ANTIOXIDANT ACTIVITY AND CITRAL CONTENT OF DIFFERENT TEA PREPARATIONS OF THE ABOVE-GROUND PARTS OF LEMONGRASS (*Cymbopogon citratus* Stapf.)

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ABSTRACT

Lemongrass (*Cymbopogon citratus* Stapf.) is a common herb used in cooking and recognized for its many health benefits. This study measured the antioxidant activity and citral content in extracts made from air-dried and fresh lemongrass tissues prepared by decoction and infusion. The plants were collected from three different areas in Iloilo (Pavia, Jaro and Lapaz) and two baranggays in Buenavista, Guimaras (Brgy. Daragan and Brgy. San Isidro). All plant extracts were found to exhibit antioxidant activity against the diphenylpicrylhydrazyl (DPPH) radical. Antioxidant activity was significantly ($p < 0.05$) higher in fresh plants than in dried plants. Fresh plants when prepared by decoction gave higher antioxidant activity than when prepared by infusion. On the other hand, antioxidant activity remained the same in dried plants, whether prepared by decoction or infusion. Citral was also found to be present in the plant extracts, but is not significantly ($p > 0.05$) affected by the kind of plant tissues used, whether fresh or dried. However, extraction by decoction gave a higher citral content than infusion. Thus, if one wanted to get the most of antioxidants and citral from lemongrass tea, fresh plants prepared by decoction must be used.

INTRODUCTION

Lemongrass (*Cymbopogon citratus* Stapf.), commonly known in the Visayan language as *tanglad*, has long been used in the Philippines for its many medicinal uses, such as for fever and as a diuretic. An infusion of the plant promotes digestion and stomach activity; it is carminative and tonic to the mucosal membranes of the intestine; it is useful for vomiting and diarrhea. An infusion made from the leaves serves as a refrigerant, and is also used as a remedy to high blood pressure, general weakness and debility. The plant, when used with ginger as a decoction, is used for stomachache, flatulence and indigestion (Quisumbing, 1978; Onaylos [1984]; Ticzon, 1996). Lemongrass tea or "infusion" is used in popular medicine in many countries. It is prepared with fresh or dried leaves and covers a wide range of indications (Negrelle & Gomes, 2007). With its versatility, ease of preparation, and ease of cultivation everywhere, lemongrass is a promising herb which can be used to help combat the harmful effects of carcinogens and free radicals, as well as promote health and wellness.

Ethanol extracts of lemongrass were found to possess antimutagenic activity (Vinitketkumnuen *et al.*, 1994) and inhibit colon carcinogenesis in rats to a significant extent (Suaeyun *et al*., 1997). Aqueous extracts of lemongrass were also found to inhibit oxidative stress (Ojo *et al*., 2006). The plant does not show any hypnotic effect and it is not toxic to test organisms (Negrelle & Gomes, 2007).

Lemongrass may contain some active compounds which may deactivate mutagens by directly trapping them or by involving liver enzymes, thus, lemongrass may serve as a source of chemo preventive agents. The mechanism for the protective effects of lemongrass against colon cancer is not clear but is suggested to be due in part to its antioxidant activity (Vinitketkumnuen *et al.*, 1994; Suaeyun *et al*., 1997). It is also suggested that

lemongrass extracts inhibit lipid peroxidation by preventing free radical attacks on bio membranes (Ojo *et al*., 2006).

Lemongrass, upon distillation, yields lemongrass oil which is one of the well-known essential oils in the world for many years. It is characterized by its yellow or amber color, and lemon-like odor. The major component of lemongrass oil is the aldehyde citral (about 70 %) which is responsible for the strong lemon-like odor of the oil. Other identified components of the oil are myrcene, geraniol, ethyl laurate, citronellol, terpineol, menthol, caryophyllene, linalool, citronellal, α-pinene, camphene and methyl heptenone (Torres & Ragadio, 1996).

Citral is chemically known as 3,7-dimethyl-2,6-octadienal. Citral (Fig. 1) derived from natural sources is a mixture of two geometric isomers, geranial (citral A) and neral (citral B). Both geranial and neral are light oily liquids. Geranial has a strong lemon odor while the lemon odor of neral is weaker but sweeter than that of geranial (Windholdz *et al*., 1983).

