Effect of temperature, time, and milling process on yield, flavonoid, and total phenolic content of *Zingiber officinale* water extract

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Abstract. Several parameters such as temperature, time of extraction, and size of simplicia play significant role in medicinal herb extraction. This study aimed to investigate the effect of those parameters on yield extract, flavonoid, and total phenolic content in water extract of *Zingiber officinale*. The temperatures used were 50, 70 and 90°C and the extraction times were 30, 60 and 90 min. *Z. officinale* in the form of powder and chips were used to study the effect of milling treatment. The correlation among those variables was analysed using ANOVA two-way factors without replication. The result showed that time and temperature did not influence the yield of extract of Powder simplicia. However, time of extraction influenced the extract of simplicia treated without milling process. On the other hand, flavonoid and total phenolic content were not influenced by temperature, time, and milling treatment.

1. Introduction

Recent trends in extraction techniques have largely focused on finding solutions that minimize the use of solvents or to find alternatives for petroleum solvents [1]. Based on The Conductor -like Screening Model for Real Solvent (COSMO-RS) approach, water is categorized as a green polar solvent due to renewable and non-toxic properties [2].

Extraction is often used for the separation of medicinally active compounds that contain in plant or animal tissues apart from the inactive or inert components. For this purpose, a suggestion temperature for drying process should not be more than 60°C and a selective solvent should be employed in standard extraction procedures[3,4].

The bioactivity analysis of water and ethanol extracts of *Zingiber officinale* has been carried out in previous study [5]. Bioactivity depends on the solvent and temperature used in extraction proces [5]. The polar solvent is used in extraction to get numerous secondary metabolites from the plant tissues such as flavonoid and phenolic compounds. Polyphenols are widely distributed in nature and found in terrestrial and marine plants or animals [6]. Among others, flavonoids are the most important component from a nutritional point of view [6]. Flavonoids are polyhydroxy chemical compounds synthesised in various plants[6]. Some of them possess medicinal properties [6]. Meanwhile, the effect of simplicia size is described in the current study. Hence, the present study aimed to investigate the effect of variable temperatures and times on extraction process of *Z. officinale* using water as solvent. Powder and chips simplicia were used in this study.The extraction method used was adapted from the methods of decoction and infusion.

2. Experimental

2.1. Material

Z. officinale was obtained from Bandung, West Java – Indonesia. The rhizome of *Z. officinale* was used for extraction. *Aqua pro distillate* was used for extraction, whereas pro analyses solvent was used for analysis.

2.2. Extraction

Z. officinale was peeled, washed, thinly sliced, and dried at 50°C for three days to get Z. officinale chips [5]. Z. officinale chips were then milled by using an electric food blender. Z. officinale of 10 g was put in 250 mL aqua pro distillate. The decoction was carried out at 90°C for 30 min whereas infusion was carried out at 90°C for 15 min. Temperature was varied at 50, 70 and 90°C whereas time was varied for 30, 60 and 90 min. The resulted extract was filtered, evaporated at 50°C into slightly viscous, and oven-dried at 50°C.

2.3. Total Flavonoid content

Total flavonoid assay was adapted from Saravanakumar and Andriyani methods [5,7]. A sample of 400 µL was put into a test tube, added with 1600 µL aquadest, 120 µL $NaNO_2$ 5%, and kept for 5 min. Further, added with 120 µL $AlCl_3$ 10% in ethanol, 800 µL NaOH 1M and 960 µL aquadest. The absorbance of the reaction mixture was measured at 420 nm. Rutine Quercetin-3-rutinoside hydrate (Sigma) was used as positive control.

2.4. Total phenolic content

Total phenolic assay was adapted from Saravanakumar and Andriyani methods [5,7]. A sample of 300 µL was put into a test tube, added with 1500 µL of Follin 10% and Na_2CO_3 75% . The mixture was incubated at 50°C for 15 min. and measured its absorbance at 760 nm. Gallic acid was used as a positive control.

3. Result and discussion

3.1. Effect of temperature and time on yield extract

As presented in Table 1, the optimal temperature and time for extraction was 70ºC and 60 min respectively, either for *Z. officinale* that was treated by milling process (powder) or not (chips). At higher temperature, some of volatile compounds might have evaporated and other compounds degraded, thus the yield extract decreased. The highest yield extract was resulted from powder simplicia ranged from 8.8 to 18.9 % compared to that from chip simplicia with a range from 8.7 to 9.6 %. Milling process caused surface area of simplicia getting much wider and made it easier for the solvent to diffuse into the tissue leading to the active compound transfer. However, statistically, time and temperature variables did not give any significant influence on the yield as indicated in Table 2 and Table 3. Statistical analysis was used to evaluate the interaction between time and temperature parameters. ANOVA two factors for milling treatment is shown in Table 2 whereas without milling treatment is presented in Table 3.

Table 2 shows that residual (error) means square (MS) in extraction of simplicia treated with milling process was lower than MS of variable time. Therefore it was concluded that there was no interaction between variables temperature and time on the yield of extract. The same result was also shown by simplicia without milling process.

As shown in Table 3, since residual (error) MS in extraction of simplicia without milling treatment was smaller than MS of variable temperature, then it was concluded that there was no interaction of variable temperature on the yield of extract. However, as residual (error) was higher than MS of variable time, thus variable time influenced the yield extraction.

Table 1. Effect of temperature and time on yield extract.

