ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT OF LEMONGRASS *(CYMBOPOGON CITRATUS)* LEAVE

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Abstract
The aim of the study was to investigate the effect of drying method, solvent concentration, extraction time and temperature on antioxidant activities and total phenolic content (TPC) of lemongrass leave extract. The antioxidant activities were evaluated using DPPH radical scavenging ability, ferric reducing antioxidant power (FRAP), and β-carotene bleaching inhibition (BCB) assays; while TPC was determined using Folin-Ciocalteau method. The optimum extraction conditions obtained were oven dried lemongrass leave extracted using 40% ethanol at 25°C for 300 min with a sample to solvent ration of 1:10 in a water bath shaker (150 rpm). Lemongrass leave extract possesses reasonably antioxidant potential with EC_{50} (DPPH) of 192 µg/ml, antioxidant activity (as measured by BCB inhibition) of 67%, and FRAP of 129 mg TE/g; as well as a TPC value of 67 mg GAE/g. However, lemongrass leave extract exhibited lower antioxidant activity in comparison with BHA and α-tocopherol. TPC was positively correlated with antioxidant activities of lemongrass leave extract assessed by FRAP (r = 0.995), BCB assay (r = 0.932), and DPPH (r = 0.777); indicating the presence of phenolic compounds contributed to the antioxidant activities of lemongrass leave. In conclusion, lemongrass leave could be a source of natural antioxidants with nutraceutical potential for food industry application.

Keywords: Antioxidant activity, Extraction conditions, Lemongrass leave, Total phenolic

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1. INTRODUCTION

Malaysia is well-known for its rich biodiversity of many indigenous fruits and vegetables grown in the wild. There are more than 120 species representing various families of traditional vegetables of the Malays in Malaysia, locally called as *"ulam"* (Mansor, 1988). Many local herbs are cooked or eaten raw as salad by dipping in shrimp and chili paste or peanut sauce to enhance flavour while some are being boiled and the extracts are used for consumption among Malaysians. Various studies reported that the consumption of these vegetables has been associated with a lower risk of degenerative diseases related to oxidative stress like cancer, diabetes, Alzheimer's disease and artherosclerosis (Kubola and Siriamornpun, 2008). Antioxidative compounds that are beneficial to human health commonly found in fruits and vegetables are phenolics, betalains, and carotenoids (Norshazila et al., 2010). Among the phytochemicals, phenolic compounds are reported to be the main contributor of antioxidant activity in plant extracts (Yoo et al., 2008).

Lemongrass is a tropical plant commonly found in Southeast Asia, which its origin can be tracked from India. Now it is being cultivated in many places, including tropical and subtropical countries. Lemongrass belongs to the family of Poaceae, and the genus, Cymbopogon. The common names are lemongrass, fever grass or *"serai"* in Malay. There are around 30 species of lemongrass found natively in Malaysia; however, there are two general types, which are the *Cymbopogon citratus* and *Cymbopogon nardus*. The latter is more commonly used as aromatic plants for extraction of citronella essential oils (FRIM, 2004). Citratus is more common for human consumption or as cooking ingredients.

Due to its aromatic and lemon scented property, lemongrass is commonly incorporated in Asian cooking. The leaves, on the other hand are

commonly used as traditional remedies. The essential oil of *C. citratus* is also used in food processes as food flavouring, perfume and cosmetic industries. There are increasing interests in discovering the antioxidant potential of medicinal plants since herbs and medicinal plant had been used for treatment since ancient times, before the development of modern medicinal knowledge.

Accumulating evidence suggested that the recovery, yield and type of phenolics in an extract are influenced by type and polarity of extracting solvents, time and temperature of extractions as well as physical characteristics of the sample (Hossain et al., 2010). Thus far, no specific or appropriate extraction solvent is recommended for optimal recovery of polyphenols for most of the plants studied. This happens because of the diverse chemical structures of phenolic compounds from simple to polymerize forms that might affect their solubility behavior (Prior et al., 2005).

With that, the objective of this study was to evaluate the effect of drying method, solvent concentration, extraction time, and temperature on antioxidant activity of lemongrass leave. Results from this study would provide a deeper understanding on the health promoting properties of lemongrass leaves so that it would be identified for further investigations and hence, developed into value-added foods and nutraceuticals for the benefit of mankind.

