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EFFECT OF DIFFERENT SOLVENTS AND TEMPERATURES OF EXTRACTION ON CITRAL CONCENTRATION AND ANTIOXIDANT PROPERTIES OF FREEZE-DRIED LEMONGRASS (*Cymbopogon citratus*) POWDER'S EXTRACT

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ABSTRACT

This study aimed to determine the effect of different solvents and temperatures on the citral concentration and antioxidant properties of freeze-dried lemongrass powder extract. Freeze-dried lemongrass powder was extracted using three solvent extractions of hexane, petroleum ether, and methanol at three different temperatures of 27°C, 40°C, and 60°C. Citral concentrations of lemongrass extract were identified and quantified by gas chromatography and flame ionization detector (GC-FID) while antioxidant properties were determined using analysis of total phenolic content, DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) assay. Results from this study show that the methanol extract at 40°C obtained the highest total yield (37.70 ± 1.78%). Hexane was found to be the most effective solvent to extract citral compound from freeze-dried lemongrass powder with 239.08 ± 1.28 mg/mL of concentration at temperature extraction of 27°C. However, the efficiency of hexane decreasing upon heating. This study suggested that the solvent extraction using methanol at 40°C is the efficient extraction for total phenolic compound (7419.33 ± 110.78 mg GAE/L), DPPH radical scavenging activity (87.45 ± 2.70%), and FRAP assay (52.11 ± 0.07 TE/mL) of freeze-dried lemongrass powder.

Key words: Extraction solvent, extraction temperature, freeze-dried lemongrass powder, citral concentration, antioxidant properties

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is an aromatic herb with a fresh grassy lemony aroma and is commonly used as a food flavoring agent. The dominant volatile compound in lemongrass is known as citral; a combination of two geometric isomers of neral and geranial in which consist of 30% and 60%, respectively in lemongrass oil (Charles, 2013). Approximately 65-85% of citral can be extracted from lemongrass stalk. This monoterpene aldehyde compound is soluble in an organic solvent but insoluble in water (Attokaran, 2011). Citral can be used to produce ionone, vitamin A and β-carotene as well as exhibit antioxidant activity and obtain antimicrobial, antibacterial, and anti-inflammatory effect (Charles, 2013). Instead of citral, lemongrass also contains tannins, flavonoids, alkaloids, steroids saponins, phenolics, glycosides, deoxy sugar, and anthraquinones (Soares *et al.*, 2013; Ekpenyong *et al.*, 2015).

Hydro distillation (HD), steam distillation, and solvent extraction are common methods used to extract the essential oil from lemongrass. Solvent extraction with low temperature was suggested to gain more yield of extract and minimal loss of heat-sensitive of bioactive components compared to steam distillation method (Suryawanshi *et al.*, 2016). The extraction temperature and solvent play important factors in affecting the quality of extract. Extraction temperature can accelerate mass transfer rates and increase the yield of extract. However, beyond optimum temperature will cause chemical and enzymatic degradation of polyphenolic constituents (Akowuah & Zhari, 2010). Different solvents extraction has a different polarity which highly selective to certain compounds. For example, non-polar solvents such as ether, petroleum ether,

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hexane, and dichloromethane are effective to extract aldehyde (Yamada & Goto, 2003) while the alcoholic solvents effective to extract the phenolic compound from the plant (Khlifi *et al.*, 2011). Selim (2011) suggested using a methanol extract of lemongrass as potential alternatives for synthetic bactericides in the food industry. According to Suryawanshi *et al.* (2016), the percentage essential oil yield for the partially dried leaves was found to be higher than that of the fresh leaves. Extensive studies have been done to determine the quality of lemongrass powder prepared with several drying methods such as sun drying, shade drying, oven drying, microwave-drying and freeze-drying (Hanaa *et al.*, 2012; Tajidin *et al.*, 2012; Hashim *et al.*, 2019; Hashim *et al.*, 2020). Previous studies on Hashim *et al.* (2019) and Hashim *et al.* (2020) also suggested that freeze-drying was the suitable method for preserving the color qualities and citral compound of lemongrass powder. Therefore, this study was conducted to determine the effect of different solvents and temperatures of extraction on the quality of extract in terms of the yield of extract, citral concentration, and antioxidant properties of freeze-dried lemongrass powder.

