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Original Research Article

Antioxidant Potential of Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract in HSC-3 Cancer Cell Line

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ABSTRACT

Oral cavity cancer is a common cancer that has a high mortality rate in humans. Owing to the high cost of the main anticancer drugs and the associated side effects, there has been ongoing research for alternative anti-cancer drugs derived from natural materials. Antioxidants are the molecules involved in scavenging reactive species, which can modify cell proliferation and apoptosis. Therefore, this research was conducted to investigate the antioxidant potential of lemongrass (*Cymbopogon citratus*) leaf ethanol extract and its cytotoxic activity against the Human Squamous Cell-3 (HSC-3) cancer cell line. This *in vitro* study used the HSC-3 cell line, which was divided into the negative, vehicle, and positive controls, as well as a lemongrass leaf extract (25, 50, 100, 200, 300, and 400 µg/mL) treatment. The antioxidant activity assay employed 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, while the cytotoxicity and proliferation assays were performed with the Cell Counting Kit-8. Migration of the HSC-3 cell line was evaluated with the scratch wound healing method. The results showed that the antioxidant activity of the lemongrass leaf ethanol extract was moderate, with an IC_{50} value of 103.65 µg/mL. Furthermore, the cytotoxicity (anticancer) activity has an IC_{50} value of 73.69 µg/mL, and there was decreased proliferation and migration in the HSC-3 cell line. The findings of this study indicate that natural antioxidants have anticancer potential because there is a strong correlation between antioxidant and anticancer activities in the HSC-3 cell line.

Keywords: Anticancer, Antioxidant activity, Ethanol extract, HSC-3 cell line, Lemongrass.

Introduction

Cancer is a deadly disease caused by mutations or changes in the biochemical structure of body cells, which are accompanied by uncontrolled cell growth and can spread.[1,2] Previous research has shown that oral cavity cancer is common in humans and ranks sixth in the world in terms of mortality rate,[3,4] with more than 90% of cases being squamous cell carcinoma.[5] Furthermore, its prediction based on populations in Japan, the United States, and Egypt is discovered on the tongue by 40, 35.2, and 50%, respectively, of total oral cavity cancers.[6,7] One of the oral cavity cancer cell lines that are widely used in *in vitro* studies is Human Squamous Cell 3 (HSC-3), which has a major predilection on the tongue.[8,9] The cells are aggressive, grow rapidly, invade surrounding tissues, and metastasize into the lymph nodes.[10-12] The main treatments for oral cavity cancer are surgery, radiotherapy, and chemotherapy.[5] They have side effects such as nausea, anorexia, diarrhea, mucositis, decreased organ function, and toxic effects on normal cells or tissues, which affect the quality of life and are relatively expensive.[13] Therefore, research into alternative anticancer drugs derived from natural materials such as lemongrass (*Cymbopogon citratus*) is ongoing.

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Lemongrass, which is a member of the Poaceae family, genus *Cymbopogon* Spreng, and species *Cymbopogon citratus* (DC) Stapf, is widely used in tropical countries.[14,15] According to research, this plant has several activities, including antioxidants,[15] anti-inflammatories,[16] antiplaque,[17] antidiarrhogenic,[18] and others. It also has anticancer,[19] analgesics,[20] sedatives,[21] decreased anxiety,[22] and antidepressant properties that are beneficial to cancer patients.[21,23] Previous research has shown that antidepressants and reducing anxiety are important because stress can lead to depression, which plays a role in the development, growth, and metastasis of cancer.[24,25] Lemongrass is only used for its stems, so its leaves are often discarded.[26] Furthermore, lemongrass leaf contains saponins, tannins, alkaloids, flavonoids, phenols, steroids, and essential oils containing citral (geranial), citral (neral), myrcene, citronellol, and linalool.[27-29] The mechanisms of action of an anticancer agent include the capacity to induce cell death (apoptosis), cycle cell arrest, immune function regulation, antiangiogenesis, antimetastasis, and antiproliferation.[30-32] Furthermore, antioxidants are molecules that are involved in scavenging reactive species that cause oxidative stress to maintain a balance between oxidation and anti-oxidation.[33,34] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also known as oxidants or pro-oxidants,[35,36] with ROS playing an important role in cell signaling, and activity.[37] Excessive production of ROS and RNS, however, can result from exposure to cigarette smoke, alcohol, radiation, or environmental toxins, resulting in the formation of many free radicals in the body and oxidative stress. They can disrupt the balance of oxidation and anti-oxidation, resulting in degenerative and chronic diseases like cancer, coronary heart disease, diabetes, and aging.[34,36,38] It is thought that reactive species can promote cancer cell invasion and alter the function of proteins in cellular processes such as proliferation and apoptosis.[33,39]
This study was conducted to investigate the antioxidant potential of lemongrass (*Cymbopogon citratus*) leaf ethanol extract and its