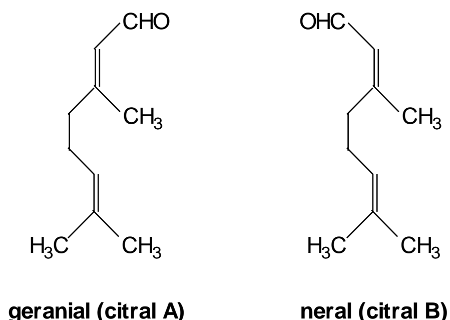

Figure 1. The two isomers of citral

Citral was found to possess anticancer property (Dubey *et al*., 1997) and anti-mutagenic activity (Rabbani *et al*., 2005). It is not mutagenic in itself but may prevent nuclear mutations by exerting anti-oxidant activity (Rabbani *et al*., 2005). Furthermore, it exerts anti-cancer activity by causing apoptosis in human and mouse leukemic cell lines but not in normal mouse cells. Apoptosis is a major form of cell death which involves the activation of caspases from their inactive forms referred to as procaspases. It is associated with many diseases including certain cancers (Dudai *et al*., 2005).

Lemongrass plants are often used by Filipinos in cooking, and the tea can be conveniently prepared by boiling the leaves (decoction) or by simply soaking them in hot water (infusion).

Objectives

This study determined how much of antioxidant activity and citral are present in the tea when lemongrass is prepared according to folk practices of decoction and infusion. Specifically, this study

1. measured antioxidant activity (in terms of percent diphenylpicrylhydrazyl or DPPH inhibition) and citral content (percent weight) on decoctions and infusions of fresh and air-dried above-ground parts of lemongrass;
2. compared antioxidant activity and citral content of lemongrass tea using fresh plants vs air-dried plants; and,
3. compared antioxidant activity and citral content of lemongrass tea using decoction vs infusion.

Scope and Limitation

This study measured citral content and antioxidant activity in lemongrass tea prepared in different ways. This involved the use of fresh and air-dried lemongrass plants at room temperature for seven days. Extraction procedures

made use of decoction by boiling the plant materials in water for three minutes, while infusion was done by steeping the plant materials in hot water for 30 minutes.

This study did not include using plants at different stages of growth. It did not determine the effect of extraction using other temperatures than what was indicated on citral content and antioxidant activity. It did not determine the effect of using other duration of extraction using decoction or infusion than what was indicated on citral content and antioxidant activity.

This study was conducted in April 2009 until March 2010 at Roblee Science Hall, Central Philippine University.

MATERIALS AND METHODS

Plant Collection, Preparation and Experimental Treatments

Mature, healthy and disease-free above-ground parts of lemongrass (*Cymbopogon citratus*) plants were collected from three different areas in Iloilo (Pavia municipality, and Districts of Jaro and Lapaz) and two baranggays in Buenavista, Guimaras (Brgy. Daragan and Brgy. San Isidro). Ten plants from each area were randomly chosen, and two stalks from each plant were taken as samples. A total of 20 stalks per area were collected. After collection, the 20 stalks from every field were randomly assigned to the four treatments with five stalks per treatment. Every treatment had a total of 25 stalks. Whole-plant specimen samples of *C. citratus* were submitted to the Department of Life Sciences, College of Arts and Sciences, Central Philippine University for identification and preservation.

Two treatments were employed in the study – treatment 1 (fresh vs air-dried) and treatment 2 (decoction and infusion). Thus the factorial experiment

was used, with four treatment combinations: (1) fresh decoction, (2) fresh infusion, (3) air-dried decoction, and (4) air-dried infusion. The completely randomized design (CRD) was used, with three replicates for each treatment combination.

The above-ground parts were used, that is, all plant parts except the roots. The wilted leaves were removed and the plants were then washed with water. The fresh plants were cut into small pieces, about one-half cm or smaller, then subjected to assays immediately. However, whole plants were stored in the refrigerator when not needed for assays. Fresh plants were used not more than three weeks after being collected and stored in the refrigerator.

Air-drying was carried out for seven days under the shade at room temperature, according to Torres and Ragadio (1996). The dried plant materials were stored in airtight containers and were used within three weeks after these were stored. The plant tissues were only cut on the day of extraction and antioxidant assay or citral content analysis. Minimal processing was applied to the plat samples to prevent the loss of citral (Barbosa *et al*., 2008).

