measure the moisture content with a *moisture analyzer* 3 times replication by weighing ginger crude drug ± 1 g; then, the tool was set at a temperature of 105°C, the time was set to auto, and it waited for constant weight (Ok & Jeong, 2012). After that, the crude drug was mashed

with a blender and sieved using a 60-mesh sieve. 5 g of red ginger rhizome powder was added with 50 mL of methanol and then extracted using the *Ultrasound-Assisted Extraction* (UAE) method with an *ultrasonic cleaner* for three replications (Murphy et al., 2020). The machine was set at 100 W of electrical power and 40 kHz of frequency for 20 minutes at room temperature. The solution mixture was then filtered using filter paper so that the extract and residue were separated, and then the extract was stored in a closed bottle tightly and kept at 4°C before further process the next day. Red ginger extract was put into a 100 mL rotary flask and concentrated using a *rotary evaporator*. After reducing the extract volume, it was transferred to a porcelain cup. The cup was weighed while empty, and the result was noted. The extract was then put into the cup and evaporated to 50°C in a water bath until it became thick. The weight of the extract was divided by the weight of the original crude drug in grams, and the result was multiplied by 100% to get the extraction yield.

Preparation of gingerol and shogaol standard solutions

An individual standard stock solution of a pure mixture of 6-gingerol (100%), 8-gingerol (99.1%), 10-gingerol (99.0%) and 6-shogaol (98.1%) with each concentration of 100 ppm was prepared by dissolving it on HPLC-grade methanol; then the solution was filtered with a 0.22 µm PTFE (Polytetrafluoroethylene) membrane filters before inserting it into the HPLC vial.

Preparation of sample for HPLC

Sample solutions were made sequentially, namely, 4000 ppm, 3385 ppm and 3956 ppm. The solution concentration was calculated after dissolving the weight of each replication's previously diluted viscous extract using HPLC-grade methanol in a 100.0 mL volumetric flask. Furthermore, samples were filtered using 0.22 µm PTFE membrane filters and put into a 1 mL HPLC vial.

Data Analysis

Methods of analysis conditions in HPLC

HPLC conditions optimized in this study include the composition of the mobile phase, the elution system, the mobile phase flow rate, and the optimum wavelength of UV light for detection. Optimization was carried out using 5 methods for separating gingerol and shogaol compounds in red ginger rhizome using the HPLC method with a UV detector. There are modifications to the flow rate of the mobile phase in methods 1 and 2; moreover, modification of the mobile phase composition was done in methods 3, 4, and 5 (Table 1).

Determination of gingerol and shogaol optimum UV wavelengths

To determine the optimum UV wavelength for 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol, mixed standard solutions were placed in a single HPLC vial and screened the wavelengths ranging from 190 nm to 300 nm by HPLC DAD detector. The wavelength at which the chromatogram peak was visible at the maximum level was then selected as the optimum UV wavelength.

Calculation of resolution (Rs), tailings factor (TF) and asymmetry (As)

The retention time was studied by separating 6-, 8-, and 10-gingerol compounds and 6-, 8-, and 10-shogaol from red ginger rhizome extract under each HPLC condition of each peak was observed. Resolution can be calculated by the formula in Equation 1. The width of the peak base on gingerol and shogaol compounds and the peak width of other compounds (ΔtR) was then divided by the total width of the peak base (W1 + W2) in gingerol, shogaol and other compounds (Gandjar & Rohman, 2007). Separation at the peak is good if the resolution value reaches ≥ 2 (Snyder et al., 2010).

For the calculation of the tailing factor (TF), as shown in Equation 2, it is first calculated at 5% of the peak height then, followed by the width of the peak on the left side (A) and the right side (B), after which both are added up and divided by twice the width of the peak on the left side (2A), as shown in Figure 1.

Table 1. HPLC condition method optimization for separating gingerol and shogaol

Method	Flow Rate (mL/min)	Time (min)	Gradient (A:B)	Eluent	UV Wavelength (nm)
1	1.0	0 - 5.0 5.0 - 18 18 - 29.5 29.5 - 30.5 30.5 - 38	40:60 22:78 22:78 0:100 0:100	Water + Buffer Phosphoric Acid (A) : Acetonitrile (B)	282
2	1.1	0 - 5.0 5.0 - 18 18 - 29.5 29.5 - 30.5 30.5 - 38	40:60 22:78 22:78 0:100 0:100	Water + Buffer Phosphoric Acid (A) : Acetonitrile (B)	282
3	1.1	0 - 1.5 1.5 - 1.8 1.8 - 5 5 - 6.5 6.5 - 9 9 - 9.1 9.1 - 12	65:35 40:60 40:60 0:100 0:100 65:35 65:35	Water (A) : Acetonitrile (B)	230
4	1.1	0 1.5 5 - 6.5 7.5 - 9 9.5 - 12	65:35 40:60 10:90 0:100 65:35	Water (A) : Acetonitrile (B)	230
5	1.1	0 1 3 4 - 6.5 7.5 - 9 9.5 - 12	65:35 40:60 30:70 10:90 0:100 65:35	Water (A) : Acetonitrile (B)	230

$$\boldsymbol{Rs} = \frac{\mathbf{2}\Delta \boldsymbol{t_R}}{(\boldsymbol{W_1}+\boldsymbol{W_2})} \quad \textbf{(1)}$$

$$\textbf{Tf} = \frac{\boldsymbol{A}+\boldsymbol{B}}{\mathbf{2}\boldsymbol{A}} \quad \textbf{(2)}$$

