Research Article

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Antioxidant, Anti-Tyrosinase and Anticancer Effects of Essential Oil From *Cymbopogon citratus*: *In Vitro* Study

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Abstract: Bioactive compounds derived from natural sources have gained increasing interest for their potential applications in skincare and therapeutic products. The essential oil of *Cymbopogon citratus* (lemongrass essential oil; LGEO) has been widely incorporated into cosmetic formulations such as soaps, balms, ointments, creams, and shampoos. This study evaluated the antioxidant, anti-tyrosinase, and cytotoxic properties of LGEO. Antioxidant activity was assessed using DPPH and ABTS assays, while anti-tyrosinase activity was assessed using dopachrome assay. Cytotoxic effects were evaluated in normal fibroblast and murine melanoma cell lines. LGEO exhibited significant antioxidant activity at 5 mg/mL, with maximal inhibition values of 56.29 ± 5.64% (DPPH) and 81.15 ± 3.45% (ABTS). At a concentration of 2.5 mg/mL, LGEO demonstrated notable anti-tyrosinase activity (52.13 ± 0.96%). Cytotoxicity assays revealed that LGEO at 0.5 mg/mL reduced murine melanoma cell viability by more than 50% without affecting normal fibroblast viability. These findings highlight the potential of LGEO as a multifunctional bioactive ingredient for skincare applications, offering antioxidant and skin-lightening effects alongside selective anticancer activity with minimal cytotoxicity toward normal cells.

Keywords: Antioxidant, Anti-tyrosinase, Lemongrass, Anti-cancer, Melanoma

INTRODUCTION

The imbalance between free radical and antioxidant leads to oxidative damage of lipid, protein and DNA resulting in cellular injury and death in various organ systems. The accumulation of free radicals in different skin cell types also contributes to hyperpigmentation through activation of the melanin production and skin deterioration by increasing the activity of degradative enzymes. These processes accelerate skin aging which is the major concern, particularly among women [1]. Recently, the exploration of natural products with antioxidant and skin-whitening properties for use as cosmetic ingredients are more popular [2,3]. *Cymbopogon citratus* (Lemongrass) is an herb traditionally used in Thai cuisine and medicine. It has been employed to relieve fever and to treat gastrointestinal disorders, such as constipation and stomach pain. Several pharmacological studies have been reported various biological activities of LGEO, including antibacterial, anti-inflammation, antioxidant, antifungal, antiviral and anticancer activity. These pharmacological properties are primarily attributed to its active

constituent, such as citral and geraniol [4]. However, the concentration of active constituents varies among different parts of the plant, resulting in differences in their biological properties. Furthermore, the extraction method can also influence the chemical composition and, consequently, the biological effects of the plant. The use of medicinal plants has gained increasing recognition in complementary and alternative medicine. Therefore, scientific studies investigating the biological activities and safety profiles of plant extracts are essential. One of the most common applications of *Cymbopogon citratus* is its essential oil, which is rich in terpenes and terpenoids. The use of essential oils has increased markedly over the past decade, particularly as ingredients in products such as soaps, ointments, balms, creams, and inhalers. These oils are claimed to provide various health benefits, including antioxidant, analgesic, anti-inflammatory, and relaxing effects [5]. Although topical application of lemongrass essential oil (LGEO) has demonstrated beneficial effects, little is known about its influence on tyrosinase activity and its effects on melanoma, a type of skin cancer.

Therefore, this study aimed to evaluate the biological activities of LGEO, including its antioxidant, anti-tyrosinase, and cytotoxic effects, using normal fibroblast cells (OUMS-36) and melanoma cells (B16F10).

MATERIALS AND METHODS

Lemongrass essential oil

Lemongrass (*Cymbopogon citratus*) essential oil (stream distillations) was purchased from Botanicessence, Thailand. The GC analysis showed the main constituents including geranial (47.46%), neral (33.34%), geraniol (5.30%), citronellal (2.53%) and etc.

Cell lines

The OUMS-36 fibroblast cell was kindly provided by Assist. Prof. Dr. Pannapa Powthong, Department of Medical Sciences, Faculty of Science, Rangsit University. The B16F10 Melanoma was purchased from ATCC (CRL-6475).

