Anti-Autooxidative and Anti-Photooxidative Effects of Lemon Grass Extracts (*Cymbopogon citratus*)

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ABSTRACT

Lemon grass (Cymbopogon citratus) is a traditional food ingredient characterized by its specific and refreshing aroma. This study was intended determine to the effect of lemon grass extract in both autooxidation and photooxidation reaction in model systems. Lemon grass was extracted sequentially with hexane, acetone and ethanol. The antioxidative effects of the extracts were determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay, reducing power, β-carotene bleaching method and linoleic acid system. The photooxidation reaction system was consisted of linoleic acid (0,03 M) in methanol containing 100 ppm erythrosine as a sensitizer and the reaction mixture was exposed under 4000 lux fluorescent light for up to 5 hours. Total phenolic content of acetone, hexane and ethanol extracts were 20.38, 7.65, and 4,97 mg/100g, respectively, which was expressed as gallic acid equivalent. The addition of acetone extracts of lemon grass at 200 and 500 ppm in the reaction mixture showed the highest scavenging activity in 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, reducing power, and β-carotene bleaching and linoleic acid system.

Key words : lemon grass, autooxidation, photooxidation, solvent extraction.

INTRODUCTION

The use of antioxidants in spice products is under extensive investigation to determine better ways of preventing oxidative deterioration. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation of propagation of oxidizing chain reaction. The use of extract from spice as a natural antioxidant was first reported by Chipault *et al.*, (1952). The data showed that some 32 different spices possess an antioxidative effect for most of the commonly-used spices. Many spices showed high antioxidant activity that an increasing consumer interest in natural food additives. Therefore, in considering these reports and the present trends toward naturally preserved food product, spices have targeted as natural antioxidant. Besides, the spice extract could acted a practical use in the actual food under realistic strorage conditions.

Lemon grass (*Cymbopogon citratus*) is one of spices widely distributed throughout the world, primarily Southeast Asia and famous for their rapid growth. Lemon grass is a potential essensial oil source for formulation of perfume and mosquito repellents (Wiart, 2002). In addition, lemon grass is used as folk medicine to treat rheumatism, skin eruptions, reduce fever, and very effective mouthwash for toothache (Perry, 1978). In. Indonesia, this spice is commonly used as flavoring of special food, such as seafood and meat product.

Furthermore, lemon grass extracts inhibit fecal â-glucuronidase and possess antioxidant property (Wiart, 2002). Miean and Mohamed (2001) reported that lemon grass extracts contents total flavonoid (178 ppm), especially kaempferol. Flavonoids are a widely distributed group of polyphenolic compounds characterized by β common benzo-g-pyrone structure, that have been reported to act as antioxidants in various biological systems (Morel *et al.*, 1993; Salah *et al.*, 1995; Whang and Zheng, 1992).

Sorata *et al.*, (1984) reported that flavonoid possess to act as quenchers of singlet oxygen. Singlet oxygen ($^1O_2$) can be formed by photosensitization with several sensitizer compound such as chlorophylls, pheophytin, myoglobin derivatives, riboflavin, erythrosine and methylene blue. These reactions involve energy transfer from the excited triplet state of the sensitizer (^{3}Sens*) to molecular triplet oxygen in the presence of light. The objectives of this research were to study the effect of lemon grass extract in autooxidation and photooxidation in linoleic acid containing mixtures.

MATERIALS AND METHODS

Material

Lemon grass obtained from a local market at Yogyakarta will be cleaned sample, freeze dried and ground to 40 mesh. Hexane, acetone, ethanol, chloroform, sodium thiosulphate, acetic acid, potassium iodide, amylum, sodium carbonate, a-tocopherol purchased from Merck (Darmstadt, Germany). Linoleic acid, DPPH, a-tocopherol and BHT were purchased from Sigma Chemical Co. (St. Lois, MO). Erythrosine were purchased from local market.

