

## Antioxidant and Anti-Glycation Activity of Ethanol Lemongrass (*Cymbopogon citratus*) Leaves Extract

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### ABSTRACT

The present study was undertaken to investigate the antioxidant and anti-glycation activity of lemongrass (*C. Citratus*) leaves extract. The antioxidant activity was measured by hydroxyl radical and hydrogen peroxide scavenging activity and chelating effect of ferrous iron. The anti-glycation activity was determined by inhibition of hemoglobin glycation method. The results of this present studies showed that the *C. Citratus* leaves extract to have hydrogen peroxide and hydroxyl radical scavenging activity, and chelating effect of ferrous iron activity. The results also showed that the *C. Citratus* leaves extract to have anti-glycation activity. The result showed that *C. Citratus* leaves extract more effective in all activity, except for hydrogen peroxide scavenging activity. The present study indicated that *C. Citratus* leaves extract showed a potential antioxidant and anti-glycation activity.

**Keywords:** *C. Citratus*, Lemongrass, Antioxidant, Anti-Glycation.

### INTRODUCTION

Oxidative and glycation stress has been implicated in several diseases including cancer, atherosclerosis, malaria, chronic fatigue syndrome, rheumatoid arthritis, diabetes Mellitus and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease<sup>1,2</sup>. Oxidative stress is defined as a misbalance in cell redox reactions which can be the result of either ROS overproduction or decreased antioxidant defense and glycation stress defined as the modification of cell proteins by non-enzymatic/irreversible reactions with reducing sugars<sup>3,4</sup>. Both oxidative and glycation stress were harmful to human health<sup>5-8</sup>.

However, both oxidative and glycation stress can prevent or retard by a compound called antioxidants<sup>2,9</sup>. Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules, and by doing so inhibit the initiation and propagation of oxidative chain reactions. They act by one or more of the following mechanisms: reducing activity, free radical scavenging, potential complexation of pro-oxidant metals and quenching of singlet oxygen<sup>10</sup>. In recent decades, there has been great interest in screening essential oils and various plant extracts for natural antioxidants because of their good antioxidant properties<sup>11</sup>.

Kalimantan is the third largest island in the world and has a very high biodiversity compared to many other areas. On this island lived about 15,000 species of flowering plants with 3,000 species of trees<sup>12</sup>. Some of them known has a

medicinal effect like lemongrass (*Cymbopogon citratus*; *C. Citratus*)<sup>7,13,14</sup>. *C. citratus* belongs to the family of Poaceae, and the genus, *Cymbopogon*<sup>15</sup>. It is an annual plant that lives in the wild, with a quasi-trunk that forms thick clumps with up to 1-2 meters height and has a strong aroma and fragrance<sup>16</sup>. This plant commonly found in Southeast Asia, which its origin can be tracked from India. Therefore, its origin is assumed to be Indonesia<sup>17</sup>. The common names for this plant are lemongrass, fever grass or "serai" in Indonesia<sup>18</sup>.

However, *C. citratus* is used as folk medicine to treat rheumatism, skin eruptions, reduce fever, and very effective mouthwash for a toothache<sup>19</sup>. Infusions of the leaves are used in traditional medicine as antimicrobial, anti-inflammatory, and sedative<sup>20</sup>. This plant extract was reported to inhibit colon cancer in animal models. The previous study also reported that ethanolic extract of lemongrass contains 535.44 to 1007.35 mg/100 g total phenolic and have 80.38 to 93.31% antioxidant activity<sup>21</sup>. The medicinal property of *C. Citratus* allegedly because of some phytochemical content in this plant extract. One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral and terpenes<sup>22,23</sup>. In addition, extracts of *C. Citratus* leaves are known also contains several other important compounds such as flavonoid, tannin, and a phenolic compound<sup>24</sup>. Some phytochemical constituents in *C. Citratus* were responsible for the antioxidant activity of this plant extract. Thus, our present study aim to investigate the antioxidant

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activity of *C. Citratus* leaves extract. Mostly, the antioxidant activity of the plant extract was measured through DPPH, ABTS, FRAP, etc methods. In this present study, we used a different approach. The antioxidant activities that investigated in this present study is hydrogen peroxide and hydroxyl radical scavenging, and chelating metal of ferrous iron activity. In addition, in this present study, we also measured the anti-glycation activity of the *C. Citratus* leaves extract.

