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Research Article

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Potential of lemongrass leaves extract (*Cymbopogon citratus*) as prevention for oil oxidation

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ABSTRACT

Cymbopogon citratus is a plant commonly used as a spice by the people in Indonesia. Utilization is limited to the stalks lemongrass only, while the leaves are still become waste. This research aims to study the potential of lemongrass leaves extract for the prevention of oil oxidation. Extraction wasused by maceration method on lemongrass leaves using ethanol 30, 70 and 96% and qualitative test of phytochemical components, determination of total phenolics and antioxidant activity on extracts of lemongrass leaves. Phytochemicals analysis results showed that the ethanol extract of leaves of lemongrass contains alkaloids, saponins, tannins, flavonoids, phenols, and steroids. The highest total phenol content was in the 30% ethanol extract of 50.017GAE (Gallic Acid Equivalent) mg/g. Ethanol extract of lemongrass leaves is potential as an antioxidant because its inhibitory against free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). The best IC_{50} values was obtained in the 70% ethanol extract of 79.444 mg/L. The highest antioxidant activity by Rancimat instrument occured in 70% ethanol extract.

Keywords: lemongrass leaves, antioxidants, total phenolic, rancimat instrument , DPPH

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) is a plant commonly used as a spice by the people in Indonesia. Spices known to contain phenolic compounds which have a strong antioxidant capacity [1].*C. Citratus* is a widely used herb in tropical countries, especially in Southeast Asia. Its oil is a yellow or amber liquid containing about 75-85% of aldehydes, chiefly citral, geranial and neral. It is used in aromatherapy.Some of the reported phytochemicals are essential oils that contain citral α, citral β, nerol geraniol, citronellal, terpinolene, geranyl acetate, myrecene and terpinol methylheptenone. Two triterpenoids, cymbopogone and cymbopogonol and flavones identified as luteolin and its 6-C-glucoside have also been isolated from leaves of *C. Citratus*[1], [2], [3].

The plant also contains reported phytochemicals such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin. The compounds identified in *C. citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. Studies indicate that it possesses various pharmacological activities such as anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal and anti-inflammatory properties. Various other effects like anti-malarial, anti-mutagenicity, anti-mycobacterial, antioxidants, hypoglycemic and neurobehaviorial have also been studied [2].

In Indonesia, the use of lemongrass as a cooking spice is only in part of the trunk, while the leaves of lemongrass still become waste. Though the leaves of lemongrass are known to have phenolcompounds which can act as

antioxidants [4], so it can be used both in the field of health and food. Antioxidants may inhibit the initiation or propagation of oxidation and effectively retard the onset of lipid oxidation in food products. Lipid oxidation is a free-radicalchain reaction which leads to a total change in the sensory properties and nutritive value of food products. In biological systems, free radicals are one of the triggers of cellular lesions in all majororgans by damaging cellular components, including proteins, lipids, carbohydrates, and DNA [4], [5],

Antoxidants were used initially to preserve fats and oils. They act by reducing the reactivity of free radicals and protecting lipids from oxidative damage.Antioxidants are commonly used in the synthetic form such as BHA (butyl hydroxyanisole), BHT (butyl hydroxy toluene), and TBHQ (terta-butyl hydroxy quinon). Nowadays, the synthetic antioxidants began to be less used due to its risk tohealth. The use of natural antioxidantshas been a new interest as it is safer for human health.

This research aims to study the potential of lemongrass leaves extract for the prevention of oxidation of oil through the test of DPPH and AOM.

EXPERIMENTAL SECTION

Sample Preparation: The materials used were lemongrass (*C. citratus*) leaves obtained from the Research Institute for Medicinal Plants and Spices, Cimanggu, Bogor, West Java, Indonesia, ± 6 months old.Lemongrass leves were washed, then dried in an oven for 3 days at a temperature of 50 °C. Once it was dried, the samples were milled and sieved to 100 meshes, packed and stored at refrigerator. The analysis of the water content was conducted by using gravimetric method (AOAC 133 925.10, 2012).[6].

Extraction of Lemongrass Leaves: Maceration of samples carried out by soaking each simplicia in 30% ethanol, 70% ethanol, and 96% ethanol with a ratio of 1:10 for 3x24 hours at room temperature (± 27 °C) and stirred with a shaker at 150 rpm, then filtered and concentrated by rotary evaporator at a temperature of 50-60 °C. Furthermore, ethanol extract obtained weighed for yield was calculated. Qualitative phytochemicals analysis was conducted using lemongrass extract [7] which includes a test alkaloids, flavonoids, phenolics, tannins, saponins, steroids and terpenoids.