MATERIALS AND METHODS

Preparation of freeze-dried lemongrass powder

Fresh lemongrass (*Cymbopogon citratus*) stalks with a moisture content of ≥80% were purchased from the local wet market at Kuala Nerus, Terengganu, Malaysia, and were then coarsely chopped before drying process using a freeze dryer (Labconco benchtop freeze dryer, USA) at a temperature of -55°C with 0.028 kPa for 48 hr following Hashim *et al.* (2019) with minor modifications. The dried sample was grounded using Waring blender for 30 sec and sieved through 0.5 mm size mesh of sieve shaker before stored in ziplock plastic covered with aluminum foil at -18.0 ± 1.0°C for further analysis.

Solvent extraction

Freeze-dried lemongrass powder was extracted using different solvents of hexane, petroleum ether, and methanol according to the modification method by Manzan *et al.* (2003). Lemongrass powder (10 g) was added with 250 mL of solvent and the mixture was then placed in a conical flask covered with aluminum foil before agitated in a water bath shaker at three different suitable temperatures for heat-sensitive compounds which included ambient (27°C) as control, 40°C (low) and 60°C (high). Two hours of maceration method of extraction was run and then kept left for another 1 hr as a function of oxidation minimization of bioactive compounds during the extraction process (Marian & Shahidi, 2006). All the residues of solvents were removed using a rotary evaporator (Eyela. N-1100s-WD, Japan) at a temperature of 35°C.

Determination of yield of extract

Yield of freeze-dried lemongrass powder extract was calculated using the following equation:

$$\text{Yield of extract (\%)} = \frac{\text{(Weight of round bottom flask + oil (g)) – Weight of round bottom flask (g)}}{\text{Weight of lemongrass powder (g)}} \times 100\%$$

Determination of citral concentration

The citral concentration of freeze-dried lemongrass powder extract was identified and quantified by Gas Chromatography-Flame Ionization Detector (GC-FID). Shimadzu gas chromatography (GC) 2010 was used in this study and the internal standard of citral with 25 ppm, 50 ppm, 75 ppm, and 125 ppm were prepared to generate the standard curve for isomers of citral compound of neral and geranial. The volume of 2 µL of an internal standard for each concentration was injected into GC. The injection port temperature of Flame Ionisation Detector was set to 250°C while a capillary column of BPX-5 with 30 m length × 0.25 mm internal diameter × 0.25 µm film thickness and the carrier gas of Helium with the flow rate of 1 mL/min were used. The oven temperature was set at 60°C for 1 min. The temperature was increased to 150°C (4°C/min), then followed by increased to 230°C (20°C/min) and hold at 230°C for 5 min. The citral compound was quantified separately into neral and geranial compound. The same procedures were carried out to quantify the citral compound from freeze-dried lemongrass powder extracted with different solvents and temperatures of extraction. Total citral concentration was calculated by summation of neral and geranial concentration.

Determination of total phenolic content (TPC)

The total phenolic content of extract from lemongrass powder was determined by using the Folin-Ciocalteu method (Mirghani *et al.*, 2012). Gallic acid with 0.5 g was placed in a 100 mL volumetric flask and was dissolved in 10 mL of ethanol before diluting to 100 mL of volume with distilled water. Standard curve was prepared by different concentrations of 0, 50, 100, 250, 500, 1000, 2000, 3000, 4000, 6000, 7000, 7500, 8000, 8500, 9000 and 100000 mg/L of gallic acid solution. In a test tube, 2.37 mL of distilled water, 0.03 mL of lemongrass extract, and 0.15 mL of Folin-Ciocalteu were added and mixed well. The mixture was then allowed to stand at 40°C for 30 min before subjected to UV-Visible Spectrophotometer (Varian Cary 50 Bio, Australia) to

determine the absorbance of the solution mixture at 750 nm. The TPC was expressed as mg/L of Gallic acid equivalents (GAE mg/L).

