Singlet Oxygen Quenching Activities Of Phenolic Extract From Lemon Grass Leaves (*Cymbopogon citratus* Stapf)

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ABSTRACT

Suryanto *et al.,* 2010. Singlet Oxygen Quenching Activities Of Phenolic Extract from Lemon Grass Leaves (*Cymbopogon citratus* Stapf).

Lemon grass (*Cymbopogon citratus* Stapf) is a traditional food ingredient characterized by its specific and refreshing aroma. This objectives of study was determined phytochemical contents and the effect of lemon grass leaves extract in photooxidation linoleic acid and ascorbic acid in system model. Lemon grass leaves was extracted sequentially with hexane and methanol. The photooxidation reaction system was consisted of linoleic acid 0.03 M in ethanol containing 0.017 mM erythrosine as sensitizer the reaction mixture was exposed under 4000 lux fluorescent light up to 5 hours while ascorbic acid 200 µg/mL in phospat buffer solution (pH 7.5) containing 0.0068 mM erythrosine as sensitizer illuminated for 20 minutes. Analyses of phytochemical based on total phenolic and flavonoid. Analyses of photooxidation of linoleic acid based on peroxide value and degradation of ascorbic acid was analyzed using the spectrophotometer at λ 265 nm. The total phenolic and flavonoid content in hexane extract showed the highest phenolic content than methanol extract. Hexane extract 1500 ppm exhibited singlet oxygen quenching effect on photooxidation linoleic acid compared with methanol extract, peroxide value 3.57 and 3.72 meq/kg after 5 hours illumination, respectively. Hexane extract 100 ppm also exhibited singlet oxygen quenching effect on photooxidation ascorbic acid compared with methanol extract, ascorbic acid content 185.44 and 76.67 mg/kg after 5 hours illumination, respectively. It's concluded that hexane extract of lemon grass leaves contain phenolic component that have singlet oxygen quencher activity.

Keywords : Lemon grass, phenolic extract, photosensitizer, singlet oxygen quencher

INTRODUCTION

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 β-glucuronidase and possess antioxidant property (Wiart, 2002). Miean and Mohamed (2001) reported that lemon grass extracts contents flavonoid, especially kaempherol. Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-γ-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.

The oxidations of biological components induced by singlet oxygen are associated with various pathological events such as pigmentation, cataract, skin aging and cancer (Davies and Goldberg, 1987; Shahidi, 1997; Haliwell and Guttridge, 2001). There are various ways to produce singlet oxygen in biological systems. Singlet oxygen can be formed chemically, enzimatically, and photochemically (Krinsky, 1977). However, the most well documented formation mechanism for singlet oxygen formation is photochemistry using sensitizers. Chlorophyll, riboflavin, myoglobin, porphyrins, food colorants and textile dyes are well-known photosensitizer and can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen (Stracke *et al*., 1999; Lledias and Hansberg, 2000; Min and Boff, 2002; DeRosa and Crutchley, 2002). These reactions involve energy transfer from the excited triplet state of the

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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 on photooxidation in linoleic acid and ascorbic acid in model system.

MATERIALS AND METHODS

Lemon grass was obtained from a local market at Malalayang, Manado. Sample then cleaned, air-dried and grounded to 40 mesh. Hexane, methanol, chloroform, sodium thiosulphate, acetic acid, ascorbic acid, di-sodium hydrogen phosphate, sodium dihydrogen phosphate, potassium iodide, amylum, sodium carbonate, erythrosine purchased from Merck (Darmstadt, Germany). Linoleic acid, gallac acid, quercetin cathecin were purchased from Sigma Chemical Co. (St. Lois, MO).

Sample preparation

Lemon grass was cleaned, air-dried and grounded to a fine powder (50 g) was extracted sequentially with 250 mL of hexane and methanol for 24 hours after filtration, the residue was extracted once more with an additional 250 mL hexane. The hexane extracts were then pooled and saved. Similary, the methanol extract 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 two extract were then weighed and store at – 20^{o}C until use.

