

## A Comparative Study on Zerumbone Concentration, Radical Scavenging Activity and Total Phenolic Content of Zingiber Zerumbet Extracted via Green and Conventional Extraction

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### ABSTRACT

Extraction is crucial for herbal extraction to ensure high quality of bioactive compounds from natural herbs. In the current work, *Zingiber Zerumbet* was extracted via subcritical water extraction (SWE) and is compared with the conventional solvent extraction, Soxhlet. The quality of the extract was investigated in terms of zerumbone concentration, radical scavenging activity (RSA) and total phenolic content (TPC). For zerumbone concentration, extraction via SWE gave  $19.82 \pm 0.004$  mg/g as compared with Soxhlet;  $28.51 \pm 0.079$  mg/g. However, the extraction time required for SWE to yield such concentration only required 40 minutes instead of Soxhlet which took 480 minutes. The same trend was recorded for RSA, which yielded  $60.70 \pm 0.070\%$  inhibition for SWE and  $68.81 \pm 0.024\%$  inhibition from Soxhlet extraction. In contrast to TPC, SWE recorded a higher response than Soxhlet extraction, which was  $19.19 \pm 0.003$  mgGAE/g DW, while Soxhlet;  $8.30 \pm 0.019$  mgGAE/g DW. Therefore, the SWE method is more favorable for obtaining a higher value of TPC, slightly good in antioxidant properties but lower zerumbone concentration value than organic solvent extraction. However, the reduced extraction time was almost 12 times quicker for SWE when compared with Soxhlet extraction. Overall, SWE is a promising alternative environmentally friendly since it only uses water as solvent and is comparable to the conventional method.

### Keywords:

Subcritical water extraction, Zingiber  
Zerumbet, zerumbone, radical scavenging  
activity, total phenolic content

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## 1. Introduction

Medicinal plants have recently received significant attention due to their extraordinary beneficial properties. For example, *Z.zerumbet*, which belongs to the ginger family, is also known as bitter ginger, shampoo ginger, and pinecone ginger[1]. This plant has long been used as a whole or by parts (leaves, rhizome, flower) as part of traditional medicine [2]–[4], food and beverage [5], [6] as

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well as personal care [1], [7]. Previous studies [8], [9] have reported the presence of high content of zerumbone (35.5-84.8%) found in *Z.zerumbet* extract, which is believed to be the significant compound to exhibit antioxidant capabilities, antiproliferative, antibacterial, anticancer among the medicinal benefit [4], [10]–[12].

However, to obtain the bioactive compound from *Z.zerumbet*, it is crucial to choose an extraction process that is efficient and safe, and cost-effective. Several extraction methods can be used, such as Soxhlet, maceration, boiling, supercritical fluid extraction, ultrasound-assisted extraction, and subcritical water extraction. SWE is a promising candidate among these methods since it only uses water as a solvent. This extraction method is different from the widely known conventional extraction; Soxhlet [13], [14] employs organic solvent and requires extended extraction time [15]. In addition, SWE has the advantage of shorter extraction time, thus reducing the overall cost [16]. Therefore, the primary aim of this paper is to critically examine the quality of *Z.zerumbet* extractant from the 1L SWE prototype and Soxhlet in terms of zerumbone concentration, radical scavenging activity, and total phenolic content.

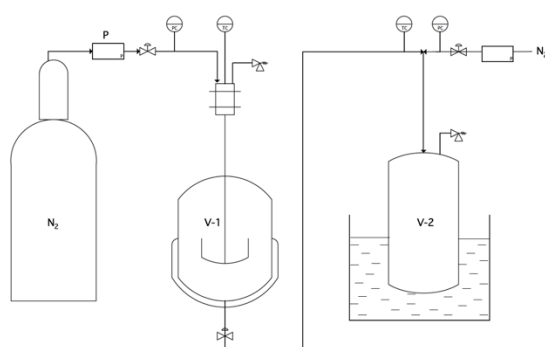
## 2. Methodology

### 2.1 Sample preparation

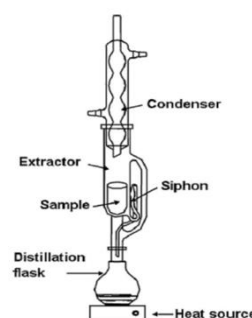
*Z.zerumbet* rhizomes used in this study were acquired from a local farmer from Kuala Krau, Pahang. The rhizomes were cleaned, sliced, dried, ground, and sieved to the following sizes: 3.36, 2.00, 1.00 and 0.50 mm. The moisture content was also measured using a moisture analyzer (OHAUS, MB 25, Switzerland) and is ensured to be lower than 10% in dry basis to prevent microbial growth [17]

