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Total Phenolic Content, Total Flavonoid Content and Radical Scavenging Activity from *Zingiber zerumbet* Rhizome using Subcritical Water Extraction

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ABSTRACT

Subcritical water extraction (SWE) is an alternative technique implemented water as a solvent. The objective of this work was to extract *Zingiber zerumbet* rhizome using SWE at a temperature range from 100ºC to 180ºC with duration from 5 to 25 min. The extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity (RSA). Soxhlet extraction using ethanol was used for a comparison purpose. Results showed the highest TPC and TFC was obtained at 180ºC and 25 min extraction, with the yield of 18.52 mg GAE/gDW and 2.34 mg QE/gDW of rhizome for TPC and TFC, respectively. RSA at peak of 83.9 % inhibition at the condition of 180ºC and 10 min extraction. In comparison to Soxhlet extraction, the extract after SWE gives the highest amount of TPC and RSA. However, the values for TFC are lower as compared to ethanolic extract. Therefore, SWE process for *Zingiber zerumbet* extract is favourable for higher TPC and RSA. A direct linear correlation between the RSA with the TPC and TFC of the extracts shows that a strong correlation was observed between TPC and the RSA with the R^2 obtained was 0.910 as compared to moderate correlation (R^2=0.785) perceived in TFC. Thus, it shows higher radical scavenging activity in *Zingiber zerumbet* was contributed by phenolic content as compared to its flavonoid content. In overall, SWE is a potential alternative extraction process that should be further explored.

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1. INTRODUCTION

Phenolic compounds are known as the most abundant secondary plant metabolites which have great potential on antioxidant activity to delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress [1]. Based on research conducted by Transparency Market Research 2016, the global demand for the polyphenol market within 2012 to 2018 was expected to reach USD 873.7 million with an annual growth rate of 6.1% [2]. This phenolic compound can be divided into several categories: phenolic acids, flavonoids, stilbenes, coumarins, stignans and lignins. In further, this active ingredients are beneficial for the formulation of natural products including as food, fragrances as well as medicinal and skin-care products.

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Zingiber zerumbet (family: *Zingiberaceae*) or 'wild ginger' are traditionally be used in foods, beverages as well as traditional medicine [3]. The species are widely distributed throughout India, tropical Asian and to northern Australia [4]. Occasionally, the rhizome was the most part of the plant been researched for its aromatic compounds. In addition, the rhizome was also reported to be rich in phenolics and flavonoids, specifically kaempferol and its glucoside along with curcumin and gingerol [3].

Previously, part of 24.21% (w/w) of the *Zingiber zerumbet* rhizome extract was reported to be water soluble, while 9.45% (w/w) was alcohol-soluble extractive [5]. However, through their preliminary phytochemical analysis, the flavonoids were not detected using ethanol and water. In contrast, reported research [6-7] have revealed the phenolic and flavonoids compounds could be extracted via organic and aqueous extraction solvent. The common solvent used in

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phenolic extraction was alcoholic, acetic and formic acid, as well as aqueous solvent [8]. Among all, alcoholic solvent shows prominent yield in *Zingiber zerumbet* rhizome phenolic content of more than 6 mg GAE/ g dry extract and antioxidant activity of more than 30% inhibition [6]. Though, the conventional extraction techniques such as soxhlet, maceration and soaking are time-consuming, limited and require relatively large quantities of solvent that might be harmful to human and environment [4].

Water is considered as one of the environmentally sustainable solvents and is generally recognised as a safe (GRAS) solvent by food and nutraceutical industries [9]. It has many advantages and plays a role as a low-cost 'green solvent' because it is natural and widely available, high in purity, non-toxic and its disposal is considered as benign with little effect on the environment. A recent study [7] showed the higher antioxidant activity of 70% inhibition was perceived from the rhizome extracted using aqueous solvent. However, in a normal condition, water has properties of high dielectric constants and not favoured to extract low-polarity compounds [9]. Hence, the enhanced technology has approved water at an elevated temperature and pressure as shown in Figure 1 could be maintained in a liquid phase to improve the efficiency of the extraction process.