In vitro antioxidant activity

The antioxidant activity was determined by using the DPPH and ABTS assay. The LGEO was freshly prepared in five concentrations (0.3125, 0.625, 1.25, 2.5 and 5 mg/mL) before the experiment. Trolox (25 µg/mL) was used as a positive control.

DPPH assay

The DPPH assay was performed according to a previously described method [6]. Briefly, 100 µL of LGEO or methanol (control) was added to each well of a 96-well plate, followed by the addition of 100 µL of 0.1 mM DPPH solution. The mixture was incubated at room temperature in the dark for 30 minutes, and the absorbance was then measured at 517 nm using a microplate reader (EZ Read 2000; Biochrome, Galapagos software). The percentage of DPPH radical scavenging was calculated using % Radical Scavenging Activity (%RSA) = [(Abs of control-Abs of sample)/Abs of control] x 100. All analyses were performed in at least three independent experiments, each conducted in triplicate.

ABTS assay

The ABTS assay was performed according to a previously described method [7]. Briefly, 100 µL of LGEO or methanol (control) was added to each well of a 96-well plate, followed by the addition of 100 µL of 7 mM $ABTS^{\bullet+}$ solution. The mixture was incubated at room temperature in the dark for 15 minutes, and the absorbance was measured at 734 nm using a microplate reader. The percentage of ABTS radical scavenging was calculated using %RSA = [(Abs of control-Abs of sample)/Abs of control] x 100. All analyses were performed in at least three independent experiments, each conducted in triplicate.

In vitro anti-tyrosinase activity assay

Tyrosinase activity was assessed using an *in vitro* dopachrome assay modified from Promden et al. [8]. Briefly, 50 µL of tyrosinase enzyme solution (100 units/mL, from mushroom) and 50 µL of 2.5 mM L-tyrosine were mixed and preincubated in a 96-well plate for 15 minutes at room temperature. Subsequently, 50 µL of phosphate-buffered saline

(PBS, pH 6.8) and 50 µL of LGEO at various concentrations or kojic acid 15.625 µg/mL (positive control) were added. The mixture was then incubated at room temperature in the dark for 30 minutes, and the absorbance was measured at 490 nm using a microplate reader. The percentage of tyrosinase inhibition was calculated using %Tyrosinase inhibition = [(Abs of control-Abs of sample)/Abs of control] x 100. All analyses were performed in at least three independent experiments, each conducted in triplicate.

MTT assay

The OUMS-36 (human fibroblast) and B16F10 (Murine melanoma) cell lines were used in this experiment. The cells were seeded at the density of $5x10^3$ cells/well in 96-well plate and allowed the cell to attach for 24 hours in 5% CO_2 incubator. Cells were then treated with various concentrations of LGEO (0.1, 0.25, 0.5, and 1 mg/mL), or left untreated as a negative control. After the incubation period, cell viability was assessed using the MTT assay. Briefly, the culture medium was carefully removed, and the cells were washed with PBS. The MTT solution was then added to each well and incubated for 3 hours. Thereafter, the MTT solution was discarded, and DMSO was added to dissolve the formazan crystal. The absorbance was measured at 570 nm by using a microplate reader. All analyses were performed in at least three independent experiments, each conducted in triplicate.

Statistical analysis

All of the experiment was expressed as mean ± SEM from at least three independent experiments. The Student's T-test was used to determine the difference between two group. The One-way ANOVA followed by Tukey's Post hoc test was used to determine the significant difference between the control and LGEO in various concentration groups using SPSS software. A *p*-value <0.05 was considered a statistically significant difference.

