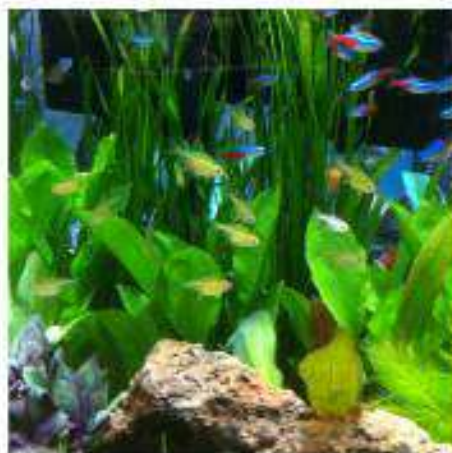


TROPICAL JOURNAL OF
**NATURAL
PRODUCT
RESEARCH**
TJNPR

www.tjnpr.org VOL. 1 NO. 1 JULY 2017



**Tropical Journal of
NATURAL PRODUCT RESEARCH**

This journal is indexed in Open J-Gate,
Crossref, Creative Commons, Index
Copernicus, Google Scholar.

All published papers are DOI assigned.

**Official Journal of Natural Product Research Group
University of Benin**

Editorial Board

Editor-in-Chief: [Professor Abiodun Falodun](#), Faculty of Pharmacy, University of Benin, Nigeria

Associate Editors:

[Professor Dr. Dr. Peter Langer](#), Institute of Organic Chemistry, University of Rostock, (Germany)

[Professor Frederick O. Ekhaie](#), Microbiology, University of Benin, Nigeria

Editorial Assistant:

[Dr. Erhanuyi Osayemwenre](#), Faculty of Pharmacy, University of Benin, Nigeria

Board Members

[Professor Ikhlas A. Khan](#), National Center for Natural Product Research, Mississippi (USA)

[Professor Nosa Egiebor](#), College of Environmental Science & Forestry, State University of New York

[Professor Samuel Qiu](#), South China Botanical Gardens, Chinese Academy of Sciences (China)

[Professor Xavier Barril](#), De Fisicoquímica-Facultat De Farmàcia Universitat de Barcelona, (Spain)

[Professor Abiodun Ogundani](#), Pharmaceutical Chemistry, OAU, Ile-Ife, (Nigeria)

[Professor Thomas Kodadek](#), The Scripps Research Institute, Scripps Florida (USA)

[Professor Anthony B Ebeigbe](#), Physiology, College of Medical Sciences, University of Benin, (Nigeria)

[Professor Eric KI Omogbai](#), Pharmacology and Toxicology, University of Benin, Nigeria

[Professor Dr. Udo Kragl](#), Institute of Organic Chemistry, University of Rostock, (Germany)

[Professor Dr. Udo Kragl](#), Institute of Organic Chemistry, University of Rostock, (Germany)

[Professor Cyril O. Usifoh](#), Faculty of Pharmacy, University of Benin, Nigeria

[Professor Azuka C Opara](#), Clinical Pharmacy & Pharmacy Practice, University of Benin, (Nigeria)

[Professor Ikhide G. Imumorin](#), Biological Sciences, Georgia Institute of Technology, Atlanta (USA)

[Professor Mark T. Hamann](#), Medical University College, South Carolina (USA)

[Professor Barbara Nebe](#), University of Rostock, (Germany)

[Professor Anthony I. Okoh](#), University of Fort Hare, Alice (South Africa)

[Professor Dr. M. Iqbal Choudhary](#), HEJ, University of Karachi, (Pakistan)

[Professor Omoanghe S. Isikhuemhen](#), North Carolina A&T State University, (USA)

[Professor Ezekiel Green](#), University of Johannesburg, (South Africa)

[Professor John Igoli](#), Strathclyde Institute of Pharmacy and Biomedical Sciences, UK

[Professor Peter Akah](#), Pharmacology & Toxicology, University of Nigeria, Nigeria

[Professor H.A.B. Coker](#), Faculty of Pharmacy, University of Lagos (Nigeria)

[Dr Kingsly Agho](#), School of Science and Health, Western Sydney University (Australia)

[Dr. E. Iqinosa](#), Microbiology, University of Benin, Nigeria



0.13

powered by scimagor.com

ISSN: 2616-0684 (Print)
ISSN: 2616-0692 (Online)
DOI: 10.26538/ijer
Index Copernicus Value (ICV) for 2017:
55.03
Scopus citation 6.3 (2020)

Indexing & Abstracting

0.3

2020
CiteScore

18th percentile

Powered by Scopus



African Index Medicus
Access to African Health Information



This work is licensed under a [Creative Commons](#)

[Attribution-NonCommercial-NoDerivatives 4.0](#)

[International License](#)



Professor Dr. M. Iqbal Choudhary, HEJ, University of Karachi, (Pakistan)

Professor Omoanghe S. Isikhuemhen, North Carolina A&T State University, (USA)

Professor Ezekiel Green, University of Johannesburg, (South Africa)

Professor John Igoli, Strathclyde Institute of Pharmacy and Biomedical Sciences, UK

Professor Peter Akah, Pharmacology & Toxicology, University of Nigeria, Nigeria

Professor H.A.B. Coker, Faculty of Pharmacy, University of Lagos (Nigeria)

Dr Kingsly Agho, School of Science and Health, Western Sydney University (Australia)

Dr. E. Igbinosa, Microbiology, University of Benin, Nigeria

Dr. Plus Fasina, School of Pharmacy, Campbell University (USA)

Professor Larry A Walker, National Center for Natural Products Research, Mississippi, USA

Prof. Dr. Alreza Haidari, Faculty of Chemistry, California South University (CSU), Irvine, California, USA

Professor Iyere O Onoagbe, Faculty of Life Sciences, University of Benin, (Nigeria)

Professor Broderick Erlbo, Department of Biology, Howard University, Washington DC, (USA)

Professor Simon Gibbons, School of Pharmacy, University College London, UK

Professor Masashi Mizuno, Laboratory of Food & Nutritional Chemistry, Kobe University, Japan



Reviews

Parquetina nigrescens Species: A Concise Review of Phytochemistry and Pharmacology

Omojumi T. Kayode, Nkechinyere J. Olanaka, Fehintolu J. Femi-Olabisi, Anthonia O. Agboola, Elizabeth O. Oyejobi

<http://www.doi.org/10.26538/ijnpv6i4.1>

pp 454-461 HTML PDF

Ginger (*Zingiber officinale* R.) as a Potent Medicinal Plant for the Prevention and Treatment of Diabetes Mellitus: A Review

Rahmad S. Siregar, Rika A. Hadiguna, Insanul Kamil, Novizar Nazir, Nofaldi Nofaldi

<http://www.doi.org/10.26538/ijnpv6i4.2>

pp 462-469 HTML PDF

An Overview of the Phenolic Constituents and Pharmacological Properties of Extracts and Compounds from *Lagerstroemia speciosa* Leaves