Determination of DPPH radical scavenging assay

Antioxidant properties of lemongrass extract were determined by 2, 2-Diphenyl-1-Pircrylhydrazyl (DPPH) free radical scavenging assay as described by Mon *et al.* (2011). A 10 µL of lemongrass extract was diluted with 10 mL of methanol. Diluted extract (2 mL) was placed in a test tube and mixed with 2 mL of DPPH in methanol solution before kept in dark conditions for 30 min. After incubation, the absorbance value was measured at 518 nm using a Spectrophotometer (Varian Cary 50 Bio, Australia). A 2 ml of methanol together with 2 mL of DPPH solution was used as a blank solution. Ascorbic acid with concentration of 0.05, 0.1, 0.2, 0.5 and 0.75 mg/mL was used to prepare the standard curve for antioxidant activity. The DPPH radical scavenging activity of lemongrass mixture was calculated by using the following equation:

$$\text{DPPH activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Determination of ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant powder (FRAP) assay of lemongrass extract was carried out according to Geetha and Geeetha (2016) method. The FRAP reagent was prepared by mixing 2.5 ml of 10 mM 2,4,6-tri [2-pyridyl-s-triazine] (TPTZ) solution in 40 mM of HCl, 25 mL of 300 mM sodium acetate buffer at pH3.6, and 2.5 mL of 20 mm Iron (III) chloride hexahydrate. The mixture solution was freshly prepared and warmed at 37°C. FRAP reagent with a volume of 900 µL was mixed with 90 µL of distilled water and 10 µL of lemongrass extract. The mixture solution was incubated at 37°C for 30 min and the absorbance was measured at 593 nm using a Spectrophotometer (Varian Cary 50 Bio, Australia) against the reagent blank of acetate buffer. Trolox (6-hydroxy-2, 5, 7, 8-tetrametylchroman-2-carboxylic acid) was used as standard. The standard calibration curve was prepared and the antioxidant potential was calculated from the Trolox standard curve and expressed as Trolox Equivalents (TE/mL).

Statistical analysis

All parameters were carried out in triplicate. Data were analyzed using one-way Analysis of Variance (ANOVA) and the significant difference between the mean values was analyzed by Fisher's Least Significant Difference (LSD) test at $p<0.05$ using MINITAB 14 software (MINITAB Inc., PA, USA).

RESULTS AND DISCUSSION

Yield of extract

Table 1 shows the extraction yield and citral concentration of freeze-dried lemongrass powder extracted with different solvents and temperatures of extraction. As can be seen, methanol extract has the highest extraction yield (32.47 to 37.70%) while hexane extract obtained the lowest ones (9.20-10.60%). This result was due to the high in polarity index of methanol to lemongrass extract when compared to petroleum ether and hexane (Zhang *et al.*, 2007) and was in good agreement with the finding obtained by Khlifi *et al.* (2011) on 37% yield of methanol extract of *Globularia alypun* L. leaves. Methanol extraction at 40°C was found to significantly increase the yield of lemongrass extract but there was a decline in the yield when the high temperature of 60°C used during the extraction process (Table 1). This finding was due to some lemongrass components of extract were lost upon heating beyond 40°C (Yang *et al.*, 2009). However, there was no significant effect ($p>0.05$) by different temperatures on the yield of hexane and petroleum ether extract.

