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 lemongrass extracts made from air-dried and fresh plants by decoction and infusion. The plants were collected from one area each from three different municipalities in Iloilo (Pavia, Jaro and Lapaz) and two baranggays in Buenavista, Guimaras (Brgy. Daragan and Brgy. San Isidro). All plant extracts have antioxidant activity against the DPPH radical. Antioxidant activity was found to be significantly higher in decoctions of 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. The kind of plant tissues used, whether fresh or dried, does not affect the citral content found in the extracts – dried plant samples, whether decocted or infused, have the same citral content than the fresh samples. However, the method of extraction affected citral content in the extracts. Extraction by decoction gave a higher citral content than infusion. Thus, if one wants to get the most of antioxidants and citral from lemongrass tea, fresh plants must be used and prepared by decoction.

INTRODUCTION

Background and Rationale

In the past years, greater attention has been given to health and wellness because of the increasing number of diseases that man face. With the advancement of science and technology comes much unwanted pollutants and waste products which contribute to sickness, such as cancer, Alzheimer's disease, cardiovascular diseases, cataracts, and inflammatory diseases. The underlying mechanism for these diseases is the formation of excessive free radicals. Production of free radicals in amounts greater than the body can neutralize leads to a phenomenon called oxidative stress. This can lead to detrimental effects including damage to cell membranes, lipoproteins, and other lipid molecules; alteration to protein structure leading to loss of activity; or oxidative damage to DNA leading to mutations. When oxidative stress is not managed, it can lead to a variety of diseases. Excessive amounts of free radicals lead to many diseases such as cancer, Alzheimer's disease, cardiovascular diseases, cataracts, inflammatory disease, as well as aging (Figure 1) (Ames, 1983; Phay-Huy *et al*., 2008).

Free radicals (Table 1) are highly reactive molecular fragments which cause damage to cell membranes and lipid molecules, alteration of protein structure leading to loss of activity, or oxidative damage to DNA leading to mutations. They are also referred to as the reactive oxygen species (ROS) and reactive nitrogen species (RNS).

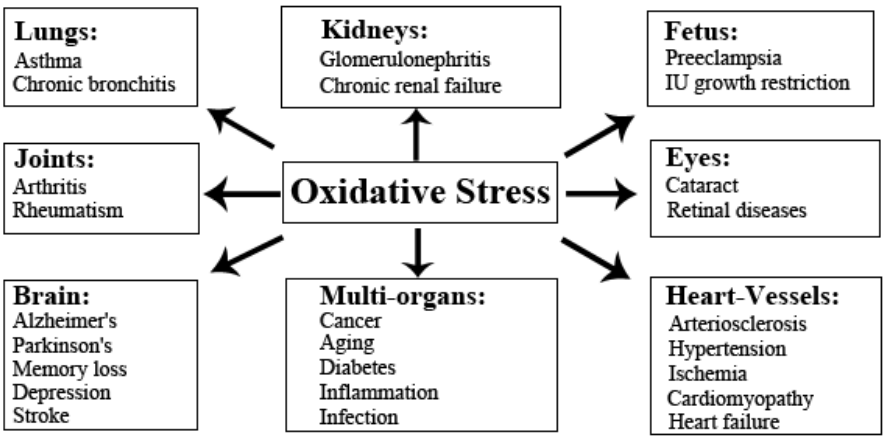

Figure 1. Oxidative Stress-induced Diseases in Humans
(Int J Biomed Sci 2008; 4 (2):89-96; p92)

They can be produced from normal metabolic processes taking place within the body during energy generation or in response to stress and infection. They can also be acquired from exposure to environmental pollutants or cigarette smoke (Phay-Huy *et al*., 2008).

Table 1. Reactive Oxygen Species and Reactive Nitrogen Species

Reactive Oxygen Species		Reactive Nitrogen Species	
Free radicals		**Free radicals**	
Hydroxyl	OH·	Nitric oxide	NO·
Superoxide	$O_2^{\cdot -}$	Nitrogen dioxide	NO_2·
Peroxyl	ROO·		
Lipid peroxyl	LOO·		
Nonradicals		**Nonradicals**	

Hydrogen peroxide	H_2O_2	Nitrous acid	HNO_2
Singlet oxygen	$^1O_2$	Peroxynitrite	$ONOO^-$
Hypochlorous acid	HOCl	Dinitrogen trioxide	N_2O_3
Lipid peroxide	LOOH		

Since free radicals are normally produced in body processes for energy generation or to produce other essential molecules, the body is also equipped with defenses to protect itself from the harmful effects of these free radicals. Such defenses are referred to as antioxidants (Gurr, 1993).