This condition was known as subcritical condition operated within a range of the boiling point of water (100ºC) to below its critical point (374ºC) and at higher than saturation pressure of within 0.1 MPa to 22.1 MPa. Principally, in subcritical condition the diffusion rate was improved and disrupted the analyte-sample interaction from van-der walls forces, hydrogen bonds and dipole interactions of water [9]. One of the significant feature of water in subcritical condition was its initial value of dielectric constant (ε) with 78.5 at 25ºC was decreased to 27.0 at the condition of 250ºC and 10MPa, which falls between those of methanol (ε=33.0) and ethanol (ε=24.0) at 25ºC.

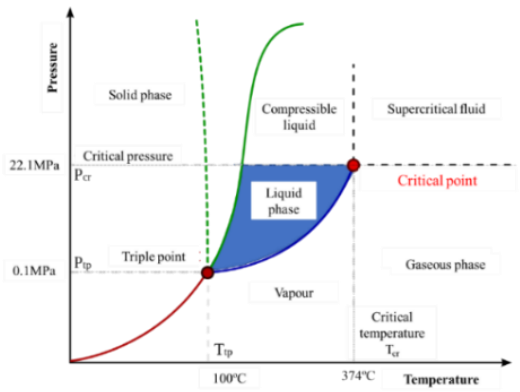

Figure 1. Water phase diagram [9]

Thus, in subcritical condition, water could be manipulated to mimic the organic solvent and selectively extracts both polar and non-polar compounds [9].

Subcritical water extraction (SWE) exhibits improvement on the issues in conventional extraction as the extraction time and operation costs were reduced, and the equipment used was simple to handle, environmental-friendly, with higher quality extracts obtained [10]. Numerous types of fruits, plants, herbs and spices have been succeeded to be extracted using SWE [4]. However, there is no report yet on the usage of water as a solvent in alternative extraction methods for *Zingiber zerumbet* rhizome of phenolic and flavonoid compounds. Thus, it shows the contribution of this research to extract its phenolic and flavonoid compounds using SWE.

Among various parameters affecting SWE, temperature and time are the key factors which have continually been studied by numerous researchers [9]. Despite the fact of higher pressure used was to maintain water in a liquid pressure at higher temperature applied. However, it has a lesser effect on the extraction efficiency and frequently to be neglected [11]. Thus, at 10.1MPa pressure applied, the temperature of higher than 100ºC was beneficial to evaluated, nonetheless, after 200ºC and longer exposure of 30 min could also form non-phenolic compounds that reacted with antioxidant compounds [12]. Whilst another research [13] have investigated from the extraction of *Phlomis umbrosa* Turcz (PT); higher phenolic and flavonoid was obtained at 200ºC and 20 min. A study reported in literature [14] have obtained maximum polyphenol of deodorised thyme at 100ºC and 5 min; nevertheless, the highest antioxidant activity was obtained at 200ºC. Thus, a suitable parameter is required to achieve a high quality of the product.

Therefore, in the current study, *Zingiber zerumbet* rhizome was extracted using SWE in a batchwise operation varied temperature of 100-180ºC with an interval of 20ºC and time within 5 to 25 min. The extracts were examined for total phenolic and flavonoid contents as well as radical scavenging activity. Finally, the correlation between radical scavenging activity with total phenolic and flavonoid contents of *Zingiber zerumbet* extracts were established. Soxhlet extraction used ethanol as a solvent was employed for comparison purposes.

2. EXPERIMENTAL

2. 1. Materials Fresh rhizome *Zingiber zerumbet* was purchased from Kiza Herbs, Pahang, Malaysia. Organic solvents applied in this study were as follows: absolute ethanol, 95% ethanol, methanol obtained from

FS (Friendmann Schmidt). Chemolab Sdn Bhd. supplied standards namely ascorbic acid, gallic acid and Quercetin with purity more than 90%. The graded analytical chemicals involved in analytical study consists of Folin-Ciocalteau reagent, sodium carbonate, potassium acetate and aluminium chloride were supplied by Chemolab Sdn Bhd whilst 1,1 diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Chemical Co. (St Louis MO, USA).

2. 2. Sample Preparation The fresh rhizome was cleaned, sliced and sun-dried for 3 days. The moisture content of the dried slices is ensured to be lower than 10% in dry basis using a moisture analyser (OHAUS MB 25, Switzerland) to protect the bioactive compounds of the sample. The dried rhizome was then grounded and sieved for the standardised size of 0.6-1.16 mm and stored in the dark storage at room temperature prior to extraction.

