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Antioxidative Properties of Essential Spices in an Indonesian Non-Alcoholic Beverage 'Bir Pletok'

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ABSTRACT

Clove, cinnamon and nutmeg are essential spices in Bir Pletok, a traditional non-alcoholic Indonesian beverage. This study aimed to determine the antioxidant activities in different aqueous extracts of clove, cinnamon and nutmeg available in Indonesia and Thailand. Extraction was performed by boiling the mixture of spice and water (25% w/v) at 95-97°C for 15-45 min. Total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC), color (L*, a*, b*) and pH values of the extracts were determined. Aqueous extracts of clove showed the highest TPC, DPPH, FRAP and ORAC, followed by cinnamon and nutmeg ($p \leq 0.05$). Extending extraction time significantly increased the antioxidative properties of the aqueous extract of all spices. Aqueous extracts of spice from Indonesia exhibited significantly higher TPC and FRAP than those obtained in Thailand ($p \leq 0.05$), but DPPH and ORAC were indifferent ($p > 0.05$). The L*, a* and pH values of the spice extracts were not affected neither by the origin nor extraction time. Meanwhile, b* values of the extracts significantly increased with the length of extraction, and those of Indonesian spices were about 40% higher than Thailand ($p \leq 0.05$). In conclusion, type and source of spices as well as extraction time were the parameters that largely affected the antioxidative properties of the hot water extracts. Thus, it might be possible to improve the potential health benefits of Bir Pletok by optimizing the proportion of spices in the recipe and the length of boiling, as well as ingredient sourcing.

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INTRODUCTION

Bir Pletok is a traditional red non-alcoholic beverage with spicy taste originated from Indonesia (Jakarta Assessment Institute for Agricultural Technology, 2016). The beverage was introduced by Betawi tribe during Dutch occupation as a halal substitute for beer. The beverage is believed to help reduce the symptoms of sprue, fatique, rheumatism and fever (Harmanto, 2000). Bir Pletok is made from different types of herb and spice, including cardamom, cinnamon, clove, ginger, kaffir lime leaves, lemongrass, nutmeg, pandan leaves, pepper and sappan wood (Jakarta Assessment Institute for Agricultural Technology, 2016). To make the beverage, herbs and spices are boiled in water for 30 min and the mixture is filtered through double-layered cheese cloth to remove the spice residue. In general, clove, cinnamon and nutmeg are essential spices presenting in the highest proportion in the recipes, although their proportions may be different from one recipe to another. Therefore these three spices are likely to majorly impart the antioxidative properties of the obtained beverage.

Antioxidant properties of clove, cinnamon and nutmeg have been reported in previous studies. Rojas et al. (2014) stated that clove is one of the richest sources of phenolic compounds such as eugenol, eugenol acetate, and gallic acid. It also contains flavonoids, hydroxybenzoic acids, hydroxycinnamic acids and hydroxyphenyl propanes. Shan et al. (2005) reported that clove buds showed the highest antioxidant activity and polyphenol content as compared with other 25 different spices; and thus it has high potential as a radical scavenger and as a commercial source of polyphenols. The result showed wide variation in Trolox equivalent antioxidant capacity (0.55-168.7 mmol/100 g) and total phenolic content (0.04-14.38 g of gallic acid equivalent/100 g). Meanwhile, Peter (2006) reported that cinnamon consists of cinnamaldehyde which is responsible for sweet taste. Dragland et al. (2003) added that intake of 1 g of cinnamon in a normal diet, might contribute to the relevant intake of plant antioxidants, and could be a better source of dietary antioxidants than many other food groups. On the other hand, Hou et al. (2012) mentioned that nutmeg contains licarin-B, dehydrodiisoeugenol, malabaricone B, malabaricone C, β-sitosterol, and daucosterol. It also has a potential to be used as a natural antioxidant in food because malabaricone C has been reported to exhibit a stronger antioxidant activity than common synthetic antioxidants. In addition, nutmeg extracted by ethyl acetate showed a strong antioxidant activities in which malabaricone C showed strongest ability reducing power as well as radical scavenging activity.