2. MATERIALS AND METHODS

Sample preparation

Fresh lemongrass (*Cymbopogon citratus*) was purchased from local market in Batu Caves, Selangor, Malaysia. The selection of leaves was based on harvesting criteria where whole plant, including the stem was harvested. Fresh lemongrass leaves gives a pungent citrus aroma when crushed or cut. The lemongrass leaves were cut 10 cm above the rhizome where the spoilt, injured or worn part was removed. The leaves were washed under running tap water to remove debris and tapped dry, equally divided into two portions, one for oven drying in convection oven (UFB 500, Memmert, Schwabach, Germany) at 40°C for 24 h until constant weight was obtained, and another one for freeze drying.

Sample extraction

Five portions of 5 g dried lemongrass leave powder (oven- and freeze-dried) were weighed using an analytical balance (AB204-S, Mettler Toledo, Switzerland). With the sample to solvent ratio fixed at 1:10, different concentrations of ethanol (v/v; 0%, 20%, 40%, 60%, 80%, and 100%) were prepared. The mixtures were shaken for 60 min at 25°C and 150 rpm in a shaking incubator. After the extraction, the extracts were filtered using Whatman No. 1 filter paper. The filtrate residue was collected and subjected for second extraction with the same conditions to increase the extraction efficiency and yield. Filtrates of both extractions were combined and centrifuged (Universal 320R, Hettich Zentrifuge, Germany) at 4500 rpm for 10 min. The supernatant was concentrated using rotary evaporator (Rotavapour R-200, BUCHI, Switzerland) at 40°C. The concentrated extract was freeze-dried, wrapped with aluminium foil, and stored at -20°C until further analysis.

Determination of optimum extraction conditions

After the best solvent concentration and drying method were determined, the extraction was repeated using the same procedures, but with different extraction time of 60, 120, 180, 240, 300 min followed by different extraction temperature of 25, 30, 40, 50, and 60°C. To proceed to the next stage, the best variable of the parameter was chosen based on the result of 2,2-diphenyl-1-picrylhdrazyl (DPPH) assay. After determining the optimized extraction conditions, the antioxidant activities of lemongrass leave were evaluated using ferric reducing antioxidant power (FRAP) and β-carotene bleaching (BCB) assays, in addition to DPPH radical scavenging and TPC assays.

Total phenolic content

The total phenolic content (TPC) was determined spectrophotometrically using Folin-

Ciocalteau reagent (FCR) according to the method described by Ferreira et al. (2007) with slight modification. Sample (1 ml) was added into test tubes, followed by 4.0 ml of FCR (diluted 10×) and 5.0 ml of sodium carbonate (7.5 g/100 ml). The contents were mixed thoroughly and stored in the dark for 30 min. The absorbance was measured at 725 nm using a spectrophotometer (PRIM, Secomam, Alés, Gard, France). TPC was expressed as mg gallic acid equivalents (GAE) per g of extract.

DPPH radical scavenging assay

The scavenging ability was determined according to the method of Xu and Chang (2007) with slight modification. To prepare DPPH reagent, 7.8 mg of DPPH powder was dissolved in ethanol and topped up to 100 ml using a volumetric flask. The flask was wrapped with aluminium foil to reduce light exposure towards DPPH solution. Sample (1 ml) was added to 500 µl of ethanolic DPPH solution in a test tube and the mixture was shaken vigorously using a vortex, and then kept in dark condition for 30 min. After incubation, the absorbance of the mixture was measured at 517 nm against ethanol blank and distilled water as negative control. Scavenging ability of sample was calculated from the equation below:

DPPH radical scavenging ability (%)
$= [1 - (Abs_{sample} / Abs_{control})] \times 100$

The percentage of scavenged DPPH was plotted against logarithm (Log) of sample concentration to calculate the EC_{50} that defined as the ability to reduce the initial DPPH concentration by 50%. BHA and α-tocopherol were used as reference.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was determined based on the reduction of Fe^{3+}-TPTZ to a blue coloured Fe^{2+}-TPTZ (Biglari et al., 2007). FRAP reagent was prepared from 10 mM 2,4,6-tri(2 pyridyl)-s-triazine (TPTZ) in 40 mM HCl with 20 mM ferric trichloride hexahydrate ($FeCl_3.6H_2O$) and 0.3 M acetate buffer (pH 3.6) at a ratio of 10:1:1 (v/v/v), and the straw coloured solution was kept in a water bath at 37°C. Then, FRAP reagent (1.5 ml) was added with 50 µl sample solution and the mixture was shaken and incubated for 4 min. Absorbance (593 nm) was determined relative to FRAP reagent blank. The result was calculated from a calibration curve plotted against various trolox concentrations (10–400 µg/ml), and expressed as trolox equivalent antioxidant capacity (TEAC).