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cytotoxic effect on HSC-3 cancer cell line in order to find a novel anticancer agent.

Materials and Methods

Source and extraction of lemongrass leaves

Lemongrass (*Cymbopogon citratus*) leaves were collected in July 2021 from Balai Penelitian Tanaman Rempah dan Obat (BALITTRO), West Java, Indonesia. They were identified with the ID number B-112/V/DI.05.07/9/2021 at Pusat Riset Biologi, Badan Riset dan Organisasi Nasional (BRIN), Cibinong, West Java, Indonesia. The leaves were dried using an oven at a temperature of 45°C for 1 week and mashed with a plant grinder. Subsequently, 1.95 kg of dried leaf samples were soaked in 10 L of 70% ethanol, agitated with a mixer (Janke and Kunkel, IKA-WERK) for 2 to 3 hours, and left for 24 hours. The preparation was filtered and concentrated using an evaporator (Buchi) for 2 hours to obtain the crude ethanol extract of lemongrass leaves.

Source and maintenance of HSC-3 cell lines

The culture medium was made from DMEM (Gibco), 10% FBS, 1% antibiotics (Penicillin-Streptomycin), and 1% antimycotics. HSC-3 cell line was obtained from the biorepository of YARSI University and were stored in liquid nitrogen tubes (Xin Guang YDS-35). They were thawed in a water-filled container at room temperature and resuspended in a culture medium. The cell concentration was determined using a 10 µL trypan blue (Gibco) and 10 µL cells in the culture medium on a hemocytometer, which was analyzed with an automatic cell counter (BIO-RAD TC20). Subsequently, 100 µL of cells were seeded on well plates, followed by the addition of a 400 µL culture medium.

Antioxidant activity assay with the 2,2- diphenyl-1- picrylhydrazil (DPPH) method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the antioxidant activity of the lemongrass leaf ethanol extract. A concentration of 160 µg/mL of DPPH solution was prepared by dissolving 4.0 mg of DPPH (Sigma-Aldrich) in methanol (25 mL) in a volumetric flask and wrapped with aluminum foil.[40] From 10 mg of crude ethanol extract diluted with 10 mL methanol, a total of 1 mg/mL stock solution was prepared in concentrations of 25, 50, 100, 200, 300, and 400 µg/mL. The extract sample, DPPH (1,000 µL) and methanol were combined to make a total solution volume of 5,000 µL. Each dilution concentration was placed into a sample tube with a micropipette, centrifuged with a centrifuge (Thermo Scientific Vortex Maxi Mix II) until a homogeneous solution was obtained, and incubated at room temperature in the dark. A UV-Vis Spectrophotometer (Shimadzu UV-1800) was used to measure absorbance at 517 nm.[41] Also, the inhibition percentage and 50% Inhibitory Concentration (IC_{50}) were calculated. The inhibition percentage was calculated using the formula below.[41,42]

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance A} - \text{Absorbance B}}{\text{Absorbance A}} \text{ x } 100\%$$

Where Absorbance A is the absorbance of the control, and Absorbance B is the absorbance of the sample

The calculation of 50% Inhibitory Concentration (IC_{50}) was determined from the results of the linear regression equation y = a + bx of the various concentrations of the sample. Therefore, the 50% Inhibitory Concentration (IC_{50}) was calculated using the formula below.[43]

$$IC_{50} = (50 - a) / b$$

Where the X-axis represents the concentration of the sample; The Y-axis represents the percentage of the inhibition; a is the intercept on the Y axis, and b is the slope.