2. 4. Soxhlet Extraction Briefly, 20 g of grounded rhizome were weighted and extracted through soxhlet extraction using Ethanol with the sample to solvent ratio of (1:17 w/v). The round bottom flask containing the solvent was heated at 76ºC for 6 hours. The extract was centrifuged for 10 min to remove insoluble solids, and the supernatant collected was stored at -20°C for further analysis. The soxhlet extractions were performed in triplicates.

2. 5. Subcritical Water Extraction (SWE) A Dionex model ASE 350 (Dionex Corporation, Sunnycale, CA, USA) was used as a subcritical water extractor to extract phenolics from *Zingiber zerumbet* rhizome. The experiments were carried out batchwise using approximately 85ml of water as the solvent. The prescribed methods used were created in the control panel based on the condition and parameter determined: one extraction cycle, 0 % flush volume and at varied extraction time (5, 10, 15, 20 and 25 min) and temperatures (100, 120, 140, 160, 180ºC).

Initially, about 5 g of *Zingiber zerumbet* dried rhizome was loaded into a 100 mL extraction cell with a cellulose paper filter inserted at the bottom of the cell. The pressure of 10.34 MPa was applied to the sample extraction cell through nitrogen tank to maintain the heated solvent in a liquid state during the extraction. The preheating time was based on the temperature set: 5 min for 100°C, 6 min for 120°C, 7 min for 140ºC, 8 min 160ºC and 9 min for 180ºC. The sample was then extracted statically at determined extraction condition: 5 to 25 min.

After completed extraction process, the extract was purged from the sample cell and collected in a standard collection bottle. At 800 rpm and 10 min, the extracts were then centrifuged, and the supernatant collected was stored at -20 °C prior to analysis. The extracts in liquid form were directly analysed using UV-Vis spectrophotometer (SP-Nano 300, Japan) for the total phenolic content, total flavonoid content as well as its radical scavenging activity. The extractions were performed in duplicates.

2. 6. Determination of Total Phenolic Content (TPC) The analysis of total phenolic content was conducted using Folin-Ciocalteu modified method [15]. The extract was initially be diluted in distilled water with the ratio of 1:10 v/v. Next, about 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 then be hand shaken vigorously. After 5 min of rest, about 2 mL of 7.5% (v/v) of sodium carbonate was added to the mixture. Later, the mixture was incubated for about 2 hours and the absorbance was measured using UV/Vis spectrometry at 750 nm.

The TPC of each extracted rhizome was initially determined from the standard curve of gallic acid prepared range from 10 to 100 mg/L solutions of gallic acid in water. Using the Equation (1), the yield in total polyphenol (Y_{TP}) was determined as mg gallic acid equivalent (GAE) per g of dry weight (DW):

$$\text{Total Phenolic Content } (Y_{TP}) = \frac{C_{TP} \times V \times d}{m} \quad (1)$$

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

2. 7. Determination of Total Flavonoid Content (TFC) Total flavonoid content was analysed using aluminium chloride colourimetric method modified from [13]. The extracts were initially diluted in distilled water (1:10v/v) as previously done in the determination of TPC procedure. Later, about 0.5 mL of diluted extract or standard was mixed with 1.5 mL of 95% ethanol followed by the addition of about 0.1 mL of 10% aluminium chloride. Next, about 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water later were added to the mixture. For 30 min, the mixture was incubated at room temperature. Finally, the absorbance of the reaction mixture was measured at 415 nm using UV/Vis spectrometry.

The TFC of each sample was determined from the generated standard curve prepared using Quercetin ranging from 10 to 100 mg/L solutions of Quercetin in water. Using the Equation (2) the yield in term of total flavonoid content (Y_{TF}) was determined as mg quercetin equivalent (QE) per g of dry weight (DW):

$$\text{Total Flavonoid Content } (Y_{TF}) = \frac{C_{TF} \times V \times d}{m} \quad (2)$$

where C_{TF} is concentration of quercetin in water from standard curve regression line (mg/L), V is the volume of the extraction solvent (L), d is the dilution factor and m is the weight of dried rhizome used (g).