RESULTS

Antioxidant activity of LGEO

In the antioxidant activity test, LGEO showed radical scavenging activity in both DPPH and ABTS assay in a dose-dependent manner (Figure 1). LGEO at the concentration of 1.25, 2.5 and 5 mg/mL

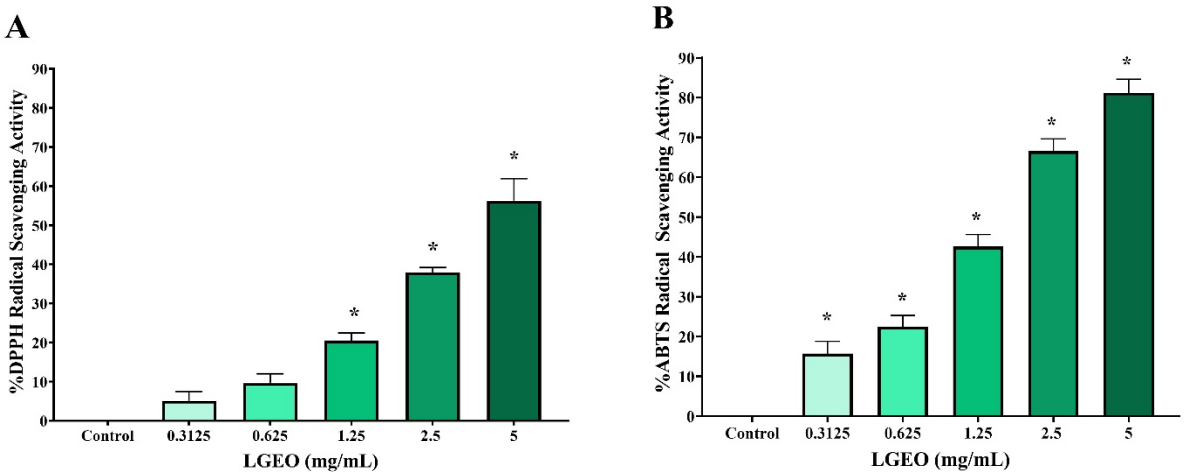

Figure 1. The antioxidant activity of LGEO on radical scavenging activity in DPPH (A) and ABTS (B) assay. *p<0.05 compared with control group. LGEO, lemongrass essential oil

Table 1. Maximum inhibition and IC50 value of LGEO in DPPH and ABTS assay

	DPPH assay		ABTS assay	
	Maximum inhibition	**IC_{50}**	**Maximum inhibition**	**IC_{50}**
LGEO	56.29 ± 5.64	4.23 ± 0.88 mg/mL	81.15 ± 3.45	1.56 ± 0.14 mg/mL

significantly increased the percentages of DPPH radical scavenging activity, with a maximum radical scavenging of 56.29 ± 5.64% at the concentration of 5 mg/mL. However, LGEO at concentrations of 0.3125 and 0.625 mg/mL also tended to increase radical scavenging activity, although the differences were not statistically significant compared with the control group (Figure 1A). In the ABTS assay, all tested concentrations of LGEO increased the percentage of ABTS radical scavenging activity compared with the control group, achieving a maximum inhibition of 81.15 ± 3.45%. The IC_{50} value of LGEO on DPPH and ABTS assay were 4.23 ± 0.88 and 1.56 ± 0.14 mg/mL, respectively (Table 1).

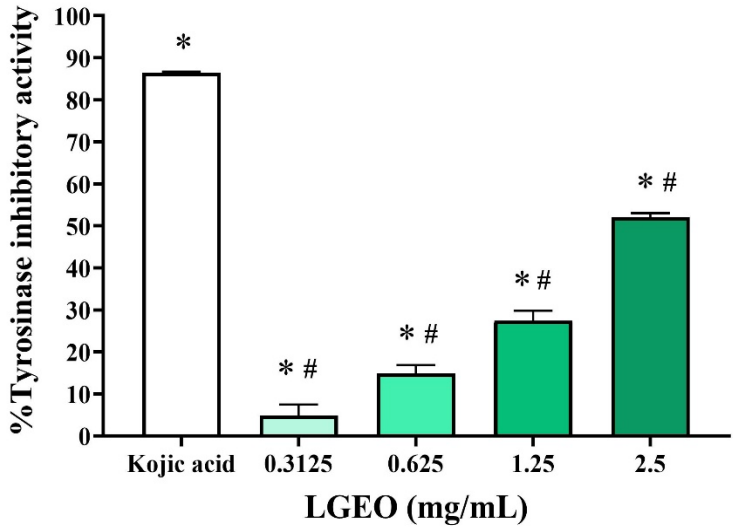

Figure 2. The inhibitory effect of LGEO on tyrosinase activity. *p<0.05 compared with control group; # p<0.05 compared with kojic acid group. LEGO, lemongrass essential oil