Determination of total phenolic

The content of total phenolic was measured using the method of Jeong *et al*., 2002. Briefly, the lemon grass extract (1 mL) was mixed with 1 mL of the 50% Folin-Ciocalteu reagen and 1 mL of 2% Na_2CO_3 and centrifuged at 13400 g for 5 min. The absorbance of extracts was read at 750 nm with Spectrophotometer Milton Roy 501 after 30 min of incubation at room temperature. The results were expressed as gallic acid equivalents.

Determination of total flavonoid

The total flavonoid content of lemon grass extracts was determined according to Zhishen *et al*., 1999. Briefly, Distilled water was added to make 5 mL and 0,3 mL $NaNO_2$ (1:20) were added to make 5 mL $AlCl_3$ (1:10) were added and the total was made up to 10 mL with distilled water. The solution was mix well again and the absorbance was measured against a blank at 510 nm with a Milton Roy 501 Spectrophotometer.

The effect of erythrosine concentration on photooxidation of linoleic acid and ascorbic acid

To study the effect of erythrosine concentration on photooxidation of linoleic acid and ascorbic acid was determine with the following method. For photooxidation of linoleic acid was evaluated with 0; 0.006; 0.011; 0.017; 0.023; 0.028 and 0.057 mM of erythrosine, while photooxidation ascorbic acid by using 0; 0.023; 0.0045; 0.0068 and 0.0091 mM of erythrosine. Ten mL of sample was transferred into a 30 mL serum bottle. The bottles were sealed air-tight with teflon septa and aluminium caps and then were placed in the light box. The light intensity at the sample level was 4,000 lux, and room temperature. Oxidation stability of linoleic acid was determined by measuring the peroxide value for 5 hours according to the AOCS (1990) method. Degradation of ascorbic acid was analysed using the spectrophotometer at λ 265 nm.

Determination of singlet oxygen quenching activity

The procedure was according to Lee *et al*. (1997) method with minor modification. To study the effects of lemon grass extract on photosensitized oxidation of linoleic acid, sample of 500-1500 ppm in 0.03 M linoleic acid were prepared in methanol that also contained 0.017 mM erythrosine as a photosensitizer. Ten mL of sample was transferred into a 30 mL serum bottle. The bottles were sealed air-tight with teflon septa and aluminium caps and then were placed in the light box. The light intensity at the sample level was 4,000 lux, and room temperature. Oxidation stability of linoleic acid was determined by measuring the peroxide value for 5 hours according to the AOCS (1990) method.

Measurement of singlet oxygen oxidation of ascorbic acid was based on the method described by Jung *et al.* (1995) with minor modification. To study the effects of lemon grass on the singlet oxygen oxidation of ascorbic acid, 0, 25, 50, 75 and 100 ppm of lemon grass extracts and 0.0068 mM erythrosine were added to 200 μg/mL ascorbic acid in 0.01 M sodium buffer phosphate. Twenty mL of the prepared sample was transferred into a 30 mL serum bottle and seal with teflon septa and aluminium caps and then were placed in the light box. The light intensity at the sample level was 4,000 lux, and room temperature. Ascorbic acid was determined by measuring the absorbance of solution at 265 nm using a spectrophotometer after 20 min of light storage at room temperature.

The light storage box consisted of two rectangular chambers: a glass chamber (60 cm x 30 cm x 50 cm) for sample storage and the wooden box (70 cm x 50 cm x 60 cm) for light sources to the glass chamber was 12 cm. Samples were placed on the a motor-driven rotating plastic disk (5 rpm) to assure uniform light exposure and 15 cm above of light source. The light source, four Sylvania 15 watt cool white fluorescence lamps were placed on the 4,000 lux. The temperature of the light storage box was kept constant at room temperature.

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. Analysis was performed for the response variables of peroxide value and ascorbic acid content.

RESULTS AND DISCUSSION

Phenolic compound was extracted sequentially from lemon grass using hexane and methanol. The extraction was done sequentially with some solvent that posseses different polarity to seperate phenolic compound in lemon grass. Extraction using hexane could dissolve less polar phenolic compounds and the use of methanol would recover the more polar compounds. The total phenolic contents of lemon grass extracts were expressed as milligrams of gallic acid equivalents per gram of dry lemon grass (mg (GAE/kg of db) and are presented in Table 1. The total phenolic contents of hexane extract (HE) and methanol extract were 72.55 ± 0.29 and 66.94 ± 0.14 mg/kg, respectively.