2. 8. Determination of Radical Scavenging Activity (RSA) The radical scavenging activity of the extract for DPPH (2,2-diphenyl-1-picrylhydrazyl) was monitored according to the method explained in literature [16]. The samples were initially being diluted at a ratio of 10:40 of extract to 80% ethanol. About 0.5 ml of each extract was then added to 3.5 ml of prepared DPPH ethanolic solutions (0.1mM). Discoloration of obtained solutions was measured at 517 nm after incubation for 30 min at room temperature in dark using the spectrophotometric method. The inhibition ability radical scavenging activity (RSA) was evaluated based on following relation:

$$\text{RSA (\% inhibition)} = \frac{A_{control}-A_{sample}}{A_{control}} \times 100 \quad (3)$$

where $A_{control}$ was defined as absorbance of the control (DPPH+ 80% EtOH) at t=0 minute whereas A_{sample} was defined as absorbance of the sample at t=30 min.

2. 9. Statistical Analysis Results were reported in the form of mean, absolute average deviation and percentage value, which was calculated using the Microsoft Excel 2017 software. The data presented were the mean values of two or more repeated experiments and the average absolute relative deviation (AAD) was calculated using the following equation:

$$AAD = \frac{1}{N}\frac{[x_i - x_{avg}]}{x_i} \quad (4)$$

where N is the number of the experimental data, x_i is the experimental point and x_{avg} is the average value at every condition.

A Tukey's multiple range tests were used for the data analysis using Minitab 17.0 software. A level of $P < 0.05$ significance was used for each extraction time and extraction temperature for TPC, TFC and RSA of the extracts.

Meanwhile, to determine the correlation between TPC, TFC and RSA of extracted rhizome from SWE, the Pearson-correlation coefficient was calculated using Minitab 17.0 software and represented in term of R^2. The correlation was also presented in the scattered plot from Microsoft Excel 17.0.

3. RESULTS AND DISCUSSION

The assessment of total phenolic content (TPC) was measured in terms of gallic acid equivalent (GAE) based on the linear standard curve equation of y=0.0118x+0.0416 with the R^2 obtained was 0.996. Meanwhile, for total flavonoid content (TFC) analysis, it was determined in term of quercetin equivalent (QE) through linear standard curve equation of y=0.0085x-0.057 with the R^2 obtained of 0.9986. The expression of TPC and TFC were standardized in term of per gram of dry weight of the rhizome.

3. 1. Total Phenolic Content (TPC) Figure 2 shows a plot of TPC of the extract versus extraction times at various temperatures.

The TPC obtained varied from 2.1236 ± 0.1358 to 18.5233 ± 0.0520 mg GAE/g DW. From the figure, each fixed extraction time of 5,10,15 and 25 min applied, showing the amount of phenolics content extracted in mg GAE/g DW which linearly increased as the temperature increased from 100ºC up to 180ºC. The incremental increase of TPC extraction temperature could be explained as at an extreme temperature of SWE, the diffusivity, solubility and mass transfer of compounds rate were also increased thus inducing the softening of polyphenol in water [17]. This is further supported by post-hoc ANOVA Tukey-test analysis that for different durations of extraction applied, the TPC was significantly increased ($p < 0.05$) by almost twofold, especially at high temperatures (T>160ºC). Similar observations of increased TPC with the rise of temperature were previously reported in *Glycyrrhiza uralensis Fisch* extracts as temperature used from 80 to 200ºC [18].

In terms of the effect of extraction time, it shows at 100ºC the TPC was slightly increased from 5 to 10 min extraction. However, the TPC decreased at 15 and 20 min and upsurged at 25 min. This trend was similarly observed at 120ºC. The observed fluctuated condition after 10 min extraction could be related to the degradation of phenolic compound induced by thermal instability, as happened in SWE [17-18]. This further supported through post-hoc Tukey's test analysis that no significant effect ($P > 0.05$) in different extraction time applied was observed between each sample for both temperatures.