Moisture content was determined by drying the plant materials at a constant temperature of 105 ± 5 ºC for nine hours according to the ASAE standards as quoted by Barbosa *et al*. (2008). Three replicates were employed for each determination.

Chemicals

All chemicals used were of analytical grade. Citral (95% pure) and barbituric acid were obtained from Sigma-Aldrich, while DPPH was obtained from Sigma. Absolute ethanol (Sharlau) was obtained from a local distributor.

Extraction of Antioxidants and Citral

The extracts were prepared in the usual way of preparing hot tea beverage. Two grams of plant material were used for antioxidant activity assay, while 10 g were used for assay of citral content. The decoction was prepared by placing the plant material in 100 mL boiling distilled water for three minutes, while the infusion was prepared by steeping the plant material in freshly boiled distilled water for 30 minutes. The extracts were cooled, filtered and the volume was brought up to 100 mL using distilled water.

Antioxidant Activity Assay

The antioxidant activity of the extracts was measured using the diphenylpicrylhydrazyl (DPPH) assay according to Zaeoung *et al*. (2005). Two milliliters of 100 µM DPPH solution in absolute ethanol was added to two mL of extract and mixed. The samples were allowed to react with DPPH for 20 minutes and the absorbance was measured at 520 nm (Lab Spectronic) after reaction was complete. Ascorbic acid (1% in distilled water) was used as positive control while distilled water was used as blank for all the samples. Preliminary analysis involved optimization of absorbance with the instrument, and the effect of dilution on antioxidant activity was monitored. The extracts were diluted four-fold and ten-fold, and the antioxidant activity was measured based on the diluted extracts.

Antioxidant activity was expressed as percent inhibition of the DPPH radical, and observed by the decolorization of the DPPH reagent from dark violet to a lighter tone or colorless solution. It was computed as follows:

$$\% \quad inhibition = \frac{A_{distilled\,water} - A_{extract}}{A_{distilled\,water}} \; x \quad 100$$

where $A_{distilled\ water}$ = absorbance of the blank
$A_{extract}$ = absorbance of the sample

This is also referred to as “quenching” of the DPPH radical (Molyneux, 2004). To obtain the correct comparison between fresh and dried plants, antioxidant activity was expressed as % inhibition per gram dry weight of plant tissue.

Assay of Citral Content

The presence of citral in the extracts was quantified using the barbituric acid condensation method according to Levi and Laughton (1959) and Laughton *et al*. (1962) with modifications. The barbituric acid reagent (0.3% by weight in 80% ethanol) was prepared as follows: 0.3 g of barbituric acid (BA) was weighed in a 100-mL volumetric flask. Then 20 mL distilled water was added and the reagent was dissolved by warming the flask in a hotplate. When all the solid had been dissolved, absolute ethanol was added to the mark and the reagent was equilibriated at 25˚C using a water bath. The volume was brought to the mark with absolute ethanol after equilibriation, and the reagent was mixed thoroughly.

The standard curve was prepared as follows: 25, 50, 75 and 100 mg of citral was weighed accurately using an analytical balance (Mettler Toledo) into four 50-mL volumetric flasks. These were then diluted to the mark using the BA reagent.

For the samples, about 20 g of the extracts were weighed in 50-mL volumetric flasks and diluted with the BA reagent. The samples, along with the standards, were incubated in a 25˚C water bath for 40 minutes to allow reaction of citral in the samples with barbituric acid. After the reaction time, 1-mL aliquots were withdrawn from each volumetric flask and diluted to 250 mL using 20% ethanol. The absorbance of the standards and samples were measured at 336 nm (Shimadzu UV-Vis), with 1 mL of the BA reagent diluted to 250 mL of 20% ethanol as blank. The standards were prepared in duplicate,

while the samples, in triplicate.

Citral content of the samples was computed based on the standard curve and expressed as g of citral per 100 g dry matter of plant sample used (percent by weight).

Statistical Analysis

Experimental data were analyzed using two-way analysis of variance (ANOVA) at 5% level of significance using SPSS 10 Package to see differences in means of citral content and antioxidant activity obtained from lemongrass plants using different methods of drying and extraction techniques.