Sample preparation

Lemon grass were cleaned, freeze dried and ground to a fine powder (50 g) were extracted sequentially with 250 mL of hexane, acetone and ethanol for 24 hours After filtration, residue was extracted once more with an additional 500 mL hexane. The hexane extracts were then pooled and saved. Similary, the acetone extract and ethanol were obtained by extracting the residue remaining after twice extraction. The solvents in the three extracts were then evaporated using a vacum evaporator. The resulting three extratcs were then weighed and store at –20°C until use.

Determination of total phenol content of andaliman extracts

The content of total phenol in lemon grass extracts was measured by Folin-Ciocalteu assay (Huang and Yen, 2002). The absorbance of extracts was read at 750 nm in a Shimadzu UV 1601 UV-Vis Spectrophotometer. Results were expressed as mg of gallic acid/100 g extract.

Determination of free radical scavencing activity (RSA)

The free radical scavenging activity of andaliman extract was measured with DPPH free radical using the method of Burda and Oleszek (2001) with minor modification. Lemon grass extracts (1 mL of 10-500 ppm ethanol solution) were added to 2 mL, 0,025 M DPPH in ethanol. After reacting for 30 minutes, the absorbance was read at 517 in a Shimadzu UV 1601 UV-Vis Spectrophotometer. Ethanol (1 mL) was mixed with 2 mL DPPH and this served as the control. Radical scavenging activity (%) was calculated as follows

$$\textit{Radical scavenging activity} = 1-\left(\frac{\textit{sampel absorbance at } 517\,nm}{\textit{control absorbance at } 517\,nm}\right)x100$$

Antioxidant activity in emulsion of b-carotene and linoleic acid

Antioxidant activity of andaliman extracts were evaluated using method of Hammerschmidt and Pratt (1978) with some modifications. The b-carotene (1.0 mg), 20 mg linoleic acid, and 200 mg Tween 20 were dissolved in 10 mL chloroform. The chloroform was subsequently evaporated in a rotary evaporator, and its remaining trace were removed by nitrogen. The model emulsion was prepared by adding 50 mL of oxygenated distilled water were added to the flask with vigorous swirling. Five mL aliquouts of this emulsion were placed in glass tubes with a stopper which contained 0.2 mL of tested

antioxidant solution, and the tubes were immediately incubated at 50°C (0.2 mL ethanol was added to the control). Destruction of b-carotene was monitored by reading the absorption at 470 nm. Reading were taken at 20 minutes interval for 120 minutes

Effect of reducing power in lemon grass extract

The reducing power of lemon grass extracts was determined according to the Yen and Chen (1995). Lemon grass extract (100 – 1000 ppm) in 1 mL methanol were mixed with phosphate buffer (2,5 mL, 0,2 M, pH 6,6) and potassium ferricyanide (2,5 mL, 1 %), the mixture was incubated at 50 °C for 20 min. A portion (2,5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2,5 mL) was mixed with distilled water (2,5 mL) and ferrichloride (0,5 mL, 0,1 %), and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Effects of lemon grass extract on erythrosine-sensitized photooxidation

Procedure is method according to Lee *et al.* (1997) with minor modification. To study the effects of andaliman extract on photosensitized oxidation of linoleic acid, sample of 500 ppm (wt/vol) in 1.0 % (wt/vol) linoleic acid were prepared in methanol that also contained 100 ppm (wt/vol) erythrosine as a photosensitizer. Samples containing 500 ppm (wt/vol) a-tocopherol were used as a positive control in the system. Ten mL of sample was transferred into a 30 mL serum bottle. The bottles were sealed aie-tight with Teflon septa and aluminium caps and then were placed in the light box (70 cm x 50 cm x 60 cm). The light intensity at the sample level was 4,000 lux, and room temperature. The experiment was carried out in duplicate.