Anticancer assay

The 10 mg/mL stock solution was made by diluting 50 mg of crude ethanol extract with 5 mL of methanol in concentrations of 25, 50, 100, 200, 300, and 400 µg/mL. The dilution volume is also listed in the supplementary data (S1). Methanol was used as the vehicle control in this study because it was used as the crude ethanol extract dissolvent and is distinct from the negative control. The vehicle control contains 0.04% methanol, while the positive control contains 10% dimethyl sulfoxide (DMSO) (Acros Organics).

Cytotoxicity and proliferation assays with cell counting kit-8 (CCK-8)

The cytotoxicity and proliferation assays were carried out using the Cell Counting Kit-8 (CCK-8) Sigma-Aldrich protocol, which was seeded at 20,000 cells/well in 96 well plates, and incubated at 37°C with 5% CO_2 for 24 hours. Subsequently, the culture medium was discarded and rinsed with 100 µL phosphate-buffered saline (PBS) (Gibco). This was followed by the addition of different concentrations of extract samples in the well and the plate was re-incubated in an incubator for 24 hours. The culture medium that had been mixed with the extract was discarded and rinsed again with 100 µL PBS. Finally, 100 µL of solution containing 10 µL of CCK-8 (Sigma-Aldrich) in 90 µL of PBS was added to each well, and the plate was re-incubated for 1 hour. The cytotoxicity and proliferation of cells were calculated based on the number of cells that died or survived in a solution of CCK-8 using a microplate reader with a wavelength of 450 nm.[44,45] The cell morphology was examined using an inverted microscope (Nikon-DS Vi1), and the cell cytotoxicity, proliferation percentage, and IC_{50} were calculated. The cell cytotoxicity percentage formula is given below.[46]

$$\text{Cell death percentage (\%)} = \frac{\text{Absorbance A} - \text{Absorbance B}}{\text{Absorbance A}} \text{ x } 100\%$$

Where Absorbance A is the absorbance of the negative control, and Absorbance B is the absorbance of the sample.

The formula for the cell proliferation percentage is stated below.[47]

$$\text{Cell growth percentage (\%)} = \frac{\text{Absorbance A}}{\text{Absorbance B}} \text{ x } 100\%$$

Where Absorbance A is the absorbance of the sample, and Absorbance B is the absorbance of the negative control.

Migration assay using scratch wound healing method

The cells were seeded at 29,000 cells/well in 24 well plates with a culture medium and incubated for 1 week until confluent. Following that, the monolayer was slowly scratched with the 10 µL tip of the micropipette, with the tip of the pipette perpendicular to the monolayer's base. The culture medium was discarded from the cells, which were then rinsed with 250 µL of PBS before the extract was added to each well. Finally, the cell migration was observed through a fluorescence microscope (EVOS FLc Cell Imaging System) and documented at 0, 2, 4, 6, and 24 hours. The closure of the wounded area was analyzed using ImageJ software.

Statistical data analysis

Shapiro-Wilk, one-way ANOVA, multivariate analysis of variance (MANOVA), Post-hoc Tukey, and Pearson correlation tests were used in the analyses, and the data were analyzed using the Statistical Package for the Social Sciences (SPSS).

Results and Discussion

Antioxidant activity of lemongrass leaf extract

The results of the antioxidant assay of the various concentrations (25, 50, 100, 200, 300, and 400 µg/mL) of lemongrass leaf extract showed that the average inhibition percentages (Table 1) were 19.61, 30.61, 64.67, 89.72, 87.95, and 86.05%, respectively. Similarly, an IC_{50} value

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of 103.65 µg/mL was obtained for the lemongrass leaf extract. To maintain the hydrophobic hydrazyl radical, the DPPH assay uses methanol as the solvent of the hydrazil radical.[48] Meanwhile, ethanol was used as a solvent in the maceration extraction because 70% ethanol has the highest antioxidant activity of lemongrass leaves.[49] The antioxidant activity of lemongrass leaf extract has an IC_{50} value in the moderate range. According to Rabima *et al.*,[50] and Purwanto *et al.*,[51] the classification of antioxidant activity is presented in Table 2.