Determination of Total Phenolic Content[8]: Lemongrass leaves extract as much as 0.5 mL with a concentration of 1000 mg/L was mixed with 2.5 mL reagent Follin Ciocalteu 10% (which had been dissolved in distilled water) and 2.5 mL of $NaHCO_3$ 7.5%. The mixture was further incubated for 45 minutes at a temperature of 45 °C. Absorbance was measured at a wavelength of 765 nm. A standard calibration curve was used gallic acid (0, 15, 20, 25, 30 and 35 mg/L). Furthermore, each standard given the same treatment with the extracts of leaves samples. The phenol content expressed in mg gallic acid/g extract.

Antioxidants Activity (DPPH method)[9]: Preparation of DPPH solution which weighed as much as 1.97 mg DPPH dissolved in ethanol to 25 mL and the obtained solution with a concentration of 0.2 mM. Ethanol extracts were diluted (concentration variation 25, 50, 75, 100, 125 and 150 mg/L). Each concentration of the solution as much as 1.5 mL pipette and added 0.75 mL of 0.2 mM DPPH. The mixture was homogenized and allowed to stand in the dark for 30 minutes. Uptake was measured by UV-Vis spectrophotometer at maximum wavelength is 517 nm DPPH. Tests performed three separate tests for each concentration of the sample solution. Reference solution used was BHT and ascorbic acid at a concentration of 2, 4, 6, 8 and 10 mg / L. Experiments performed three separate tests and calculated in the inhibition using the formula:

% Inhibition = ($Abs_{blank} - Abs_{sample}$) / Abs_{blnko} x 100.

The IC_{50} values were calculated by linear regressionof plots where the abscissa represented the concentrationof tested lemongras extracts and the ordinate the averagepercent of antioxidant activity from three separate tests.

Rancimat (METRHOM, Switzerland) experiment [10]: A total of 10 mL of soybean oil added 1 mL of lemongrassleaves extract with a concentration of 200 mg/L and tween 80 as much as 3 mL. Negative controls were made without the addition of the extract, while the positive control is made by adding BHT 200 mg/L. The mixture is then weighed as much as 2.5 g in a test tube and placed in a heating block. Set air speed of 18-20 L / h and a temperature of 110 ^oC. Measurements were made during the induction period measured, which means the time when the rapid increase happens in electrical conductivity. This test allows the determination of induction period(IP) or oil/oxidative stability index (OSI), which is the timebefore rapid deterioration of the oil occurs. The induction time ina sense could be considered a parameter to measure theoxidative stability of lipids. The higher the induction time, the better the quality of the antioxidants.

OSI= [Induction time of soybean oil + sample extract (h)] / [induction time of soybean oil (h)]

Protection factor (%) = [Induction time of soybean oli + sample extract (h)] / [induction time of soybean oil + BHT] X 100.

Statistical Analysis: The data were analyzed using analysis of variance 139 (ANOVA) to see the effect of the treatment of the relevant test parameters, which then followed byDuncan test. The statisticalanalysis was performed using SPSS v.20 (SPSS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Based on the analysis conducted in this study, the sample contained water up to 3.98±0.50%. Quality standards from Material Medika Indonesia (MMI) determined that the maximum water content is 10% [11]. Thus, the water content of the lemongrass leaves simplicia fulfilled the standards.The yield of 70% ethanol extract (7.66%) had the highest value compared to the two other extracts. Based on statistical analysis, it appears that the yield of ethanol extract 30% and 96% ethanol extracts were not significantly different, but the yield of both extracts were significantly different with 70% ethanol extract (Table 1).

Table 1 Moisture content of simplicia and yield lemongrass leaves extract

Samples	Solvent	Yield corrected (%)
Lemongrass leaves	30% ethanol	6.19[b]
	70% ethanol	7.66[a]
	96% ethanol	6.66[b]

[a, b] *show different correlation or not significantly different between data*

Phytochemical Componouds

Phytochemicals analysis of samples results showed that all the positive extract contains alkaloids, saponins, tannins, flavonoids, phenolic, steroids, except triterpenoids (Table 2). Results of this study was supported by research Nambiar and Matela [4] which states that the ethanol extract of leaves of lemongrass contains alkaloids, saponins, tannins, flavonoids, phenols, and steroids. The study also showed that the ethanol extract of the leaves of lemongrass does not contain triterpenoids. Triterpenoid, which is a building block of essential oil, was not identified in this study, since it has been lost during the process of drying the sample [12].