It is known that antioxidant activities of spice extracts largely depend on growing areas, processing and storage (Das and Sharangi, 2018). Moreover, the conditions of extraction such as solvent, temperature and time largely affect the amount and activities of antioxidant in the extracts. In most of the aforementioned studies (Hossain et al., 2008 and Shan et al., 2005), extractions of clove, cinnamon and nutmeg were conducted using 80% methanol under ambient temperature; whereas, in the production of *Bir Pletok*, spices are commonly boiled in water (Jakarta Assessment Institute for Agricultural Technology, 2016). The study on antioxidant properties of hot water extracts of these three important spices is still limited. Therefore, this study aims to determine the antioxidant activities of hot water extracts from clove, cinnamon and nutmeg obtained from different sources, i.e., Indonesia and Thailand, and extraction conditions. The results obtained from this study will be useful in improving the antioxidative properties of *Bir Pletok* by optimizing the source of ingredients, processing parameters, as well as the recipes.

MATERIALS AND METHODS

Materials

Dry spices, including clove, cinnamon and nutmeg were purchased, each in 3 separated batches, from local supermarkets in Bangkok, Thailand and Semarang, Indonesia during January to March 2018. The spices were vacuum-packed in aluminum foil bags and kept at room temperature in a desiccator. Moisture content of spice samples, analyzed according to AOAC Official Method (AOAC, 2016), are listed in Table 1. All chemical, unless stated otherwise, were obtained from Sigma-Aldrich (St. Louis, Missouri, U.S.A.).

Table 1. Moisture content of clove, cinnamon and nutmeg samples

Spice	Moisture content (g/100 g)[1]	
	Thailand	Indonesia
Clove	26.65±1.59	27.10±0.41
Cinnamon	12.72±0.37	14.37±1.77
Nutmeg	9.28±0.28	11.33±0.33

[1]Means±standard deviations from 3 purchasing batches

Preparation of hot water-extract

Each spice was mixed with deionized (DI) water at a ratio of 25 g of spice in 100 ml of water without size reduction or grinding into powder. Extraction was performed using the modified method from Jakarta Assessment Institute for Agricultural Technology (2016) by heating the spice-water mixture on a hot plate until the temperature reached 95-97°C for 15, 30 or 45 min. Then, the mixture was filtered through double-layered cheese cloth to remove the spice residue. The obtained hot-water extract was hot-filled in appropriate container, immediately cooled down in an ice bath, and kept frozen at -20°C in a freezer for further analyses.

Determination of pH

The pH value was measured using a pH meter (EcoMet P25, Istek, Seoul, Korea) at 25°C. Calibration with standard buffers pH 4, 7, and 10 had been conducted before measurements on each day.

Determination of color

Color values (L*, a*, b*) were measured using a spectrocolorimeter (ColorFlex EZ, Hunter Associates Laboratory, Reston, Virginia, U.S.A.) which was pre-calibrated with black- and white-colored tiles. L* represents the lightness while a* and b* are color coordinates (+a*=red, -a*=green, +b*=yellow and -b*=blue).

Determination of total phenolic content (TPC)

TPC was analyzed according to the method of Ainsworth and Gillespie (2007). Twenty-five microliters of sample was mixed with 50 µl of 10% (v/v) solution of 2 N Folin-Ciocalteau reagent in deionized water in a 96-well microplate. After 5 min incubation, 200 µl of 7.5 % (w/v) Na_2CO_3 was added. The mixture was incubated in the dark at room temperature for 2 h. The absorbance at 765 nm was measured by using a microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, Vermont, U.S.A). The results were calculated as gallic acid equivalent (mg GAE/g dry wt) prepared from gallic acid (10-200 µg/ml).

Determination of 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method (DPPH)

DPPH assay was performed according to Fukumoto and Mazza (2000) with modification. Twenty two microliters sample and 200 µl

solution of DPPH prepared in 95% of ethanol were transferred into 96-well microplate and incubated at room temperature for 30 min. The reaction was monitored using the microplate reader at 520 nm wavelength. The results were calculated as µmol Trolox equivalent (TE)/g dry wt prepared from Trolox solution (0.01- 0.64 µM).