Cytotoxicity, proliferation and migration effects of lemongrass extract as measure of anticancer activity

The lemongrass leaf extract has cytotoxicity in HSC-3 cells with an IC_{50} value of 73.69 µg/mL. The calculation of the IC_{50} was obtained from the regression equation with the value of $y = 0.1341x + 40.118$ and $R^2 = 0.6661$. The results of the cytotoxicity and proliferation assays (Figures 1-3) revealed that the best concentration was achieved at 300 µg/mL, although not significantly different from the 100, 200, and 400 µg/mL treatments and the positive control. The inhibitory percentages of the positive control on cytotoxicity and proliferation were 83.40 and 16.60%, respectively, while the inhibitory percentages of concentration of 300 µg/mL were 87.00 and 13.00%. As a positive control, 10% DMSO was used, and 0.04% methanol was used as a vehicle control. This is because DMSO concentrations below 10% are not toxic to cells, despite some reports claiming that it is still toxic to various cells.[52] The use of DMSO in culture medium at concentrations of 0.04% is thought to not affect the viability of HSC-3 cells, and its application as a solvent is limited to 0.1%.[53,54] According to previous research, the critical concentration of DMSO use is 1%.[55] Moreover, DMSO, or dimethyl sulfoxide, is a colorless liquid derived from wood pulp byproducts in the papermaking process.[56] It is an aprotic polar organic molecule that can be used as a solvent in biological and medical research for polar and non-polar compounds that are difficult to dissolve.[57,58] For this study, methanol was chosen as the crude ethanol extract dilution because it has been reported to have low toxicity and is not toxic within the concentration limit of 0.15 to 1.25% in some cell lines, such as HepG2, MDA-MB-231, MCF-7, and VNBRCA1.[55]

Table 1: Inhibition percentage of lemongrass leaf extract using the DPPH assay

Concentration of extract (µg/mL)	Inhibition percentage (%)			Mean ± SD
	1	2	3	
25	21.46	17.16	20.21	19.61 ± 1.81
50	39.53	37.26	15.03	30.61 ± 11.06
100	65.09	60.53	68.39	64.67 ± 3.23
200	89.73	89.47	89.95	89.72 ± 0.19
300	88.09	87.68	88.08	87.95 ± 0.19
400	86.04	85.79	86.32	86.05 ± 0.22

DPPH: 2,2- diphenyl-1- picrylhydrazyl

Table 2: Classification of antioxidant activity

IC_{50} Value	Category
<50 µg/mL	Very strong
50-100 µg/mL	Strong
101-150 µg/mL	Moderate
151-200 µg/mL	Weak
>200 µg/mL	Very weak

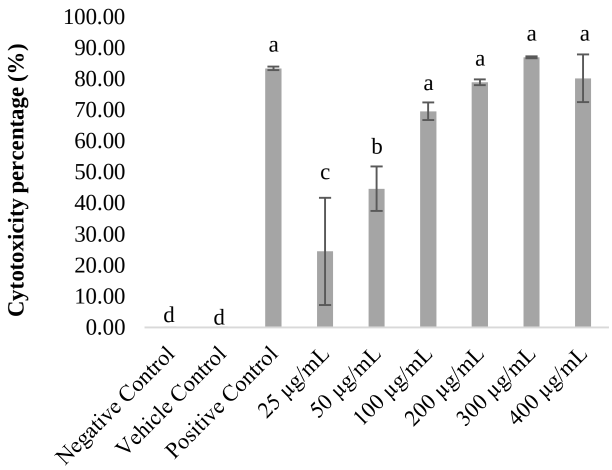

Figure 1: Effect of lemongrass leaf extract on cytotoxicity percentage of HSC-3 cell line.