β-Carotene linoleate bleaching assay

β-Carotene bleaching assay was conducted using a method by Nsimba et al. (2008) with slight modification. To prepare the working reagent, 0.2 mg of β-carotene was dissolved in 1 ml of chloroform, then added with 0.02 ml linolenic acid and 0.2 ml Tween 40. The chloroform in the mixture was removed under vacuum. Oxygenated water (50 ml) was added followed by 0.5 ml sample extract and 4 ml reagent and shaken until liposome was formed. Absorbance (470 nm) was read at 0 min and every interval of 20 min for 2 h (t = 0, 20, 40, 60, 80, 100, and 120 min). The anti-bleaching rate of sample was calculated based on the formula as below:

β-Carotene bleaching rate (R)
$= \ln (abs_{t=0} / abs_{t=20, 40, 60, 80, 100, \text{and } 120 \text{ min}}) / t$

Where, ln is natural log and t = 0 is the initial absorbance at time 0 and so-forth.
Antioxidant activity (AA) was calculated as percentage of inhibition relative to control using equation below:

$\%AA = 100 \times (R_{control} - R_{sample}) / R_{control}$

Statistical analysis

All data were expressed as mean ± standard deviation and were statistically analyzed using the SPSS statistical software version 20 (SPSS Inc, Chicago, Illinois, USA). All analyses were done in triplicate. One-way analysis of variance (ANOVA) and Tukey's test were used to compare means among groups. Pearson correlation test was used to assess the

relationships between TPC and antioxidant activities. The significance level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

Extraction of lemongrass leave using different ethanol concentration (0, 20, 40, 60, 80, and 100%) was tested to evaluate its free radical scavenging potential. Figure 1 shows the EC_{50} value for both oven-dried and freeze-dried samples extracted by 40% ethanol concentration had the lowest value of 212 and 229 µg/ml, respectively, but freeze-dried was significantly higher that oven-dried ($p < 0.05$). Therefore, 40% ethanol oven-dried sample was selected as the best extraction condition to proceed with the subsequent stage of experiment that is to determine the optimal extraction time.

DPPH radical scavenging activity can be influenced by extraction time; the EC_{50} of sample extracted with 40% ethanol extraction for 300 min (253 µg/ml) was significantly lower compared to others (Figure 1). With that, 300 min was selected for the determining of optimum extraction temperature that ranges from 25–60°C. The EC_{50} value of 25°C showed significantly lower value (288 µg/ml) compared to other temperatures, with the decreasing trend of EC_{50} value was 40°C > 60°C > 50°C = 30°C > 25°C. Thus, 25°C was selected as the optimal extraction temperature for high DPPH radical scavenging ability of lemongrass leave. Overall, the optimum extraction conditions were oven-dried lemongrass leave extracted using 40% ethanol for 300 min at 25°C.

With the determined optimum extraction conditions, an array of antioxidant capacity assays by means of ferric reducing antioxidant power (FRAP), β-carotene bleaching assay, and DPPH radical scavenging ability, as well as TPC were carried out (Table 1). The TPC of lemongrass extract stands at 67 mg GAE/g extract. The reducing power can serve as a significant reflection of the antioxidant activity. The reducing power of lemongrass leave extract was 129 mg TE/g and was significantly lower compared to BHA and α-tocopherol.

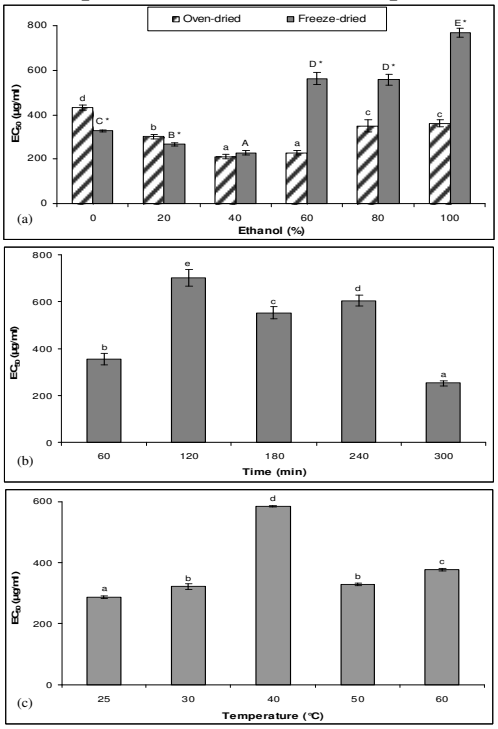

Fig. 1 – The effect of (a) solvent concentration (and drying method), (b) extraction time and (c) temperature on DPPH radical scavenging ability of lemongrass leave extract