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 (Gurr, 1993; Shahidi, 2000).

The lack of antioxidants in the diet, not only because of exposure to mutagens or carcinogens, may lead to diseases due to oxidative damage. Intake of natural antioxidants in the diet is considered an important aspect of strengthening our body against free radicals and oxidative damage (Ames, 1979; Ames, 1983). Thus the inclusion of certain fruits and vegetables which are rich in antioxidants is essential for a healthy diet.

Plants are very good sources of natural antioxidants. These antioxidants are mostly produced via the secondary metabolism of plants and are referred to as secondary products. They include the following: plant phenolics particularly phenylpropanoids, coumarins, flavonoids; the polyphenolic compounds tannins and proanthocyanidins; nitrogen-containing compounds including the alkaloids, nonprotein amino acids, isothiocyanate and indoles; phytosterols; carotenoids; and the chlorophyll derivatives (Mimika-Dukic, retrieved May 30, 2008). Furthermore, herbs that contain phytosterols, triterpenes, flavonoids, saponins and carotenoids have been found to be cancer preventive by acting as antioxidants in one way. Such herbs include garlic, onions, turmeric, basil, celery seed, rosemary, coriander, ginseng, and lemongrass (Craig, 1999).

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).

Many studies were conducted on lemongrass to show that it exhibits various biological activities including anticancer activity and antioxidant activity. The plant does not show any hypnotic effect and it is not toxic to test organisms (Negrelle & Gomes, 2007). Ethanol extracts of lemongrass were found to possess antimutagenic activity against mutation in *Salmonella typhimurium* strains TA98 and TA100 induced by chemicals such as aflatoxin (Vinitketkumnuen *et al.*, 1994) and inhibit colon carcinogenesis in the rat to a significant extent (Suaeyun *et al*., 1997). Aqueous extracts of lemongrass were also found to inhibit oxidative stress particularly lipid peroxidation, as well as alteration of lipid membrane systems, caused by paracetamol (Ojo *et al*., 2006).

These results suggest that lemongrass contain some active compounds which may deactivate mutagens by directly trapping them or by involving liver enzymes. Thus lemongrass may serve as a source for chemopreventive agents (Vinitketkumnuen *et al.*, 1994; Suaeyun *et al*., 1997). 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 (Suaeyun *et al*., 1997). It is also suggested that lemongrass extracts inhibit lipid peroxidation by preventing free radical attacks on biomembranes (Ojo *et al*., 2006).

Lemongrass oil is one of the well-known essential oils in the world for many years. It is obtained by distillation of lemongrass from two of its species, *Cymbopogon flexuosus* (Steud) Wats. and *C. citratus* (DC) Stapf. It is characterized by its yellow or amber color, and lemon-like odor. It also has a herbaceous verbena-like odor not possessed by lemon oil. Identified components of the oil are myrcene, geraniol, ethyl laurate, citronellol, terpineol, menthol, caryophyllene, linalool, citronellal, α-pinene, camphene and methyl heptenone. The major component of lemongrass oil is the aldehyde citral (about 70 %) which is responsible for the strong lemon-like odor of the oil (Torres & Ragadio, 1996).

Citral is chemically known as 3,7-dimethyl-2,6-octadienal. Citral (Figure 2) 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 geranial. Both are insoluble in water but miscible in alcohol, ether, benzyl benzoate, diethyl phthalate, glycerol, propylene glycol, mineral oil and essential oils (Windholdz *et al*., 1983). However, liquid-liquid equilibria studies on water, ethanol and citral shows that citral is soluble in water to a small extent at 303.15 K (about 30˚C) with neral having greater affinity for the aqueous phase than geranial. Furthermore, when citral (neral + geranial) is extracted using water, ethanol must be added since the results show that ethanol enhances the solubility of citral in water (Gramajo de Doz *et al*., 2007).