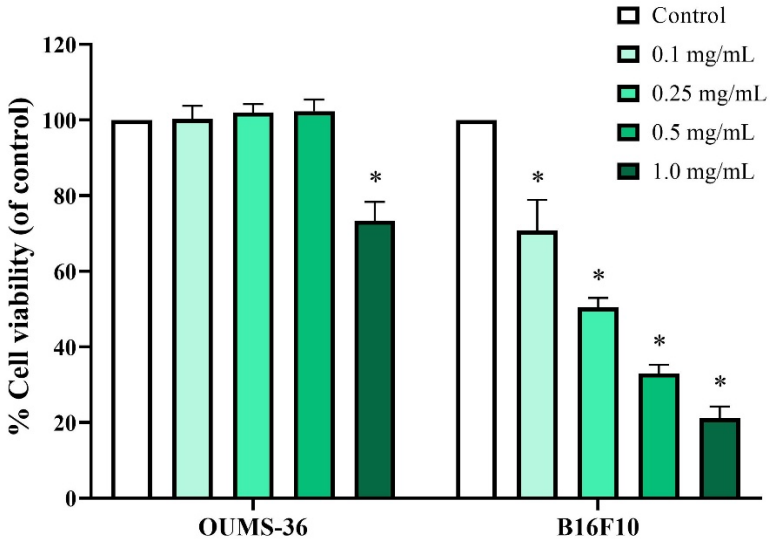

Figure 3. The effect of LGEO on the percentage of cell viability of OUMS-36 (A) and B16F10 (B) cells following 24 hours exposure. *p<0.05 compared with untreated control group. LEGO, lemongrass essential oil; OUMS-36, Human fibroblast cell; B16F10, Murine melanoma cell

Anti-tyrosinase activity of LGEO

LGEO showed the anti-tyrosinase activity in a dose-dependent manner (Figure 2). LGEO at the concentration of 0.3125, 0.625, 1.25 and 2.5 mg/mL significantly increased the percentages of tyrosinase inhibitory activity, with a maximum inhibition of 52.13 ± 0.96% observed at 2.5 mg/mL. The anti-tyrosinase activity of LGEO at 5 mg/mL could not be determined in this experiment due to turbidity observed in the assay system. Moreover, a significant difference was observed when compared with the positive control (kojic acid), which showed a maximum inhibition of 86.44 ± 0.27%.

The effect of LGEO on cell viability of OUMS-36 and B16F10 cell line

The cytotoxic effect of 24-hour exposure of LGEO was determined using MTT assay in OUMS-36 (fibroblast) and B16F10 (melanoma) cell lines. In the fibroblast cells, LGEO at 1 mg/mL significantly reduced cell viability to 73.33 ± 5.00%, whereas concentration below 0.5 mg/mL showed no observable effect (Figure 3). In the B16F10 cell lines, all tested concentrations of LGEO significantly reduced cell viability in a dose-dependent manner. LGEO at a concentration of 1 mg/mL decreased cell viability to 25.65 ± 5.05% as shown in Figure 3.

DISCUSSION

Free radicals are unstable atom that caused of cell damage and death. Under normal physiological conditions, cells can neutralize free radicals through enzymatic and non-enzymatic antioxidant systems. However, excessive accumulation of free radicals leads to oxidative stress. The explore of the supplement that has antioxidant activity from natural resource are interest. It is known that individual experiment *in vitro* assay for screening the antioxidant activity is not capable to cover all of the antioxidant profile. Therefore, in this study two different assays were employed. The DPPH assay is suitable for antioxidants soluble in organic solvents, whereas the ABTS assay can assess both hydrophilic and lipophilic antioxidants [8]. Our results demonstrated that LGEO exhibited antioxidant activity in a dose-dependent manner. However, in the ABTS assay, LGEO showed greater free radical scavenging activity, because ABTS radicals are more reactive and have a faster reaction rate than DPPH radicals. The antioxidant activity of LGEO may be attributed to its high content of monoterpenes, particularly geranial and neral, which are the trans- and cis-isomers of citral, respectively. Citral, a well-known acyclic monoterpene, has been reported to possess strong antioxidant activity in several studies [10].