Citral concentration

Citral concentration of hexane, petroleum ether, and methanol extract at 27°C, 40°C, and 60°C are shown in Table 1 while the gas chromatogram of neral and geranial compounds (isomers of citral) of hexane, petroleum ether, and methanol extract are shown in Figure 1, 2 and 3, respectively. Hexane showed to be an efficient solvent to extract citral compound from freeze-dried lemongrass powder followed by petroleum ether and methanol (Table 1). Lemongrass powder extracted by hexane at 27°C obtained the highest citral concentration (239.08 ± 1.28 mg/mL). Citral is an aldehyde compound and this aldehyde can be extracted by non-polar solvents like hexane due to the high selectivity of non-polar solvent as well as the low polarity of hexane when compared to both petroleum ether and methanol (Yamada & Goto 2003). This result was in line with a study done by Nur Ain *et al.* (2013) who reported that extraction of lemongrass at ambient temperature by n-Hexane obtained the highest amount of both citral isomers of neral (87.56 ± 0.5 mg/100 mg) and geranial (220.32 ± 0.3 mg/100 g) when compared to ethanol and water extract. The significantly decreased concentration of citral for both 40°C and 60°C of hexane extraction might due to thermal degradation of citral isomers of neral and geranial at higher temperatures. This study also indicated that methanol extract at 27°C and 40°C have low in citral concentration with 7.16 ± 0.78 mg/mL and 6.80 ± 0.83 mg/mL, respectively. According to Vellore *et al.* (2016) methanol solvent has a high

Table 1. Total extraction yield and citral concentration of freeze-dried lemongrass powder extracted with different solvents and temperatures of extraction

Lemongrass extract with solvent (temperature) of extraction	Yield of extract (%)	Citral concentration (mg/mL)
Hexane (27°C)	10.60 ± 0.53^{d}	239.08 ± 1.28^{a}
Hexane (40°C)	9.50 ± 0.46^{d}	20.97 ± 0.04^{f}
Hexane (60°C)	9.20 ± 0.46^{d}	39.63 ± 0.41^{d}
Petroleum ether (27°C)	17.74 ± 1.27^{c}	80.30 ± 2.40^{c}
Petroleum ether (40°C)	17.90 ± 0.40^{c}	97.97 ± 3.04^{b}
Petroleum ether (60°C)	16.87 ± 0.25^{c}	37.90 ± 1.05^{d}
Methanol (27°C)	33.33 ± 1.17^{b}	7.16 ± 0.78^{g}
Methanol (40°C)	37.70 ± 1.78^{a}	6.80 ± 0.83^{g}
Methanol (60°C)	32.47 ± 0.86^{b}	24.58 ± 0.36^{e}

Mean values are expressed as mean ± standard deviation (n=3). Mean values with a different superscript letter in the same column are significantly different at p<0.05.