Statistical analysis

Experimental data were analyzed by the analysis of variance (ANOVA) and the significant differences among means were determined by Duncan's multiple range test (DMRT) using SPSS version 10 for windows and $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Phenolic compound was extracted sequentially from lemon grass using hexane, acetone and ethanol. The extraction was done sequentially with solvents that posseses different polarity to seperate phenolic compound in lemon grass. Extraction using hexane could dissolve less polar phenolic compounds. Whereas acetone could dissolve semi polar compounds and the use of ethanol would recover the more polar compounds.

Figure 1. shows total phenol content in the three solvents. The total phenol of acetone extract (EA) gave higher yields from sequential extraction than hexane extract (HE) and ethanol extract (EE). According to Peri and Pompei (1971), the total phenol content can be resulted from the sum of phenolic compounds such as simple phenolics (derivatives of hydroxybenzoic and hydroxycinnamic acid), non tannin flavans (anthocyanins, catechins and leucoanthocyanins), hydrolysable tannins gallic and ellagic acid), and condensed tannins (polymers and copolymers of catechins and leucoanthocyanins). Miean and Mohamed (2001) reported that lemon grass extracts containing total flavonoid of 178 ppm, which was mosthy kaempferol.

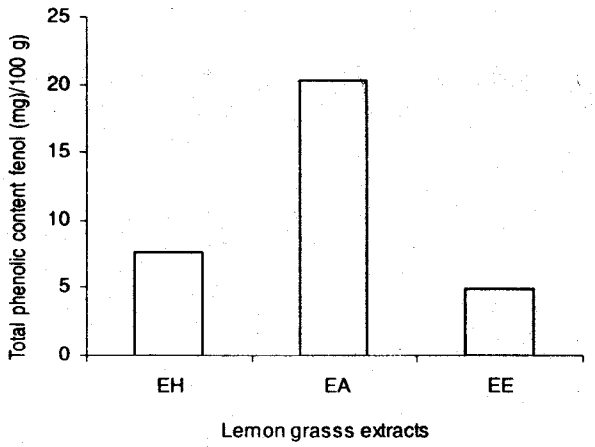

Figure 1. Total phenolic content of lemon grass extracts (mg/100 g). (HE: hexane extract, AE: acetone extract, EE: ethanol extract)

DPPH radical scavenging activity of the lemon grass extracts

The result of radical scavenging activity test (RSA) in DPPH from the three extracts showed all extract posses ability as radical scavenger (Figure 2). In other hand, extracts prepared from lemon grass have radical scavenging activity. The result shows that there was positiv relationship between the amounts of total phenolic compounds in lemon grass and radical scavenging activity. For example, acetone extract (AE) showed highest phenolic compounds and posseed higher scavenger activity followed a hexane extract (HE) and ethanol extract (EE). Many research showed that extract of plant as fruits, leaves, and vegetables have positive corellation between total phenol content and antioxidant activity (Velioglu *et al.*, 1998; Duh and Yen, 1997).

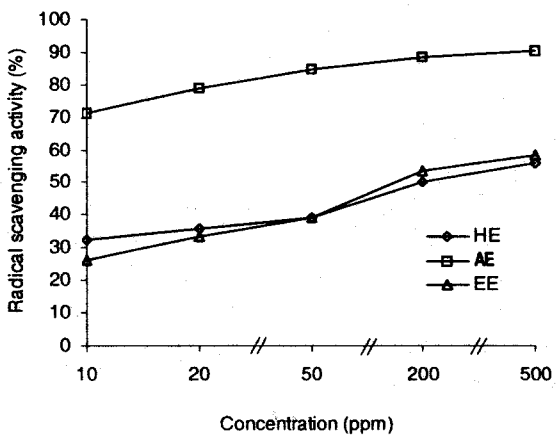

Figure 2. Effects of lemon grass extract at different concentrations on scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Figure 2 shows that all extracts examined were found to possess DPPH-scavenging activity, indicating that these three extracts of phenolic compounds were used the most potent DPPH scavengers. For hexane extract (HE) and ethanol extract (EE), as the DPPH concentrations increased from 10 ppm to 500 ppm, scavenging activity of both extracts were increased significantly ($p<0,05$). However, radical scavenging activity between HE and EE obtained from the same material were statistically not significant. Hexane Extract (HE) was assumed containing essential oils and oleoresin which consisted mainly of terpenes and sesquiterpenes were present in the extract. The presence of chlorophill in hexane extract and acetone extract was responsible for the deep-green coloration.