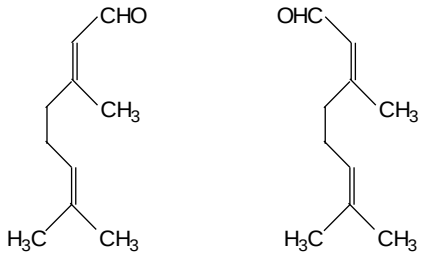

Figure 2. The Two Isomers of Citral

Dubey *et al*. (1997) found citral to possess anticancer property. Citral was found to be cytotoxic to P_{388} mouse leukaemia cells at an IC_{50} value of 7.1 µg/mL. In another study, citral was found to possess anti-mutagenic activity against three known mutagens cyclophosphamide, motimycin-C and nickel metal ($NiCl_2$) using the micronucleus anti-mutagenic assay. The mutagenic potential of citral was also tested at a high dose but there was no significant increase in the frequencies of micronucleus in erythrocytes, thus citral is not mutagenic in itself. Furthermore, this study suggests that citral attenuates nuclear damage induced by the clastogens by exerting anti-oxidant activity (Rabbani *et al*., 2005).

The first report to examine the mechanism of action of citral as an anti-cancer agent was made by Dudai *et al*. (2005). Apoptosis is associated with many diseases including certain cancers. It is a major form of cell death which involves the activation of caspases from their inactive forms referred to as procaspases. Treatment of human and mouse

leukemic cell lines with citral for 4 to 24 hr at a concentration of 45 μg/mL led to the activation of caspase-3 enzyme activity, but not in normal mouse cells. This was further supported by the processing of the procaspase-3 protein, and the appearance of the DNA ladder when human leukemic cells were treated with citral.

Considering the beneficial effects established in lemongrass extracts and citral, this study aimed to determine how much of citral and the antioxidant activity of lemon grass extract are present when lemon grass is prepared based on folk practices. It is often used by Filipinos in cooking, and tea can be conveniently prepared by boiling the leaves or by simply soaking them in hot water.

If significant amounts are found to be present, this might lead to the development of aqueous extract- and citral-based products considering that lemongrass is abundant everywhere in the country, and it can be easily grown in the backyard.

With its versatility and ease of preparation, tanglad 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.

Objectives

This study aimed to compare the levels of citral and antioxidant activity on several ways employed in the preparation of tanglad tea.

Specifically, this study aimed to:

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

Scope and Limitation

This study was concerned with the measurement of two parameters, citral content and antioxidant activity, on the different ways of preparing tea. This involved the use of fresh lemongrass plants and air-dried plants at room temperature for 7 days. Extraction procedures made use of decoction by boiling the plant material in water for 3 min, while infusion was done by steeping the plants in hot water for 30 min.

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 lengths time of extraction using decoction or infusion than what was indicated on citral content and antioxidant activity.

MATERIALS AND METHODS

Experimental Treatments and Design

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

Type of Lemongrass Tissue			
Tea Preparation		Air-dried	Fresh
	Decoction	air/decoction	Fresh/decoction
	Infusion	Air/infusion	Fresh/infusion

Figure 3. Experimental Treatment

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*) plants were collected from one area each from three different municipalities in Iloilo (Pavia, Jaro and Lapaz) and two baranggays in Buenavista, Guimaras (Brgy. Daragan and Brgy. San Isidro). 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. After collection, 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.

The above-ground parts were used. 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.

Air-drying was carried out for 7 days under the shade at room temperature. The dried plants were stored in airtight bottle containers. The dried plants were used within three weeks after these were stored. The dried plants were cut on the same day of extraction and antioxidant assay or citral content analysis. Minimal processing were applied to the plants so as to prevent the loss of the essential oil and citral (Barbosa *et al*., 2008).

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. Distilled water (Wilkins) was obtained from a local supplier.

Extraction

The extracts were prepared in the usual way of preparing hot tea beverage. Two grams of plant material was used for antioxidant activity assay, while 10 g was used for assay of citral content. The decoction was prepared by placing the plant material in 100 ml boiling distilled water for 3 minutes. 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.