Eric Wei Chiang Chan, Siu Kuen Wong, Hung Tuck Chan

<http://www.doi.org/10.26538/ijnpv6i4.3>

Short Communication

Isolation and Characterization of Cellulose from Siwajan *Borassus flabellifer* Fruit Peel Fiber

Sentot Purwandi, Mohammad Yuvono, Esti Handradi

<http://www.doi.org/10.26538/ijnpv6i4.4>

pp 480-483 HTML PDF

Total Phenolic, Total Flavonoid Contents, and Radical Scavenging Activity of Different Concentrations of Ethanol Extract of *Pyrrosia piloselloides* (L.) M. G. Price

Djarot S.H. Seno, Dewi P.D.B. Sembiring, Popi A. Kurniatin, Waras Nurcholis

<http://www.doi.org/10.26538/ijnpv6i4.5>

pp 484-487 HTML PDF

Original Research Articles

Modulating Effect of Vincamine on the Oxidative Stress Markers and Lipid Profile in High Fat Diet and Streptozotocin-Induced Type 2 Diabetic Rats

Nasreena Shaban, Chakkravarthy Elanchezhian, Shanmugam Manoharan

<https://doi.org/10.26538/ijnpv6i4.14>

pages 545 - 551 HTML PDF

A Novel Broad-Host-Range Phage for Treatment of Mouse Model of *Escherichia coli* Urinary Tract Infection

ISSN 2616-0634 (Print)
ISSN 2616-0632 (Online)
DOI: 10.26538/ijnpv
Index Copernicus Value (ICV) for 2017
18.11
Scopus citation 8.1 (2020)

Indexing & Abstracting

0.3 CiteScore

18th percentile

Powered by Scopus



African Index Medicus
Access to African Health Information



This work is licensed under a Creative Commons

Attribution-NonCommercial-NoDerivatives 4.0

International License



[A Novel Broad-Host-Range Phage for Treatment of Mouse Model of Escherichia coli Urinary Tract Infection](#)

Suhad Y. Abed, Ali H. Alsakini, Manal K. Mohammad, Sundus Q. Mohammed, Sadeq A.G. Kaabi
<http://www.doi.org/10.26538/tjnpr/v6i4.6>

pp 488-493 [HTML](#) [PDF](#)

[Physicochemical Analysis of Leachates from the Public Landfill of Guercif City Before Restoration and Impact on the Tafrata Water Table, Soil, and River Moulouya in Morocco](#)

Nora Zamani, Abdellatif Janati-Iddrissi, Khalid Bouraada, Mohammed Merzouki, Mariam Essafi
<http://www.doi.org/10.26538/tjnpr/v6i4.7>

pp 494-501 [HTML](#) [PDF](#)

[Pharmacokinetics and Molecular Docking Study of Siddha Polyherbal Preparation Shailam Against COVID-19 Mutated s Gene](#)

Rajalakshmi Shanmugavelan, Mohamed Musthafa M
<http://www.doi.org/10.26538/tjnpr/v6i4.8>

pp 502-513 [HTML](#) [PDF](#)

[Assessment of Frequency and Consumption Pattern of Some Indigenous Soups in Delta State](#)

Omajuwa E. Ejueyitsi, Betty O. George, Akpovwehwee A. Anigboro, Joel Okpoghono
<http://www.doi.org/10.26538/tjnpr/v6i4.9>

[Assessment of Groundwater Quality for Irrigation Purposes in Sidi Slimane, Morocco Using the Irrigation Water Quality Index](#)

Imane Bedoui, Sarra EL-Haji, Ghizlane Houzi, Nadia Aghrib, Dounia Challi, Samiha Kaioua, Hefdhallah Alaizari, Mohamed Fadli
<http://www.doi.org/10.26538/tjnpr/v6i4.21>

pp 514-519 [HTML](#) [PDF](#)

[Antioxidant Potential of Lemongrass \(Cymbopogon citratus\) Leaf Ethanol Extract in HSC-3 Cancer Cell Line](#)

Felicia Felicia, Komariah Komariah, Indra Kusuma
<http://www.doi.org/10.26538/tjnpr/v6i4.10>

pp 520-528 [HTML](#) [PDF](#)

[Evaluation of the Therapeutic Effect of Curcumin Phytosomes on Streptozotocin-Induced Diabetic Rats](#)

Wasel Alhabashneh, Khaled M. Khleifat, Moath Alqaraleh, Laila Al-Omari, Nidal Qinna, Muhamad O. Al-Ilmoun, Haitham Qaralleh, Husni S. Farah, Talal Alqaisi
<http://www.doi.org/10.26538/tjnpr/v6i4.11>

pp 529-536 [HTML](#) [PDF](#)

[Combination of High-Performance Liquid Chromatography \(HPLC\) Methods for Isolation of Ampelopsin C, a Trimeric Oligostilbene](#)

pp 537-541 [HTML](#) [PDF](#)

[Estimation of Oxidative Stress Enzymes and Vitamins Levels in Acute Lymphocytic Leukemia Patients in the Governorate of Basrah, Iraq](#)

Alaa A.H. Al-Maliki, Abbas D.M. Al-Maliki

<http://www.doi.org/10.26538/tjnpr/v6i4.13>

pp 542-545 [HTML](#) [PDF](#)

[Comparative Evaluation of Different Extraction Techniques on Phytochemicals and Antioxidant Activity of Psidium Guajava L. Leaves](#)

Shanthirasekaram Kokilananthan, Vajira P. Bulugahapitiya, Harshi Manawadu, Chinthaka S. Gangabadage

<http://www.doi.org/10.26538/tjnpr/v6i4.15>

pp 552-557 [HTML](#) [PDF](#)

[Assessing the Differences in Phenolics, Antioxidant and Anti-Tyrosinase Activities of Spent Coffee Ground Fractions](#)

Danh Vu,

<http://www.doi.org/10.26538/tjnpr/v6i4.16>

pp 558-562 [HTML](#) [PDF](#)

[Date Seeds \(*Phoenix dactylifera* L.\) in Morocco: Phenolic Profiling and In vitro Antioxidant Potency](#)

Hamza Ourradi, Said Ennahli, Lahcen Hssaini, Marto-Viuda Martos, Francisca Hernandez, Hafida Hanine

pp 563-571 [HTML](#) [PDF](#)

[Synthesis of Endophytic Fungi Metabolites, Antimicrobial Potentials, and Detection of their Bioactive Molecules Using Gas Chromatography-Mass Spectrometry](#)

Olusola Majolagbe, Tomiwa Adeniji, Daniel Aina, Iyabo Omomowo, Abimbola Owoseni, Ezekiel Adeyeni,

Olusola Ogunmodede

<http://www.doi.org/10.26538/tjnpr/v6i4.18>

pp 572-579 [HTML](#) [PDF](#)