In Figure 2, acetone extract at concentration 10 ppm to 500 ppm exhibited excellent antiradical activities (71.32 % to 90.41 %). Effect of ethanol extract at 500 ppm on radical scavenging activity was compared to that a-tocopherol and BHT as α positive control. Ethanol extract exhibited the greatest antiradical activity than 200 ppm of α tocopherol and BHT. However, scavenging activity of extracts at the level of 10-200 ppm was lower than that positive control.

Antioxidant activity of lemon grass extract using β-carotene/linoleic acid method

The antioxidant activity of lemon grass extracts was evaluated using a β-carotene-linoleic acid model system. Lemon grass extract prepared using different solvent is shown in Figure 3. The antioxidant activity, expressed as accumulated in absorbance over 120 minutes, was proportional to the oxidative loss of β-carotene. The calculated concentration of the lemon grass extract in the β-carotene assay was 200 mg in 5 mL of emulsion.

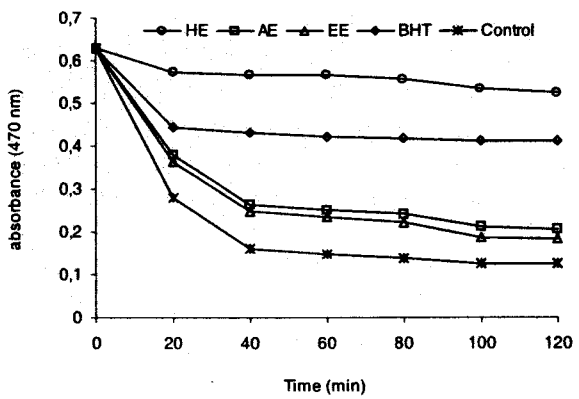

Figure 3. Antioxidant activity of lemon grass extracts at 200 ppm in the β-carotene/linoleic acid system. (HE: hexane extract, AE: acetone extract, EE: ethanol extract)

All the lemon grass extract examined were found to possess good antioxidant activity. From three extracts of lemon grass to β-carotene in emulsion with linoleic acid, hexane extract showed highest than acetone extract, and ethanol extract. Hexane extract compared to that BHT as standard showed that BHT lower than hexane extract ($p<0,05$). This result was different from the scavenging activity was determined by the DPPH test, in which acetone extract exhibited excellent scavenging activity than hexane extract. One possible explanation is that due to its poor solubility in water, hexane extracts accumulated in the hydrophobic sites of the lemon grass extract. According to Porter (1980), small oil droplets and the lipophilic nature of antioxidants would improve their effeciencies in oil-in-water emulsions. On the other hand, high surface to volume ratio, where the surface is ready to the whole phase and poor solubility of lipophilic antioxidant in the aqueous phase may explain the contradictory results (Porter, 1980; von Gadow *et al.*, 1997).

Reducing power of lemon grass extracts

Reducing power of three extracts was determined by reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Lai *et al.* (2001) reported that increased absorbance at 700 nm indicated an increase in reduding power. As shown in Figure 4, the reducing power of acetone extract , hexane extract and ethanol extract were 0.78; 0.74 and 0,31 at concentration of 500 ppm, respectively. The results indicate that acetone extract and hexane extract showed a greater reducing power than ethanol extract. Reducing power of acetone extract and hexane extract was compared with BHT and α-tocopherol at 200 ppm. Acetone extract and hexane extract showed greater reducing power the greatest than α-tocopherol, but the absorbance value was lower than BHT.