Table 2 Phytochemical extracts of lemongrass

Samples	Phytochemicals						
	Alkaloid	Saponin	Tanin	Flavonoid	Fenolic	Triterpen	Steroid
30% ethanol	+	+	+	+	+	-	+
70% ethanol	+	+	+	+	+	-	+
96% ethanol	+	+	+	+	+	-	+

Total Phenolic Contents

Total phenolic content of the three lemongrass leaves extract was determined by using the data from gallic acid standard curve. The equation of gallic acid standard curve obtained in this study was $y = 0.02x - 0.077$ (R^2 value of 0.991). The statistical analysis showed that there was no significant difference in total phenolic content of 30% ethanol extract (50.017 mg GAE/g) and 70%ethanol (49.317 mg GAE/g), but total phenolic of both extract was significantly different with that of 96% ethanol extract (GEA 43.433 mg/g) as seen in Fig 1. The results of this study were lower than the results of Sah et al. [9] in 40% ethanol extract of the leaves of lemongrass with total phenolic content of 67.28 mg GAE/g. While Suryanto et al. [13] obtained results of phenol hexane in lemongrass leaves at 72.55 mg GAE/g and a methanol extract is equal to 66.94 mg GAE/g. Total phenolic assay is not able to distinguish the types of phenols contained in the extract (monomer / dimer / trimer) [14]. The content of phenolic compounds in plant can be affected by the following: sample preparation (drying time, temperature), growing condition of the plant, the extracting method, and the technique of analysis.

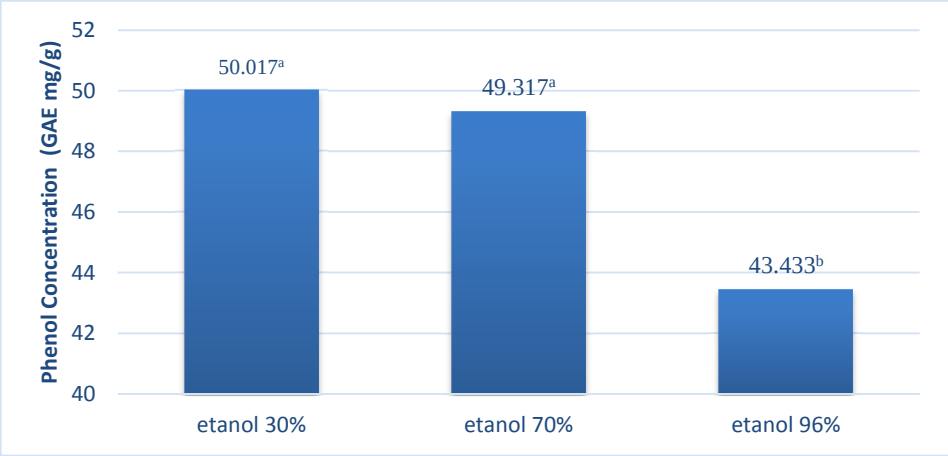

Figure 1 Total phenolic extract of the leaves of lemongrass
a, b show different correlation or not significantly different data

Antioxidant Activity (DPPH method)

The antioxidant activity of lemongrass leaves extracts were determined using DPPH method with two standard antioxidants, ascorbic acid and BHT. The results showed that $IC_{50}$of BHT was lower than that of ascorbic acid (Figure 2). The data also showed that the highest antioxidant activity was found in samples extracted with 70% ethanolbutit was not significantly different with ascorbic acidas the reference (Fig. 2).