### 2.2 Extraction of *Z.zerumbet* using SWE

The schematic diagram of 1 L SWE prototype (CLEAR, UTM KL) was shown in Fig. 1. Briefly, 25 grams of the ground rhizome material were placed in the extraction vessel filled with 500 ml of water and subjected to subcritical conditions. The processing parameters were optimized based on previous study [18] (temperature: 170° C, time: 40 minutes; mean particle size: 2.36 mm, pressure: 2.0 MPa; p-value<0.05) by employing Design Expert Software Version 12. The extraction time started once the target temperature was reached, as indicated by the temperature indicator in the extraction cell. The extract was immediately transferred to the cooling vessel when the extraction was completed. Finally, the crude extract was collected and further subjected to analysis.



**Fig. 1:** Schematic diagram of 1L SWE prototype



**Fig. 2:** Schematic diagram of soxhlet apparatus

### 2.3 Soxhlet Extraction

25 g of dried *Z.zerumbet* with a mean particle size of 2.36 mm was weighed and extracted with 500 ml ethanol for 8 hours. The best operating condition was based on the previous study [19], [20]. The extraction temperature was kept constant at the boiling point of ethanol (78.10°C) and was monitored using an infrared laser thermometer (AR300, China). As illustrated in Fig. 2, the sample was gradually filled with condensed new ethanol from the distillation flask. A siphon aspirates the extracted sample from the sample matrix and unloads it into the distillation flask whenever the liquid overflows [21]. This is a repetitive process until the extraction is completed. Afterward, the ethanol was left to evaporate in the oven at 40°C overnight before analysis. From the soxhlet extraction, the percentage recovery of *Z.zerumbet* was calculated using Eq (1);

$$\text{Percentage of recovery} = \frac{C_i \frac{\mu\text{g of bioactive}}{\text{g dried Z.zerumbet}}}{C_{oi} \frac{\mu\text{g of bioactive}}{\text{g dried Z.zerumbet}}} \times 100 \quad (1)$$

Whereby  $c_i$  is the sample concentration in the bulk solution,  $c_{oi}$  is the initial sample concentration from soxhlet extraction.

### 2.4 HPLC Analysis

The targeted marker compound for *Z.zerumbet* was zerumbone. HPLC analysis was conducted using Waters (e2695, Waters, USA) with a photodiode array detector (PDA) on a C18 column (Symmetry®). Before analysis, the extracted samples were homogeneously dissolved in 10mL of methanol, followed by filtration through 0.45 μm membrane filter (Nylon, Waters Corporation). The mobile phase was methanol: acetonitrile (35:65 v/v) and was carried out in isocratic elution. The total running time was 10 minutes with a flow of 1 ml/min at the wavelength of 254 nm. This is a modified method as conducted by [22]. The standard calibration curve was established by diluting the standard zerumbone with methanol to six concentrations: 5 ppm, 10 ppm, 20 ppm, 50 ppm, 100 ppm, and 500 ppm. Then, the absorbance was plotted against the concentration to obtain the equation of a straight line.

### 2.5 Radical Scavenging Activity

The RSA of the extract was analyzed against the stable DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma Aldrich, Germany). According to previous literature [19], [23]. Initially, the samples were diluted at a ratio of 10:40 of extract to 80% ethanol. Next, about 0.5 ml of each extract was added to 3.5 ml of prepared DPPH ethanolic solution (0.1 mM). The solution was incubated for 30 min at room temperature in the dark and the discoloration was measured at 517 nm using the UV-VIS spectrophotometer. The inhibition ability radical scavenging activity (RSA) was calculated using Eq (2):

$$\text{RSA (\% inhibition)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (2)$$

$A_{\text{control}}$  is the absorbance of the control (DPPH+80%EtOH) at t=0 min and  $A_{\text{sample}}$  was the absorbance of sample at t=30 min.

