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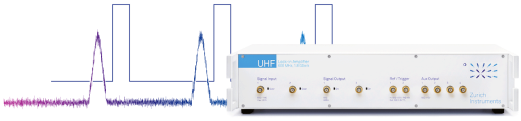
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Bioactivity of Essential Oil from Lemongrass (*Cymbopogon citratus* Stapf) as Antioxidant Agent

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Abstract. Free radical induced oxidative stress that influences the occurrence of various degenerative diseases such as cancer, coronary heart disease and premature aging. In the case that body's antioxidant defense system does not have excessive antioxidants, additional natural antioxidant via food or other nutrients intake is needed. Stems of lemongrass *Cymbopogon citratus* Stapf are known to contain phenolic compounds that are known to have antioxidant activity. Lemongrass (*Cymbopogon citratus* Stapf) plant is well known herb in Asia, espesially in Indonesia and used for cooking and has many health benefits. A study has been carried out to determine antioxidant potential of stems of lemongrass. In this the primary study is to examine essential oil *Cymbopogon citratus* Stapf from Cileles Jatinangor as an antioxidant agent. Essential oil of *Cymbopogon citratus* Stapf was isolated from 1272 g of dried stem by using Karlsruhe steam distillation methods with 0.24% in yield. The product of essential oil was also tested against antioxidant activity DPPH and resulted low activity compare to ascorbic acid and lemongrass oil standard as reference material.

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INTRODUCTION

Antioxidants are substances which delay or prevent the oxidation of an oxidizable substrate they can either be natural antioxidants or synthetic antioxidants. Natural antioxidants are produced by biological systems. They exist as small molecules such as Vitamin A, Vitamin C, Vitamin E, the carotenoids, or as complex enzyme systems such as catalase, superoxide dismutase, glutathione peroxidase and the transition metal binding proteins [1]. The Lemongrass (*Cymbopogon citratus* Stapf), is of great interest due to its commercially valuable essential oils and widely used in functional food as well as in traditional medicines. Owing to the new attraction for natural products like essential oils, despite their wide use and being familiar to be used for fragrance, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment [1].

Cymbopogon citratus commonly is an aromatic, perennial grass belonging to the family grimneae [2]. It is a tropical plant, grown as an ornamental plant in many temperate areas with maximum a height of about 1.8 m and its leaves 1.9 cm wide covered with a whitish bloom [3]. In certain medications, it is used for mental illness. It is an antifungal, antitoxicant and deodorizing agent. In combination with other herbs, it has large use as cure for Malaria [4]. One of the main constituents of the many different species of lemongrass (genus Cymbopogon) is citral (3,7-dimethyl-2,6-octadien-1-al) [5-6]. Lemongrass oil has been found to contain up to 75-85% citral [7]. Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral), beta-myrcene (MYR, 7-methyl-3-methylene-1,6 octadiene), limonenepiperitone, citronellal, citrat-2, alpha-terpineole, pinene, farnesol, proximadiol and (+)-cymbodiacetal [3]. The volatile oil from the roots contains 56.67% longifolene-(V4) and 20.03% selina-6-en-4-ol

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[8]. In particular, a study of Cymbopogon citrate isolated fatty acids contains common sterols and 16hydroxypentacos-14(z)-enoic acid [9].

Chemical structure of major constituent bioactive of lemongrass *Cymbopogon citratus* can be seen in Fig. 1 below.

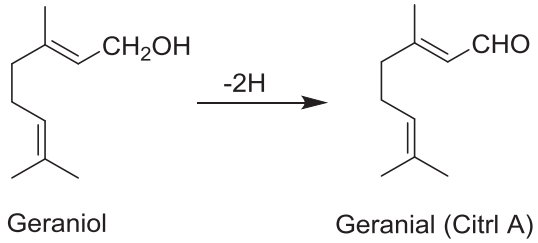

FIGURE 1. Chemical structure of the major constituents of lemonggrass Geraniol and Geranial [11]

Phytochemical studies showed that natural essential oil have attracted for their usefulness, not only nutraceutical and pharmaceutical, but also in the food. *Cymbopogon* is a genus of about 55 species, which are indigenous in tropical and semi-tropical areas of Asia and are cultivated in South and Central America, Africa and other tropical countries. These are tuff ted perennial C4 grasses with numerous stiff stems arising from a short, rhizomatous rootstock with citrus flavor and can be dried and powdered or used fresh. The name *Cymbopogon* is derived from the Greek words "kymbe" (boat) and "pogon" (beard), referring to the flower spike arrangement [10]. Lemon grass is commonly used in teas, soups and curries. It is also suitable for poultry, fish and seafood. People nowadays are more aware on health issue due to the development of new diseases.