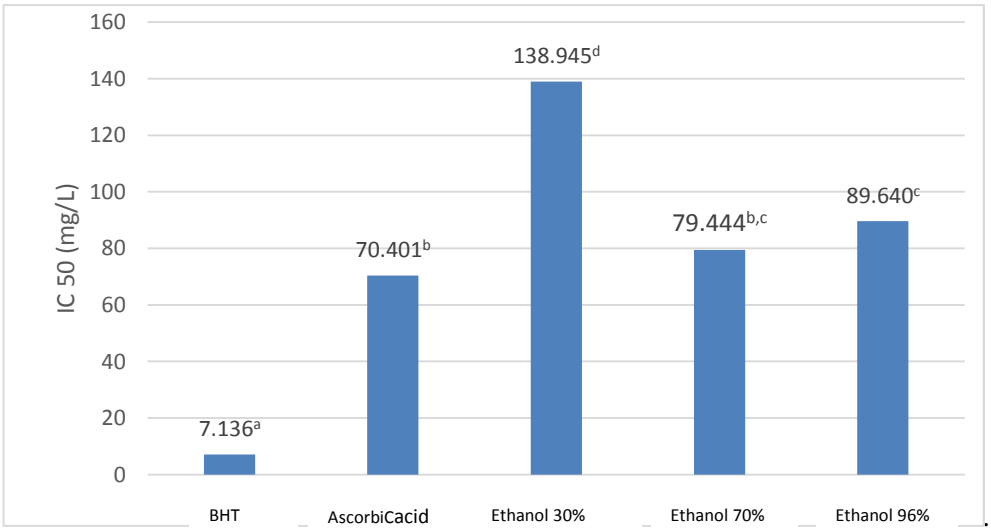

Figure 2. IC_{50} value BHT, ascorbic acid and ethanol extract of lemongrass leaves
a, b show different correlation or not significantly different between data

The antioxidant activity in this method stated by the percent inhibition, which is the percentage of inhibition against free radical DPPH. The percent inhibition values can be determined by IC_{50} which is the concentration of antioxidants that can produce the percent inhibition of DPPH by 50%. IC_{50} values are determined based on the concentration and the percent inhibition using linear regression equation obtained. Antioxidant activity can be classified based on the IC_{50}value. A compound isconsidered a very powerful antioxidant when IC_{50}<50 mg/L, a strong if IC_{50} values worth 50-100 mg/L, was medium when the IC_{50} value worth 100-150 mg/L, and weak when the IC_{50} value worth 150-200 mg / L [15].

Antioxidant activity of ethanol extract of lemongrass leavewas compared with synthetic antioxidants such as BHT and ascorbic acid. BHT has the lowest IC_{50} value that was equal to 7.136 mg/L, which indicatingthe highest inhibition of BHT against DPPH. As for the leaves extract of the lemongrass, the lowest IC_{50} values obtained in 70% ethanol extract (79.444 mg/L) suggested highest inhibition than that of both other extracts (Fig 2). Results obtained in this study werelower than the results reported by Mongkolsilp *et al*.[16](1140 mg/L), using fresh lemongrass extracted with 80% methanol. BHT could be classified as a highly potent antioxidant compounds because it had IC_{50}

values below 50 mg/L. As for ascorbic acid, 70% ethanol extract and ethanol extract 96% were classified into a powerful antioxidant compounds whereas, 30% ethanol extract was classified as weak antioxidants [15].Sah *et al.*[9] described that 40% ethanol extract of the leaves of lemongrass has a IC_{50} value of 191.97 mg/L and therefore was classified as weak antioxidants. Their antioxidant activity in the lemongrass allegedly because the leaves have bioactive compounds, such as phenols, flavonoids, tannins, as well as compounds that have many groups sulphide and alkaloids. Kanopa et al. [17] stated that the presence of these compounds has the potential as an antioxidant.

Oxidative stability(Rancimat experiment)

The important parameter for the quality assessment of animal and vegetable fats and oils is oxidative stability. The oxidation process is initiated by radical reactionsinvolving unsaturated fatty acids. Rancimat test is used to determine oxidative stability, a fat isexposed to a stream of dry air at a temperature of100-140 ^{o}C. This method developed by Hadorn and Zurcher. Itutilizes thefact that the greater part of the volatile productsconsists of formic acid . These volatile components trapped in distilled water, measured conductometricallyand the conductivity plotted automatically. The t, (point of greatest inflection} isdetermined graphically after completion of the experiment. This tehnique don't need supervision during the course ofan experiment [10].

In this research, pure soybean oil presented airflow at 110^{o}C temperature. Induction period associated with the formation of volatile carboxylic acid is the end product of oxidation [18]. This method used soybean oil as a negative control and soybean oil plus BHT as a positive control. Oxidative stability was determined by comparing the time of induction of soybean oil which was added to the sample extract with induction time of soybean oil (Table 3).