Determination of ferric reducing antioxidant power activity (FRAP)

FRAP assay was performed according to the method of Benzie and Strain (1996) with slight modification. Twenty microliters of sample was mixed with 150 µl FRAP reagent containing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-striazine in 40 mM HCl and $FeCl_3.6H2O$ (10:1:1) in 96-well microplate and incubated at room temperature for 8 min. The reaction was monitored using the microplate reader at a 595 nm wavelength. The results were calculated as FRAP values (mmol TE/g dry wt).

Determination of oxygen radical absorbance capacity (ORAC)

ORAC assay was performed according to Ou et al. (2001). A hundred and fifty microliters of fluorescein working solution (30 nM) and 25 µl sample or Trolox (3.125-100 µM) dissolved in 75 mM phosphate buffer pH 7.4 were transferred into 96-well microplate and incubated at 37°C for 15 min. After adding 25 µl of 2,2'-azobis (2-amidinopropane) dihydrochloride known as AAPH (150 mM) to the microplate, the fluorescence was recorded under constant shaking at 1 min intervals for 90 min at excitation and emission wavelengths of 485 and 528 nm, respectively. The results were calculated based on the differences in areas under the sodium fluorescein decay curve (AUC) and expressed as Trolox equivalent (mmol TE/g dry wt). The AUC was calculated as:

$$AUC = 0.5 + \sum_{i=1}^{i=90} \frac{f_i}{f_0}$$

where fo is the initiation fluorescence reading at 0 minute and f1,2,…,90 is the fluorescence reading at 1,2,…,90 minute(s).

Statistical analysis

The experiment was conducted in 2x3 factorial in Completely Randomized Design of three replicates. Data was processed using a computer software (IBM SPSS Statistics 19.0, IBM, Armonk, New York, U.S.A.). Mean difference was carried out by independent sample t-test or one-way Analysis of Variance and Duncan's multiple range test at p≤0.05.

RESULTS AND DISCUSSION

pH

The pH values of all hot water extracts ranged from 3.89 up to 5.70 (Table 2). Hot water extracts of clove were the most acidic, followed by those of cinnamon and nutmeg. This was consistent to the pH values reported elsewhere of clove, cinnamon and nutmeg, i.e., 3.80, 4.80, and 5.75, respectively (Baby and Girish, 2010). There was no significant difference (p>0.05) in pH value of hot water extracts of each similar spice obtained from different extraction durations. The acidic pH of the spice extracts was possibly caused by phenolic acid in spices that were solubilized during the hot water extraction (Guldiken et al., 2018). Besides that, some bioactive compounds such as gallic acid and p-coumaric acid in clove, protocatechuic acid and caffeic acid in cinnamon and nutmeg might leach into the extract as well (Santos et al., 2017 and Guldiken et al., 2018). At any similar extraction duration, the pH values of hot water extract of all spices, except nutmeg, from both countries were not significantly different (p>0.05). Nutmeg from Thailand gave hot water extract with significantly higher pH values than that from Indonesia. This might cause by the lower antioxidant content of Thai nutmeg which means there were less phenolic acid, protocatechuic acid and caffeic acid solubilized in hot water extracts (Guldiken et al., 2018).

Table 2. pH values of the hot water extracts of clove, cinnamon and nutmeg

Spice	Extraction time (min)	pH[1]	
		Indonesia	Thailand
Clove	15	3.94±0.00^{aA}	4.00±0.00^{aA}
	30	3.98±0.08^{aA}	3.95±0.03^{aA}
	45	3.89±0.01^{aA}	4.00±0.00^{aA}
Cinnamon	15	5.22±0.02^{aA}	5.06±0.00^{aB}
	30	4.99±0.04^{aA}	4.85±0.09^{aA}
	45	5.05±0.02^{aA}	4.90±0.02^{aA}
Nutmeg	15	5.17±0.14^{aB}	5.70±0.14^{aA}
	30	5.38±0.04^{aB}	5.51±0.05^{aA}
	45	5.15±0.02^{aB}	5.51±0.02^{aA}

[1]Means±standard deviations of 3 replicates

$^{a-c}$Means within the same column of the same spice with different superscripts are significantly different (p≤0.05).

^{A,B}Means within the same row with different superscripts are significantly different (p≤0.05).