The effect of dilution on antioxidant activity was also monitored, thus the extracts were diluted four-fold and ten-fold, and the antioxidant activity was measured 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 ligher tone or colorless solution. It was computed as follows:

$$\% \quad inhibition = \frac{A_{distilledwater} - A_{extract}}{A_{distilledwater}} \; x \quad 100$$

where $A_{\text{distilled water}}$ is the absorbance of the blank and A_{extract} is the 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% in 80% ethanol) was prepared as follows: 0.3 g of barbituric acid (BA) was weighed in a 100-mL volumetric flask. Twenty 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 (Shimidzu UV-Vis spectrophotometer), 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 or percentage by weight.

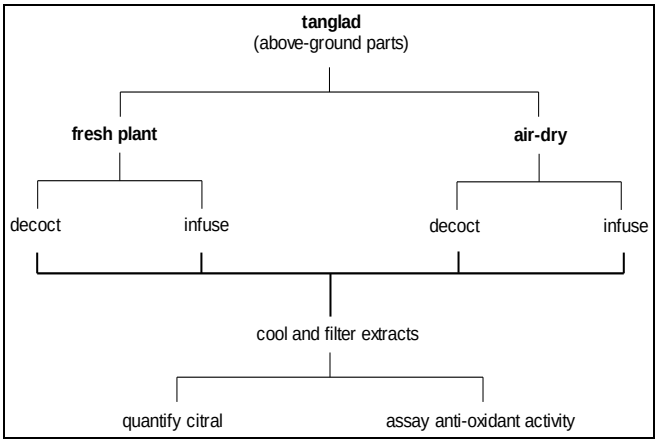

Figure 4. Overview of Experiments

Statistical Analysis

Experimental data were analyzed using two-way analysis of variance (ANOVA) at a confidence level of 95% to see differences in means of citral content and antioxidant activity obtained from lemongrass plants using different methods of drying and extraction techniques. Differences among treatment means were determined.

RESULTS AND DISCUSSION

Antioxidant Activity

Preliminary analysis. It has been observed during preliminary experiments of antioxidant activity (data not shown) that extracts of fresh and dried lemongrass plant samples possess antioxidant activity against ascorbic acid, which is a widely-used standard. Pure citral (obtained from Sigma) 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 5 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 for the infusions were almost the same, regardless of the extent of dilution. Thus, the final assay for the antioxidant activity of the lemongrass extracts were set at using 2 g of plant material at 100 mL water and these extracts were further diluted ten-fold (1:10 dilution, or 10 mL diluted to 100 mL).

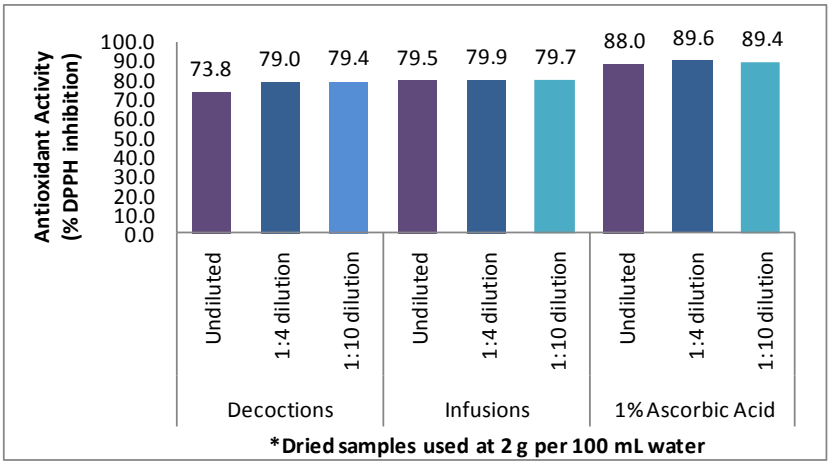

Figure 5. Effect of Dilution on Antioxidant Activity

Antioxidant Activity of Lemongrass Extracts. As shown in Figure 6, the antioxidant activity of dilute lemongrass extracts is significantly higher in fresh plants (mean 63.3%) than in dried plants (mean 44.3%). Table 2 shows that fresh plants when prepared by decoction gave higher antioxidant activity (68.8%) than those prepared by infusion (57.7%). On the other hand, antioxidant activity remained the same in dried plants, whether prepared by decoction or infusion.