Table 1. Total phenolic and flavonoid content of lemon grass extracts

Extracts	Total phenolic content (mg/kg)	Total flavonoid content (mg/kg)
Hexane extract (HE)	72.55 ± 0.29	3.53 ± 0.05
Methanol extract (ME)	66.94 ± 0.14	3.35 ± 0.05

Data are presented as means with standard deviation obtained from duplicate analysis

The total phenolic of hexane extract (HE) gave higher yields from sequential extraction than methanol extract (ME). Different solvent extracts showed significant ($p<0.05$) different in their total phenolic contents. The high total phenolic content in HE were predicted cause its phenolic compounds in extract was less polar, so it would recover and soluble in hexane solvent. According to Peri and Pompei (1971), the total phenolic 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). It is interesting to note that hexane extract (HE) and methanol extract (ME) which showed no significance between both total flavonoid content ($p>0.05$). The total flavonoid content of HE and ME were 3.53 ± 0.05 dan 3.35 ± 0.05 mg/kg, respectively. The present results suggest that may be flavonoid compound was soluble in polar and less polar solvents.

The effect of erythrosine on photooxidation linoleic acid and ascorbic acid

The effect of various concentration of erythrosine 0; 0.006; 0.011; 0.017; 0.023; 0.028 and 0.057 mM on photooxidation linoleic acid 0.03 M in methanol for 5 hours illuminated with fluorescent light 4000 lux showed on Figure 1. Erythrosine effectively act as singlet oxygen initiator at all level of concentration. In contrast with, linoleic acid that illuminated with light without sensitizer (0 mM) or without light (dark) did not show any increasing of peroxide value significantly ($p>0,05$). It proved that linoleic acid that illuminated without erythrosine can't produce singlet oxygen from triplet oxygen. Min and Bob (2002) declared that singlet oxygen were produce from triplet oxygen with the presence of sensitizer and light.

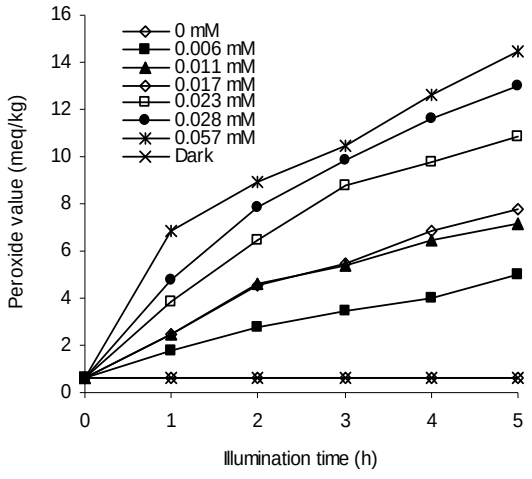

Figure 1. The effect of erythrosine concentration on photooxidation linoleic acid 0.03 M for 5 hours fluorescent light storage.

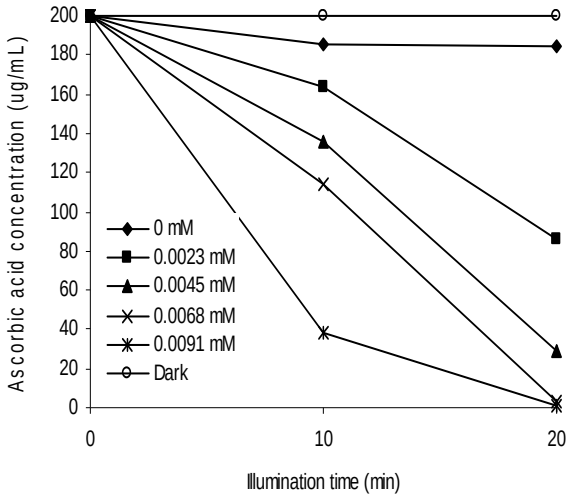

Figure 2. The effect of erythrosine concentration on photooxidation ascorbic acid 200µg/mL for 20 min fluorescent light storage.