Color

Table 3 reveals the minimal difference in color, especially L* and a* values, of hot-water extracts of similar spice obtained from different sources and extraction duration. L* and a* values of all hot water extracts ranged from 88.58 to 91.15 and -1.55 to 0.90, respectively. Meanwhile, b* values were more differ among the spices, i.e., 3.20 to 5.94, 2.67 to 4.87, and 0.11 to 0.35 for the aqueous extract of clove, cinnamon, and nutmeg, respectively. Extracts of Indonesian clove were more yellow in color than Thai clove; while for cinnamon and nutmeg, the extracts of Indonesian spices tended to be less yellow. It is known that spices with more yellow color might consist of more phenolic acid and thus have higher antioxidant content (Rubio-Perez et al., 2014 and Guldiken et al., 2018).

Comparing with the color values of dry spices, it was found that all aqueous extracts were generally darker, less red and less yellow. L* values of cloves, cinnamon and nutmeg were 20.00–20.70, 28.59–29.43 and 34.60–36.42, respectively. The a* values were 6.32-6.86, 7.21-8.12, and 5.55-5.88, respectively for cloves, cinnamon and nutmeg. In addition, b* values of the three dry spices were about similar at 9.73-9.91, 10.06-10.55, and 9.84-10.61, respectively. There was almost no difference in each color value of the same spice obtained from different countries, except that dry cinnamon from Thailand was redder and the Thai nutmeg was darker in color than that obtained in Indonesia (Table 4).

Total phenolic content (TPC)

Hot water extract of clove showed the highest TPC, followed by cinnamon and nutmeg (Table 5), which correlated with the acidity of the water extracts (Table 2). Significant differences (p≤0.05) in TPC were also observed in the extracts obtained from clove of different sources and extraction duration. Longer extraction duration increased TPC of the extract of all spices; while at any similar extraction time the TPC of extracts of Indonesian spices tended to be higher than that of Thai spices.

Table 3. Color values of the hot water extracts of clove, cinnamon and nutmeg

Spice	Color value	Extraction time (min)	Value[1]	
			Indonesia	Thailand
Clove	L*	15	88.75±0.01^{bA}	88.71±0.01^{cB}
		30	89.89±0.09^{aA}	89.78±0.04^{bB}
		45	88.58±0.01^{cB}	90.52±0.01^{aA}
	a*	15	-1.39±0.01^{aA}	-1.42±0.01^{cB}
		30	-1.55±0.01^{bB}	-1.18±0.01^{aA}
		45	-1.37±0.03^{aA}	-1.33±0.01^{bA}
	b*	15	5.40±0.01^{bA}	4.10±0.06^{aB}
		30	4.64±0.13^{cA}	3.80±0.09^{bB}
		45	5.94±0.05^{aA}	3.20±0.10^{cB}
Cinnamon	L*	15	89.70±0.03^{aA}	89.14±0.01^{aB}
		30	90.02±0.30^{aA}	89.23±0.26^{aA}
		45	89.35±0.07^{aA}	89.11±0.07^{aB}
	a*	15	-1.05±0.01^{bA}	-1.07±0.01^{bB}
		30	-1.00±0.05^{abA}	-0.99±0.06^{bA}
		45	-1.02±0.02^{aB}	-0.90±0.03^{aA}
	b*	15	2.67±0.08^{bB}	4.87±0.04^{aA}
		30	3.55±0.27^{bB}	4.53±0.24^{abA}
		45	3.84±0.19^{aB}	4.45±0.15^{bA}
Nutmeg	L*	15	91.16±0.01^{aA}	91.10±0.01^{aB}
		30	91.15 ±0.01^{aA}	91.13±0.01^{aA}
		45	91.13 ±0.04^{aA}	91.13±0.02^{aA}
	a*	15	-1.20±0.01^{aA}	-1.23±0.02^{aA}
		30	-1.19±0.01^{aA}	-1.21±0.01^{aA}
		45	-1.18±0.02^{aA}	-1.21±0.01^{aA}
	b*	15	0.14±0.01^{bB}	0.31±0.01^{aA}
		30	0.11±0.01^{cB}	0.35±0.01^{bA}
		45	0.20±0.01^{aB}	0.32±0.01^{aA}

[1]Means±standard deviations of 3 replicates

[a-c]Means of each color value within the same column of the same spice with different superscripts are significantly different (p≤0.05).