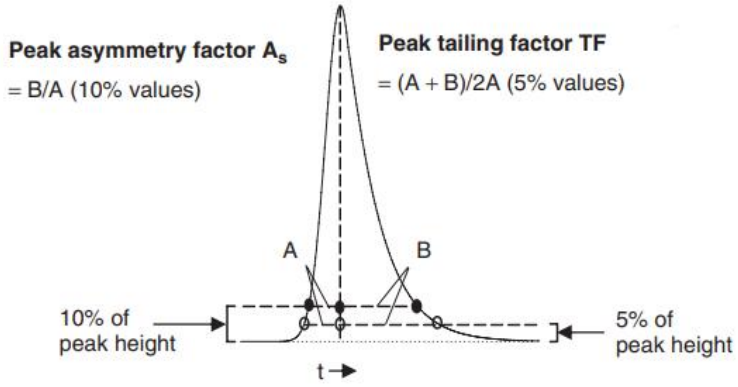

Figure 1. Calculating the chromatogram's Tailings factor and Asymmetry factor (Snyder et al., 2010)

Asymmetry (As) is calculated by determining 10% of the peak height first and then proceeding with the division between the right side (B) and the left side (A) width of the chromatogram peak, as shown in Equation 3 and Figure 1 (Snyder et al., 2010).

$$As = \frac{B}{A} \qquad (3)$$

RESULT AND DISCUSSION

At first, 99.5 g of dried red ginger was obtained (Figure 2A) with a moisture content of 5.23 ± 0.83% (Table 2), which means it has met the drying requirements because the value is not more than 10% (Direktorat Jenderal Kefarmasian dan Alat Kesehatan, 2017). If the water content is still a lot (more than 10%), then crude drug is easily damaged due to microbial growth (Sembiring, 2022). The next step is making crude drug powder. The rhizome is mashed with a blender and sieved using a 60 mesh, obtaining a powder weight of 29.7 g (Figure 2B).

Table 2. The moisture content of red ginger crude drug

Replication	Weight (g)	Moisture content (%)	Average ± SD (%)
1	1.540	4.38	
2	1.514	5.27	5.23 ± 0.83
3	1.567	6.03	

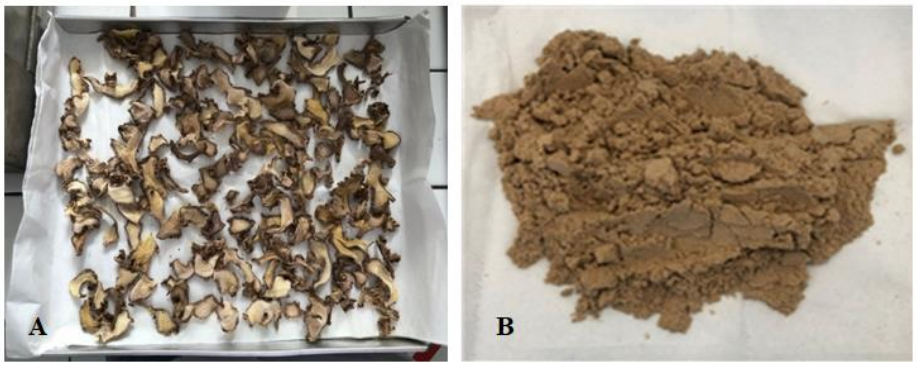

Figure 2. The results of drying red ginger rhizomes (A) and red ginger crude drug powder (B)

The extraction method used in this study is the Ultrasound-Assisted Extraction (UAE) method. It was chosen because it uses modern technology with the help of ultrasonic waves; in addition to its simple operation, time efficiency and temperature can be adjusted depending on the compound to be analyzed (Ok & Jeong, 2012; Vankar & Srivastava, 2010). The resulting filtrate from the UAE was then calculated as the extract yield; the results obtained were the % yield of the replicated thick extract 1, 2 and 3, namely 8.00%, 6.74% and 7.91%, an average of 7.55 ± 0.70% (Table 3). Then, qualitative organoleptic observations were carried out on the concentrated extract, including the color of the thick ginger extract, characteristic odor and the viscosity of the extract (Figure 3). From the results of organoleptic observations; it was found that the extracts obtained were following the criteria of Indonesian Herbal Pharmacopoeia (Direktorat Jenderal Kefarmasian dan Alat Kesehatan, 2017).