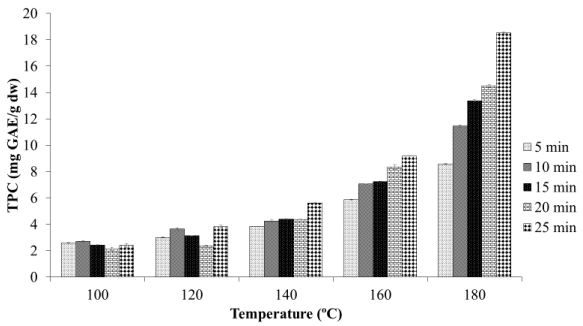

Figure 2. Variation of total phenolic content (TPC) of *Zingiber zerumbet* rhizome

Therefore, as the instability of the TPC was observed after 10 min extraction, hence, for a lower temperature less than 120ºC, 10 min extraction was sufficient to extract high phenolic content in *Zingiber zerumbet* rhizome.

On the other hand, a similar trend was perceived at 140,160 and 180ºC, as the TPC was gradually increased as time increased from 5 to 25 min. Previous research in the SWE of pomegranate residues has approved the upsurge of TPC with 30 min extraction relatively at the temperature higher than 140ºC [10]. It could be supported by Tukey's test at 140ºC where a significant difference existed at 25 min extraction ($P < 0.05$). At a temperature of 160ºC, the TPC was not dependent on extraction time. Meanwhile, at 180ºC extraction time was significantly different ($P > 0.05$) at the shortest and longest extraction time applied. However, at 25 min the TPC was relatively high (18.5233±0.0519 mgGAE/gDW) compared to 5 min extraction (8.5778±0.0353 mgGAE/gDW).

3. 2. Total Flavonoid Content (TFC) The variation on TFC of the *Zingiber zerumbet* SWE extract is depicted in Figure 3.

The TFC obtained varied from 0.9567 ± 0.0154 to 2.3403± 0.0453 mg QE/g DW. In terms of the effect of extraction temperature on total flavonoid content (TFC), from the figure, it shows that at 5 min, the TFC extracted rapidly increased from 100ºC to 180ºC. These findings were in agreement with reported data [19] which showed a positive linear effect of TFC extracted as temperature increased from grape byproducts. However, as observed through Tukey's test analysis, the TFC yield was not significant ($P > 0.05$) at 120 and 140ºC.

Meanwhile, at 10 and 15 min extraction, the TFC was increased from 100ºC until 160º. However, it could be seen at 180ºC that the TFC in *Zingiber zerumbet* extracts had degraded. These findings further support the idea that degradation and hydrolysis of flavonoids happen at high temperatures [17].

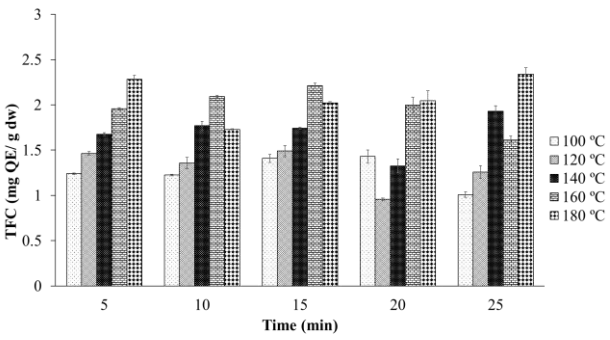

Figure 3. Variation of **t**otal flavonoid content (TFC) of *Zingiber zerumbet* rhizome

Through Tukey's test analysis for the effect of extraction temperature on TFC, it shows for 10 min extraction, the temperature of 140ºC and 160ºC were significant ($P < 0.05$), whilst for 15 min extraction, the TFC was significantly different ($P < 0.05$) at 160ºC. Thus, it could be perceived that for both extraction times, the moderate temperature was preferred. Similar situation was approved by other investigator [20] that reported from the range of 100-300ºC studied, the flavonoid compounds from *Momordica foetida* were preferable to be extracted at a moderate temperature of 150°C.

In contrast, at 20 and 25 min extraction times, the TFC extracted at different temperatures fluctuated. At 20 min, the TFC was decreased at 120ºC and afterwards gradually increased until 180ºC. Meanwhile, at 25 min extraction time, the TFC rapidly increased from 100ºC until 140ºC and decreased at 160ºC. This might be due to the composition of *Zingiber zerumbet* extracted at different temperatures, thus affecting the TFC.