The preparation of the plant materials did not significantly affect antioxidant activity, that is, preparation by decoction (mean 56.4%) gave the same antioxidant activity as with infusion (mean 51.1%). However, it can be seen in Table 2 that the antioxidant activity of decoctions is significantly higher in fresh plants than in dried plants. Similarly, the antioxidant activity of infusions is significantly 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.

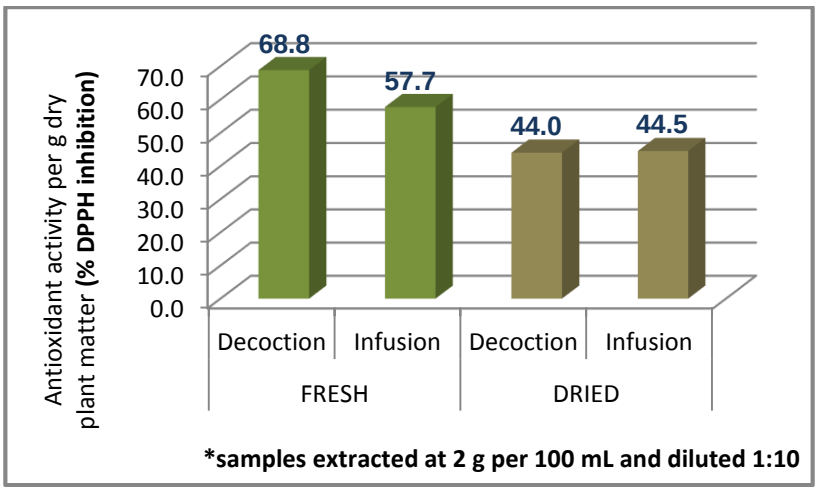

Figure 6. Antioxidant activity of fresh and dried lemongrass extracts prepared by decoction and infusion

Table 2. Cross-tabulation of Antioxidant Activity using Fresh vs Dried Plant Tissues and Decoction vs Infusion Tea Preparation

Type of lemongrass 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 lemongrass extracts highlights some considerations in preparing the plant extracts. Cheel *et al.* (2005) prepared the decoction by boiling 10 g of air-dried, powdered plant material (aerial parts) in 250 mL of water for 2.5 h. The infusion was prepared by soaking 10 g of air-dried, powdered plant material in 250 mL boiling water for 15 min. Their results on the antioxidant activity of the decoctions and infusions were 41.9% and 40.2% decolorization of the DPPH radical, respectively. In this study, decoctions and infusions of air-dried samples gave 75.6% and 76.6% DPPH decolorization, respectively, which are almost double than what Cheel *et al.* has reported in their paper. This difference suggests that when the plants are extracted by decoction, the boiling must not be very long and the plant samples must not be ground very finely since this leads to loss of components of the plant material in the extracts. Furthermore, the industrial and drying processes also affect the essential oil content of the plant samples (Barbosa *et al*., 2008).

Citral Content

Figure 7 shows citral content in the extracts prepared from 10 g plant material. The kind of plant tissues used, whether fresh or dried, does not affect the citral content found in the extracts – dried plant samples, whether decocted or infused (mean of 0.9265), have the same citral content than the fresh samples (mean of 1.0273). However, the method of extraction affected citral content in the extracts. Extraction by decoction (mean of 1.1174) gave a higher citral content than infusion (mean of 0.8364). It is shown in Table 3 that both fresh and dried plants gave higher citral contents when prepared by decoction as compared to infusion.