Results from this study would provide a deeper understanding of the health promoting properties of *C. Citratus* leaves so that it would be identified for further investigations and hence, developed into value-added foods and nutraceuticals for the benefit of mankind.

## MATERIAL AND METHODS

### Collection and Identification of Plant materials

The fresh leaves of *C. Citratus* were collected from Tangkiling Village, Central Kalimantan, Indonesia. Before use, it were ensured that the leaves was free from contamination, sand and no microbial growth. The leaves were shade dried and were made into coarse powder using a commercial blender.

### Preparation of Extracts

Four portions of 5 g dried *C. Citratus* leave powder (oven- and freeze-dried) were weighed using an analytical balance. With the sample to solvent ratio fixed at 1:10, different concentrations of ethanol (v/v; 0%, 10%, 20%, and 30%) 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 centrifuged at 4500 rpm for 10 min. The supernatant was concentrated using rotary evaporator at 40°C. The concentrated extract was freeze-dried, wrapped with aluminum foil, and stored at -20°C until further analysis. In addition, all experimental models and measurement were done in Medical Chemical/Biochemical Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia.

### Hydrogen Peroxide Scavenging Activity Analysis

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank.<sup>7</sup> The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$\% \text{ Hydrogen Peroxide Scavenging Activity} = \left( 1 - \frac{\text{Absorbance (test)}}{\text{Absorbance (blank)}} \right) \times 100$$

### Hydroxyl Radical Scavenging Activity Analysis

The scavenging activity for hydroxyl radicals was measured by Fenton reaction.<sup>7</sup> Reaction mixture contained 60 µL of 1.0 mM FeCl<sub>2</sub>, 90 µL of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H<sub>2</sub>O<sub>2</sub>, and 1.0 ml of extract. Adding

H<sub>2</sub>O<sub>2</sub> started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured using spectrophotometer. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula:

$$\% \text{ Hydroxyl Radical Scavenging Activity} = \left( 1 - \frac{\text{Absorbance (test)}}{\text{Absorbance (blank)}} \right) \times 100$$

### Chelating Effect of Ferrous Iron Activity Analysis

The chelating effect of ferrous ions was estimated by the method of Lin et al.<sup>7</sup> Briefly, the extracts were added to a solution of 2 mM FeCl<sub>2</sub> (0.02 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.04 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the mixture was measured at 562 nm. Chelating effect was calculated using the equation:

$$\% \text{ Chelating of Ferrous Iron} = \left( 1 - \frac{\text{Absorbance (test)}}{\text{Absorbance (blank)}} \right) \times 100$$

### Anti-glycation Activity Analysis

Evaluation of anti-glycation activity was estimated by the method of Adisa et al.<sup>25</sup> The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. Hemolysate was prepared based on the principle of hypotonic lysis. The red blood collected were washed thrice with 0.14 M NaCl solution and one volume of red blood cells suspension was lysed with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl<sub>4</sub>. The haemolysate was then freed from the debris by centrifugation at 2300 rpm for 15 min at room temperature. The haemoglobin rich fraction in the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use.

To 1 mL of haemoglobin solution, 5µL of gentamycin and 25 µL of the plant extracts with different concentrations (0, 10, 20, 30%) were added. The reaction was started by the addition of 1 mL of 2% glucose in 0.01M phosphate buffer (pH 7.4) and incubated in the dark at room temperature. The concentrations of glycated haemoglobin at the incubation period of 24 hrs were estimated spectrophotometrically at 443nm

### Data Analysis

Each experiment was carried out in triplicates and results were recorded as mean % antioxidant and anti-glycation activity ± SD. IC<sub>50</sub> values were calculated from the plotted graph of each activity activity against the concentrations of the samples. IC<sub>50</sub> is defined as the concentration of extract necessary causing 50 per cent of each activities that measured in this study. Ascorbic acid was used as the reference compound (positive control) with the same concentrations as the plant extract. IC<sub>50</sub> was calculated using regression analysis in MS excel 2010 fro windows 10.