Tyrosinase is a key enzyme involved in melanin production and skin pigmentation. Increased tyrosinase activity can lead to elevated free radical generation, which in turn may further stimulate tyrosinase activity. Several studies have reported that compounds possessing antioxidant properties can also inhibit tyrosinase activity, a desirable effect for applications in skincare formulations [11]. Therefore, the anti-tyrosinase activity of LGEO was investigated in this study. LGEO demonstrated a dose-dependent reduction in tyrosinase activity, likely due to its monoterpene constituents. These compounds may directly bind to the enzyme and inhibit its function. Both geranial and neral appear to exert synergistic effects on tyrosinase inhibition. Previous studies have shown that citral, composed of these two isomers, can inhibit tyrosinase through its reactive aldehyde group, which interacts with specific amino acid residues near the enzyme's active site. Thus, citral acts as a competitive inhibitor of tyrosinase [12].

The anticancer potential of compounds derived from natural products has gained increasing attention in recent years. In this study, fibroblast and melanoma cell lines were used to evaluate both the anticancer efficacy and safety of LGEO, considering its common topical application in skincare products. Oxidative stress has been documented as a contributing factor in melanoma development; therefore, normal fibroblast cells were used to assess the cytotoxicity of LGEO. The results showed that LGEO decreased the viability of melanoma cells in a dose-dependent manner. In contrast, fibroblast viability was only reduced at a concentration of 1.0 mg/mL, while concentrations between 0.1 and 0.5 mg/mL did not exhibit cytotoxic effects. These findings demonstrate that LGEO possesses selective anticancer activity against melanoma cells, with normal fibroblasts showing greater resistance to LGEO-induced toxicity. Previous

studies have reported the anticancer activity of LGEO in various cancer types, including colorectal, breast, liver, cervical, and prostate cancers. Moreover, citral, one of the major constituents of LGEO, has been shown to exert cytotoxic effects against melanoma cells, with an IC_{50} value of 1.04 μM [13]. The underlying mechanisms proposed for its anticancer activity include the induction of apoptosis, enhancement of oxidative stress, inhibition of cell proliferation pathways such as the PI3K/AKT pathway, and suppression of HSP90 activity [14–16]. It is well established that free radicals play a dual role in cancer cell survival and growth. Moderate levels of reactive oxygen species (ROS) typically promote cancer cell survival and proliferation, whereas high levels induce cell death. The antioxidant effect of LGEO, observed at concentration causing a reduction in viability of melanoma cells, is likely one of the underlying mechanisms. However, further investigation is warranted, as its anticancer activity may involve additional pathways. Furthermore, the results of this study differ from previous report utilizing pure citral for anticancer activity. It is generally recognized that while pure compounds often exhibit a greater cytotoxic effect on cancer cells, they may simultaneously affect normal cells. Several studies support the use of extract containing components other than the pure compound, demonstrating that certain coexisting substances can mitigate toxicity and enhance the efficacy of the primary active ingredient [17, 18]. In the present study, LGEO exhibited anticancer activity against melanoma cells while showing minimal toxicity toward normal fibroblasts. The observed differences from previous reports may be attributed to the synergistic effects of multiple bioactive constituents in the essential oil, including geranial, neral, geraniol, citronellal, myrcene, and linalool. Additionally, at non-toxic concentrations in normal cells, LGEO also demonstrated antioxidant and anti-tyrosinase activities, supporting its potential as a multifunctional skincare ingredient.

CONCLUSION

In this study, lemongrass essential oil (LGEO) demonstrated significant antioxidant, anti-tyrosinase, and anticancer activities while exhibiting minimal cytotoxicity toward normal fibroblast cells. These findings collectively suggest that LGEO has potential as a multifunctional agent for cosmeceutical applications, including anti-aging, skin-brightening, and dark-spot–correcting products. Furthermore, its minimal toxicity combined with demonstrated anticancer effects shows significantly promise for therapeutic development against skin cancer. However, as the present study was limited to *in vitro* assays, the results primarily serve as preliminary evidence of LGEO's biological potential. Further in vivo investigations are necessary to confirm its efficacy, safety, and underlying molecular mechanisms before clinical or commercial applications can be established.

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