Treatment using plant-based medicine appears to be an alternative approach due to the adverse effects associated with the use of synthetic drugs. The genetic diversity of plants has provided us not only survival, but a high degree of comfort and the most important thing of all is the potential treatment for various diseases [11].

The anti-oxidant properties of essential oils might be encouraging to consider them as natural oxidant in nutraceuticals functional food and pharmaceutical preparations. In recent years, there is an increasing interest in finding antioxidant photochemically, because they can inhibit the propagation of free radical reactions, protect the human body from diseases [12] and retard lipid oxidative rancidity in foods. The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO) and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2-) [13]. The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins [14].

This study aim to test antioxidant, from essential oil of *Cymbopogon citratus* Stapf isolated from dried stem by using Karlsruhe steam distillation. The product of essential oil tested against antioxidant activity DPPH.

MATERIALS AND METHODS

Cymbopogon citratus Stapf was harvested and collected freshly from a native farms Cileles Jatinangor and authenticated in Environmental Biology Laboratory, Department of Biology FMIPA-UNPAD. The completely randomized design (CRD) was used, with three replicates for each treatment and two instrument readings per replicate. All experiments were repeated at a different plant sampling time for reproducibility.

Plant Material and Preparation

Mature, healthy and disease-free lemongrass (*Cymbopogon citratus* Stapf) plants were collected from one area each froma native farms Cileles Jatinangor. Nine plants from each area were randomly chosen and two stalks from each plant were taken as samples. Thus, a total of 18 stalks per area were collected. Then, the 18 stalks from every field were randomly assigned to the six treatments (three stalks per treatment). Thus every treatment had a total of 15 stalks and the above-ground parts were used for the process. The wilted leaves were removed and the plants were then washed with water. The fresh plants were cut into small pieces, about one and half cm or smaller, then subjected to assays immediately. However, whole plants were stored in the refrigerator when not required for assay process. Fresh plants were used not more than three weeks after being collected and stored. Air-drying was carried

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out for 7 days under the shade at room temperature and dried plants were stored in airtight bottle containers. The dried plants were used within three weeks after these were stored.

Chemicals

All chemicals used were of analytical grade. Citral and barbituric acid were obtained from Sigma-Aldrich, while DPPH (diphenyl picril hydrazine) was obtained from Sigma. Absolute ethanol was obtained from a local distributor. Distilled water was obtained from a local supplier.

Isolation of Essential Oil of *Cymbopogon citratus* Stapf by Steam Distillation

Four batch dried sample of *Cymbopogon citratus* Stapf were prepared for steam distillation using Karlsruhe apparatus. Each batch was isolated from 283 g (batch 1), 242.5 g (batch 2), 335 g (batch 3) and 332 g (batch 4), respectively. Then each product was purified from water and the results were presented in Table 1.

Antioxidant Acctivity Assay (DPPH Methods)

Preparation of DPPH solution 0.4 mM: 1 mg DPPH dissolved and stirred with methanol 6.25 mL. Essential oil *C. citratus* was diluted in methanol (stock solution 20000 mg/L and concentration variations were used for stock dilution) as displayed in Table 1. Each concentration from the solution was pipetted and added to 1 mL of 0.4 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 of 517 nm DPPH. Test performed two separate tests for each concentration of the sample solution. Reference solution used was ascorbic acid and essential oil standards at concentration of 2, 4, 6, 8 and 10 mg/L. Experiments were performed for three separate test and% inhibition is calculated using the formula:

$$\% \text{ inhibition} = (A_{blank-} A_{\text{sample}})/A_{blank} \text{ x } 100 \quad (1)$$

The IC50 values were calculated by linear regression from plots where the abscissa represents the concentration of tested *C. citratus* oil and the ordinate the average inhibition of antioxidant activity from two separate tests.

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RESULT AND DISCUSSION

Isolation of Essential Oil of Stem *Cymbopogon citratus* Stapf by Steam Distillation

Steam distillation is the most commonly used method for collecting essential oils. Four batch dried samples of *Cymbopogon citratus* Stapf, were isolated by steam distillation using Karlsruhe apparatus. Each batch was isolated and the results are presented in Table 1. The total yield from all batch dried sample of *Cymbopogon citratus* Stapf (1272 g) were collected to obtain product yield of 3.013 g (3.5 mL, 0.24%), while yield percentage in literatures is average of about 0.20-0.37%.