Table 3. The yield of red ginger methanolic extract

Replication	Weight of crude drug (g)	Volume of solvent (mL)	Thick extract weight (g)	Yield (%)	Average± SD (%)
1	5.0022	50	0.4000	8.00	
2	5.0229	50	0.3385	6.74	7.55 ± 0.70
3	5.0038	50	0.3956	7.91	

Figure 3. The observation results of thick red ginger extract with methanol as solvent

After the yield of each extract was obtained, a sample dilution solution was made, which would be used for *running* HPLC in the three replications. Then observations were made at UV wavelengths, which were seen in the spectrum of the standard solution having good absorption at λ 200 nm, 224 nm, 226 nm and 282 nm. The initial stage was performed for HPLC analysis, namely dilution of the sample resulting from the extract yield. Then the research continued with determining the optimum wavelength in HPLC using a UV detector. In this study, the wavelength was detected, and then the peaks on the spectrum were λ 200 nm, 224 nm, 226 nm and 282 nm (Liu et al., 2014). The selected wavelengths for optimizing the separation of gingerol and shogaol compounds were 230 nm (Figure 4A) and 282 nm (Figure 4B). The wavelength of 200 nm was not chosen because there is a lot of matrix interference when screening samples with complex matrices. The matrix in ginger samples refers to the complex mixture of compounds present in the sample, which can interfere with detecting gingerols and 6-shogaol. The interference can be caused by other compounds in the sample, such as lipids, pigments, and other phenolic compounds. In previous studies, a comparison of the maximum wavelength of 282/280 according to United States Pharmacopeia (USP) (United States Pharmacopeia, 2023) and International Organization for Standardization (ISO) (International Organization for Standardization, 1997) with the optimized wavelength of 230 nm was done, and the results of 230 nm show higher sensitivity and better peak resolution values (You et al., 2019).

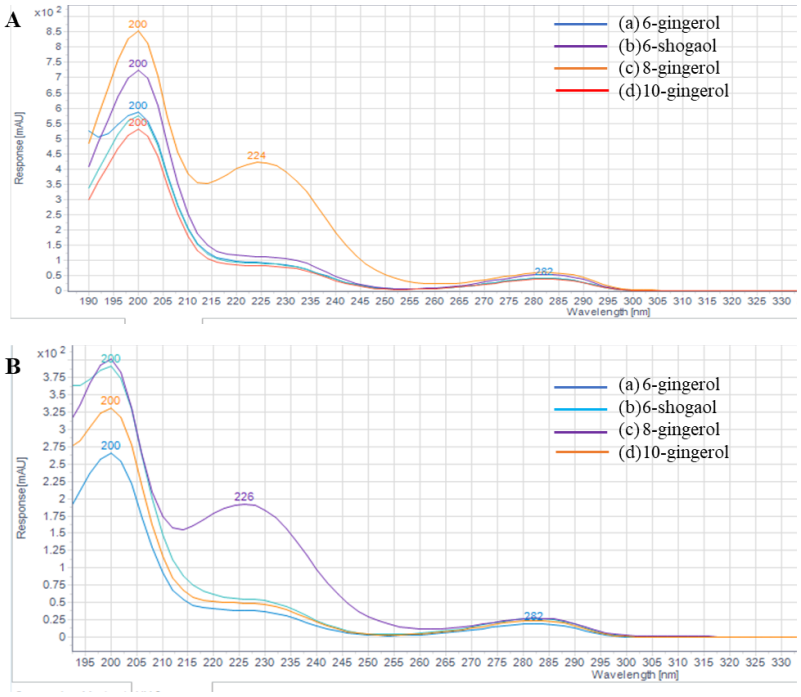

Figure 4. UV spectrum of (a) 6-gingerol; (b) 6-shogaol; (c) 8-gingerol; and (d) 10-gingerol at a wavelength of 230 nm (A) and 282 nm (B)

After determining the wavelength, it was continued with optimization of HPLC conditions for separating a mixture of standard gingerol and shogaol. The conditions used in this study were to modify several methods, including the composition of the mobile phase, the mobile phase flow rate, the mobile phase elution system and the maximum wavelength of the compound (Yang et al., 2017). Furthermore, the separation parameters used in this study were to determine whether a compound was properly separated based on the resolution value, tailing factor and asymmetry factor. The resolution value is said to be qualified if its value (Rs $\geq$ 2), as well as the tailing factor and asymmetry, is said to be eligible if the value has reached As = Tf = 1 or Tf < 1.2 and As < 1.3 (Snyder et al., 2010).

Based on the research that has been done, the experiment was carried out using 5 methods, with each method having different conditions of analysis. For Method 1 and Method 2 (Table 4), as followed by Zhang et al. (2022), with the modification, HPLC was running using different analytical conditions at the flow rate of 1.0 mL/min and 1.1 mL/min (Zhang et al., 2022). Based on the HPLC chromatogram results, the flow rate of 1.1 mL/min (method 2) resulted in a shorter retention time than that of 1.0 mL/min using a detection wavelength of 282 nm. The chromatogram methods 1 (Figure 5A) and method 2 (Figure. 5B) observed that there had not been a good separation for each standard compound of gingerol and shogaol, so HPLC was continued again using the composition of the mobile phase, flow rate and different wavelengths. In Method 1, the retention times for the compounds are reasonable, indicating some separation. However, the resolution values are moderate, suggesting that the peaks may not be well resolved (Figure 5A). The tailing factors and asymmetry values are within acceptable ranges, indicating symmetrical peak shapes. One limitation of this method is the relatively lower resolution, which may impact the accuracy and reliability of compound identification and quantification, likewise the previously reported results (Feng et al., 2014). Method 2 shows slightly shorter retention times than Method 1 (Table 4). The resolution values are comparable, suggesting similar separation efficiency. The

tailing factors and asymmetry values are acceptable, indicating symmetrical peak shapes. However, one limitation is the possibility of overlapping peaks (Figure. 5B), which may affect quantification accuracy if the compounds of interest are not well resolved, similar to previous results (Feng et al., 2014).