[The Physicochemical and Microbiological Characteristics of Fermented Hibiscus sabdariffa Calyxes Using Probiotics Starter Cultures](#)

Ufuoma Omole, Solomon Oranusi, Eze F. Ahuekwe, Ayomikun Kade

<http://www.doi.org/10.26538/tjnpr/v6i4.19>

pp 580-586 [HTML](#) [PDF](#)

[Proximate, Physicochemical and Sensory Properties of Millet \(*Pennisetum glaucum*\)-Based Kunun-zaki with Groundnut \(*Arachis hypogaea*\) Inclusion](#)

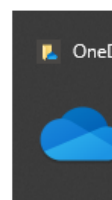
Towobola Michael, Stephen O. Owa, Ayoyinka O. Olojede, James A. Ndako, Emmanuel O. Oludipe,

Ewhoritsemogha P. Dottie, Remilekun M. Thomas

<http://www.doi.org/10.26538/tjnpr/v6i4.20>

pp 587-591 [HTML](#) [PDF](#)

[Physico-Chemical Properties, Chemical Composition and Antimicrobial Activity of *Adenidia merillii* Kernel Seed Oil](#)



pp 599-605 [HTML](#) [PDF](#)

[Corrosion Behaviour of Mild Steel in the Presence of Ceiba pentandra Extract in Acidic Solution: Adsorption and Mechanism](#)

Christogonus O. Akalezi, Nwanneamaka R. Oze, Patience Usman, Emeka E. Oguzie

<http://www.doi.org/10.26538/tjnpr/v6i4.23>

pp 606-614 [HTML](#) [PDF](#)

[Antioxidant Property and Lipid Profile Effects of Aqueous and Ethanol Root Extracts of Gnetum africanum Welw](#)

Callistus I Iheme, Chekwube K. Nwifo, Uchechi K. Obasi, Chidi U. Igwe, Cosmas O. Ujowundu, Chieme S. Chukwudoruo, Raymond C. Ibeh, Thaddeus O. Ukwueze, Moshood A. Haruna

<http://www.doi.org/10.26538/tjnpr/v6i4.24>

pp 615-620 [HTML](#) [PDF](#)

[Analogues of Dipicolinic acid, Phenanthroline, and N-ethylmaleimide as Potential Inhibitors of Plasmodium falciparum falcilysin](#)

Abduljabbar I. Dankani, Suleiman Aminu, Umar Saidu Mukhtar J. Ladan

<http://www.doi.org/10.26538/tjnpr/v6i4.25>

pp 621-627 [HTML](#) [PDF](#)

[Acute and Sub-Acute Effects of Morinda lucida Benth Stem Bark Methanolic Extract on Blood Cytometry, Blood Chemistry and Histoarchitecture of Wistar Rats](#)

Jeremiah J. Oloche, Adedayo F. Asalu, Grace O. Gbotosho

<http://www.doi.org/10.26538/tjnpr/v6i4.26>

pp 628-636 [HTML](#) [PDF](#)

[Gastroprotective Effect of Methanol extracts of Sphenocentrum jollyanum Pierre and Curculigo pilosa \(Schumacher & Thonn.\) Engl. in Wistar Rats](#)

Idayat A. Akinwumi, Mubo A. Sonibare, Adeola T. Salami

<http://www.doi.org/10.26538/tjnpr/v6i4.27>

pp 637-644 [HTML](#) [PDF](#)

[Phytochemicals of Cynodon dactylon and the Toxicological Effect of its Aqueous Extract on Wistar Rats](#)

Doris Akachukwu, Joel O. Anah, Prince O. Alaebo, Chidiebere R. Onwuegbu, Patricia Wokoma

<http://www.doi.org/10.26538/tjnpr/v6i4.28>

pp 645-649 [HTML](#) [PDF](#)

[Investigation of the Antitrypanosomal Activity of Hydromethanol Extract of Olax subscorpioidea Root in Rats Experimentally Infected with Trypanosoma brucei](#)

Amaechi Onyeabor, Samuel O. Onoja, Emmanuel C. Uwalaka, Chukwunonso F. Obi, Bonaventure O. Eze

<http://www.doi.org/10.26538/tjnpr/v6i4.29>

[Curculigo pilosa \(Schumach. & Thonn.\) Engl. in Wistar Rats](#)

Idayat A. Akinwumi, Mubo A. Sonibare, Adeola T. Salami

<http://www.doi.org/10.26538/tjnpr/v6i4.27>

pp 637-644 [HTML](#) [PDF](#)

[Phytochemicals of Cynodon dactylon and the Toxicological Effect of its Aqueous Extract on Wistar Rats](#)

Doris Akachukwu, Joel O. Anah, Prince O. Alaebo, Chidiebere R. Onwuegbu, Patricia Wokoma

<http://www.doi.org/10.26538/tjnpr/v6i4.28>

pp 645-649 [HTML](#) [PDF](#)

[Investigation of the Antitrypanosomal Activity of Hydromethanol Extract of Olax subscorpioidea Root in Rats Experimentally Infected with Trypanosoma brucei](#)

Amaechi Onyeabor, Samuel O. Onoja, Emmanuel C. Uwalaka, Chukwunonso F. Obi, Bonaventure O. Eze

<http://www.doi.org/10.26538/tjnpr/v6i4.29>

pp 650-653 [HTML](#) [PDF](#)

[Effects of Costus afer Extract in Mouse Models of Anxiety and Depression and Its Possible Mechanisms of Action](#)

Akanji A. Murtala, Abidemi J. Akindele, Ibrahim A. Oreagba

<http://www.doi.org/10.26538/tjnpr/v6i4.30>

pp 654-660 [HTML](#) [PDF](#)

Antioxidant Potential of Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract in HSC-3 Cancer Cell LineFelicia Felicia¹, Komariah Komariah^{2*}, Indra Kusuma³¹Undergraduate student, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia²Department of Histology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia³Department of Physiology, Faculty of Medicine, Yarsi University, Jakarta, Indonesia

ARTICLE INFO

Article history:

Received 18 February 2022

Revised 26 March 2022

Accepted 04 April 2022

Published online 03 May 2022

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 IC₅₀ value of 103.65 µg/mL. Furthermore, the cytotoxicity (anticancer) activity has an IC₅₀ 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.

Copyright: © 2022 Felicia *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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.