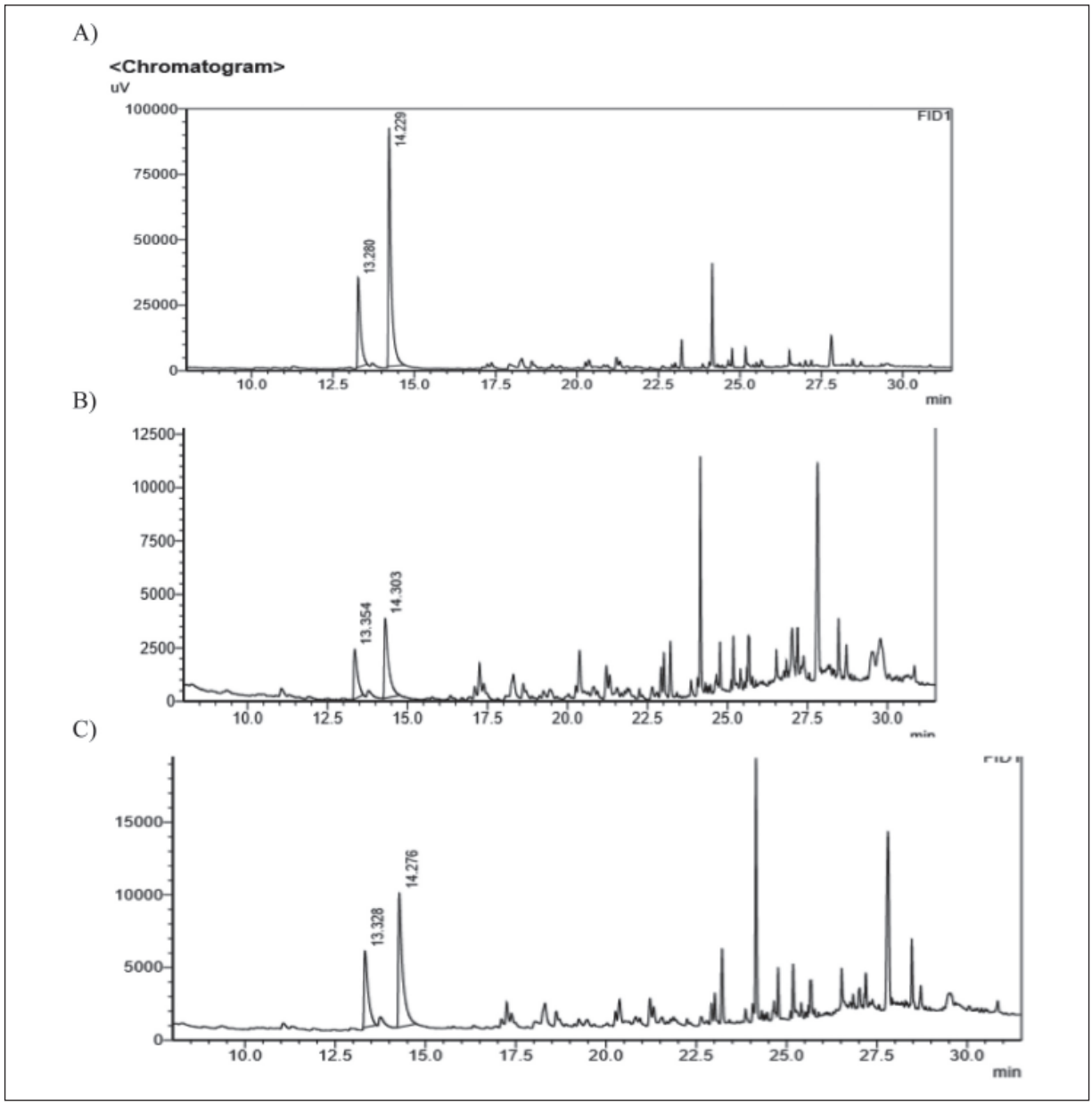

Fig. 1. Gas chromatogram of citral concentration (mg/mL) of hexane extract at different extraction temperatures of 27°C (A), 40°C (B) and 60°C (C), in where neral peak at a retention time of 13.3 min and geranial peak at a retention time of 14.3 min.