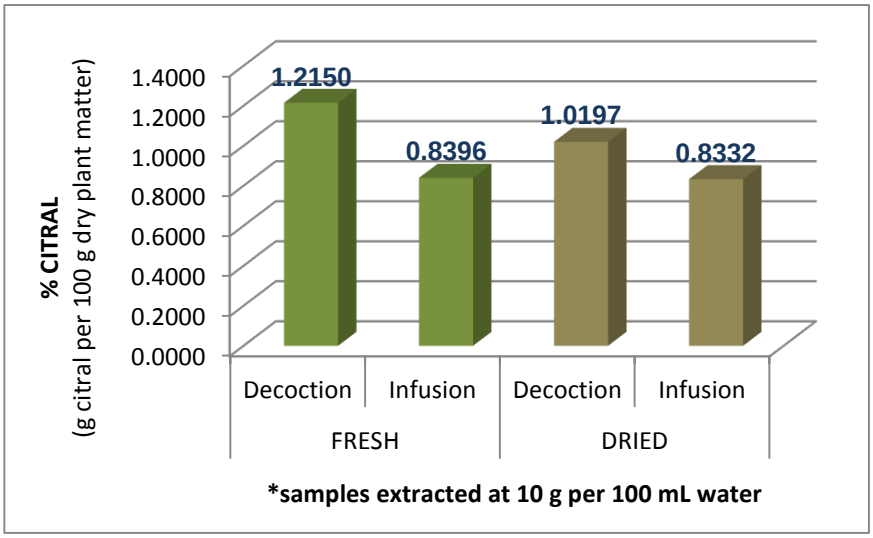

Figure 7. Citral content of fresh and dried lemongrass extracts prepared by decoction and infusion

Table 3. Cross-tabulation of Citral Content using Fresh vs Dried Plant Tissues and Decoction vs Infusion Tea Preparations

Type of Lemongrass 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 at the 5% level of probability

Thus, if one wants 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. However, it was observed that the tea prepared from fresh plants tastes better and more delicious than that prepared from dried plants.

It has been reported that citral concentrations of 44.5 μM induced apoptosis in several hematopoietic cancer cell lines (Dudai *et al.*, 2005). In their article, Dudai *et al.* (2005) explicitly stated that 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 to DNA fragmentation, and eventually cell death.

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 which present in 1 g of plant material is computed as 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 670 µM using dried plants. Appendix D shows the computations for converting % wt to µM. Figure 8 shows the comparison of citral content obtained by different preparations. Comparing the amount of citral which can be obtained by boiling 1 g of plant material in 100 mL of water, it can be seen that the extraction procedure used in this research gave a greater amount than what is needed to induce apoptosis for cancel cells.

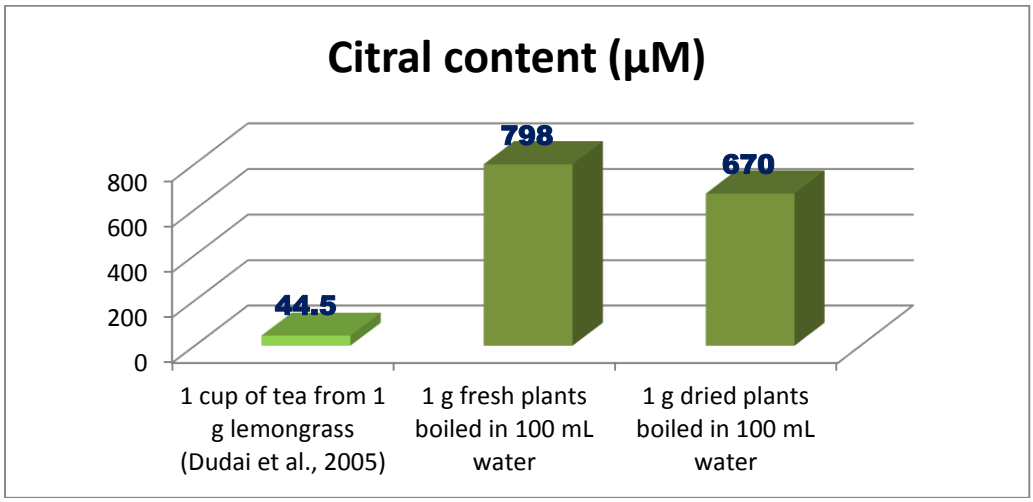

Figure 8. Comparison of Citral Content Obtained by Different Preparations

Since citral content is shown to be high in the prepared extracts, a concern which 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.

Function of Citral in Lemongrass

Citral is a volatile compound, and many plant volatiles exhibit anti-microbial and anti-herbivore activity, which serve as indirect plant defenses. Other plant volatiles have the ability to combine with reactive oxygen species, and serves to protect the plant against internal oxidative damage, or externally induced oxidative stress. Other volatiles act as direct defenses by repelling or intoxicating pathogens or herbivores (Dudareva *et al*., 2004).

This study shows citral to possess antioxidant activity, and it may serve as one of the antioxidant defenses of the plant against harmful free-radicals or reactive oxygen species.