*Corresponding author. E mail: Komariah@trisakti.ac.id
Tel: +62 821-1116-3806

Citation: Felicia F, Komariah K, Kusuma I. Antioxidant Potential of Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract in HSC-3 Cancer Cell Line. Trop J Nat Prod Res. 2022; 6(4):520-528. doi.org/10.26538/tjnpr/v6i4.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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 antiarrhythmic,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

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-picrylhydrazyl (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 (IC₅₀) were calculated. The inhibition percentage was calculated using the formula below.^{41,42}

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

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

The calculation of 50% Inhibitory Concentration (IC₅₀) 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 (IC₅₀) was calculated using the formula below.⁴³

$$\text{IC}_{50} = (50 - a) / b$$

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% CO₂ 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 IC₅₀ were calculated. The cell cytotoxicity percentage formula is given below.⁴⁶

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

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

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

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 IC₅₀ value

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 hydrazyl 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 IC₅₀ 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 IC₅₀ value of 73.69 µg/mL. The calculation of the IC₅₀ 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 VNBRC1.⁵⁵

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

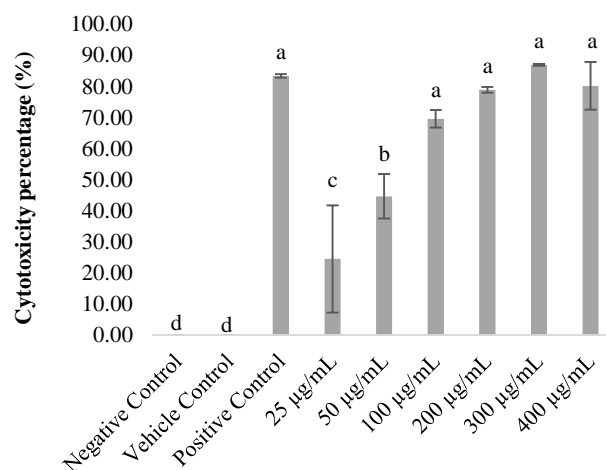


Figure 1: Effect of lemongrass leaf extract on cytotoxicity percentage of HSC-3 cell line.

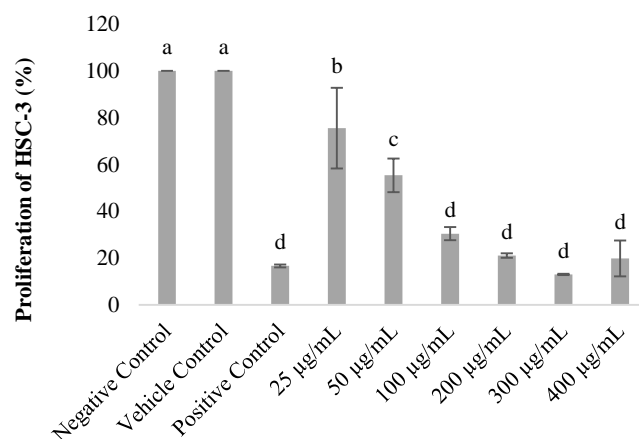


Figure 2: Effect of lemongrass leaf extract on proliferation percentage of HSC-3 cell lines.

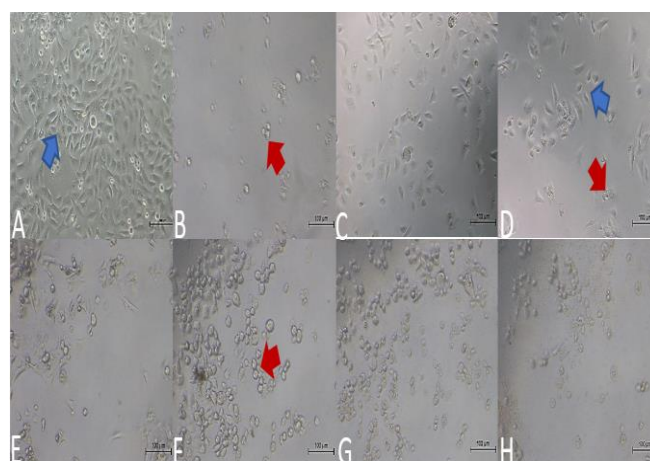


Figure 3: HSC-3 cell morphology after 24 hours of lemongrass leaf extract treatment.

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.

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 $\mu\text{g/mL}$; chronic myeloid erythroleukemia (K562) cancer cell line with an IC_{50} value of 57.9 $\mu\text{g/mL}$; and neuroblastoma cancer cell line (SH-SY5Y) with an IC_{50} value of 97.8 $\mu\text{g/mL}$.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 $\mu\text{g/mL}$, respectively); glioblastoma cancer cell lines (SF-767 and SF-763 with IC_{50} values of 45.13 and 172.05 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ indicated that the wound area was still visible. Furthermore, at 25, 50, and 100 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$	High
21-200 $\mu\text{g/mL}$	Moderate
201-500 $\mu\text{g/mL}$	Low
>500 $\mu\text{g/mL}$	Not Toxic

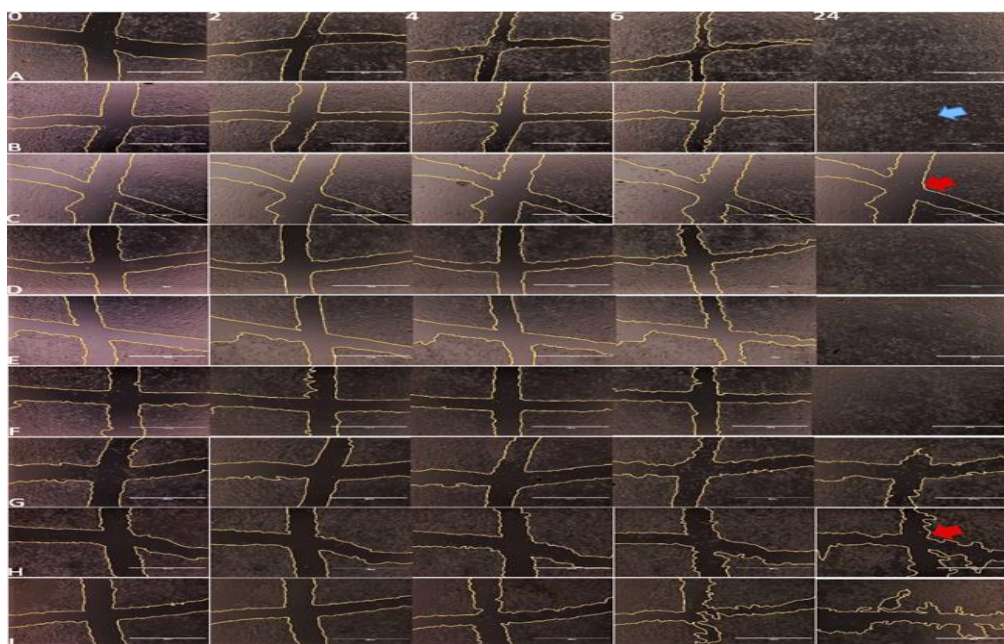


Figure 4: Wound closure in the migration test.

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 $\mu\text{g/mL}$ (D); extract 50 $\mu\text{g/mL}$ (E); extract 100 $\mu\text{g/mL}$ (F); extract 200 $\mu\text{g/mL}$ (G); extract 300 $\mu\text{g/mL}$ (H); and extract 400 $\mu\text{g/mL}$ (I); Observation was made with a magnification of 40x on a scale of 1000 μm .