[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

Table 4. Color values of clove, cinnamon and nutmeg

Spice	Color value	Value[1]	
		Indonesia	Thailand
Clove	L*	20.00±0.99^{A}	20.70±1.25^{A}
	a*	6.86±0.61^{A}	6.32±0.30^{A}
	b*	9.91±0.31^{A}	9.73±0.93^{A}
Cinnamon	L*	28.59±1.04^{A}	29.43±0.66^{A}
	a*	8.12±0.20^{A}	7.21±0.99^{B}
	b*	10.06±0.77^{A}	10.55±0.62^{A}
Nutmeg	L*	34.60±0.56^{B}	36.42±1.90^{A}
	a*	5.88±0.51^{A}	5.55±0.29^{A}
	b*	9.84±0.55^{A}	10.61±0.64^{A}

[1]Means±standard deviations of 3 purchasing batches

[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

Phenolic compounds are known to have antioxidant activity that provide health benefits, especially those present in spices (Vaya et al., 1997; Mihaylova et al., 2015). They donate hydrogen to radicals and break the oxidation reaction at the initiation step (Agbor et al., 2006). The higher TPC in hot water extracts obtained from longer extraction durations was due to the fact that the tissue structure of the spices was softened by heat applied during boiling so that phenolic compounds could leach out of the spices more easily (Chipurura et al., 2010). TPC content of ethanol and ethanol/water extracts of clove have been reported to be higher than nutmeg and comparable to cinnamon (Przygodzka et al., 2014). The TPC of water extracts obtained in this study was lower than those reported previously, i.e., 180–230 mg GAE/g dry wt for clove extracted by ethanol (El-Maati et al., 2016), 186 mg GAE/g for cinnamon extracted by 50% acetone (Su *et al*., 2007) and 33 mg GAE/g dry wt for nutmeg extracted by DI water (Aliakbarlu et al., 2014). Such differences were mainly due to the extracting solvent and condition of extraction. Moreover, the differences in source, handling and storage conditions of spices might also impart (Peter, 2006).

Table 5. Total phenolic content of the hot water extracts of clove, cinnamon and nutmeg

Spice	Extraction time (min)	TPC (mg GAE/g dry wt)[1]	
		Indonesia	Thailand
Clove	15	24.90±0.15^{cA}	20.80±0.34^{cB}
	30	29.75±0.05^{bA}	26.07±0.03^{bB}
	45	54.43±0.63^{aA}	49.61±0.92^{aB}
Cinnamon	15	15.99±0.05^{cA}	15.49±0.11^{cB}
	30	18.38±0.17^{bB}	19.29±0.09^{bA}
	45	25.53±0.46^{aA}	24.15±0.19^{aB}
Nutmeg	15	0.29±0.00^{cA}	0.26±0.02^{cB}
	30	0.42±0.01^{bA}	0.37±0.00^{bB}
	45	0.58±0.01^{aA}	0.48±0.01^{aB}

[1]Means±standard deviations of 3 replicates

[a-c]Means within the same column of the same spice with different superscripts are significantly different (p≤0.05).

[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

2,2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH)

Considering DPPH assay (Table 6), hot water extracts of clove showed the highest DPPH activity, which was slightly higher than those of cinnamon and nutmeg. Modest increases in DPPH values were observed for the extracts of cinnamon and nutmeg when the extraction duration was extended to 45 min; while longer extraction times tended to lower the DPPH value of clove extract. Unlike TPC, extracts of spices from both countries at any similar extraction time showed no difference in their DPPH; except for clove and nutmeg extracted for 45 min. However, such differences were in small extents though they were statistically significant (p≤0.05). Assefa et al. (2018) reported that DPPH values of methanolic extract of cinnamon, clove and nutmeg were 91.09, 55.84 and 7.53 mg TE/g, respectively. However, the hot water extracts in this study exhibited much lower DPPH values.