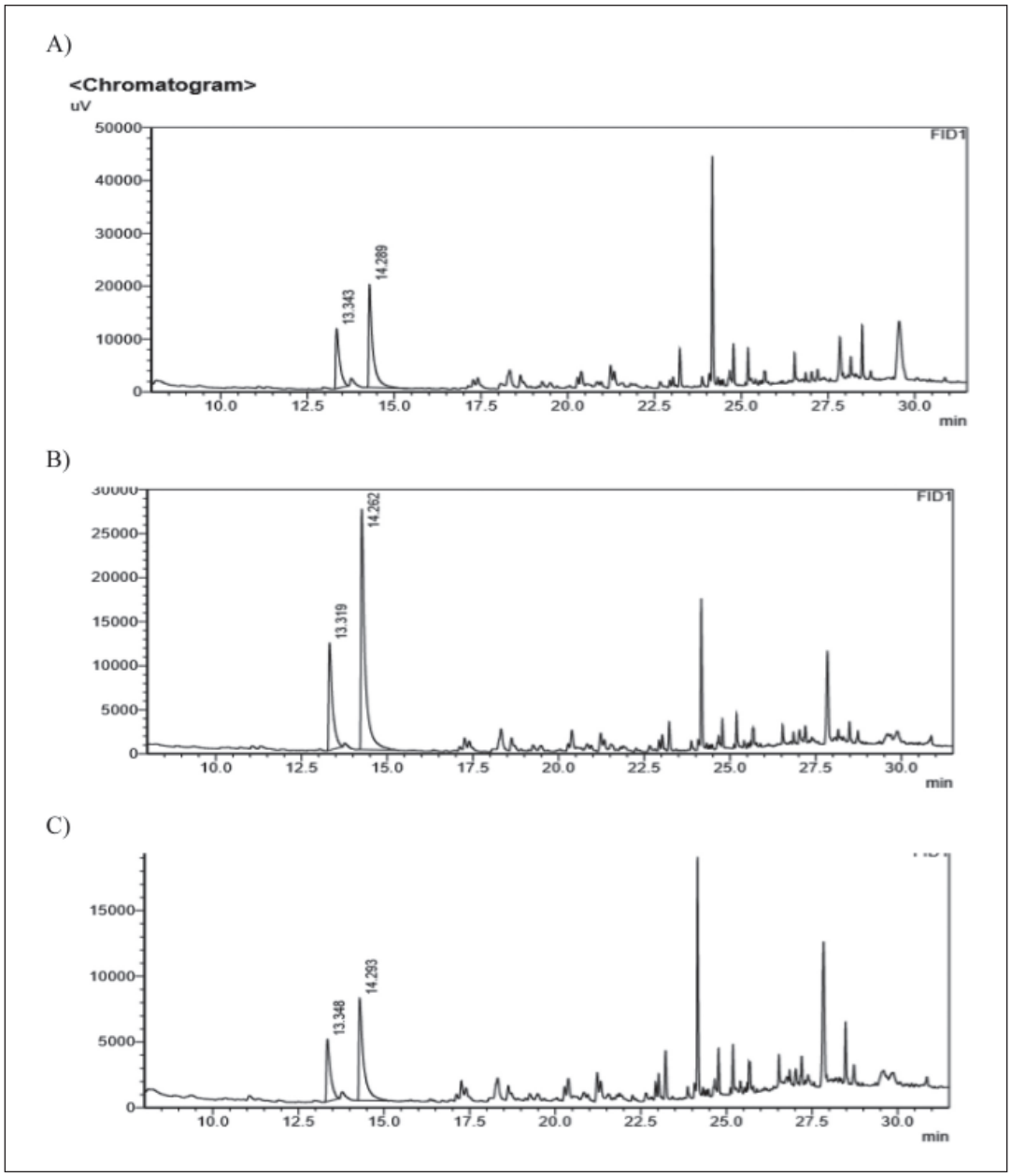

Fig. 2. Gas chromatogram of citral concentration (mg/mL) of petroleum ether extract at different extraction temperatures of 27°C (A), 40°C (B) and 60°C (C), in where neral peak at a retention time of 13.3 min and geranial peak at a retention time of 14.3 min.

ability to extract other phytochemicals such as alkaloids, flavonoids, phenols, tannins, and terpenoids but low extraction ability for aldehyde compound.

Total phenolic content (TPC)

Table 2 shows the total phenolic content and antioxidant properties of freeze-dried lemongrass powder extracted with different solvents and temperatures of extraction. Methanol extract shows the highest value in TPC (6849.33 to 7419.33 mg GAE/l) among other extracts (Table 2). Phenolic acids are the main compounds that contributed to antioxidant activity (Xu & Chang, 2008). As can be seen, the total phenolic content of all lemongrass extracts was significantly increased (p<0.05) when the temperature of extraction increased from 27°C to 40°C but there is decreasing in the trend of TPC when a temperature of 60°C was used. This finding was in line with a study done by Chew *et al.* (2011) who reported that recovery of phenolic compounds had decreased when *Centella asiatica* leaves were extracted beyond 45°C. At high temperatures, phenolic compounds will undergo degradation and causing a loss in antioxidant capacities (Chan *et al.*, 2009).