Table 4. Optimization of HPLC condition

Method	Compound	Retention time (min)	Resolution (Rs)	Tailing Factor (TF)	Asymmetric (As)
1	6-gingerol	2.991 ± 0.001	0.522 ± 0.416	0.989 ± 0.163	0.977 ± 0.119
	6-shogaol	4.877 ± 0.001	1.492 ± 0.525	0.993 ± 0.151	0.956 ± 0.095
	8-gingerol	5.489 ± 0.001	1.056 ± 0.438	0.929 ± 0.144	0.937 ± 0.129
	10-gingerol	7.546 ± 0.001	1.324 ± 0.615	0.998 ± 0.089	0.986 ± 0.095
2	6-gingerol	2.762 ± 0.001	1.398 ± 0.534	0.988 ± 0.153	0.987 ± 0.134
	6-shogaol	4.538 ± 0.001	1.053 ± 0.379	0.983 ± 0.142	0.976 ± 0.122
	8-gingerol	5.133 ± 0.001	0.798 ± 0.218	0.939 ± 0.184	0.927 ± 0.117
	10-gingerol	7.122 ± 0.002	1.654 ± 0.542	0.995 ± 0.114	0.986 ± 0.125
3	6-gingerol	6.324 ± 0.001	0.798 ± 0.010	1.040 ± 0.011	1.049 ± 0.014
	6-shogaol	8.148 ± 0.001	1.193 ± 0.875	0.919 ± 0.174	0.907 ± 0.139
	8-gingerol	8.431 ± 0.001	0.898 ± 0.318	0.994 ± 0.110	0.906 ± 0.075
	10-gingerol	8.988 ± 0.001	1.761 ± 0.325	1.088 ± 0.049*	1.059 ± 0.023*
4	6-gingerol	4.947 ± 0.001	2.267 ± 0.019*	1.008 ± 0.024*	1.048 ± 0.032*
	6-shogaol	6.168 ± 0.001	1.575 ± 0.144	1.040 ± 0.043*	1.023 ± 0.018*
	8-gingerol	6.554 ± 0.001	1.315 ± 0.141*	1.086 ± 0.014*	1.069 ± 0.014*
	10-gingerol	7.412 ± 0.002	2.215 ± 0.085*	0.913 ± 0.036	0.964 ± 0.042
5	6-gingerol	4.777 ± 0.023	1.684 ± 0.051	1.008 ± 0.030	1.058 ± 0.015
	6-shogaol	5.981 ± 0.003	1.926 ± 0.191*	1.008 ± 0.031	1.078 ± 0.031
	8-gingerol	6.286 ± 0.014	0.946 ± 0.155	1.130 ± 0.061	1.086 ± 0.021
	10-gingerol	7.060 ± 0.013	1.864 ± 0.226	0.936 ± 0.067	0.997 ± 0.059

Asterisk annotation (*) indicates the optimum result, compared between different methods on the same compound, considering each parameter criteria.

The separation of the red ginger extract sample using method 3 is still not optimal because the value of the resolution still does not meet the separation requirements (Figure 5C), namely, for all compounds in the sample, the value of Rs < 2, then the value of the tailings is good because the Tf value in method 3 is close to 1 as a condition for a good Tf value as well as an asymmetry value that is close to 1 and meets the requirements. In the experimental method, 3 compounds/analytes that have met the separation requirements include 6-gingerol, 6-shogaol and 10-gingerol (Table 4). In the 8-gingerol compound, the tailing factor and asymmetry values have met the requirements, but the resolution value is still not eligible, namely RS ≥ 2. The results for method 3 are the retention times of all compounds that appear quite short, in the range of 6 to 8 minutes, which contradicts previous results (Gupta et al., 2013). The average for the results of the resolution values for all replications of method 3 with a value less than 2 means that it does not yet meet the resolution value requirements.

In method 4, optimization was done by changing the mobile phase's composition again, emphasizing adding acetonitrile at each elution time since it has a greater comparison value than in the method. Then, acetonitrile was added at 5 minutes, and the initial ratio of water: acetonitrile was from 40:60 to 10:90. The results of these additions caused the retention time to decrease and the resolution of other compounds to increase as well so that it meets the requirements for the resolution value (Table 4). It was found that the shorter time for each compound was 4 to 6 minutes later for the resolution increased in

the 6-gingerol and 10-gingerol compounds (Figure 5D), namely, the resolution value that exceeded 2 and the value of tailings. The factor appears to increase in compounds that have not previously reached a value of 1. Thus, method 4 demonstrates higher resolution values than Method 2, indicating an improved separation between the peaks. The tailing factors and asymmetry values are generally acceptable, suggesting symmetrical peak shapes. However, one limitation is that the retention times are similar to method 2, which may imply longer analysis time without significant improvement in separation (Foudah et al., 2020; Gupta et al., 2013).