The presence of sensitizer such as erythrosine can increase oxidation, it caused that sensitizer possess activity to absorb light energy and produced hidroperoxide by photooxidation. Photooxidation of singlet oxygen in linoleic acid can produce hidroperoxide at conjugated double bond 9-OOH and 13-OOH and not conjugated 10-OOH and 12-OOH. Autooxidation of triplet oxygen on linoleic acid produce hidroperoxide at conjugated 9-OOH and 13-OOH (Neff and Frankel, 1980). There's a correlation that the increasing of erythrosine can increase peroxide value. Peroxide value depending on concentration of erythrosine. McLearie *et al.* (1992) reported that erythrosine possess to produce singlet oxygen on photooxidation phenyl linoleic and phenyl arachidonat.

The effects of different erythrosine concentration on photooxidation of 200 µg/mL ascorbic acid in 0.02 M phosphate buffer (pH 7.5) during 20 min light storage were shown in Figure 1. The data are shown that erythrosine accelerated the degradation of ascorbic acid during 20 min light storage. As the concentration of erythrosine increased from 0; 0.023; 0.0045; 0.0068 and 0.0091 mM, the decomposition of ascorbic acid incresed by 92.14 to 0.59% during 20 min light storage ($p<0.05$). When 0.0091 mM erythrosine was added, 1.17 µg/mL of ascorbic acid was degradated after 20 min of light strorage. Without addition of erythrosine, after 20 min of light storage, 184.17 µg/mL of ascorbic acid remained unphotosensitized. That is, the erythrosine did not affect ascorbic acid concentration of sample during storage in the dark ($p>0.05$). These results clearly showed that erythrosine acted as a photosensitizer to accelerate the degradation of ascorbic acid. According to Jung *et al*. (1995), the oxidation of ascorbic acid might be due to the generation of singlet oxygen or excited triplet erythrosine.

The effect of lemon grass extract on photooxidation linoleic acid

After study about erythrosine that can act as photosensitizer under fluorescent light illumination, the addition of extract was done at various concentration into a sample to study hows hexane (HE) and methanol (ME) extract of lemon grass leaves can act as singlet oxygen quencher in exhibit photooxidation of linoleic acid. Extract concentration that was added 500, 1000 and 1500 ppm. The effect of both extract on photooxidation linoleic acid that sensitized by erythrosine (15 ppm) for 5 hours fluorescent light exposure 4000 lux showed on Figure 2. The addition of some concentration both of Lemon grass leaves extract was significantly can exhibit singlet oxygen in photooxidation linoleic acid sensitized by erythrosine ($p<0.05$). This data presented with peroxide value both of extract were less than peroxide value control. Control (erythrosine illuminated with light) show peroxide value changing that continuous increase under 5 hour illumination. Its indicated that erythrosine as sensitizer can act as singlet oxygen oxidation initiator on photooxidation of linoleic acid and it's proved with the increasing of linoleic acid peroxide value, although sample without light did not show any peroxide value changing that significant for 5 hours illumination by fluorescent light. Therefore, the production of singlet oxygen can not be happen without the presence of sensitizer and light to initiate photooxidation process of unsaturated fatty acid (Min and Boff, 2002).

Based on peroxide value (converted in percent inhibition) that controled for 5 hours, the inhibition effect of extract that had been extracted with hexane on photooxidation of linoleic acid show singlet oxygen inhibition activity less more than methanol solvent. Concentration effect 500, 1000 and 1500 ppm from HE has inhibition effect were 50.19; 54.05 and 57,92%, although ME extract were 46.33; 52.12 and 54.05%. From data above, HE extract show inhibition activities more strong at all level of concentration extract on singlet oxygen oxidation.