Overall, it could be seen that in different SWE temperature conditions applied, an increase of extraction time had reduced the TFC particularly at the longer exposure of subcritical condition. For example, at 100ºC, the TFC slowly increased from 5 to 20 min and decreased at 25 min extraction. Meanwhile, at 120ºC and 140ºC the TFC rapidly decreased after 20 min extraction and upsurged at 25 min. On the other hand, at 160ºC, it showed that the TFC rapidly increased with time and had optimum flavonoid content at 15 min extraction, while it reduced after 20 min extraction. Whereas at the highest temperature utilized at 180ºC, the TFC rapidly decreased after 5 min extraction and steeply increased as time prolonged.

It could be described as the stability of flavonoids compounds in subcritical condition were dissimilar based on their melting points and molecular weight [21]. In addition, at static extraction mode application, the efficiencies of SWE were strongly dependant on the partition-equilibrium constant as well as on the solubility of compounds [9]. Thus, the fluctuated flavonoid content with extraction time might be due to the different nature of bioactive compound extracted in *Zingiber zerumbet* rhizome.

3. 3. Radical Scavenging Activity (RSA) The radical scavenging activity (RSA) of *Zingiber zerumbet* rhizome after SWE represented in term of % inhibition is shown in Figure 4. The RSA obtained varied from 8.101 ± 0.0684 % inhibition to 83.924 ± 0.0683 % inhibition.

From the figure, the RSA depicts at 100ºC is gradually decreased at 10 and 15 min. Meanwhile, at 15 and 25 min the RSA steeply upsurged. In contrast, a sudden increase of the RSA at 25 min was similarly observed at 120, 140 and 160ºC.

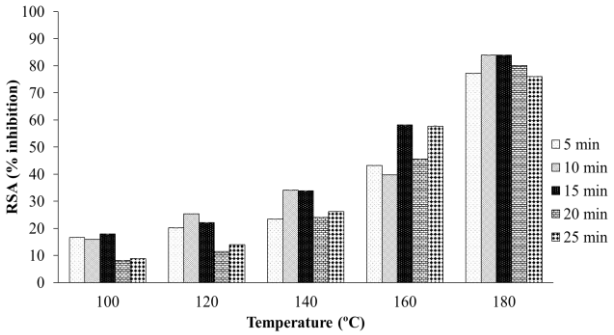

Figure 4. Variation of **r**adical scavenging activity (RSA) of *Zingiber zerumbet* rhizome

The previous study had discovered that the occurrence of hydrolyzation and Maillard reaction happened at long exposure and extreme temperature thus formed another new compound such as hydroxymethylfurfural and melanoidin that could affect the increment of antioxidant activity [12]. This further supported by Tukey's test analysis as lower extraction time were significantly different ($P < 0.05$). Thus, as a whole, it shows that for a lower temperature applied, shorter exposure in subcritical condition was preferred.

In contrast, the reduction of RSA happened at 20 min for all temperatures applied from 100 to 180ºC that might attribute to the possible degradation of the thermo-sensitive related antioxidant compound at longer extraction times [22]. Previously, the occurrence of longer extraction time of more than 20 min had diminished the RSA in *Tagetes erecta L* [17]. As a whole, it could be deduced from all temperatures tested, that 10 to 15 min of SWE was preferred to have higher antioxidant activity in *Zingiber zerumbet* extract.

In terms of the effect of extraction temperature at different times used, the tendency of radical scavenging activity in *Zingiber zerumbet* rhizome appeared to steadily increase as the temperature increased from 100ºC until 180ºC. Meanwhile, the RSA reached 50% inhibition at each extraction time applied at a temperature of more than 160ºC. This supported by Tukey's test as higher temperatures of 140, 160 and 180ºC applied, the RSA was significantly different ($P < 0.05$) for different time used.

The positive effects of increased temperature of more than 120ºC on the RSA using SWE have been reported in *Crocus sativus* petals [22]. This is proven by the theory of water in a subcritical condition wherein decreased properties of viscosity and surface tension of water while observing an increase of the diffusivity had affected the mass transfer rate and induced the antioxidant compounds [11]. In addition, the water polarity was reduced and acted as a relative polar to extract both polar and non-polar compounds that could affect the increment of antioxidant activity [18, 22].

3. 4. Correlation between Radical Scavenging Activity with Total Phenolic Content and Total Flavonoid Content Figure 5 shows the plotted graph of the linear relationship between total phenolic content (TPC) and total flavonoid content (TFC) with radical scavenging activity (RSA) of the SWE extracts.