In the last method, or method 5, the retention time decreased more than in the previous method, but the resolution value decreased, and the tailing factor value and asymmetry remained at ±1. The results obtained are not much different from the analysis in method 3 using the same other conditions. The 8-gingerol compound is still not completely separated. It can be seen that the resolution value does not meet the requirements (Table 4). Furthermore, for the results of the resolution value in this method, the average seems to have decreased because the distance between the peaks looks closer (Figure 5E). A decrease in resolution indicates reduced separation between the peaks of interest. Several factors could contribute to this observation, such as column selectivity, flow rate, column temperature and mobile phase composition (Zhang et al., 2022). Considering that the first three factors were the same between methods 3 to 5, then mobile phase composition may be the key reason. The composition of the mobile phase, including solvent type and concentration, can affect the separation of compounds. Changes in the mobile phase composition might alter the interactions between the analytes and the stationary phase, potentially leading to closer elution of peaks and reduced resolution (Snyder et al., 2010).

From the data from the optimization results in Table 4, method 4 was selected as the optimum condition. The reason was due to the resolution values ranging from 1.315 to 2.267, indicating better separation compared to the previous methods; the tailing factors range from 0.913 to 1.086, indicating reasonably symmetrical peaks; and the asymmetry values range from 0.964 to 1.069, indicating relatively symmetrical peaks. However, the retention time of method 4 is not the shortest among all 5 methods. However, it is still faster than previous results (Feng et al., 2014; Foudah et al., 2020; Gupta et al., 2013).

After optimization of the standard solution, further optimization of HPLC conditions was carried out to separate samples of red ginger extract simultaneously. We used method 3, method 4 and method 5 as the reference for the analysis method. When the red ginseng extract was analyzed with those 3 methods, other compounds potentially named 8-shogaol and 10-shogaol were also detected (Figure 6). gingerol and shogaol are the main compound content of ginger oil, with the amount of gingerol ranging from 23-25% and shogaol ranging from 18-25% (Ok & Jeong, 2012). Like the standard solution chromatograms, method 4 gave a shorter retention time, with a good resolution and tailing factor (Table 5). The HPLC chromatogram analysis results of the red ginger extract are presented in the table. Among all methods, Method 4 showed the resolution values range from 1.575 to 2.267, indicating improved separation compared to Method 3. The tailing factors range from 1.010 to 1.086, suggesting slightly distorted peak shapes. The peak asymmetry is indicated by the asymmetric values, which vary from 1.024 to 1.069 (Ok & Jeong, 2012).

Overall, the results indicate that Method 4 generally provides the highest resolution among the three methods, implying better separation between the compounds. Similar to previous research on shogaol analysis, a shorter retention time is preferred because it allows for faster analysis and reduces the risk of peak broadening (Ok & Jeong, 2012). Good resolution is important because it ensures the peaks are well separated and distinguishable. The tailing factor is another important factor to consider because it indicates the symmetry of the peak, and a high tailing factor can result in inaccurate quantification (Cafino et al., 2016; Foudah et al., 2020). Therefore, the best method of HPLC is the one that provides the shortest retention time with good resolution and tailing factor.