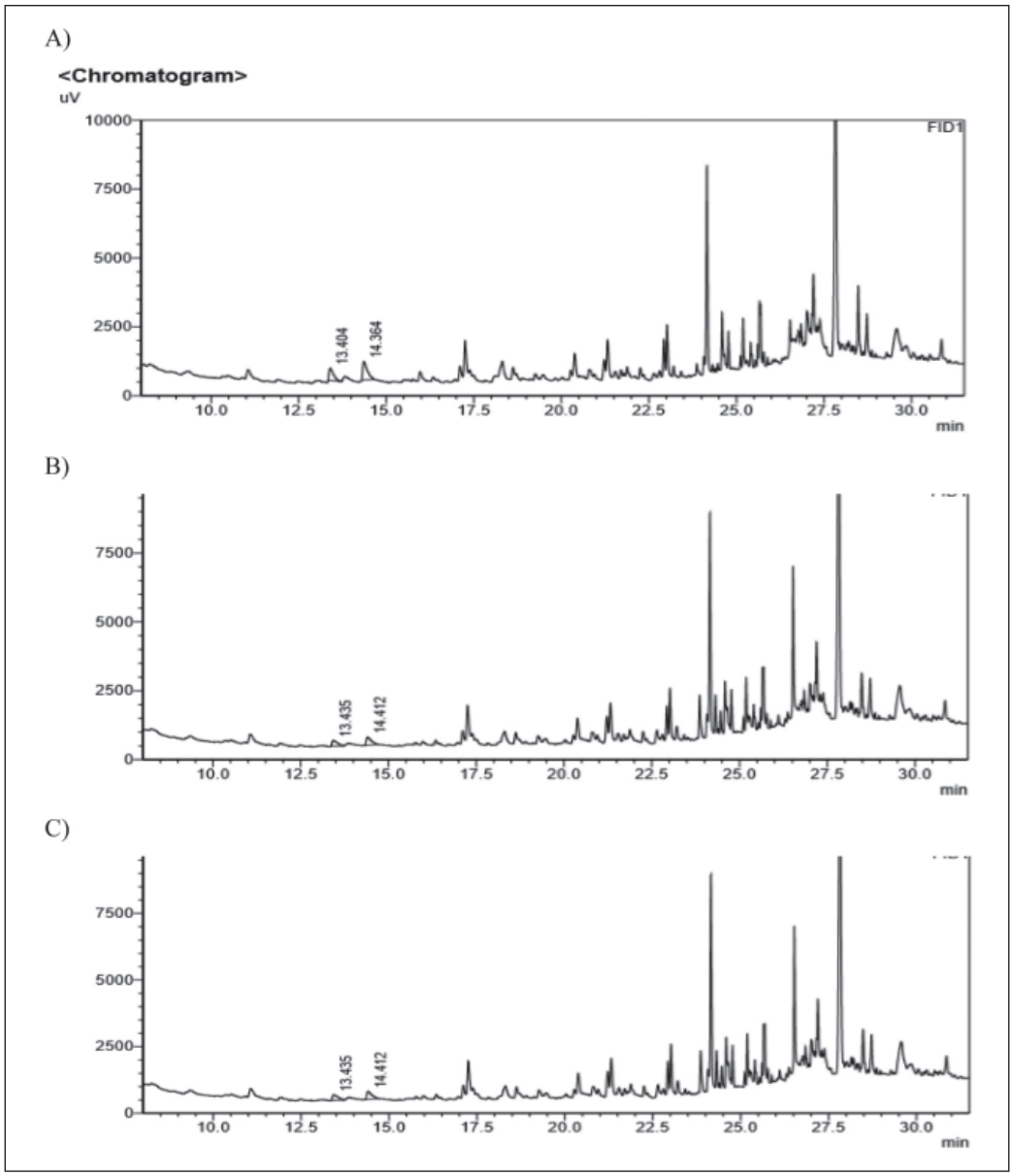

Fig. 3. Gas chromatogram of citral concentration (mg/mL) of methanol extract at different extraction temperatures of 27°C (A), 40°C (B) and 60°C (C), in where neral peak at a retention time of 13.4 min and geranial peak at a retention time of 14.4 min.