In other studies, citral was demonstrated to serve as a plant defense against the damaging effects of microorganisms. Citral was found to exert antifungal activity against *Aspergillus flavus* spores by damaging the cell wall and membrane of the spore. After entering the cell, citral causes further damage by interfering with DNA and mitochondrial processes, and also the aggregation of protein-like molecules. This leads to metabolic disorder and eventually loss of the capacity of the spores to germinate (Luo *et al.*, 2004). Citral showed potent antifungal activity against three fungi which cause severe postharvest diseases in fruits – *Colletotrichum musae*, *Colletotricum gloeosporioides*, and *Fusarium subglutinans* f. sp. *ananas* – by altering the hyphae morphology of the three fungi (Garcia *et*

al., 2008). Citral also showed antifungal activity against a postharvest pathogen, *Penicillium digitatum* in lemon fruit (Ben-Yehoshua *et al*., 1995).

Green and Berenbaum (1994) suggest that since citral is a volatile compound, it may function in nature to protect the plant from insect predators by repelling the insects rather than killing them. They found citral to be toxic to the insect *Trichoplusia ni* (cabbage loopers), and its toxicity is enhanced when exposed to ultraviolet light, thus making it phototoxic. The phototoxicity of citral, however, is reduced by Vitamin A (an antioxidant) in the insect by 50%. This suggests that citral can be converted as a pro-oxidant or harmful free-radical (or reactive oxygen species) in some organisms under certain conditions.

Thus citral may act as internal plant defense from oxidative stress by acting as an antioxidant, but it may also act as an external plant defense from predators by acting as a pro-oxidant under specific conditions.

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study measured the antioxidant activity and citral content in lemongrass (*Cymbopogon citratus*) extracts made from air-dried and fresh plants by decoction and infusion. The plants were collected from one area each from three different municipalities in Iloilo (Pavia, Jaro and Lapaz) and two baranggays in Buenavista, Guimaras (Brgy. Daragan and Brgy. San Isidro).

Summary and Conclusions

Lemongrass, a common plant, has long been used in the Philippines as a flavoring ingredient in cooking, and in tea preparations, because of its wide availability and many reported health benefits. The tea extracts of fresh and dried plants prepared by decoction and infusion showed high antioxidant activity against the DPPH radical. Antioxidant activity was found to be significantly higher in decoctions of 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. The kind of plant tissues used, whether fresh or dried, does not affect the citral content found in the extracts – dried plant samples, whether decocted or infused, have the same citral content than the fresh samples. However, the method of extraction affected citral content in the extracts. Extraction by decoction gave a higher citral content than infusion.

Thus, if one wants to get the most of antioxidants and citral from lemongrass tea, fresh plants must be used and prepared by decoction.

From the results obtained, the following conclusions are drawn from the study:

1. Antioxidant activity and citral were found to be present in the water extracts of lemongrass;
2. Antioxidant activity of dilute extracts were significantly higher in fresh plants than in dried plants;
3. Citral content is significantly higher in decoctions than infusions, regardless of whether fresh or dried plants are used;
4. The preparation of lemongrass tea which gives the highest antioxidant activity and citral content is that which is prepared by boiling fresh plants.

Recommendations

On measurement of antioxidant activity. In determining the antioxidant activity, it is important to consider the clarity of the solutions for spectrophotometric measurement. It is ideal to use dilute samples so as to define clear differences of values between the samples. Thus, optimization of the reaction conditions as well as the concentration of the extracts is strongly recommended.

On citral content assay. The assay procedure for citral content analysis using the barbituric acid condensation method needs further optimization to achieve uniformity in the parameters (y-intercept and slope) of the standard curve when doing measurements from time to time since it was observed that the y-intercept of the standard curves have slight variations. This may be due to the citral standard used which is liquid, and more difficult to handle as compared to using a standard which is solid. Optimization is also needed to assess the time it will take for the absorbance to decline, which is an estimate of the decomposition of the chromophore. This will be helpful in determining the maximum number of samples which can be assayed in one run with the same standard curve.

On future studies. It is recommended that a more detailed study be conducted on the citral content of the water extracts of lemongrass using different parts of the plants, such as leaf sheath versus leaf blade. This is based on the observation that tea prepared using the bulb tastes better than that using the leaves, as well as more of the lemon odor is found in the stalk. Furthermore, the effect of soil and environmental conditions on antioxidant activity and citral content must be taken into consideration.

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