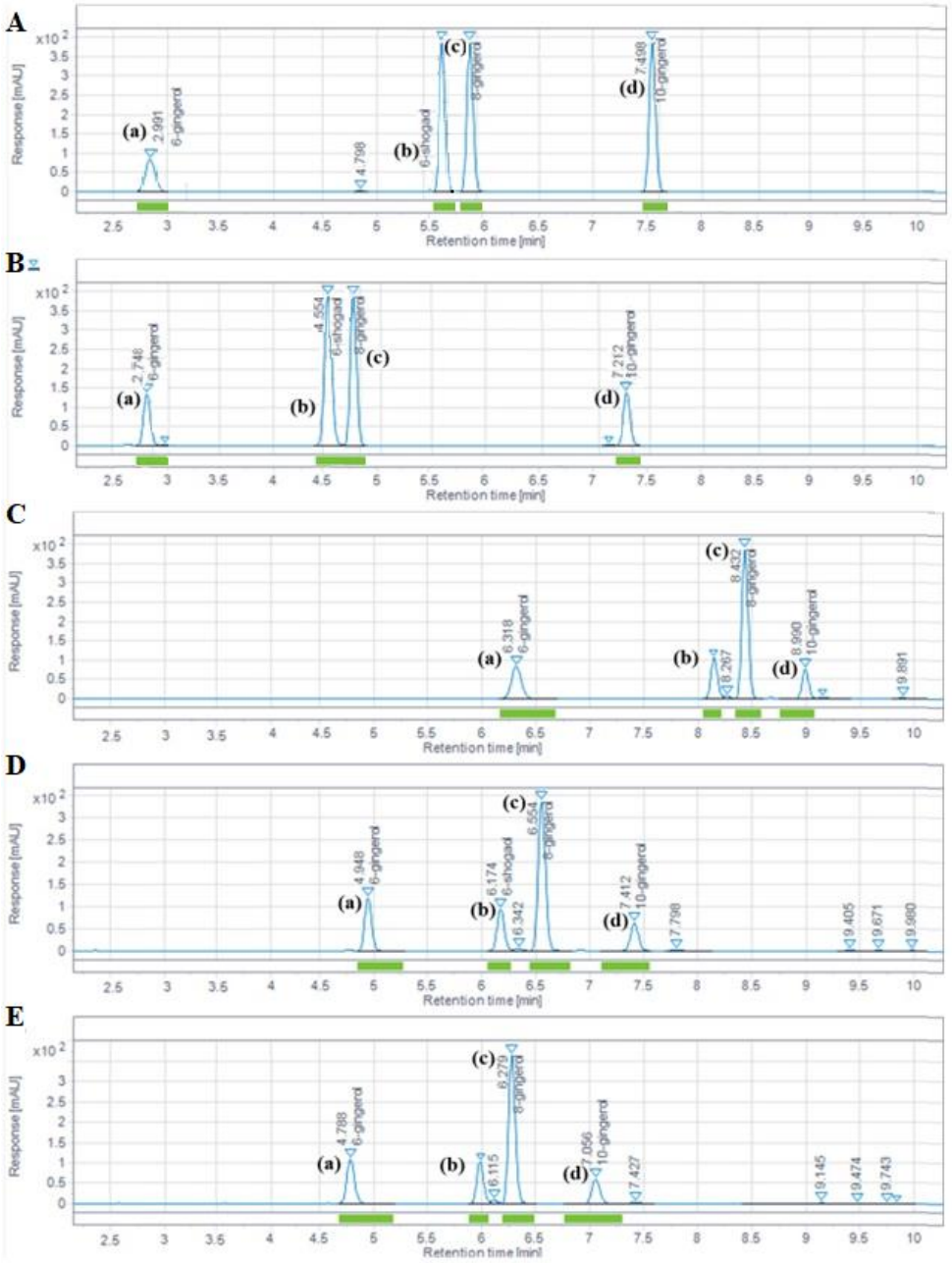

Figure 5. Chromatogram results of standard solution by using method 1 (A); method 2 (B); method 3 (C); method 4 (D), and method 5 (E); whereas (a) 6-gingerol; (b) 6-shogaol; (c) 8-gingerol; (d) 10-gingerol

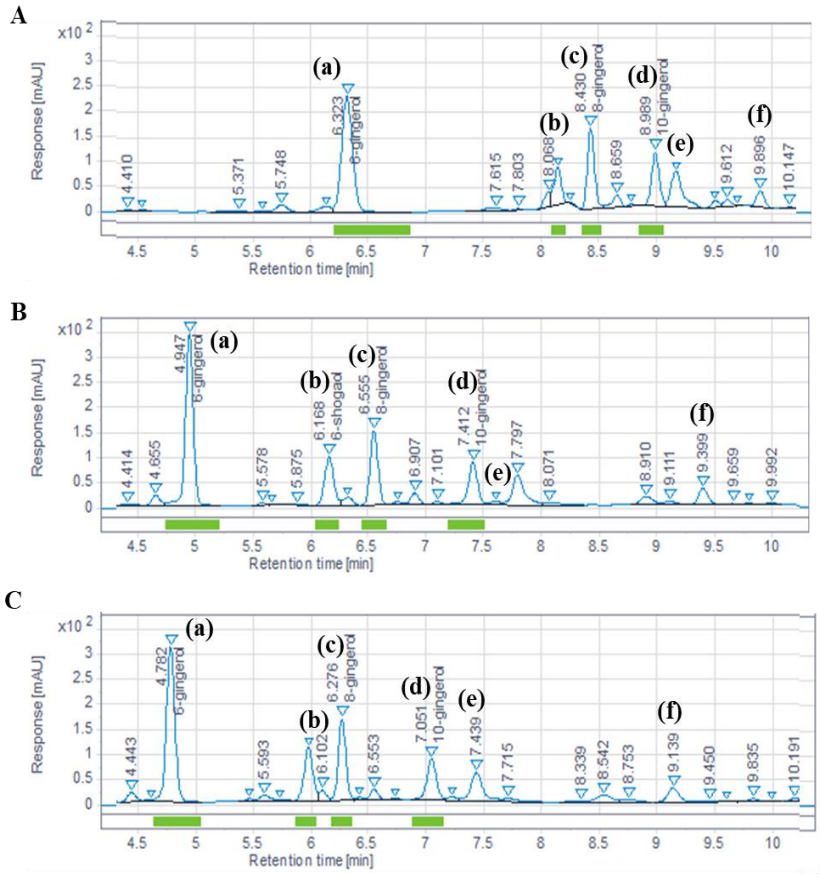

Figure 6. Chromatogram results of extract sample by using method 3 (A); method 4 (B); and method 5 (C). (a) 6-gingerol; (b) 6-shogaol; (c) 8-gingerol; (d) 10-gingerol; (e) 8-shogaol; (f) 10-shogaol