Table 4: Wound closure of migration assay

Group	Repetition (n)	Migration \pm SD (million μm^2)				
		0 Hour	2 Hour	4 Hour	6 Hour	24 Hour
Negative control	3	1.70 \pm 0.13 ^{ab}	1.33 \pm 0.13 ^{bc}	1.09 \pm 0.17 ^c	0.91 \pm 0.14 ^d	0.00 \pm 0.00 ^e
Vehicle control	3	1.49 \pm 0.06 ^{ab}	1.33 \pm 0.10 ^{bc}	1.22 \pm 0.12 ^{bc}	1.07 \pm 0.14 ^d	0.00 \pm 0.00 ^e
Positive control	3	1.76 \pm 0.03 ^a	1.76 \pm 0.03 ^a	1.82 \pm 0.08 ^a	1.85 \pm 0.11 ^a	1.87 \pm 0.10 ^a
25 $\mu\text{g/mL}$	3	1.56 \pm 0.10 ^{ab}	1.39 \pm 0.07 ^{bc}	1.25 \pm 0.08 ^{bc}	1.12 \pm 0.07 ^{bcd}	0.00 \pm 0.00 ^e
50 $\mu\text{g/mL}$	3	1.45 \pm 0.09 ^b	1.30 \pm 0.14 ^c	1.20 \pm 0.20 ^{bc}	1.09 \pm 0.15 ^{cd}	0.00 \pm 0.00 ^e
100 $\mu\text{g/mL}$	3	1.51 \pm 0.11 ^{ab}	1.43 \pm 0.17 ^{bc}	1.28 \pm 0.11 ^{bc}	1.09 \pm 0.15 ^{cd}	0.04 \pm 0.01 ^c
200 $\mu\text{g/mL}$	3	1.74 \pm 0.09 ^a	1.64 \pm 0.09 ^{ab}	1.49 \pm 0.02 ^{ab}	1.44 \pm 0.00 ^b	1.36 \pm 0.13 ^b
300 $\mu\text{g/mL}$	3	1.63 \pm 0.06 ^{ab}	1.55 \pm 0.09 ^{abc}	1.48 \pm 0.05 ^{ab}	1.42 \pm 0.02 ^{bc}	1.36 \pm 0.14 ^b
400 $\mu\text{g/mL}$	3	1.69 \pm 0.14 ^{ab}	1.61 \pm 0.13 ^{abc}	1.42 \pm 0.15 ^{bc}	1.21 \pm 0.02 ^{bcd}	1.19 \pm 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 (saikosaponin, ginsenosides, 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, xanthonenes, 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 (FI-O \cdot) 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 (%)	<i>r</i>	<i>P-value</i>
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 IC₅₀ 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.

Acknowledgments

The authors wish to express their deepest gratitude to Professor Rahmi Amtha (Department of Oral Medicine), Professor Melanie Sadono Djamil (Department of Biochemistry and Molecular Biology), Doctor Monica Dewi Ranggani (Department of Physiology), and Doctor

Moehamad Orliando Roeslan (Department of Oral Biology), at the Trisakti University, for all the support and advice. We are also grateful to the Faculty of Dentistry at Trisakti University for their assistance, as well as the Cell Culture Laboratory at YARSI University and BALITTRO for the materials.

References

1. Rahayuwati L, Rizal IQ, Pahria T, Lukman M, Juniarti N. Pendidikan kesehatan tentang pencegahan penyakit kanker dan menjaga kualitas kesehatan. Media Karya Kesehatan. 2020; 3(1):59-69.
2. Wijaya CA and Muchtaridi M. Pengobatan kanker melalui metode gen terapi. Farmaka. 2017; 15(1):53-68.
3. Lan Z, Jia Z, Guo H, Yang Z, Yang Z, Pan X. Model of human tongue squamous cell lines stably transfected with human papillomavirus (HPV)16 E6 and E7 genes and biological characteristic analysis. Biomed Res Int. 2021; 2021:9968691.
4. Xie S, Zhang XY, Shan XF, Yau V, Zhang JY, Wang W, Yan YP, Cai ZG. Hyperion image analysis depicts a preliminary landscape of tumor immune microenvironment in OSCC with lymph node metastasis. J Immunol Res. 2021; 2021:9975423.
5. Langlais RP, Miller CS, Gehrig JS. Atlas berwarna lesi mulut yang sering ditemukan. 5th ed. Translator: Gunardi I. Jakarta: EGC; 2020; 162p.
6. Yamamoto N and Shibahara T. Epidemiology of oral cancer. In: Kirita T and Omura K (editors). Oral cancer diagnosis and therapy. Tokyo: Springer; 2015. 3p.
7. Hamdy R and Halim A. Squamous cell carcinoma of the oral tongue: a single institution retrospective cohort study from Mansoura university hospital. Indones J Cancer. 2019; 12(4):102-108.
8. Ribeiro IP, Rodrigues JM, Mascarenhas A, Kosyakova N, Caramelo F, Liehr T, Melo JB, Carreira IM. Cytogenetic, genomic, and epigenetic characterization of the HSC-3 tongue cell line with lymph node metastasis. J Oral Sci. 2018; 60(1):70-81.
9. Komariah K, Ageng A, Kusuma I. Efek kombinasi asam valproat dan nano kitosan kumbang tanduk (*Xylotrupes gideon*) terhadap viabilitas dan sitotoksitas sel kanker lidah (HSC-3). Proceedings of the 2nd national expert seminars; 2019 Mar 2; Jakarta: Lembaga Penelitian Universitas Trisakti; 2019.
10. Vilen ST, Salo T, Sorsa T, Nyberg P. Fluctuating roles of matrix metalloproteinase-9 in oral squamous cell carcinoma. Sci World J. 2013; 2013:920595.
11. Kurihara-Shimomura M, Sasahira T, Nakashima C, Kuniyasu H, Shimomura H, Kirita T. The multifarious functions of pyruvate kinase M2 in oral cancer cells. Int J Mol Sci. 2018; 19(10):2907.
12. Rahmawati D, Anggraini W, Djamil M. Cytotoxicity of red fruit ethyl acetate extract (*Pandanus conoideus* lam.) on squamous cell carcinoma cell line (HSC-3). Sci Dent J. 2021; 5(1):42-46.
13. Ohnishi S and Takeda H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. Front Pharmacol. 2015; 6:14.
14. Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (lemongrass). J Adv Pharm Technol Res. 2011; 2(1):3-8.
15. Chen Z, Ye X, Qingkui G, Wenliang Q, Wen Z, Ning W, Min Z. Anticancer activity of green synthesized AgNPs from *Cymbopogon citratus* (LG) against lung carcinoma cell line A549. IET Nanobiotechnol. 2019; 13(2):178-182.
16. Li M, Liu B, Bernigaud C, Fischer K, Guillot J, Fang F. Lemongrass (*Cymbopogon citratus*) oil: a promising mitocidal and ovidical agent against *Sarcoptes scabiei*. PloS Negl Trop Dis. 2020; 14(4):e0008225.