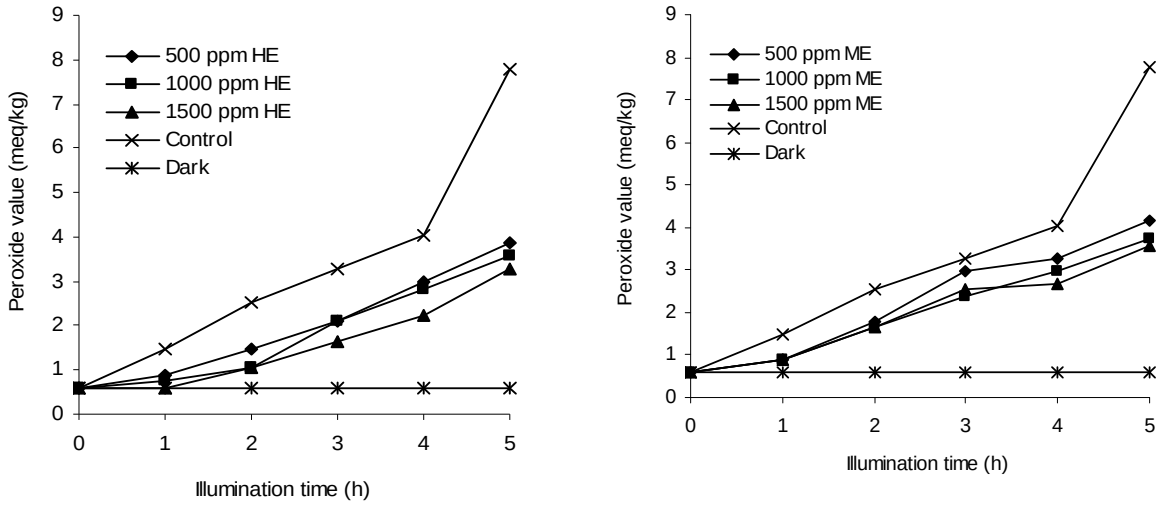

Figure 3. The effect of concentration Lemon grass leaves extract on singlet oxygen inhibition on photooxidation linoleic acid that sensitized by erythrosine for 5 hours illuminated with fluorescent light (4000 lux). Hexane extract (HE) and methanol extract (ME).

Figure 3 shows that HE extract possess singlet oxygen quenching activity on photooxidation linoleic acid sensitized by erythrosine under fluorescent light exposured. Singlet oxygen quencher can decrease photooxidation by three methods such as quenching exited triplet sensitizer, physically quenching singlet oxygen and chemically quenching singlet oxygen. Quenching mechanism by a compound was determined by measuring total quenching mechanism constant, physically and chemically quenching (Min and Boff, 2002).

A compound in extract was predicted act as singlet oxygen quencher, it was phenolic compound from simple phenolic compound group, flavonoid and tannins. Based on its total phenolic content, HE extract have higher yield of phenolic content compared with ME extract (Table 1), although both of extract possess flavonoid content that not significantly different. Flavonoid was reported can react with singlet oxygen ($^1O_2$). Mukai *et al*. (2005) report that cathecin in tea and epimer can act as singlet oxygen quencher physically. Tournaire *et al.* (1993) study about the reactivity of 13 selected flavonoid (from flavonol, flavon, flavanon and flavan group) with singlet oxygen and try to determine the correlation between structure and activity. They found out that the efficiency of physical quencher determined by the presence of functional cathecol on ring B, even ring C structure (especially hydroxyl functional group that activated the double bond) was the main factor that determined the efficiency of chemical reactivity of this compound with singlet oxygen. The reactivity of flavonol group was stronger on singlet oxygen than flavon, flavanon and flavan. Besides that, they also found that flavonoid more effectively act as physical quencher than chemical.

The effect of lemon grass extract on photooxidation ascorbic acid

The effect of hexane extract from Lemon grass (*Cymbopogon citratus* Stapf) leaves and methanol extract as singlet oxygen quencher on ascorbic acid oxidation with erythrosine (6 ppm) as sensitizer showed on Figure 4. The result showed that HE extract (accept 25 ppm) possess act as singlet oxygen quencher in 200 ppm ascorbic acid for 20 minutes illuminated with fluorescent light. HE can exhibit ascorbic acid oxidation compared with control ($p<0.05$). It means that HE can quench singlet oxygen. From this data, sample in condition without light did not show any decreasing on ascorbic acid concentration for 20 minutes without light at room temperature ($p>0.05$). It means that singlet oxygen did not performed in condition without light. Singlet oxygen did not presence without sensitizer and light (Rawls and van Santen, 1970; Jung *et al*., 1999; Yang *et al*., 2002).