## 2.6 Total Phenolic Content

TPC was evaluated using Folin-Ciocalteu modified method [19], [24]. Initially, the extract was diluted in distilled water with a ratio of 1:10 v/v. Afterward, 0.5 mL of diluted extract or standard prepared was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent in distilled water (1:10 v/v). The mixture was hand-shaken vigorously. The mixture was left for 5 min rest before 2 mL of 7.5% (v/v) sodium carbonate was added. Next, the mixture was incubated for about 2 hours and was measured using UV/VIS Spectrometry at 750 nm. The TPC for each sample was determined from a standard curve of gallic acid ranging from 10 to 100 mg/L solutions of gallic acid in water. The yield in total polyphenol ( $Y_{TP}$ ) was calculated using Eq (3).

$$\text{Total phenolic content, } (Y_{TP}) = \frac{C_{TP} \times V \times d}{m} \quad (3)$$

Where  $C_{TP}$  is the concentration of gallic acid in water from the standard curve regression line ( $y=mx+c$ ) and is represented in mg/L,  $V$  is the volume of extraction solvent (L),  $d$  is the dilution factor and  $m$  is the weight of dried rhizome used (g)

## 2.7 Statistical Analysis

The extraction process was conducted in triplicate and standard deviations were calculated. Results were expressed in the form of mean, absolute average deviation (AAD) and percentage calculated using Microsoft Excel 2021.

## 3. Results

The standard calibration curve of zerumbone concentration gave good  $R_2$  value of 0.9998 and linear equation of  $y=17130x + 17719$ . The total phenolic content (TPC) evaluation was measured in terms of gallic acid equivalent (GAE) based on the linear standard curve equation  $y=0.0078x - 0.0099$  and  $R_2$  value of 0.9993.

### 3.1 Zerumbone Concentration

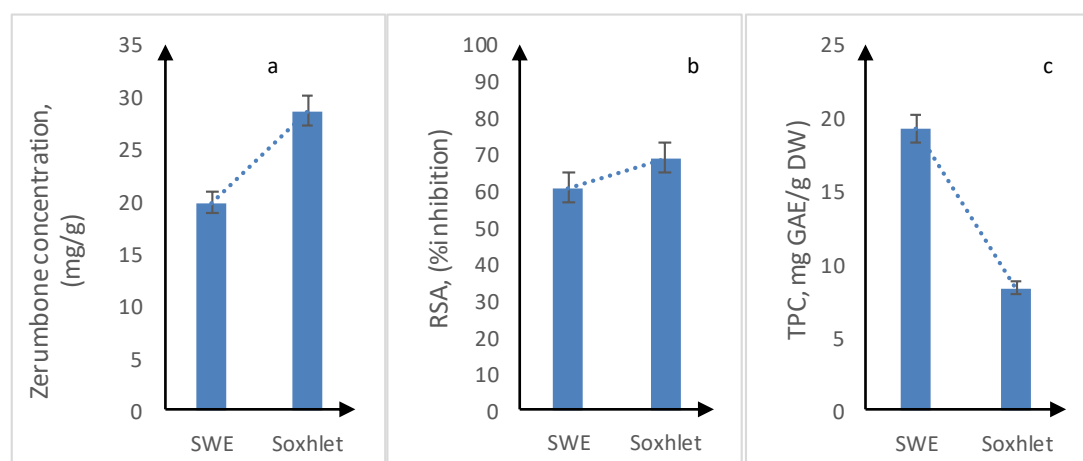
For zerumbone concentration, it is apparent that the SWE method reported significantly lower than Soxhlet, which is depicted in Fig. 3 (a). This finding is consistent with that of Tzeng *et al.* [25] who found the highest amount of zerumbone content from ethanol solvent. Zerumbone has been confirmed to be a polar compound in a study conducted by Rosnani *et al.* [26]. Following the "like dissolve like" principle [27], zerumbone is more likely to be extracted with a polar solvent. This is reflected in the findings whereby the zerumbone content via SWE is comparable to that of Soxhlet extraction by 70% recovery rate which is in the acceptable recovery rate of 60-140% based on [28], [29]. This is a significant finding since Soxhlet has always been used as the benchmark for any extraction methods. Under SWE condition, water can mimic the characteristic of organic solvent via the decrease in dielectric constant [30]. Therefore, it is possible in this study that water in SWE behaved like organic solvent from the result attained. In addition, it is worthy to point out that the extraction time taken for SWE to complete one cycle was 12 times faster than Soxhlet. This is a remarkable outcome of SWE as it could produce comparable extract properties with a significantly reduced in time. Consequently, the operational expenses are greatly reduced due to the decreased in the extraction time [31], [32].