Table 2. Antioxidant properties of freeze dried lemongrass powder extracted with different solvents and temperatures of extraction

Lemongrass extract with solvent (temperature) of extraction	Total phenolic content (mg GAE/L)	DPPH radical scavenging activity (%)	FRAP assay (TE/mL)
Hexane (27°C)	176.00 ± 2.36^{f}	12.05 ± 0.33^{ef}	4.64 ± 0.07^{d}
Hexane (40°C)	274.33 ± 18.86^{e}	15.92 ± 2.66^{de}	6.92 ± 0.07^{c}
Hexane (60°C)	82.67 ± 7.07^{g}	10.81 ± 1.71^{f}	4.31 ± 0.32^{e}
Petroleum ether (27°C)	246.00 ± 11.79^{ef}	39.71 ± 1.69^{c}	4.84 ± 0.05^{d}
Petroleum ether (40°C)	382.67 ± 16.50^{d}	20.02 ± 0.30^{d}	4.04 ± 0.07^{e}
Petroleum ether (60°C)	21.00 ± 4.71^{g}	18.04 ± 2.63^{d}	3.32 ± 0.17^{f}
Methanol (27°C)	7171.00 ± 14.14^{b}	40.51 ± 2.72^{c}	51.85 ± 0.07^{a}
Methanol (40°C)	7419.33 ± 110.78^{a}	87.45 ± 2.70^{a}	52.11 ± 0.07^{a}
Methanol (60°C)	6849.33 ± 25.93^{c}	55.28 ± 0.52^{b}	51.34 ± 0.10^{b}

Mean values are expressed as mean ± standard deviation (n=3). Mean values with a different superscript letter in the same column are significantly different at p<0.05.

DPPH radical scavenging assay

The antioxidant capacity of lemongrass extracts was determined using two methods of DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay and the mean values for both analyses are shown in Table 2. Methanol extract has the highest percentage of DPPH radical scavenging activity, followed by petroleum ether and hexane extract. According to Kusmardiyani *et al.* (2016), the DPPH radical scavenging activity of lemongrass extract by methanol was higher than lemongrass essential oil. A high in polarity index of 5.1 of methanol contributed to give effective impact on DPPH radical scavenging activity of lemongrass extract when compared to the efficiency of the low polarity of petroleum ether and hexane. Flavonoid and polyphenol compounds of lemongrass which are highly extracted by methanol act as a free radical scavenger while phenolic compounds are promoted directly to antioxidant activity and also acts as a hydrogen-donating radical scavenger (Chew *et al.*, 2011). As can be seen in Table 2, more antioxidants can be extracted when the temperature of extraction increased to 40°C due to the acceleration of the mass transfer rate. However, beyond this suitable temperature may result in the decomposition of antioxidants. This finding is also supported by Akowuah and Zahri (2010) who found that the stability of polyphenolic constituents was affected by extraction temperature. The author and co-worker reported that methanol extract at 40°C of *Orthosiphon stamineus* leaf obtained the highest level of polyphenolic markers compared to extract at 60°C, 80°C, and 100°C.

Ferric reducing antioxidant power (FRAP) assay

As expected, a similar trend with DPPH radical scavenging activity was observed on FRAP values of lemongrass extract. The methanol extract of lemongrass powder obtained the highest value of FRAP (51.34-52.11 TE/mL) compared to hexane (4.31-6.92 TE/mL) and petroleum ether extract (3.32-4.84 TE/mL). The amounts of polyphenols were increased with increasing temperature of extraction but only until 40°C. The FRAP capacity of all extracts was found to be decreased when the extraction temperature increased from 40°C to 60°C. This result might be due to the degradation of polyphenol compounds at the high temperature of extraction (Yang *et al.*, 2009).

CONCLUSION

Different temperatures and solvents of extraction gave a significant impact on to yield, citral concentration, and antioxidant properties of the extract from freeze-dried lemongrass powder. Methanol extract at 40°C obtained the highest yield of extract (37.70 ± 1.78%) while hexane extraction at 27°C is the most effective solvent to extract citral compound from lemongrass powder with the highest concentration of 239.08 ± 1.28 mg/mL. However, the efficiency of hexane decreasing upon heating. In terms of antioxidant properties, freeze-dried lemongrass powder extracted by methanol at 40°C is efficient for total phenolic content, DPPH radical scavenging activity, and FRAP assay.

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