Table 5. HPLC chromatogram analysis results of red ginger extract

Method	Compound	Resolution (Rs)	Tailing Factor (TF)	Asymmetric (As)
3	6-gingerol	0.799 ± 0.019	1.040 ± 0.018	1.049 ± 0.023
	6-shogaol	1.193 ± 1.015	0.919 ± 0.176	0.907 ± 0.136
	8-gingerol	0.899 ± 0.513	0.994 ± 0.162	0.906 ± 0.150
	10-gingerol	1.761 ± 0.518	1.088 ± 0.067*	1.059 ± 0.052*
4	6-gingerol	2.267 ± 0.031*	1.010 ± 0.027*	1.048 ± 0.031*
	6-shogaol	1.575 ± 0.144*	1.014 ± 0.040*	1.024 ± 0.023*
	8-gingerol	1.315 ± 0.124*	1.086 ± 0.026*	1.069 ± 0.029*
	10-gingerol	2.215 ± 0.155*	0.913 ± 0.063	0.964 ± 0.072
5	6-gingerol	1.641 ± 0.943	1.018 ± 0.052	1.048 ± 0.035
	6-shogaol	1.534 ± 0.849	1.016 ± 0.038	1.078 ± 0.038
	8-gingerol	0.946 ± 0.141	1.131 ± 0.086	1.086 ± 0.042
	10-gingerol	1.864 ± 0.748	0.937 ± 0.077	0.997 ± 0.101

Asterisk annotation (*) indicates the optimum result, compared between different methods on the same compound, considering each parameter criteria.

CONCLUSION

Of the five analytical methods, the best analytical conditions were found in method 4 with a mobile phase flow rate of 1.1 mL/min, an optimum UV wavelength of 230 nm, gradient elution system of 0 min (65:35); 1.5 min (40:60); 5 – 6.5 min (10:90); 7.5 – 9 min (0:100); 9.5 – 12 min (65:35), which fulfilled separation parameters such as resolution, tailing factor and asymmetry values. Further optimization of the analytical conditions can be carried out by modifying several other conditions to achieve even better separation parameters and then proceed with method validation of several analytical parameters (accuracy, linearity, selectivity, precision, and sensibility).

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REFERENCES

Cafino, E. J. V, Lirazan, M. B., & Marfori, E. C. (2016). A simple HPLC sethod for the analysis of [6]-Gingerol Produced by Multiple Shoot Culture of Ginger (*Zingiber officinale*). *International Journal of Pharmacognosy and Phytochemical Research*, *8*(1), 38–42.

Direktorat Jenderal Kefarmasian dan Alat Kesehatan. (2017). *Farmakopeherbal Indonesia edisi II Tahun 2017*. Kementrian Kesehatan RI, Jakarta. https://farmalkes.kemkes.go.id/unduh/farmakope-herbal-indonesia-edisi-ii-tahun-2017/.

Fadaki, F., Modaresi, M., & Sajjadian, I. (2017). The effects of Ginger extract and diazepam on anxiety reduction in animal model. *Indian Journal of Pharmaceutical Education and Research*, *51*(3s), s159–s162. https://doi.org/10.5530/ijper.51.3s.4.

Feng, X., Kong, W., Wei, J., Ou-Yang, Z., & Yang, M. (2014). HPLC fingerprint analysis combined with chemometrics for pattern recognition of ginger. *Pharmaceutical Biology*, *52*(3), 362–367. https://doi.org/10.3109/13880209.2013.837493.

Foudah, A. I., Shakeel, F., Yusufoglu, H. S., Ross, S. A., & Alam, P. (2020). Simultaneous determination of 6-Shogaol and 6-Gingerol in various Ginger (*Zingiber officinale* Roscoe) extracts and Commercial formulations using a green RP-HPTLC-densitometry method. *Foods*, *9*(8), 1136. https://doi.org/10.3390/foods9081136.

Gandjar, I. G., & Rohman, A. (2007). *Kimia Farmasi Analisis*. Yogyakarta : Pustaka Pelajar.

Gupta, A., Gupta, S., Datt, P., Pandotra, P., Sharma, R., & Kushwaha, M. (2013). Ultrasound-assisted extraction and fast chromolithic method development, validation and system suitability analysis for 6, 8, 10-gingerols and shogaol in rhizome of *Zingiber officinale* by liquid chromatography-diode array detection. *International Journal of Green Pharmacy*, *7*(3), 189. https://doi.org/10.4103/0973-8258.120197.

Herawati, I. E., & Saptarini, N. M. (2020). Studi Fitokimia pada Jahe Merah (*Zingiber officinale* Roscoe Var. Sunti Val). *Majalah Farmasetika.*, *4*. https://doi.org/10.24198/mfarmasetika.v4i0.25850.