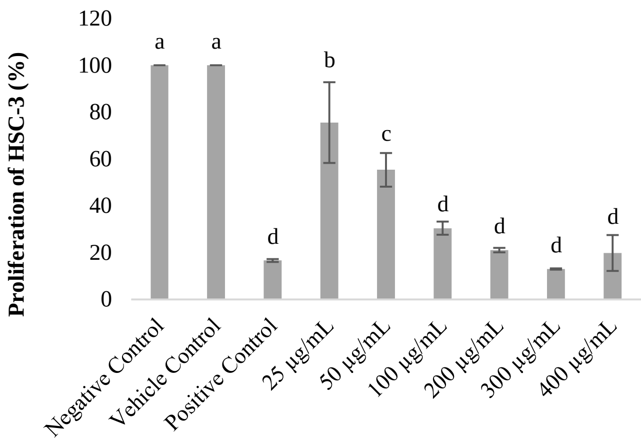

Figure 2: Effect of lemongrass leaf extract on proliferation percentage of HSC-3 cell lines.

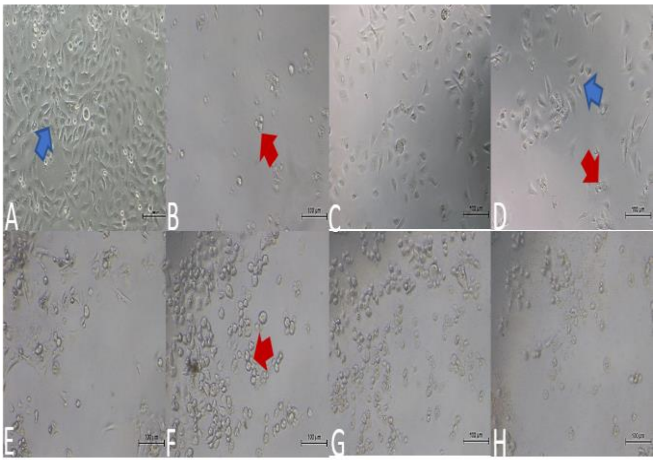

Figure 3: HSC-3 cell morphology after 24 hours of lemongrass leaf extract treatment.

The blue arrow shows live cells and the red arrow indicates dead cells with morphological changes. Observation of the morphological structure of HSC-3 cells in groups, namely vehicle control (A); positive control (B); extract 25 µg/mL (C); extract 50 µg/mL (D); extract 100 µg/mL (E); extract 200 µg/mL (F); extract 300 µg/mL (G); and extract 400 µg/mL (H); Observation was made with a 100x magnification on a scale of 100 µm.

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The cytotoxicity test results of the ethanol extract from lemongrass leaves revealed cytotoxicity to HSC-3 cells with an IC_{50} in the medium range. According to Damasuri *et al*.,[59] the classification of cytotoxicity is shown in Table 3. Several reports support these findings, including the cytotoxic activity of breast cancer cell lines (MCF-7, T47D, MDA-MB-231) with IC_{50} values of 98.7, 109.5, and 38.4 μg/mL; chronic myeloid erythroleukemia (K562) cancer cell line with an IC_{50} value of 57.9 μg/mL; and neuroblastoma cancer cell line (SH-SY5Y) with an IC_{50} value of 97.8 μg/mL.[60]

In addition, lemongrass extract has been shown to induce apoptosis in prostate cancer cells (PC-3, DU-145) and selectively in normal cells,[19] induce apoptosis in colon cancer cell lines (HT-29, HCT-116), but not in normal colon mucosa cells (NCM-460).[61] The report indicated cytotoxic effects in prostate cancer cell lines (LNCaP and PC-3 with IC_{50} values of 6.36 and 32.1 μg/mL, respectively); glioblastoma cancer cell lines (SF-767 and SF-763 with IC_{50} values of 45.13 and 172.05 μg/mL, respectively);[62] and inducing apoptosis in small cell lung cancer cell line (LU135-wt-src).[63] Trang *et al*.,[64] also showed that the essential oils of lemongrass have cytotoxic activity through induction of apoptosis and cell cycle arrest. Furthermore, Philion *et al*.,[65] stated that apoptosis induction of cancer cells by lemongrass depends on ROS generation. The proliferation assay of lemongrass leaf ethanol extract showed a decrease in proliferation ability. These results are consistent with the findings of Halabi and Sheikh,[66] that the lemongrass extract has anti-proliferative effects on HCT-16, MCF-7, MDA-MB 231, SKOV-3, and COAV cancer cells. Several reports also show antiproliferative activity such as decreased proliferation and necrosis of breast cancer-induced with DMBA in mice,[67] inhibited growth of cervical cancer cell lines (HeLa and ME-180) with an IC_{50} value of 200 μg/mL in both cancer cell lines,[68] induced apoptosis, and inhibited proliferation of Hodgkin lymphoma cells (L540, KMH2, HDMYZ), as well as non-Hodgkin lymphoma cells (U-937).[65] The results of the migration assay (Table 4 and Figure 4) revealed a decreased acceleration of HSC-3 cell migration. During the 24-hour observation period, concentrations of 200, 300, and 400 μg/mL indicated that the wound area was still visible. Furthermore, at 25, 50, and 100 μg/mL, the concentrations differ significantly from the negative and positive controls. Although the mechanism of inhibition requires further investigation, cancer cell migration inhibition can be based on the content of active compounds with anti-metastasis and invasion activity, such as alkaloids, flavonoids, and steroids.[69-71]