RESULTS AND DISCUSSION

Antioxidant Activity

Preliminary analysis. It was observed that extracts of fresh and dried lemongrass plant samples possess antioxidant activity (data not shown) against ascorbic acid, which is a widely-used standard. Citral (95%, Sigma-Aldrich) also has antioxidant activity which is almost the same as that of ascorbic acid.

Effect of dilution. It is of interest to determine the effect of dilution on antioxidant activity of the extracts since both fresh and dried samples have antioxidant activities. Figure 2 shows the antioxidant activity values of 2 g dried plant samples extracted by decoction. The decoctions were diluted to 1:4 and 1:10 from the original concentration. The undiluted sample for decoction showed 73.8% activity which is slightly lower than those of the samples diluted at 1:4 and 1:10 which are 79% and 79.4%, respectively. The antioxidant activity of the infusions were almost the same, regardless of the extent of

dilution. Thus, subsequent assays for the antioxidant activity of the lemongrass extracts utilized 2 g of plant material extracted with 100 mL water. These extracts were further diluted ten-fold (1:10).

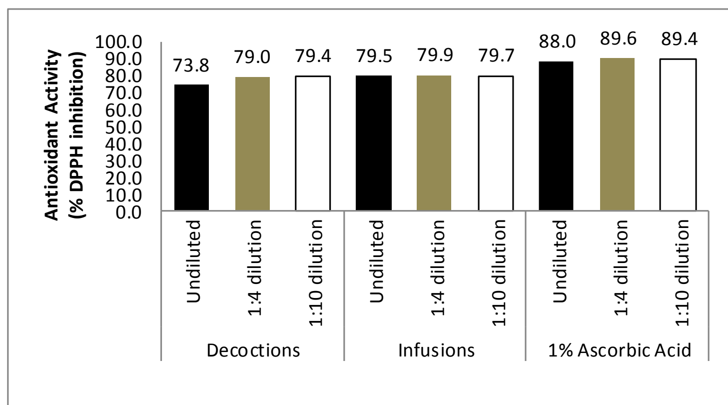

Figure 2. Antioxidant activity of air-dried lemongrass extracts at different dilutions

Antioxidant activity of dilute lemongrass extracts. As shown in Table 1 and Figure 3, the antioxidant activity of lemongrass extracts is higher in fresh plants ($\bar{x}$ = 63.3%) than in dried plants ($\bar{x}$= 44.3%). Fresh plants extracted by decoction had higher antioxidant activity (68.8%) than those extracted by infusion (57.7%). On the other hand, antioxidant activity remained the same in air-dried plants, whether extracted by decoction or infusion.

These results show that the preparation of the plant materials did not significantly ($p > 0.05$) affect antioxidant activity, i.e., decoctions ($\bar{x}$ = 56.4%) had the same antioxidant activity as infusion ($\bar{x}$ = 51.1%). However, the antioxidant activity of decoctions is significantly ($p < 0.05$) higher in fresh plants than in dried plants. Similarly, the antioxidant activity of infusions is significantly ($p < 0.05$) higher in fresh plants than in dried plants. Thus, to get the optimal

benefit of antioxidants from lemongrass tea, fresh plants should be used and prepared by decoction. However, infusions of fresh plants are better than any of the tea preparations using dried plants.

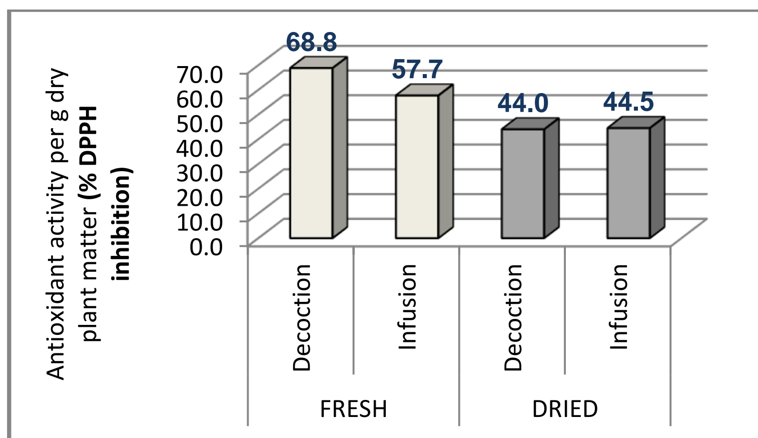

Figure 3. Antioxidant activity of fresh and dried lemongrass extracts prepared by decoction and infusion, and further diluted ten-fold