## RESULTS

### Hydrogen Peroxide Scavenging Activitiy

The scavenging ability of ascorbic acid and ethanol extracts of *C. Citratus* on hydrogen peroxide is shown Figure 1. The *C. Citratus* extracts are capable of

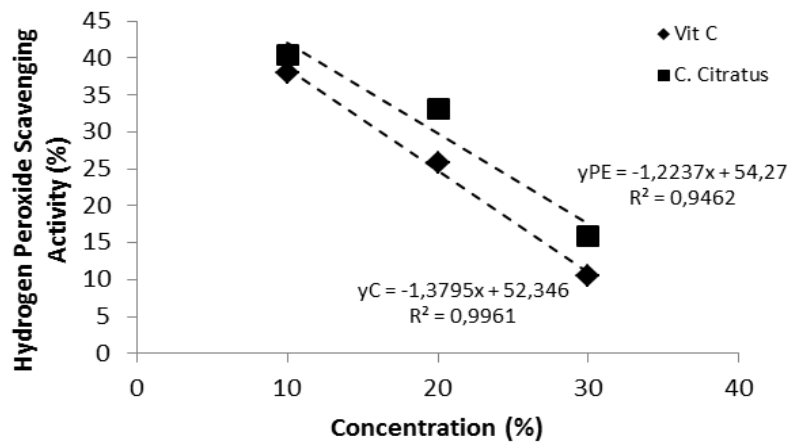


Figure 1: Hydrogen peroxide scavenging activity of *C. Citrus* leaves extract and ascorbic acid. yPE represent *C. Citrus* leaves extract; and yC represent ascorbic acid.

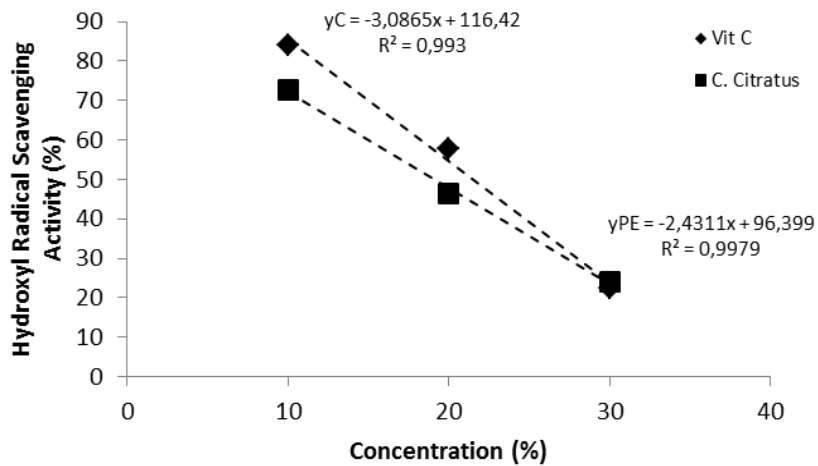


Figure 2: Hydroxyl radical scavenging activity of *C. Citrus* leaves extract and ascorbic acid. yPE represent *C. Citrus* leaves extract; and yC represent ascorbic acid.

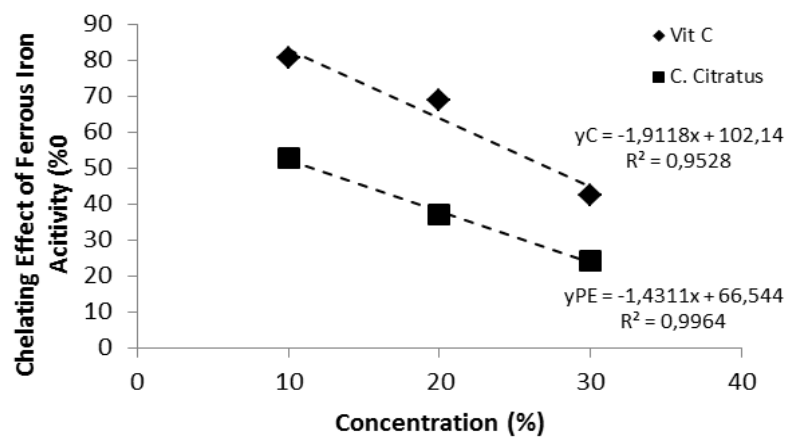


Figure 3: Chelating effect of ferrous iron activity of *C. Citrus* leaves extract and ascorbic acid. yPE represent *C. Citrus* leaves extract; and yC represent ascorbic acid.