Table 6. 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of the hot water extracts of clove, cinnamon and nutmeg

Spice	Extraction time (min)	DPPH (µmol TE/g dry wt)[1]	
		Indonesia	Thailand
Clove	15	3.44±0.03^{aA}	3.48±0.07^{aA}
	30	3.31±0.10^{aA}	3.39±0.01^{aA}
	45	2.38±0.22^{bB}	3.21±0.30^{aA}
Cinnamon	15	2.68±0.08^{bA}	2.70±0.14^{aA}
	30	2.76±0.03^{bA}	2.78±0.01^{aA}
	45	2.89±0.07^{aA}	2.78±0.13^{aA}
Nutmeg	15	1.18±0.04^{cA}	1.26±0.08^{aA}
	30	1.68±0.04^{bA}	1.66±0.14^{aA}
	45	2.21±0.15^{aA}	1.74±0.18^{aB}

[1]Means±standard deviations of 3 replicates
[a-c]Means within the same column of the same spice with different superscripts are significantly different (p≤0.05).
[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

Ferric reducing antioxidant power activity (FRAP)

Based on Table 7, cloves extracts showed the highest FRAP values among the three spices. Hossain et al. (2008) mentioned that clove extracted by 80% methanol showed the highest FRAP for about 61.63 g Trolox/ 100 g dry wt, followed by cinnamon for about 24.27 g Trolox/ 100 g dry wt and nutmeg for about 4.31 g Trolox/ 100 g dry wt. The result showed the higher extraction time, the higher FRAP values in all spices. Moreover, extraction at 45 min showed the highest FRAP values. The results was consistent with that previously reported for hot water extracts of fresh leaves of lemon balm that FRAP values increased with duration of extraction (Mihaylova et al., 2015). Meanwhile, extracts of spices from Indonesian spices had slightly higher FRAP values than Thai spices.

Table 7. Ferric reducing antioxidant power of the hot water extracts of clove, cinnamon and nutmeg

Spice	Extraction time (min)	FRAP (mmol TE/g dry wt)[1]	
		Indonesia	Thailand
Clove	15	319.65±1.57^{cA}	297.31±2.87^{cB}
	30	419.64±17.80^{bA}	308.35±2.87^{bB}
	45	568.36±14.19^{aA}	556.86±6.90^{aA}
Cinnamon	15	79.48±1.54^{cA}	54.41±1.25^{cB}
	30	112.08±3.75^{bA}	96.38±4.16^{bB}
	45	177.88±3.27^{aA}	137.55±3.77^{aB}
Nutmeg	15	0.82±0.01^{cA}	0.54±0.01^{cB}
	30	1.59±0.05^{bA}	1.15±0.02^{bB}
	45	1.82±0.08^{aA}	1.27±0.10^{aB}

[1]Means±standard deviations of 3 replicates
[a-c]Means within the same column of the same spice with different superscripts are significantly different (p≤0.05).
[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

Oxygen radical absorbance capacity (ORAC)

Hot water extract of all spices exhibited higher ORAC values when extraction was performed for longer duration (Table 8). ORAC values of the extracts obtained from 45 min extraction were about 2 times higher than those extracted for 15 min, regardless of the type and source of spices. Like other antioxidant parameters, the greatest ORAC value was observed in clove extracts, followed by those of cinnamon and nutmeg. The greater ORAC value of cinnamon than nutmeg was also reported for extracts prepared using 50% acetone or 80% ethanol (Mariutti et al., 2008). This study is synchronized with the previous study of Haytowitz and Bhagwat (2010), in which ORAC values of cloves, cinnamon and nutmeg prepared by a mixed solvent of hexane, ethanol and water were reported to be 290.28, 131.42 and 69.64 mmol TE/ 100 g, respectively. Dudonné et al. (2009) added that water extract of cinnamon also possessed ORAC value of 8.52 mmol TE/g dry wt. Interestingly, the result from this study showed much higher ORAC value for all spices than those two studies. Based on the effect of source, aqueous extracts of cinnamon and nutmeg obtained from Indonesia showed higher ORAC values than those purchased in Thailand. However, such difference was minimally observed for the extracts of clove from both countries.