Antioxidant and anticancer active compounds in lemongrass extract

The active compounds in lemongrass leaves, such as alkaloids, saponins, tannins, triterpenoids, flavonoids, phenols, and steroids were found to have antioxidant and anticancer properties.[72-74] There are also active compound derivatives such as quercetin, luteolin, apigenin, kaempferol, and major terpene. Citral α or geranial (10 – 48%), citral β or neral (3 – 43%), geraniol (2.6 – 40%), geranyl acetate (0.1 – 3%), borneol (5%), linalool (1.2 - 3.4%), citronellal, pinene, estragole, 1,8-cineole, and limonene are all included.[75,76] Meanwhile, differences in extract effectiveness are caused by differences in cell characteristics.[11,77] Table 5 depicts the antioxidant and anticancer mechanisms of some of the active compounds.

Antioxidant and anticancer correlation analysis

The antioxidant and anticancer correlation results were obtained using the Pearson correlation test, and the values obtained are shown in Table 6. According to the findings, there is a strong link between antioxidants and anticancer activity, which is supported by previous research. According to Grigalius and Petrikaite,[88] trihydroxyflavone antioxidants have a moderate correlation with anticancer activity in the cancer cell lines A549 and U87. Zhang *et al*.,[89] also reported that antioxidants from active compounds in plants correlate with anticancer activity. Furthermore, quercetin, an antioxidant compound, has a strong toxic activity that inhibits cancer progression through mechanisms such as cell cycle inhibition in the G2/M phase and apoptosis based on p53.[90,91]

Table 3: Classification of anticancer (cytotoxicity) activity

IC_{50} Value	Category
< 20 μg/mL	High
21-200 μg/mL	Moderate
201-500 μg/mL	Low
>500 μg/mL	Not Toxic