17. Rajesvari R and Lakshmi T. Lemon grass oil for improvement of oral health. *Dent Hypotheses*. 2013; 4(4):115-117.
18. Rubin CCB, Horn RC, Hirsch G, Gellatti GT, Tissiani AC, Calçada JPC, Heringer T, Cunha A. Redox alterations in pregnant women: antioxidant effect of lemongrass (*Cymbopogon citratus* (DC.) Stapf). *Braz J Dev*. 2020; 6(9):67171-67187.
19. Nguyen C, Mehaidli A, Baskaran K, Grewal S, Pupulin A, Ruvinov I, Scaria B, Parashar K, Vegh C, Pandey S. Dandelion root and lemongrass extracts induce apoptosis, enhance chemotherapeutic efficacy, and reduce tumour xenograft growth *in vivo* in prostate cancer. *Evid-Based Compl Altern Med*. 2019; 2019:2951428.
20. Devi RC, Sim SM, Ismail R. Effect of *Cymbopogon citratus* and citral on vascular smooth muscle of the isolated thoracic rat aorta. *J Evid-Based Compl Altern Med*. 2012; 2012:539475.
21. Umukoro S, Ben-Azu B, Ajayi AM, Adebesein A, Emokpae O. *Cymbopogon citratus* aqueous leaf extract attenuates neurobehavioral and biochemical changes induced by social defeat stress in mice. *Chin Herb Med*. 2020; 12(3):303-309.
22. Al Sears MD. *Healing Herbs of Paradise: Discover useful, practical cures and treatments from a rich herbal tradition almost unknown in the western world*. Florida: Wellness Research & Consulting; 2016. 218p.
23. Dudhgaonkar S, Mahajan M, Deshmukh S, Admane P, Khan H. Evaluation of anti-depressant effect of lemon grass (*Cymbopogon citratus*) in albino mice. *Int J Basic Clin Pharmacol*. 2014; 3(4):656-660.
24. Kruk J, Aboul-Enein BS, Bernstein J, Gronostaj M. Psychological stress and cellular aging in cancer: a meta-analysis. *Oxid Med Cell Longev*. 2019; 2019:1270397.
25. Kim YK, Na KS, Myint AM, Leonard BE. The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016; 64:277-284.
26. Nuryadin Y, Naid T, Dahlia AA, Dali S. Kadar flavonoid total ekstrak etanol daun serai dapur dan daun alang-alang menggunakan spektrofotometri UV-VIS. *Window of Health: J Kesehatan*. 2018; 1(4):337-345.
27. Basera P, Lavania M, Agnihotri A, Lal B. Analytical investigation of *Cymbopogon citratus* and exploiting the potential of developed silver nanoparticle against the dominating species of pathogenic bacteria. *Front Microbiol*. 2019; 10:282.
28. Majewska E, Kozłowska M, Gruczyńska-Sekowska E, Kowalska D, Tarnowska K. Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation – a review. *Pol J Food Nutr Sci*. 2019; 69(4):327-341.
29. Ranitha M, Nour AH, Sulaiman ZA, Nour AH, Thana Raj S. Comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) method. *Int J Chem Eng Appl*. 2014; 5(2):104-108.
30. Lu JJ, Bao JL, Chen XP, Huang M, Wang YT. Alkaloids isolated from natural herbs as the anticancer agents. *Evid-Based Compl Altern Med*. 2012; 2012:485042.
31. Rayan A, Raiyn J, Falah M. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. *PLoS ONE*. 2017; 12(11):e0187925.
32. El-Nabi SH, El-Garawani IE, El-Berry A. The anticancer potential of lemongrass and cinnamon extracts on human hepatocellular carcinoma cell line (HepG2): the possible effectiveness of combined treatment. *Egypt J Exp Biol (Zoo)*. 2017; 13(2):139-148.
33. Sarangarajan R, Meera S, Rukkumani R, Sankar P, Anuradha G. Antioxidants: friend or foe? *Asian Pac J Trop Med*. 2017; 10(12):1111-1116.
34. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. *Int J Mol Sci*. 2017; 18(1):96.
35. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem*. 2015; 30(1):11-26.
36. Chusri S, Singthong P, Kaewmanee T. Antioxidant, anticancer, and cytotoxic effects of Thai traditional herbal preparations consumed as rejuvenators. *CyTA J Food*. 2015; 13(1):40-48.
37. Wang J, Luo B, Li X, Lu W, Yang J, Hu Y, Huang P, Wen S. Inhibition of cancer growth *in vitro* and *in vivo* by a novel ROS-modulating agent with ability to eliminate stem-like cancer cells. *Cell Death Dis*. 2017; 8(6):e2887.
38. Kawamura T, Tanisawa K, Kawakami R, Usui C, Ito T, Tabata H, Nakamura N, Kurosawa S, Choi W, Ma S, Radak Z, Sawada SS, Suzuki K, Ishii K, Sakamoto S, Oka K, Higuchi M, Muraoka I. Determinants of resting oxidative stress in middle-aged and elderly men and women: WASEDA'S health study. *Oxid Med Cell Longev*. 2021; 2021:1-11.
39. Wang S, Meckling KA, Marcone MF, Kakuda Y, Tsao R. Can phytochemical antioxidant rich foods act as anti-cancer agents? *Food Res Int*. 2011; 44(9):2545-2554.
40. Maesroh K, Kurnia D, Al-Anshori J. Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et Natura Acta*. 2018; 6(2):93-100.
41. Marvibaigi M, Amini N, Supriyanto E, Abdul Majid FA, Kumar Jaganathan S, Jamil S, Hamzehalipour Almaki J, Nasiri R. Antioxidant activity and ROS-dependent apoptotic effect of *Scurrula ferruginea* (Jack) Danser methanol extract in human breast cancer cell MDA-MB-231. *PLoS ONE*. 2016; 11(7):e0158942.
42. Wang WH, Hsuan KY, Chu LY, Lee CY, Tyan YC, Chen ZS, Tsai WC. Anticancer effects of *Salvia miltiorrhiza* alcohol extract on oral squamous carcinoma cells. *Evid-Based Compl Altern Med*. 2017; 2017:1-9.
43. Wilujeng S, Laksitarahmi IP, Suharnanik, Tiaranisa P. Antioxidant maja fruit (*Aegle marmelos* (L.) Carrea) lowering blood sugar mus musculus. *Bir Ex J*. 2020; 2(3):362-367.
44. Dona R, Frimayanti N, Ikhtiarudin I, Iskandar B, Maulana F, Silalahi NT. Studi *in silico*, sintesis, dan uji sitotoksik senyawa p-metoksi kalkon terhadap sel kanker payudara MCF-7. *J Sains Farm Klin*. 2019; 6(3):243-249.
45. Cai H, Li J, Zhang Y, Liao Y, Zhu Y, Wang C, Hou J. LDHA promotes oral squamous cell carcinoma progression through facilitating glycolysis and epithelial-mesenchymal transition. *Front Oncol*. 2019; 9:1446.
46. Wahjuningrum DA, Elizabeth ME, Puteri FH, Mardiyah AA, Subiyanto A. Cytotoxicity assay of sodium hypochlorite and Qmix on cultured human periodontal ligament fibroblast cells. *J Int Oral Health*. 2019; 11:204-207.
47. Orellana EA and Kasinski AL. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. *Bio Protoc*. 2016; 6(21):1-9.
48. Nicklisch SC and Waite JH. Optimized DPPH assay in a detergent based buffer system for measuring antioxidant activity of proteins. *MethodsX*. 2014; 1:233-238.
49. Hasim H, Nasution SP, Kurniawati SO, Rachmawati I. Aktivitas sitotoksik sitral serai sebagai antikanker payudara MCM-B2. *Curr Biochem*. 2020; 7(1):29-36.
50. Rabima R, Pangaman SD. Formulasi dan uji aktivitas antioksidan dari sediaan masker *peel-off* ekstrak umbi wortel varietas chantenay (*Daucus carota* L.). *Indones Nat Res Pharm J*. 2020; 5(2):135-148.