In the same concentration HE possess singlet oxygen quenching activities that significantly high compared with ME extract. Besides that, singlet oxygen quenching depending on extract concentration. HE extract showed the increasing of singlet oxygen quenching activity from 25 ppm until 100 ppm extract ($p<0.05$). This data showed a tendency that the increasing of extract indicating to the increasing of extract activity in exhibit singlet oxygen oxidation.

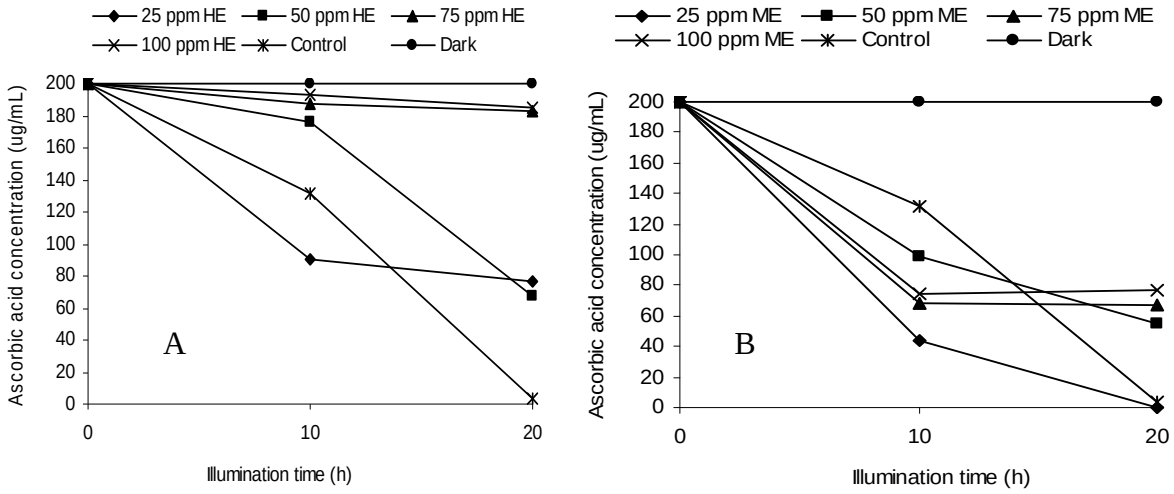

Figure 4. The effect of concentration HE (A) and ME (B) on singlet oxygen inhibition from ascorbic acid oxidation that sensitized with erythrosine for 5 hours fluorescent light storage (4000 lux). Hexane extract (HE) and methanol extract (ME).

From data above, need to be noted that ME extract at all level of concentration did not show singlet oxygen quenching activities for 10 minutes, but have singlet oxygen quenching activities for 20 minutes. Its cause in model system, ascorbic acid act as singlet oxygen quencher for 10 minutes, although finally ascorbic acid were degradated and just remain 3.11 ppm. Ascorbic acid possess as singlet oxygen quencher, peroxil radical scavenger and can reduct ascorbic acid become active form condition, but ascorbic acid can oxidized by singlet oxygen by its doble bond (Ingold, 1962). Sahbaz and Somer (1993) declared that phytochemical decomposition of ascorbic acid related with the absorption of light energy process. The sources and intensity of light also related with ascorbic acid oxidation (Jung *et al*., 1995).

CONCLUSION

Lemon grass leaves extract showed significance having phenolic component. Extracts obtained from hexane were characterized that having a high content of total phenolic compared to those of extracts obtained from methanol. The sequential extraction of lemon grass using hexane solvent possessed quencher of singlet oxygen on linoleic acid and ascorbic acid photooxidation with the presence of erythrosine as sensitizer, compared to methanol extract. As the concentration of lemon grass leaves extract, reduction of singlet oxygen formation in linoleic acid and ascorbic acid increased.

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