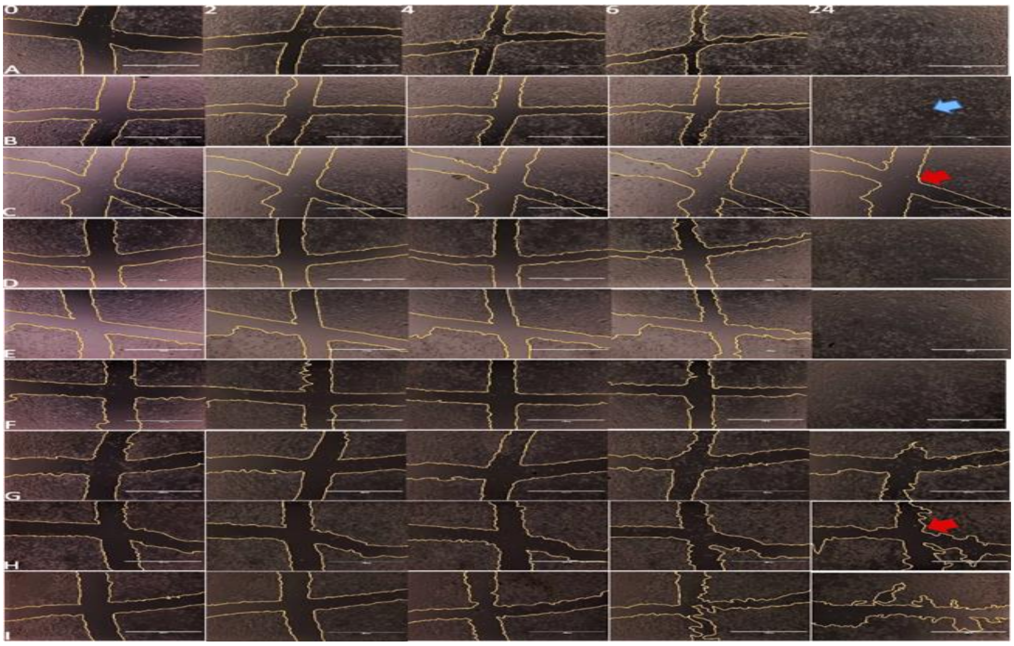

Figure 4: Wound closure in the migration test.

The blue arrow shows that there was a good closure of the wound, therefore, there was no visible area of the wound. The red arrow shows inhibition of cell migration, indicating that the wounded area was still visible. Observation of closure of wounded areas or migration of HSC-3 cells in the group, negative control (A); vehicle control (B); positive control (C); extract 25 μg/mL (D); extract 50 μg/mL (E); extract 100 μg/mL (F); extract 200 μg/mL (G); extract 300 μg/mL (H); and extract 400 μg/mL (I); Observation was made with a magnification of 40x on a scale of 1000 μm.

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Table 4: Wound closure of migration assay

Group	Repetition (n)	Migration ± SD (million μm^2)				
		0 Hour	2 Hour	4 Hour	6 Hour	24 Hour
Negative control	3	1.70 ± 0.13^{ab}	1.33 ± 0.13^{bc}	1.09 ± 0.17^{c}	0.91 ± 0.14^{d}	0.00 ± 0.00^{c}
Vehicle control	3	1.49 ± 0.06^{ab}	1.33 ± 0.10^{bc}	1.22 ± 0.12^{bc}	1.07 ± 0.14^{d}	0.00 ± 0.00^{c}
Positive control	3	1.76 ± 0.03^{a}	1.76 ± 0.03^{a}	1.82 ± 0.08^{a}	1.85 ± 0.11^{a}	1.87 ± 0.10^{a}
25 μg/mL	3	1.56 ± 0.10^{ab}	1.39 ± 0.07^{bc}	1.25 ± 0.08^{bc}	1.12 ± 0.07^{bcd}	0.00 ± 0.00^{c}
50 μg/mL	3	1.45 ± 0.09^{b}	1.30 ± 0.14^{c}	1.20 ± 0.20^{bc}	1.09 ± 0.15^{cd}	0.00 ± 0.00^{c}
100 μg/mL	3	1.51 ± 0.11^{ab}	1.43 ± 0.17^{bc}	1.28 ± 0.11^{bc}	1.09 ± 0.15^{cd}	0.04 ± 0.01^{c}
200 μg/mL	3	1.74 ± 0.09^{a}	1.64 ± 0.09^{ab}	1.49 ± 0.02^{ab}	1.44 ± 0.00^{b}	1.36 ± 0.13^{b}
300 μg/mL	3	1.63 ± 0.06^{ab}	1.55 ± 0.09^{abc}	1.48 ± 0.05^{ab}	1.42 ± 0.02^{bc}	1.36 ± 0.14^{b}
400 μg/mL	3	1.69 ± 0.14^{ab}	1.61 ± 0.13^{abc}	1.42 ± 0.15^{bc}	1.21 ± 0.02^{bcd}	1.19 ± 0.06^{b}

Alphabets on values in the column are post hoc Tukey test that show the difference of significance;
SD: Standard deviation