The degree of correlation between TPC, TFC and RSA using Pearson-correlation coefficient shows that a strong correlation was observed between TPC and RSA (R^2=0.910; $P < 0.05$) compared to moderate correlation (R^2=0.785; $P < 0.05$) perceived in TFC. This relationship revealed that phenolics demonstrate the highest relativity with antioxidant, followed by flavonoids content.

This result was in accordance with a previous study that observed good and moderate linear correlation of *C. sativum* extracts for its antioxidant activity with the TPC of $R^2 = 0.965$ and TFC of $R^2 = 0.709$ [23]. The high correlation coefficient of $R^2 > 0.9$ between TPC and antioxidant activity has been previously reported in saffron petal and licorice extract [18, 22]. This finding was confirmed in literature [16] where phenolic compounds were considered to be the major groups acting as a primary antioxidant or free radical terminators which constitutes on the most important antioxidants of plant materials.

Overall, it shows that using SWE, the phenolic and flavonoid compounds contributed towards radical scavenging activity of *Zingiber zerumbet* rhizome. It could be deduced that the increase of radical scavenging activity in subcritical water condition was affected by the reaction of polyphenols which are polar compounds with various OH groups that are soluble in water, and trapped the DPPH free radicals [3].

Thus, based on the result, high correlations could support the hypothesis of the contribution of phenolics and flavonoids content towards antioxidant capacity in terms of DPPH radical scavenging activity.

3. 5. Comparison with Soxhlet Extraction Soxhlet extraction employed ethanol as a solvent yielded total phenolic content (TPC) of 8.376 mg GAE/ g DW, total flavonoid content (TPC) of 6.948 mg QE/g DW and radical scavenging activity (RSA) gained at 60.45 % inhibition. The comparison of the maximum yield of total phenolic content (TPC), total flavonoid content TFC and radical scavenging activity (RSA) of the extracted rhizome after subcritical water extraction and ethanolic soxhlet are shown in Table 1. A comparison with the ethanolic soxhlet extract shows that SWE gives a better result on TPC and RSA. Using SWE at 160ºC and 10 min, the TPC extracted was the same as SE of 6 hours using ethanol as the solvent. While at 180ºC and 25 min, SWE gave more than 100% recovery or more than twofold of TPC as compared to 5 min of extraction.

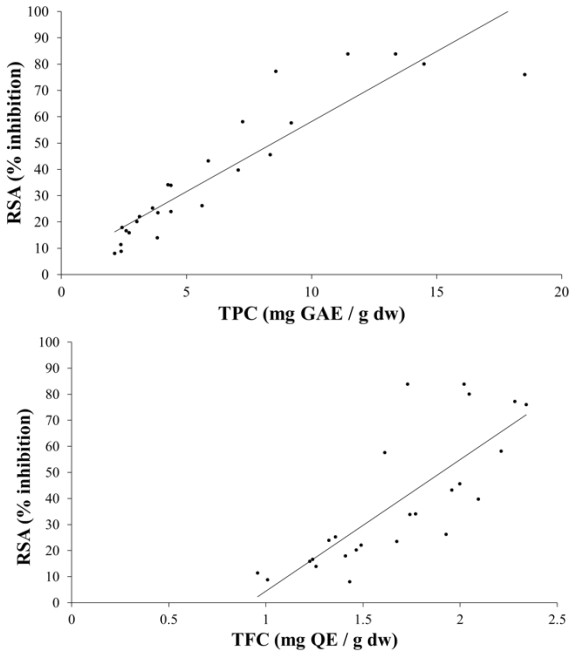

Figure 5. Correlation graphs between TPC and TFC with RSA of *Zingiber zerumbet* rhizome

TABLE 1. TPC, TFC and RSA of *Zingiber zerumbet* rhizome using SWE and soxhlet extraction

	SWE	Soxhlet[a]
TPC (mgGAE/gdw)	18.5233 ± 0.0520[b]	8.376 ± 0.020
TFC (mgQE/gdw)	2.3403± 0.0453[b]	6.948 ± 0.012
RSA (% inhibition)	83.924 ± 0.0683[c]	60.453 ± 0.008

[a] Ethanolic, 6 hour
[b] Water:180ºC,25min
[c] Water: 180ºC,10min

In addition, the RSA also shows better inhibition at 180ºC although it was extracted at a much reduced time. In contrast, the maximum TFC of *Zingiber zerumbet* extracted using SWE was lower by half than the flavonoid extract using soxhlet extraction method at 6.95 mg QE/g dry sample. However, despite the ethanolic extract gave the highest TFC, an extraction time of 6 hours was required.