TABLE 1. Yield percentage of essential oil of stem *Cymbopogon citratus* Stapf by steam distillation.

Dried *C. citratus S*tapf (g)	Essential oil yield (mL)	Yield (%)
283	0.5	0.15
342.5	0.8	0.20
335	1	0.26
312	1.2	0.33

Antioxidant Activity of Essentials oil of *Cymbopogon citratus* Stapf

It has been observed during preliminary experiments of antioxidant activity that essential oil possess antioxidant activity against DPPH, using ascorbic acid as standard and also compared with commercially of *Cymbopogon*

citratus essential oil. The sample of lemongrass essential oil were subjected for the anti-oxidant activity test namely DPPH free radical scavenging assay and the results are shown in Fig. 2.

TABLE 2. Antioxidant activity of essential oil of *Cymbopogon citratus* Stapf against DPPH

Sample (ppm)	Absorbance		% Inhibition		IC_{50} (ppm)		IC_{50} (ppm)
	1	2	1	2	1	2	
0	0.699	0.706	0	0			
5000	0.595	0.586	14.88	17.00			
10000	0.480	0.468	31.33	33.71	16082.9	15745.2	15914.0
15000	0.356	0.357	49.07	49.43			
20000	0.277	0.270	60.37	61.76			

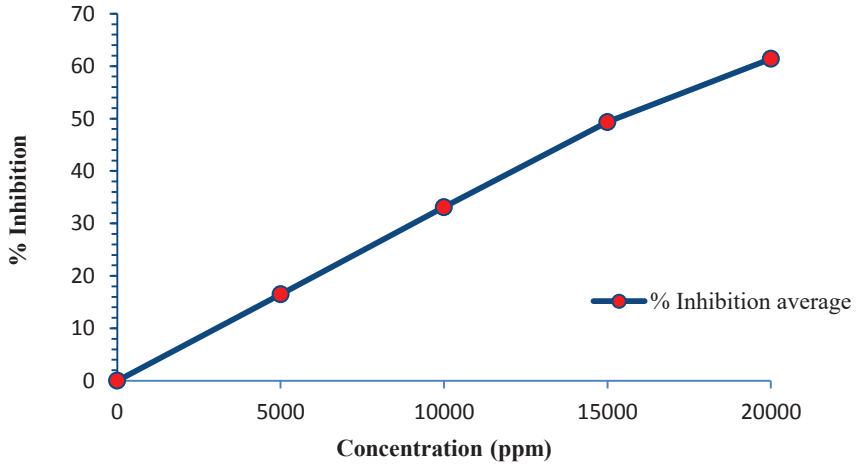

FIGURE 2. Antioxidant activity of essential oil *Cymbopogon citrus* Stapf against radical DPPH

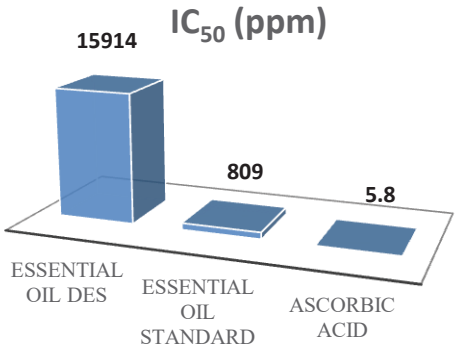

FIGURE 3. Comparation of IC_{50} (ppm) values between Essential oils of *Cymbopogon citrus* distillation standard and ascorbic acid

From Table 2, it can be summarized that the essential oil extracted from the lemongrass stalk has antioxidant activity with up to 809 ppm inhibition at the ratio of 1:2 for the volume concentration of essential oil per volume of solvent. As illustrated in the Fig. 3, we can see that lemongrass stalk essential oil inhibit almost the same rate with the standard essential oil bought from Sigma-Aldrich Chemistry which is used as the control.

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CONCLUSIONS

Essential oil from *Cymbopogon citratus* Stapf was isolated from 1272 g of dried stem by using Karlsruhe steam distillation methods with 0.24% as yield. The product of essential oil was also tested against antioxidant activity DPPH and resulted in low activity compared to ascorbic acid and lemongrass oil standard as references.

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