51. Purwanto D, Bahri S, Ridhay A. Uji aktivitas antioksidan ekstrak buah purnajiwa (*Kopsia arborea* Blume.) dengan berbagai pelarut. Kovalen. 2017; 3(1):24-32.
52. Galvao J, Davis B, Tilley M, Normando E, Duchon MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. FASEB J. 2014; 28(3):1317-1330.
53. Chen JL, Lai CY, Ying TH, Lin CW, Wang PH, Yu FJ, Liu CJ, Hsieh YH. Modulating the ERK1/2-MMP1 axis through corosolic acid inhibits metastasis of human oral squamous cell carcinoma cells. Int J Mol Sci. 2021; 22(16):8641.
54. Kim LH, Khadka S, Shin JA, Jung JY, Ryu MH, Yu HJ, Lee HN, Jang B, Yang IH, Won DH, Kwon HJ, Jeong JH, Hong SD, Cho NP, Cho SD. Nitidine chloride acts as an apoptosis inducer in human oral cancer cells and a nude mouse xenograft model via inhibition of STAT3. Oncotarget. 2017; 8(53):91306-91315.
55. Nguyen S, Nguyen H, Truong K. Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. BMRAT. 2020; 7(7):3855-3859.
56. Capriotti K and Capriotti JA. Dimethyl sulfoxide: history, chemistry, and clinical utility in dermatology. J Clin Aesthet Dermatol. 2012; 5(9):24-26.
57. Yuan C, Gao J, Guo J, Bai L, Marshall C, Cai Z, Wang L, Xiao M. Dimethyl sulfoxide damages mitochondrial integrity and membrane potential in cultured astrocytes. PloS ONE. 2014; 9(9):e107447.
58. Verheijen M, Lienhard M, Schrooders Y, Clayton O, Nudischer R, Boerno S, Timmermann B, Selevsek N, Schlapbach R, Gmuender H, Gotta S, Geraedts J, Herwig R, Kleinjans J, Caiment F. DMSO induces drastic changes in human cellular processes and epigenetic landscape *in vitro*. Sci Rep. 2019; 9(1):4641.
59. Damasuri AR, Sholikhah EN, Mustofa M. Cytotoxicity of ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) on hela cell line. Indon J Pharmacol Ther. 2020; 1(2):54-59.
60. Thangam R, Sathuvan M, Poongodi A, Suresh V, Pazhanichamy K, Sivasubramanian S. Activation of intrinsic apoptotic signaling pathway in cancer cells by *Cymbopogon citratus* polysaccharide fractions. Carbohydr Polym. 2014; 107:138-150.
61. Ruvinov I, Nguyen C, Scaria B, Vegh C, Zaitoon O, Baskaran K, Mehaidli A, Nunes M, Pandey S. Lemongrass extract possesses potent anticancer activity against human colon cancers, inhibits tumorigenesis, enhances efficacy of FOLFOX, and reduces its adverse effects. Integr Cancer Ther. 2019; 18:1-13.
62. Bayala B, Bassole IHN, Maqdasy S, Baron S, Simporé J, Lobaccaro JA. *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule. Biochimie. 2018; 153:162-170.
63. Maruoka T, Kitanaka A, Kubota Y, Yamaoka G, Kameda T, Imataki O, Dobashi H, Bandoh S, Kadowaki N, Tanaka T. Lemongrass essential oil and citral inhibit Src/Stat3 activity and suppress the proliferation/survival of small cell lung cancer cells, alone or in combination with chemotherapeutic agents. Int J Oncol. 2018; 52(5):1738-1748.
64. Trang DT, Hoang TKV, Nguyen TTM, Van Cuong P, Dang NH, Dang HD, Nguyen Quang T, Dat NT. Essential oils of lemongrass (*Cymbopogon citratus* Stapf) induces apoptosis and cell cycle arrest in A549 lung cancer cells. Biomed Res Int. 2020; 2020:5924856.
65. Pillion C, Ma D, Ruvinov I, Mansour F, Pignatelli C, Noel M, Saleem A, Arnason J, Rodrigues M, Singh I, Ropat J, Pandey S. *Cymbopogon citratus* and *Camellia sinensis* extracts selectively induce apoptosis in cancer cells and reduce growth of lymphoma xenografts *in vivo*. Oncotarget. 2017; 8(67):110756-110773.
66. Halabi MF and Sheikh BY. Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. Biomed Res Int. 2014; 2014:906239.
67. Rojas-Armas JP, Arroyo-Acevedo JL, Palomino-Pacheco M, Herrera-Calderón O, Ortiz-Sánchez JM, Rojas-Armas A, Calva J, Castro-Luna A, Hilario-Vargas J. The essential oil of *Cymbopogon citratus* Stapf and carvarol: an approach of the antitumor effect on 7,12-Dimethylbenz-[a]-anthracene (DMBA)-induced breast cancer in female rats. Molecules. 2020; 25(14):3284.
68. Ghosh K. Anticancer effect of lemongrass oil and citral on cervical cancer cell lines. Pharmacogn Commun. 2013; 3(4):41-48.
69. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins (Basel). 2019; 11(11):656.
70. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutr. 2020; 12(2):457.
71. Dey P, Kundu A, Chakraborty HJ, Kar B, Choi WS, Lee BM, Bhakta T, Atanasov AG, Kim HS. Therapeutic value of steroidal alkaloids in cancer: current trends and future perspectives. Int J Cancer. 2019; 145(7):1731-1744.
72. Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Sci Afr. 2019; 6:e00137.
73. Andikoputri SF, Komariah K, Roeslan MO, Ranggaini D, Bustami DA. Nano chitosan encapsulation of *Cymbopogon citratus* leaf extract promotes ROS induction leading to apoptosis in human squamous cells (HSC-3). Curr Issues Pharm Med Sci. 2021; 34(3):134-137.
74. Veronica G, Komariah K, Gabriella Clara Maria L, editors. Microencapsulation of lemongrass leaves effects on reactive oxygen species (ROS) fibroblasts. 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce); 2021 July 14-16; 2021.
75. Shendurse AM, Sangwan RB, Amit K, Ramesh V, Patel AC, Gopikrishna G, Roy SK. Phytochemical screening and antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves essential oil. J Pharmacogn Phytochem. 2021; 10(2):445-449.
76. Madivoli ES, Gita L, Gumba E. Isolation and identification of essential oils from *Cymbopogon citratus* (Stapf) DC using GC-MS and FT-IR. Chem Mater Res. 2012; 2(4):13-22.
77. Kiseleva LN, Kartashev AV, Vartanyan NL, Pinevich AA, Samoilovich MP. A172 and T98G cell lines characteristics. Cell Tissue Biol. 2016; 10(5):341-348.
78. Macáková K, Afonso R, Saso L, Mladěnka P. The influence of alkaloids on oxidative stress and related signaling pathways. Free Rad Biol. 2019; 134:429-444.
79. Elekofehinti OO, Iwaloye O, Olawale F, Ariyo EO. Saponins in cancer treatment: current progress and future prospects. Pathophysiol. 2021; 28(2):250-272.
80. Sieniawska E. Activities of tannins – from *in vitro* studies to clinical trials. Nat Prod Commun. 2015; 10(11):1877-1884.
81. Ghante MH and Jamkhande PG. Role of pentacyclic triterpenoids in chemoprevention and anticancer treatment: an overview on targets and underlying mechanisms. J Pharmacopunct. 2019; 22(2):55-67.
82. Bouriche H, Kada S, Senator A, Demirtas I, Ozen T, Toptanci BÇ, Kizil G, Kizil M. Phenolic content and biomolecule oxidation protective activity of *Globularia alypum* extracts. Braz Arch Biol Technol. 2017; 60:e17160409.
83. Anantharaju PG, Gowda PC, Vimalambike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutr J. 2016; 15(1):99.
84. Zeng S, Kapur A, Patankar MS, Xiong MP. Formulation, characterization, and antitumor properties of trans- and cis-citral in the 4T1 breast cancer xenograft mouse model.