Table 3. Induction time by Rancimat test

Samples	Induction time (h)
Soybean oil	5.18
Soybean oil+BHT	7.92
Soybean oil+30% ethanol	5.43
Soybean oil+70% ethanol	6.15
Soybean oil+ 96% ethanol	5.74

Soybean oil was added antioxidant BHT hadoxidative stability at 1.53 and the 70% ethanol of lemongrassleavesextract samples that had antioxidant activity at 1.19. Based on statistical analysis (Fig. 3) the value of the oxidative stability of 70% ethanol extract was significantly different from the BHT, but not significantly different from the two other extracts. The oxidative stability in this study was higher when compared with the results of research Tensiska *et al*. [19], in ethanol extract of the fruit andaliman at 1.18, and lower than the research Zahidah et al. [20]in the extract of guava leaves at 1.72.

Oxidative stabilityusing Rancimat test in the ethanol extract of the lemongrass leaves wascausedby the content of phenolic compounds which is an oxidation-preventing compounds [17]. Phenolic compounds contained in the leaves of lemongrass inhibit the auto-oxidation process in fats and oils. The others compounds such as flavonoids, tannins, alkaloid, saponin had the potential as an antioxidant[21]. Lack of activity of ethanol extract of lemongrass leaves than BHT in oil system presumably caused by the lower solubility in oil [19].

Antioxidant activity IC_{50} with DPPH method (inhibition of the oxidation reaction) aligned with a protection factor results result Rancimat test which is the highest antioxidant activity of 70% ethanol extract> extract 96%> 30% ethanol. The higher the value obtained protection factor, the higher the antioxidant activity in the samples. When connected between the levels of total antioxidant activity of phenols, it can be seen that the total phenolic content was not directly proportional to the antioxidant activity. Levels of total phenol extract 30%> extract 70%> 96% extract. Different concentrations of ethanol will produce phenolic component of different profiles so that the antioxidant activity will also be different [22]. This was presumably because DPPH method is a method that allows the radical DPPH reacts with all kinds of antioxidant compounds present in the sample, not only the phenolic compounds [23]. Other than that, Zuhra et al. [24] states bioactive compounds such as flavonoids may also act as an antioxidant.

Table 3 Protection factors of ethanol extract of lemongrass leaves

Samples	Protection factor (%)*
30% ethanol	68.56
70% ethanol	77.65
96% ethanol	72.47

calculated based on correction factor BHT = 100%

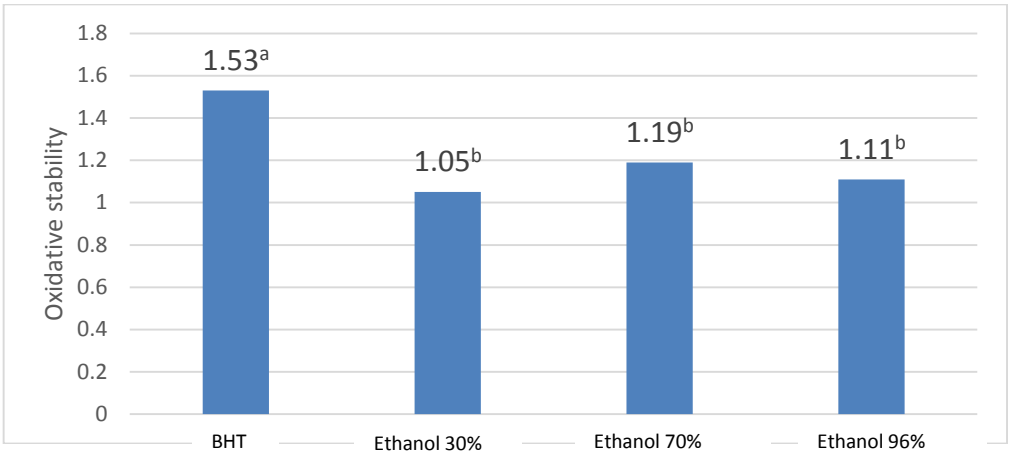

Figure 3 The oxidative stability from Rancimat test results of various extracts of lemongrass leaves and BHT
[a, b] show different correlation or not significantly different datas

CONCLUSION

Ethanol extract of lemongrass leaves contains alkaloids, saponins, tannins, flavonoids, phenols, and steroids. 70% ethanol extracts may be selected to obtain the highest antioxidant activity that is IC_{50} of 79.444 mg/L,but it was not significantly different with ascorbic acid as the reference and 77.65% with a protection factor of BHT (100%) as a comparison.

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