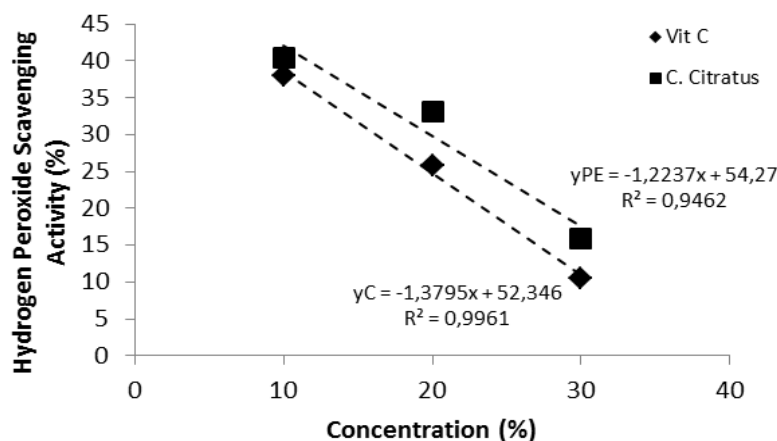


Figure 4: Anti-glycation activity of *C. Citratus* leaves extract and ascorbic acid.  $y_{PE}$  represent *C. Citratus* leaves extract; and  $y_C$  represent ascorbic acid.

scavenging hydrogen peroxide in an amount dependent manner. The ethanol extract show a good scavenging ability compare to the ascorbic acid. From the figure 1, regression and correlation coefficient ( $R^2$  and  $r$ ) and IC 50 were evaluated. The results shows in table 1. The ethanol plant extract shows a strong correlation with the scavenging ability on hydrogen peroxide. However, the IC50 for plant extract is found higher than ascorbic acid.

#### Hydroxyl Radical Scavenging Activity

Figure 2 represented the mean values  $\pm$  standard error (Mean  $\pm$  SE) of hydroxyl radical scavenging activity of ethanol extract of *C. Citratus* and ascorbic acid. The result shows that the *C. Citratus* extract can scavenge hydroxyl radical. Ascorbic acid is found to have a better activity in all concentration compare to plant extract. Furthermore, the value of  $R^2$ ,  $r$ , and IC50 for plant extract and ascorbic acid were evaluated. Both plant extract and ascorbic acid shows a strong correlation with scavenging activity (table 1). The IC50 of plant extract is found lower than ascorbic acid.

#### Chelating Effect of Ferrous Iron Activity

Figure 3 represented the mean values  $\pm$  standard error (Mean  $\pm$  SE) of chelating effect of ferrous iron activity of ethanol extract of *C. Citratus* and ascorbic acid. The result shows that the *C. Citratus* extract have chelating effect of ferrous iron activity. Ascorbic acid is found to have a better activity in all concentration compare to plant extract. Furthermore, the value of  $R^2$ ,  $r$ , and IC50 for plant extract and ascorbic acid were evaluated. Both plant extract and ascorbic acid shows a strong correlation with scavenging activity (table 1). The IC50 of plant extract is found lower than ascorbic acid.

#### Anti-Glycation Activity

The anti-glycation ability of ascorbic acid and ethanol extracts of *C. Citratus* on hydrogen peroxide is shown Figure 4. The *C. Citratus* extracts are capable of inhibit glycation reaction in an amount dependent manner. The ethanol extract show a same potential of anti-glycation activity as ascorbic acid. From the figure 4, regression and correlation coefficient ( $R^2$  and  $r$ ) and IC 50 were evaluated. The results shows in table 1. The ethanol plant extract

shows a strong correlation with the anti-glycation ability. However, the IC50 for plant extract is found lower than ascorbic acid.