Table 5: Antioxidant and anticancer mechanisms from active compounds

Active compound	Activity	Mechanism	Reference
Alkaloids (berberine, sanguinarine, aporphine, etc.)	Antioxidant	Scavenging free radicals and also can have pro-oxidant activity	69,78
	Anticancer	Inhibit G1 cell cycle, angiogenesis, invasion, and metastasis	
Saponins (saicosaponin, ginsenoides, etc.)	Anticancer	Induce apoptosis and immunomodulatory effects	79
Tannins (corilagin, ellagitanin, etc.)	Antioxidant	Scavenging free radicals and inhibiting lipid peroxidation.	80
	Anticancer	Release tumor necrosis factors alpha (TNF-α), antitumor, and suppress epidermal growth factor receptors.	
Triterpenoids (betulinic acid, etc.)	Anticancer	Activate p38 mitogen-activated protein kinase (MAPK) and stress-activated protein kinases (SAPK)/ jun amino-terminal kinases (JNK) that are involved in cell regulation such as mitosis and apoptosis.	81
Flavonoids (flavanones, anthocyanidins, etc.)	Anticancer	Induce apoptosis, autophagy, and suppress cell invasion.	70
	Antioxidant	Scavenging ROS and maintaining ROS homeostasis by working as antioxidants under normal circumstances and pro-oxidants specifically in cancer cells for occurring apoptosis.	
Phenols (lignins, xanthones, coumarins, etc.)	Antioxidant	ROS scavengers, chelators of metal cations and induce ROS	82,83
	Anticancer	Inhibit the cascade of oncogenic signaling proliferation and angiogenesis controllers, promoting P53 and increasing the ability to transform in to normal cells.	
Steroids (steroidal alkaloids, etc.)	Anticancer	Induce apoptosis, cycle cell arrest, antiproliferation, and antimetastasis.	71
Citral	Anticancer	Inhibit tumor growth, induce caspase 3 activity, activate p53, inhibit the expression of B-cell lymphoma 2, and induce apoptosis.	49,84
Linalool	Anticancer	Arrest cell cycle in phase G0/G1 on U037 cell and phase G2/M on HeLa cell, increased natural killer cell and induce apoptosis.	85,86,87

Natural antioxidants, such as polyphenols and flavonoids, can generate pro-oxidants or ROS and cause DNA damage, which activates tumor suppressors p21, p27, and p53, inducing caspase 3, 8, and 9, resulting in cancer cell apoptosis.[92,93] Because of Cu and Fe metals in the biological system that cause fenton reactions, antioxidants can also undergo transformation to become pro-oxidants.[94] It was assumed that because cancer cells have higher concentrations of Cu and Fe than normal cells, the possibility of antioxidant to pro-oxidant changes in cancer cells is greater. Pro-oxidants derived from natural antioxidants have selective cytotoxicity against cancer cells.[93,95] Flavonoids are thought to form a highly reactive radical phenoxyl flavonoid (Fl-O·) as a result of scavenging ROS. However, antioxidant networking mechanisms such as ascorbate or glutathione, which can recycle oxidized antioxidants, can overcome this.[94] The limitations of the present study include only observing proliferation for 24 hours and not performing fractionation of the lemongrass leaf ethanol extract to determine the active components responsible for the anticancer and antioxidant activities in the HSC-3 cell line.

Table 6: Pearson correlation between antioxidant and anticancer activities

Extract conc. (μg/mL)	Antioxidant inhibition (%)	Anticancer inhibition (%)	*r*	*P-value*
25	21.46	6.67		
	17.16	41.03		
	20.21	25.71		
50	39.53	45.33		
	37.26	37.18		
	15.03	51.43	0.905	<0.001
100	65.09	68.00		
	60.53	67.95		
	68.39	72.86		
200	89.73	80.00		
	89.47	78.21		
	89.95	78.57		
300	88.09	86.67		
	87.68	87.18		
	88.08	87.14		
400	86.04	72.00		
	85.79	87.18		
	86.32	81.43		

r: Correlation coefficient; Correlation is significant at the 0.01 level (1-tailed)

Conclusion

The findings of this study revealed that lemongrass leaf ethanol extract has moderate antioxidant and anticancer activities with an IC_{50} values of 103.65 μg/mL and 73.69 μg/mL, respectively, which reduces the proliferation and migration of HSC-3 cells. The highest antioxidant and anticancer activities were achieved at concentrations of 200 μg/mL and 300 μg/mL, respectively with a strong correlation between the antioxidant activity and cytotoxic activity on the HSC-3 cell line. Natural antioxidants have the potential to be anticancer agents. More research on ROS generation from natural antioxidants as anticancer agents is recommended, especially in oral cavity cancer.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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