Many investigators have shown that SWE using water were more efficient in the extraction of bioactive compounds from various types of sources. The previous study has shown that SWE of lower than 150ºC and 120 min was efficient to extract essential oil from *Matricaria Chamomilla* L. compared with hydrodistillation [24]. Meanwhile, SWE also shows prominent application to extract phenolic compounds as compared with soxhlet extraction in pomegranate and *Phlomis umbrosa* Turcz [10, 13]. Therefore, this suggests that SWE of *Zingiber zerumbet* rhizome had also improved the recovery of TPC, TFC and RSA.

4. CONCLUSION

In conclusion, high temperatures and high exposure times used in SWE give the highest amount of TPC, TFC and RSA. The highest TPC and TFC obtained using SWE is 180 and 25 min, giving the values of 18.52 mg GAE/g dry sample and 2.34 mg QE/g dry sample for TPC and TFC, respectively. However, as compared with soxhlet extraction using SWE, the TFC of the extracts is reduced. At the optimum temperature of 180ºC and 10 minutes of SWE, it shows the highest RSA of 83.90%. SWE process for *Zingiber zerumbet* extract is favourable for TPC and anti-oxidant properties, however, the values for TFC, in general, are lower compared to organic solvent extraction.

6. ACKNOWLEDGEMENT

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Total Phenolic Content, Total Flavonoid Content and Radical Scavenging Activity from *Zingiber zerumbet* Rhizome using Subcritical Water Extraction

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چکیده

استخراج آب زیر بحرانی (SWE) یک تکنیک جایگزین است که آب را به عنوان یک حلال عمل می کند. هدف از این کار مقایسهٔ کارایی جذب آب اضافی بحرانی (SWE) در محدوده دما از 100 ºC تا 180 ºC در زمان استخراج در محدوده 5 تا 25 دقیقه با استخراج اتانولی سوکسله از نظر مقدار کل ترکیبات فنلی (TPC)، محتوای فلاونوئیدها (TFC) و فعال شدن فعالیت رادیکال ها (RSA) از ***Zingiber zerumbet*** نتایج نشان داد که در محدوده 180 درجه و 25 دقیقه، مقدار کل فنولیک عصاره بالاترین مقدار در نمونه خشک 19.88 میلی گرم GAE / g است. مقدار کل فلاونوئید مشاهده شده با زمان کمی در دمای مربوطه تغییر می کند. فعالیت آنتی اکسیدان به حداکثر بازداری 83.9 درصد در دمای 180 درجه سانتیگراد و 10 دقیقه SWE رسیده است. در مقایسه با استخراج سوکسله، استخراج آب زیر بحرانی مقدار بیشتری از محتوای فنل و فعالیت آنتی اکسیدانی را به ارمغان می آورد. با این حال، محتوای فلانوبید عصاره ها کاهش یافت. یک همبستگی مستقیم خطی بین فعالیت تخلیه رادیکال با محتوای کل فنولیک و فلاونوئیدها عصاره ها نشان می دهد که بین محتوای فنلی و فعالیت فاضلاب رادیکال (R^2 = 0.910) در مقایسه با همبستگی متوسط (R^2 = 0.785) مشاهده شده در محتوای فلاونوئیدها مشاهده شد. نتایج نشان می دهد که محتوای فنول در ***Zingiber zerumbet*** موجب افزایش فعالیت آنتی اکسیدانی عصاره ها نسبت به مقدار فلاونوئید آن شده است. روند SWE برای عصاره ***Zingiber zerumbet*** برای خواص TPC و آنتی اکسیدانی مناسب است. با این حال، مقادیر TFC به طور کلی نسبت به استخراج حلال های آلی کمتر است. SWE فرایند استخراج جایگزین بالقوه است که باید بیشتر مورد بررسی قرار گیرد.

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