#### DISCUSSION

Ethanol extract of *C. Citratus* leaves demonstrated potential hydrogen peroxide scavenging and activity. However, the ascorbic acid was slightly more effective to scavenge hydrogen peroxide than the plant extract. Hydrogen peroxide is regarded as less reactive because of its weak oxidizing ability and relatively less toxicity but as it is stable under physiological pH and temperature with the capacity to penetrate membrane as well as diffuse to long distances, it plays a key role in pathogenesis of many diseases<sup>26</sup>. Furthermore, in presence of transition metal, it is converted to more toxic hydroxyl radical which may be the origin of many of its toxic effects. It can also generate singlet oxygen by reacting with superoxide anion or with hypochlorous acid (HOCl) or chloramines in living systems<sup>26,27</sup>.

Hydroxyl radical is highly reactive oxygen centre radical formed from the reaction of various hydroperoxides with transition metal ions<sup>28</sup>. Hydroxyl radical directly cause lipid peroxidation and is the most harmful among the ROS to damage the cellular components. It also participates in DNA damage and cause carcinogenesis, mutagenesis and cytotoxicity<sup>29</sup>. The result shows that ethanolic extract of *C. Citratus* leaves shows a hydroxyl radical scavenging activity. This result is also similar to Oboh et al.<sup>30</sup> in Nigeria who were found that hot and cold water extract of the *C. Citratus* leaves have a hydroxyl radical scavenging activity. The ability of *C. Citratus* to scavenge hydroxyl radical was found more effective compare to ascorbic acid. It is based by IC50 values that were found to be lower than ascorbic acid.

Ferrous ions are one of the most effective pro-oxidants; their interaction with hydrogen peroxide in biological systems can lead to formation of highly reactive hydroxyl radicals<sup>31</sup>. According to the result, the presence of ethanolic extract of *C. Citratus* leaves interfere the formation of the ferrozine, indicating that the extract have

Table 1: Regression coefficient (R<sup>2</sup>), correlation coefficient (r), and IC50 value of ascorbic acid (AA) and *C. Citratus*.

Parameters	Hydrogen Peroxide		Hydroxyl Radical		Chelating Metal		Anti-Glycation	
	AA	<i>C. Citratus</i>	AA	<i>C. Citratus</i>	AA	<i>C. Citratus</i>	AA	<i>C. Citratus</i>
R <sup>2</sup>	0,996	0,946	0,993	0,998	0,953	0,996	1,000	0,957
R	0,998	0,973	0,996	0,999	0,976	0,998	1,000	0,978
IC50	1.620	3.979	21.533	19.095	27.263	11.558	12.537	9.959

chelating activity. It is in line with Halabi and Sheikh<sup>32</sup> results study that indicated a potential iron chelating activities of *C. Citratus* leaves extract. The result also shows that plant extract is more effective to chelate ferrous than ascorbic acid which can be seen from the IC50 value that found lower than the ascorbic acid. The result indicated that *C. Citratus* leaves extract is effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion<sup>33</sup>.

Glycation is a non-enzymatic reaction between free amino groups of proteins and reducing sugars. This reaction is known as Maillard reaction<sup>25</sup>. The reaction occurs when the amino group of amino acid reacts with a carbonyl group of reducing sugar<sup>8</sup>. Accumulation of glycation products is associated with various diseases including, first of all, diabetes and diabetic nephropathy, microangiopathy and atherosclerosis<sup>2</sup>. The results shows that the presence of *C. Citratus* leaves extract could inhibit the glycation reaction. The inhibition effect of *C. Citratus* leaves extract was found more effective than ascorbic acid.

The antioxidant and anti-glycation activity of *C. Citratus* leaves extract was believed because some phytochemical constituents contain in these extract. It shows that *C. Citratus* leaves contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids<sup>30</sup>. Those phytochemical constituents contribute directly to antioxidant activity. These metabolites could act as radical scavengers, reducing agents, hydrogen donors and singlet oxygen quenchers<sup>17</sup>.

In conclusion, the present study concluded that the ethanol extract of leaves of *C. Citratus* showed antioxidant and anti-glycation activity, which could be helpful in preventing various human oxidative stress and glycation related disease.

#### CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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