### 3.2 Radical Scavenging Activity

Zerumbone has been widely recognized to have a wide range of pharmaceutical activities including antioxidant activity. The RSA results are in agree with the zerumbone content, whereby the RSA % inhibition increases accordingly with the zerumbone content obtained via both extraction methods. In general, the RSA from SWE was slightly lower than Soxhlet by 12%, as shown in Fig. 3 (b). The RSA was also compared with ultrasound-assisted extraction (UAE) from previous study which suggested the frequency has little effect on the RSA value. By comparison, the RSA from UAE was two times lower than SWE in this study. This suggests the efficiency of SWE 1L prototype in comparison to Soxhlet method. Meanwhile, it is clearly elucidated that temperature and time played a significant role in higher RSA value. From the results, the use of UAE at lower temperature and time gave low RSA value in comparison to SWE and Soxhlet. The possible explanation on the narrow margin between SWE and Soxhlet might be contributed by the high temperature of SWE (170° C). Previous study by Plaza *et al.* [33] has suggested the formation of new compounds such as hydroxymethylfurfural and melanoidin formed through hydrolyzation and Maillard reaction under extreme temperature and long exposure. These new compounds might have an impact on the increase in antioxidant activity.

**Table 1**  
Zerumbone concentration, RSA and TPC of *Z.zerumbet* using SWE, Soxhlet and UAE extraction

		SWE	Soxhlet	UAE[19]
<b>Conditions</b>	Solvent	Water	Ethanol	Water
	Temperature	170	78.4	60
	Time (min)	40	480	25
	Pressure (MPa)	2.0	0.1	0.1
	Frequency (kHz)	n.a	n.a	25
<b>Response</b>	Zerumbone concentration	19.82 ± 0.004	28.51 ± 0.79	n.a
	RSA	60.70 ± 0.070	68.81 ± 0.024	28.01 ±
	TPC	19.19 ± 0.003	8.30 ± 0.019	2.48 ± 0.20



**Fig. 3:** Zerumbone concentration, RSA and TPC of SWE and Soxhlet in this study

### 3.3 Total Phenolic Content

The total phenolic content gave a surprising result whereby, a different trend was observed for TPC. The SWE extract gave more than 100% which is over twofold compared to Soxhlet extraction. Figure 3 (c) shows the TPC from SWE and Soxhlet showed immense difference. UAE in comparison still gave lower value of TPC which was 9 times lower than SWE and almost four times lower compared to Soxhlet. The high value of TPC from SWE may be explained by the softening of polyphenol in water which was influenced by the increase in the diffusivity, solubility, and mass transfer of compound rate [34]. However, this outcome is contrary to that of [35] which concludes that the TPC decreases with the increase in water content. Various studies have demonstrated the success of using SWE as an efficient method for extracting bioactive compound from various resources. Previous study conducted by Vladić *et al.* [31] has found significant improvement on *S. montana* extracts in terms of increasing antioxidant activity and TPC compared to soxhlet extraction. Thus, the findings reported here suggest that SWE can be used to extract *Z.zerumbet* and has improved TPC while having similar value of zerumbone concentration and RSA compared with solvent extraction.

### 4. Conclusions

In conclusion, the present study has demonstrated the potential of SWE to produce a high quality of *Z.zerumbet* extracts comparable with the established conventional extraction method; Soxhlet. For the investigated response of zerumbone concentration, the value was  $19.82 \pm 0.004$  mg/g and  $28.51 \pm 0.79$  mg/g for SWE and soxhlet extraction, respectively. In terms of RSA, the value for SWE and Soxhlet were somewhat close to one another with  $60.70 \pm 0.070$  and  $68.81 \pm 0.024$ , respectively. In contrast, SWE yielded the highest value of TPC compared to Soxhlet extraction with  $19.19 \pm 0.003$  and  $8.30 \pm 0.019$  mg GAE/gDW, respectively. Overall, SWE is a promising alternative extraction method that can safely and efficiently extract high-quality bioactive compounds.

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