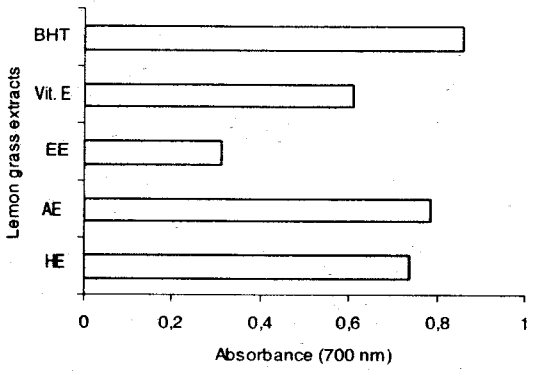

Figure 4. Reducing power of lemon grass extract at 500 ppm (Hexane extract, Acetone extract, Ethanol extract)

Therefore, the phenolic presence in lemon grass extract are good electron donors and could react with free radicals by converting free radical to more stable products. The result in Figure 4 showed corellation between total phenol content (Figure 1.) and scavenging activity (Figure 2).

Effect of lemon grass extract on photooxidation of linoleic acid

Effect of concentration at 500 ppm of hexane extract (HE), acetone extract (AE), and ethanol extract (EE) on peroxide value of 0.03 M linoleic acid in methanol and exposed to light at room temperature is presented in Figure 5. The peroxide value increase in linoleic acid was due to addition of erythrosine as photosensitizer. A probable explanation was that erythrosine may produce singlet oxygen from triplet oxygen when it was exposed to light. The formation of singlet oxygen by photosensitizer accelarated lipid peroxidation. Therefore, erythrosine effectively act as photosensitizer to accelarate linoleic acid oxidation in model system exposed to light (Yang *et al.*, 2002). The peroxide value of linoleic acid were relative similar for 5 hours under light without sensitizer (C-2) and dark (C-3). Without the presence of sensitizer in linoleic acid or in dark system triplet oxygen could not be converted to singlet oxygen.

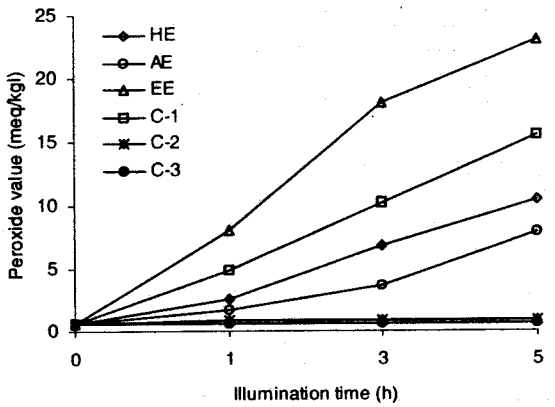

Figure 5. Effect of 500 ppm lemon grass extract on oxidation of singlet oxigen in linoleic acid for 5 hours. ((Hexane extract, Acetone extract, Ethanol extract,C-1: with light and sensitifer, C-2: light without sensitifer, C-3: donk with sensitifer)

Acetone extract showed very strong antiphotooxidative activity on erythrosine – sensitized photooxidation of linoleic acid for 5 hours exposure to light ($p<0.05$). However, ethanol extract indicate increasing peroxide value. This is probably due to catalytic effect of chlorophyll or nature sensitizer can act synergistically with erythrosine to generate singlet oxygen so that oxidation rate of peroxide formation increase. Endo *et al.* (1985) had reported that chlorophyll and its derivate can promote lipid oxidation during storage. Wanasundara and Shahidi (1998), in their study showed that etanol-aqueous extract possesed effect of prooxidant in edible oil, perhaps caused effect of catalitic from chlorophyll.

CONCLUSION

The acetone extract of lemon grass seems to contain phenolic compound showed scavenging activity in DPPH and reducing power test. This study showed that acetone extract could act as quencher of singlet oxygen on linoleic acid photooxidation with the presence of erythrosine as sensitizer.

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