(a) Different small letters (a-d) for oven-dried and capital letters (A-E) for freeze-dried samples denote significantly different ($p < 0.05$). *Denotes significantly different ($p < 0.05$) between oven- and freeze-dried samples, except for 40% ethanol.
(b) & (c) Different small letters (a-e) denote significantly different ($p < 0.05$).

Table 1 – Total phenolic content and antioxidant activities of *Cymbopogon citratus* leave extract at optimized extraction conditions (40% ethanol, 5 h, 25°C, oven-dried)

Sample	TPC (GAE, mg/g)	FRAP (TEAC, mg/g)	ANT, % (BCB)	DPPH radical scavenging EC_{50}† (µg/ml)
Crude extract	67.28 ± 0.86	128.88 ± 3.16^{c}	67.41 ± 5.31^{b}	191.97
BHA	NA	3528.79 ± 101.71^{a}	98.91 ± 0.26^{a}	<1
α-Tocopherol	NA	1434.02 ± 109.40^{b}	96.33 ± 0.23^{a}	<1

Each value was expressed as mean ± standard deviation (n=2), except for DPPH EC_{50} value. NA: not applicable. TPC=Total phenolic content; FRAP=Ferric reducing antioxidant power; ANT (BCB)=Antioxidant activity as measured by β-carotene bleaching inhibition (at 500 ppm sample concentration).
*Different superscripts within the column (FRAP and BCB) denote significantly different ($p < 0.05$).
†Defined as effective concentration that was able to scavenge 50% of the total DPPH radicals; EC_{50} was calculated by interpolation of linear regression analysis (based on concentration-dependent result – data not shown).

β-Carotene bleaching mechanism is based on hydroperoxides formed from linoleic acid which then attacks the highly unsaturated β-carotene molecules. The absence of antioxidant causes lipid oxidation to occur that result in the decolouration of orange-yellowish β-carotene. The antioxidant activity as measured by β-carotene bleaching inhibition was found significantly higher in BHA and α-tocopherol (both > 95%) compared to lemongrass leave extract (67%) ($p < 0.05$). Lemongrass leave extract possesses moderate antioxidant activity (60–70%) according to Kaur and Kapoor (2002). The EC_{50} value for DPPH radical scavenging ability of lemongrass extract (192 µg/ml) was found to be significantly higher than that of BHA and α-tocopherol (both < 1 µg/ml).

Many authors had stressed the need to perform more than one type of antioxidant activity measurement to take into account the various mechanisms of antioxidant action and limitation of each method (Rufino et al., 2010). This occurs because different antioxidant assays available showed different antioxidant activities based on different mechanisms. Different methods might be measuring different kinds of antioxidant present in the plants studied.

Positive correlations were observed between TPC and FRAP ($r = 0.995$); with BCB ($r = 0.932$); and DPPH ($r = 0.777$) (Table 2). These support the results reported by Leontowicz et al. (2003) that significant correlations between TPC and antioxidant activities were found in apple, pear peel, and pulp extracts; as well as in oat (Serea and Barna, 2011). On the contrary, no such correlations were found in bitter gourd extracts, as reported by Kubola and Siriamornpun (2008).

Table 2 – Correlations between TPC and DPPH, FRAP, BCB assays of *Cymbopogon citratus* leave extract at optimized extraction conditions (40% ethanol, 5 h, 25°C, oven-dried)

DPPH	FRAP	BCB
0.777	0.995	0.932

All values are significant at $p < 0.05$.

4. CONCLUSION

The optimized extraction parameters of oven-dried lemongrass leave were 40% ethanol concentration, with 25°C extraction temperature for 5 h. The optimized extraction conditions influence the antioxidant capacity of lemongrass leave extract. However, the antioxidant activities were found to be lower than the standards (BHA and α-tocopherol). Nonetheless, further studies are needed for the optimization of recovery of antioxidant contents and identification potent antioxidant present in *C. citratus* leave.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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