International Organization for Standardization (1997). ISO 13685:1997. Ginger and its oleoresins — Determination of the main pungent components (gingerols and shogaols) — Method using high-performance liquid chromatography. https://www.iso.org/standard/22613.html

Liu, M., Xia, X., Chou, G., Liu, D., Zuberi, A., Ye, J., & Liu, Z. (2014). Variations in the contents of Gingerols and chromatographic fingerprints of Ginger root extracts prepared by different preparation methods. *Journal of AOAC International*, *97*(1), 50–57. https://doi.org/10.5740/jaoacint.12-437

Murphy, A., Norton, E., Montgomery, F., K. Jaiswal, A., & Jaiswal, S. (2020). Ultrasound-assisted extraction of polyphenols from Ginger (*Zingiber officinale*) and evaluation of its antioxidant and antimicrobial properties. *Journal of Food Chemistry & Nanotechnology*, *6*(2). https://doi.org/10.17756/jfcn.2020-088.

Navni, S., Arora, K., & Aniket, P. (2020). A systematic review on extraction methods, pharmacology, pharmacokinetics and clinical studies of bioactive lead: 6-gingerol. *Plant Cell Biotechnology and Molecular Biology*, *21*(15), 161–174.

Ok, S., & Jeong, W.-S. (2012). Optimization of extraction conditions for the 6-Shogaol-rich Extract from Ginger (*Zingiber officinale* Roscoe). *Preventive Nutrition and Food Science*, *17*(2), 166–171. https://doi.org/10.3746/pnf.2012.17.2.166.

Pradhita, F., Aulia, M. P., & Hargono. (2012). Optimasi proses ekstraksi Gingerol dari rimpang Jahe segar menggunakan pelarut N-hexane secara Batch. *Jurnal Teknologi Kimia Dan Industri*, *1*(1), 468–473.

Purwakusumah, E. D., Rafi, M., Syafitri, U. D., Nurcholis, W., & Adzkiya, M. A. Z. (2014). Identification and authentication of Jahe Merah using combination of FTIR spectrocopy and chemometrics. *Jurnal Argitech*, *4*(1), 82–87.

Salmon, C. N. A., Bailey-Shaw, Y. A., Hibbert, S., Green, C., Smith, A. M., & Williams, L. A. D. (2012). Characterisation of cultivars of Jamaican ginger (*Zingiber officinale* Roscoe) by HPTLC and HPLC. *Food Chemistry*, *131*(4), 1517–1522. https://doi.org/10.1016/j.foodchem.2011.09.115.

Sembiring, P. (2022). Formulasi dan uji ektivitas antibakteri sediaan lotion ekstrak etanol daun Sawao terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Penelitian Farmasi & Herbal*, *4*(2), 20–28. https://doi.org/10.36656/jpfh.v4i2.853.

Snyder, L. R., Kirkland, J. J., & Dolan, J. W. (2010). *Introduction to Modern Liquid Chromatography (3rd ed.)*. (pp. i–xli). John Wiley & Sons, Inc. https://doi.org/10.1002/9780470508183.fmatter.

Srikandi, S., Humaeroh, M., & Sutamihardja, R. (2020). Kandungan gingerol dan Shogaol dari ekstrak Jahe Merah (*Zingiber officinale* Roscoe) dengan metode Maserasi bertingkat. *Al-Kimiya*, *7*(2), 75–81. https://doi.org/10.15575/ak.v7i2.6545.

United States Pharmacopeia (2023). Dietary supplement monographs, ginger. USP-NF. Rockville, MD: United States Pharmacopeia. https://doi.org/10.31003/USPNF_M34965_02_01

Usama I. Aly, Mohamed S. Abbas, Hussein S. Taha, & El-Sayed I. Gaber. (2022). Characterization of 6-Gingerol for In Vivo and In Vitro Ginger (*Zingiber officinale*) Using High Performance Liquid Chromatography. *Global Journal Of Botanical Science*, *1*(1), 9–17. https://doi.org/10.12974/2311-858X.2013.01.01.2.

Vankar, P. S., & Srivastava, J. (2010). Ultrasound-assisted extraction in different solvents for Phytochemical study of *Canna indica*. *International Journal of Food Engineering*, *6*(3). https://doi.org/10.2202/1556-3758.1599.

Yang, Y., Zhang, G., Sun, Q., Liu, L., Peng, H., Wang, J., & Xiang, L. (2017). Simultaneous determination of 8 compounds in Gancao-Ganjiang-tang by HPLC-DAD and analysis of the relations between compatibility, dosage, and contents of medicines. *Evidence-Based Complementary and Alternative Medicine*, *2017*, 1–8. https://doi.org/10.1155/2017/4703632.

You, H., Ireland, B., Moeszinger, M., Zhang, H., Snow, L., Krepich, S., & Takagawa, V. (2019). Determination of bioactive nonvolatile ginger constituents in dietary supplements by a rapid and economic HPLC method: Analytical method development and single-laboratory validation. *Talanta*, *194*, 795–802. https://doi.org/10.1016/j.talanta.2018.10.075.

Zhang, S., Mak, K.-K., Marappan, P., Balijepalli, M. K., Chong, G.-H., & Rao Pichika, M. (2022). Simple Hplc method for the Quantification Of Gingerols (4-, 6-, 8-, And 10-) and Shogaols (6-, 8-, And 10-) In *Zingiber officinale* var. Rubrum Supercritical carbon dioxide (Sc-co2) extract. *Rasayan Journal of Chemistry*, *15*(01), 65–71. https://doi.org/10.31788/RJC.2022.1516682.