Time (min)	Temperature (°C)					
	50	70	90	50	70	90
	Yield Extract of *Z. officinale* Chips (%, w/dw)			Yield Extract of Powder *Z. officinale* (%,w/dw)		
30	8.7 ± 0.6	9.6 ± 0.4	8.9 ± 0.8	17.0 ± 0.4	16.1 ± 0.9	12.0 ± 0.5
60	9.2 ± 0.4	9.6 ± 0.4	9.1 ± 0.5	16.8 ± 1.5	18.9 ± 1.8	15.3 ± 1.2
90	8.9 ± 0.6	9.5 ± 0.6	9.6 ± 0.4	16.9 ± 0.2	18.4 ± 1.6	8.8 ± 0.2

w: weight; dw: dry weight

Table 2. ANOVA two factors for powder *Z. officinale*.

Source of Variation	Mean Square
Time	4.8
Temperature	29.2
Error	4.1

Table 3. ANOVA two factors of *Z .officinale* chips.

Source of Variation	Mean Square
Time	0.06
Temperature	0.30
Error	0.07

3.2. Effect of milling treatment on total flavonoid and phenolic content

The results of milling treatment on total flavonoid and total phenolic content of *Z.officinale* extract are presented in Table 4. It shows that total flavonoid and total phenolic content of the extract from powder simplicia was higher than that from the chips form. The highest total flavonoid and phenolic content was obtained at 90ºC for both form of simplicia, but a longer time was needed for the chips. These results indicated that extraction of bioactive compounds from simplicia in the form of powder was more effective compared to that from the chips form due to the small size and the wide surface of simplicia. The result of ANOVA two factors for flavonoid is presented in Table 5.

The same as yield extract, the residual (error) MS value was also smaller than either MS of time and temperature or milling treatment and without milling treatment. This result indicated that the effect of time-temperature did not interact with the milling treatment. Moreover, time and temperature variables as well as milling treatment have no effect on flavonoid content. Further, the result of ANOVA two factors for total phenolic content is presented in Table 6.

Table 6 shows that since residual (error) MS was smaller compared to both of the MS variables, therefore the effect of time-temperature did not interact with the milling treatment. Moreover, time and temperature variables have no effect on total phenolic content. In addition, milling/without milling did not affect the phenolic content.

Table 4. Effect of milling treatment on total flavonoid and phenolic content of extract.

Temperature (°C)	Time (min)	*Z. officinale* Chips		Powder *Z. officinale*	
		Total Flavonoid (%)	Total Phenolic Content (%)	Total Flavonoid (%)	Total Phenolic Content (%)
50	30	3.3 ± 0.12	0.6 ± 0.03	5.9 ± 0.13	1.3 ± 0.02
	60	3.9 ± 0.04	1.2 ± 0.18	5.4 ± 0.04	1.2 ± 0.04
	90	2.9 ± 0.02	0.8 ± 0.01	5.6 ± 0.17	1.3 ± 0.01
70	30	3.8 ± 0.20	1.2 ± 0.01	5.2 ± 0.08	1.1 ± 0.05
	60	4.0 ± 0.01	1.2 ± 0.01	6.0 ± 0.04	1.6 ± 0.03
	90	5.1 ± 0.03	1.3 ± 0.00	4.9 ± 0.03	1.4 ± 0.01
90	30	4.3 ± 0.11	1.0 ± 0.01	7.6 ± 0.01	1.8 ± 0.05
	60	5.9 ± 0.65	1.4 ± 0.08	7.0 ± 0.55	1.8 ± 0.03
	90	5.3 ± 0.07	1.4 ± 0.04	6.9 ± 0.09	1.9 ± 0.03

Table 5. ANOVA two factors of total flavonoid of powder/chips.

Source of Variation	Mean Square
Time and temperature	1.26
Powder and Chips	14.09
Error	0.56

Table 6. ANOVA two factors of total phenolic content of powder/chips.

Source of Variation	Mean Square
Time and temperature	0.12
Powder and Chips	0.67
Error	0.04

4. Conclusion

Z. officinale has been extracted in water through milling and without milling treatment. Even though statistically all the variables did not give any interaction, the optimum condition to obtain high yield extract was achieved at 70°C for 60 min. However, the highest total flavonoid and phenolic content was obtained at 90ºC for 30 min for powder simplicia and 60 min for the chips.

Acknowledgement

The authors are grateful to Indonesian Institute of Sciences for financial support for this research.

References

[1] Chemat F, Vian MA 2014 *Alternative Solvents for Natural Products Extraction* 5.
[2] Laurianne Moity, Morgan Durand, Adrien Benazzouz VM, Aubry and J-M 2014 *Alternative Solvents for Natural Products Extraction: In Silico Search for Alternative Green Solvents* 1–24.
[3] Handa 2008 *International Centre For Science And High Technology*: *An Overview of Extraction Techniques for Medicinal and Aromatic Plan* 21–54.

[4] Anonim 2008 *Farmakope Herbal Indonesia I*. Departemen Kesehatan Republik Indonesia XXV.
[5] Andriyani R, Budiati TA and Pudjiraharti S 2015 *Procedia Chemistry* **(16)**149–54.
[6] Somasundaram SG and Oommen B 2013 *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases: Antioxidant Flavonoids for Arthritis Treatment Human and Animal Models* 1-16.
[7] Saravanakumar A 2009 *Pak. J. Pharm. Sci.* **22(3)**:282–6.