Table 8. Oxygen radical absorbance capacity of the hot water extracts of clove, cinnamon and nutmeg

Spice	Extraction time (min)	ORAC (mmol TE/g dry wt)[1]	
		Indonesia	Thailand
Clove	15	682.21±14.98^{cA}	698.94±50.41^{bA}
	30	951.99±64.57^{bA}	752.73±56.84^{bB}
	45	1,357.91±60.75^{aA}	1,377.52±120.95^{aA}
Cinnamon	15	250.58±24.39^{bA}	206.87±14.07^{cA}
	30	285.16±6.73^{bA}	259.50±9.58^{bB}
	45	448.96±10.28^{aB}	546.68±6.45^{aA}
Nutmeg	15	5.52±0.33^{cA}	4.33±0.34^{cA}
	30	9.77±0.24^{bA}	8.00±0.26^{bB}
	45	11.72±0.27^{aA}	9.64±0.58^{aB}

[1]Means±standard deviations of 3 replicates
[a-c]Means within the same column of the same spice with different superscripts are significantly different (p≤0.05).
[A,B]Means within the same row with different superscripts are significantly different (p≤0.05).

The three antioxidant assays used for determination of antioxidant activities in this study is based on different mechanisms. DPPH assay measures the ability of antioxidant activity of substance in donating hydrogen atom (El-Maati et al., 2016). In FRAP assay, the antioxidant capacity is measured on the basis of the ability to reduce ferric(III) ions to ferrous(II) ions (Song et al., 2010). Meanwhile, ORAC measures antioxidant inhibition of peroxyl radical induced oxidation and thus reflects radical chain breaking antioxidant activity by hydrogen atom transfer (Ou, et al., 2001). However, this study did not determine the metal chelating activity of the spices.

Water extracts of clove showed the highest result of TPC value and antioxidant activities compared to those of the other two spices. Guldiken et al. (2018) and Peter (2006) stated that the polyphenolic compounds present in spices of clove is phenolic acid with strong

antioxidant potential such as gallic acid, protocatechuic acid, syringic acid and p-coumaric acid. The antioxidant potential of clove extracts may be due to its strong hydrogen-donating and metal chelating ability, as well as its effectiveness as a scavenger of hydrogen peroxide, superoxide and free radicals (Hossain et al., 2008). Abdelfadel et al. (2016) also added that clove extract showed the highest TPC and antioxidant activity both for hot and cold extraction among herbs and spices such as thyme, cumin, ginger, and cinnamon. However, extraction using boiling water (95-97oC), especially for longer period of time, led to the decrease in antioxidant activity of clove extract, possibly due to the lower phenolic content (Table 5). Liu et al. (2008) supported that clove extract showed the highest TPC value, the second highest for FRAP among 68 spices and exhibited high antioxidant potential with scavenging activity for DPPH assay. It could also be inferred that the acidic pH of the water extracts of spices correlated with their antioxidative properties, especially TPC (Table 2 and Table 5).

Source of spices is one of the important factors for antioxidant activities. The result from this study showed that TPC, FRAP and ORAC of hot water extracts of spices obtained from different sources were significantly different in ($p \leq 0.05$). Indonesian spices showed greater antioxidative properties than Thai spices. Besides source of raw materials, there are some factors that could affect antioxidative properties of spices, particularly the conditions for growing, processing and storage (Das and Sharangi, 2018). Gibson and Newsham (2018) added that spices are also sensitive to oxygen, heat, light, moisture, and temperature. Therefore, improper storage of spices might increase their moisture content, decrease their volatile compound, resulting in the changes in color and antioxidative properties (Santos et al., 2017). It is known that Indonesia is one of the biggest producing and exporting countries of spices in the world while most spices available in Thailand are imported from elsewhere (Chomchalow, 2001). So, in this study, the lower antioxidant properties of water extracts of spices from Thailand might be due to the oxidation occur during shipping and storage from the exporting country (Peter, 2006).

Extraction duration also affected the TPC, FRAP and ORAC values of water extracts of spices. The longer extraction time provided extract with the higher TPC, FRAP and ORAC values, except for cloves that DPPH slightly decreased when extraction was extended from 30 to 45 min. The optimum extraction time to obtain the highest TPC, DPPH, FRAP, and ORAC values was 30 min. Effect of extraction time on TPC and antioxidant activities of herb and spice extracts has been reported in several studies (Mihaylova et al., 2015; Toh et al., 2016).

CONCLUSIONS

Type and source of spices as well as extraction time largely affected the antioxidative properties of the hot water extracts. Thus, consideration should be taken on further investigation to improve the potential health benefits of *Bir Pletok* by optimizing the length of boiling, as well as ingredient sourcing.

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