Table 1. Antioxidant Activity using Fresh and Dried Lemongrass Tissues Prepared by Decoction and Infusion

Type of tissue	Tea preparation		**Mean**
	Decoction	Infusion	
Dried	44.0^{c}	44.5^{c}	**44.3**
Fresh	68.8^{a}	57.7^{b}	**63.3**
Mean	**56.4**	**51.1**	

cv = 7.46%

^{abc} Means followed by the same letter superscript are not significantly different at the 5% level of probability.

Comparison of these results with those reported by Cheel *et al.* (2005) on

dried lemongrass extracts suggests that antioxidant activity is not affected by the preparation of the plant material and the length of decoction or infusion. Their results on the antioxidant activity of the decoction (boiled for 2.5 h) and infusion (soaked for 15 min) of aerial powdered, air-dried lemongrass plants were 41.9% and 40.2% DPPH decolorization, respectively. In this study, decoctions and infusions of air-dried samples gave 44.0% and 44.5% DPPH decolorization, respectively, which are comparable with what Cheel *et al.* had reported.

Most antioxidant activity in leaves and fruits are due to the total phenolic content in these plants (Grzeszczuk *et. al*.; Roidaki *et.al*., 2015). These phenolics are water-soluble and are conveniently extracted using hot water, which supports the findings that antioxidant activity is the same in infusions and decoctions. Degradation of phenolics due to exposure to heat and light takes place when the plant materials are dried, leading to loss in antioxidant activity. Thus decoctions of fresh plants materials provide better extraction of phenolics, which was reflected in the high antioxidant activity in the extracts of fresh lemongrass through decoction.

Citral Content

Figure 4 shows citral content in the extracts prepared from 10 g plant material. The kind of plant tissues used, whether fresh or dried, did not affect the citral content found in the extracts – dried plant samples, whether decocted or infused ($\bar{x}$ = 0.9265), had the same citral content with the fresh samples ($\bar{x}$ = 1.0273). However, the method of extraction affected citral content in the extracts. Extraction by decoction ($\bar{x}$ = 1.1174) gave a higher citral content than infusion ($\bar{x}$ = 0.8364). Table 2 indicates that both fresh and dried plants gave higher citral contents when prepared by decoction as compared to infusion. Thus, if one wanted to get the highest citral content from lemongrass plants, the tea must be prepared by decoction regardless of whether the plant used is fresh or dried.

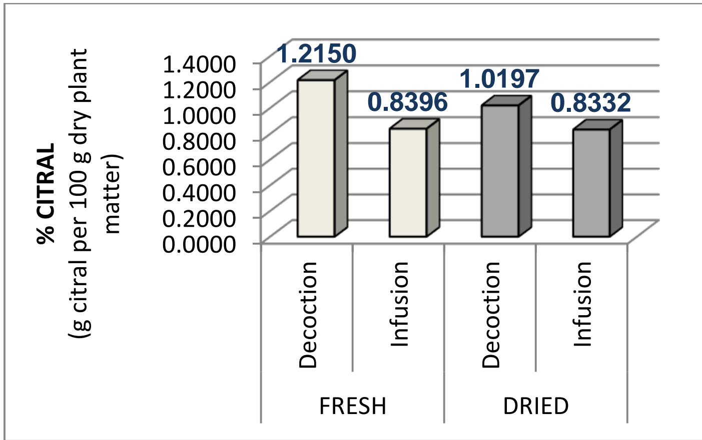

Figure 4. Citral content of fresh and dried lemongrass extracts prepared by decoction and infusion.

Table 2. Citral content of fresh and air-dried lemongrass tissues prepared as decoction and infusion

Type of Tissue	Tea Preparation		Mean
	Decoction	Infusion	
Dried	1.0197	0.8332	**0.9265**[ns]
Fresh	1.2150	0.8396	**1.0273**
Mean	**1.1174**[a]	**0.8364**[b]	

cv = 7.79%

[abc] Means followed by the same letter superscript are not significantly different at the 5% level of probability.

[ns] Not significantly different at the 5% level of probability

Lemongrass oil, comprised mostly of citral, is extracted using steam distillation. This involves high temperature to break the oil glands in order to

release the oil (Schaneberg & Khan, 2002). Decoction involved placing plant materials in vigorously boiling water, which allows more extraction of essential oil through contact of the plant materials with the steam produced at the surface of the boiling water. This gave a greater yield of citral compared to simply soaking the plant materials in hot water. The essential oil of mint leaves are traditionally extracted by steam distillation and hydro distillation.

Hydro distillation extracts the oil faster and produces a greater yield since the leaves are in contact with boiling water, allows the leaves to move more freely as well as provides a greater surface area for heat to extract the oil from oil glands (Gavahian *et. al*., 2015).

It has been reported that citral concentrations of 44.5 μM induced apoptosis in several hematopoietic cancer cell lines. The concentration of 44.5 μM is comparable to the amount of citral found in 1 cup (approximately 100 mL) of tea prepared from 1 g of lemongrass. Furthermore, a lesser concentration of 22.25 μM of citral was as effective as the standard staurosporine in activating caspase-3 enzymatic activity, which resulted in DNA fragmentation, and eventually cell death (Dudai *et al.,* 2005).

In this study, the amount of citral obtained by boiling 10 g of fresh plant samples in 100 mL of water was 1.2150 % by weight (or 1.2150 g citral per 100 g dry weight of plant material), while dried samples gave 1.0197 % by weight. Furthermore, the amount of citral present in 1 g of plant material was computed to be 0.012 g for fresh plants and 0.010 g for dried plants. Decoction using 1 g plant material in 100 mL water will give about 798 μM of citral using fresh plants, while it will be about 670 μM using dried plants. Figure 8 shows the comparison of citral content obtained by different preparations, and the extraction procedure used in this research gave a greater amount than what is needed to induce apoptosis for cancel cells according to Dudai *et al*.

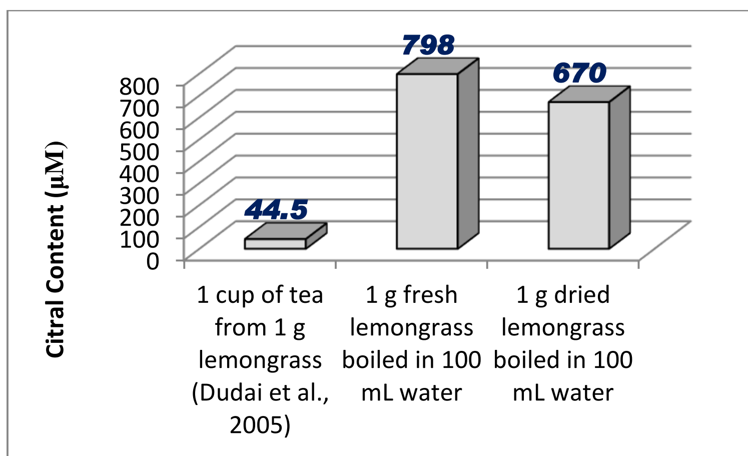

Figure 5. Citral content of lemongrass from different decoctions

Since citral content is shown to be high in the prepared extracts, a concern that may arise is whether or not citral may be toxic to an organism when taken in high amounts. Rabbani *et al*. (2005) tested the mutagenic potential of citral based on suggested reports that drugs administered above the therapeutic concentration might cause damage to the nucleus and result in mutagenicity in the organism. They found out from their results that citral has no mutagenic potential when tested in mice at a high dose of 50 mg citral per kg body weight. That is, citral in itself poses no harm, even when ingested in high doses.

CONCLUSIONS AND RECOMMENDATIONS

Antioxidant activity was present in decoctions and infusions of fresh and dried lemongrass plants, with decoctions of fresh plants having the highest value. On the other hand, citral was present in all extracts and the highest content was found in decoctions of the plants. The best way to prepare lemongrass tea is by boiling fresh plants.

It is recommended that a more detailed study should be conducted on the citral content of the water extracts of lemongrass using different parts of the plants, such as leaf sheath and leaf blade. The effect of soil and environmental conditions on antioxidant activity and citral content should be further studied.

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