- Pharm Res. 2015; 32(8):2548-2558.
85. Zhao Y, Meng X, Zeng Y, Wang C, Chen J, She Z. Linalool inhibits MCF-7 tumor growth in a xenograft model by apoptosis induction and immune modulation. *Nat Prod Commun.* 2021; 16(5):1-4.
 86. Chang MY, Shieh DE, Chen CC, Yeh CS, Dong HP. Linalool induces cell cycle arrest and apoptosis in leukemia cells and cervical cancer cells through CDKIs. *Int J Mol Sci.* 2015; 16(12):28169-28179.
 87. Iwasaki K, Zheng YW, Murata S, Ito H, Nakayama K, Kurokawa T, Sano N, Nowatari T, Villareal MO, Nagano YN, Isoda H, Matsui H, Ohkohchi N. Anticancer effect of linalool via cancer-specific hydroxyl radical generation in human colon cancer. *World J Gastroenterol.* 2016; 22(44):9765-9774.
 88. Grigalius I and Petrikaite V. Relationship between antioxidant and anticancer activity of thihydroflavones. *Molecules.* 2017; 22(12):2169.
 89. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules.* 2015; 20(12):21138-21156.
 90. Rauf A, Imrah M, Khan IA, Ur-Rehman M, Gilani SA, Mehmood Z, Mubarak MS. Anticancer potential of quercetin: a comprehensive review. *Phytother Res.* 2018; 32(11):2109-2130.
 91. Herni K, Subarnas A, Diantini A, Iskandar Y. Cytotoxicity of quercetin and quercetin-3-O-rhamnoside of *Etlingera elatior* (Jack) R.M.Sm. leaves against HeLa cervical cancer cells. *J Appl Pharm Sci.* 2021; 11(05):085-090.
 92. Eghbaliferiz S and Iranshahi M. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanisms and catalyzing metals. *Phytother Res.* 2016; 30(9):1379-1391.
 93. Shin J, Song MH, Oh JW, Keum YS, Saini RK. Pro-oxidant actions of carotenoids in triggering apoptosis of cancer cells: a review of emerging evidence. *Antioxid (Basel).* 2020; 9(6):532.
 94. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. *Fitoter.* 2011; 82(4):513-523.
 95. Koçyiğit A and Selek Ş. Exogenous antioxidants are double-edged swords. *Bezmialem Science.* 2016; 2:70-75.

TJNPR-3 Mei 2022

by Komariah Komariah

Submission date: 07-Apr-2023 02:37PM (UTC+0700)

Submission ID: 2058252963

File name: TJNPR-3_Mei_2022.pdf (800.96K)

Word count: 7991

Character count: 42081



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

Felicia Felicia¹, Komariah Komariah^{2*}, Indra Kusuma³

6
¹Undergraduate student, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia

²Department of Histology, Faculty of Dentistry, Trisakti University, Jakarta, Indonesia

³Department of Physiology, Faculty of Medicine, Yarsi University, Jakarta, Indonesia

ARTICLE INFO

Article history:

Received 18 February 2022

Revised 26 March 2022

Accepted 04 April 2022

Published online 03 May 2022

Copyright: © 2022 Felicia *et al.* This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 IC₅₀ value of 103.65 µg/mL. Furthermore, the cytotoxicity (anticancer) activity has an IC₅₀ 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.

6
*Corresponding author. E mail: Komariah@trisakti.ac.id

Tel: +62 821-1116-3806

1
Citation: Felicia F, Komariah K, Kusuma I. Antioxidant Potential of Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract in HSC-3 Cancer Cell Line. Trop J Nat Prod Res. 2022; 6(4):520-528. doi.org/10.26538/tjnpr/v6i4.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

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 anti-diarrhoeal,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

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 (Bnke 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-picrylhydrazyl (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 (IC₅₀) were calculated. The inhibition percentage was calculated using the formula below.^{41,42}

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

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

The calculation of 50% Inhibitory Concentration (IC₅₀) 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 (IC₅₀) was calculated using the formula below.⁴³

$$\text{IC}_{50} = (50 - a) / b$$

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% CO₂ 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 IC₅₀ were calculated. The cell cytotoxicity percentage formula is given below.⁴⁶

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

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

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

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 IC₅₀ value

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 hydrazyl 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 IC₅₀ 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 IC₅₀ value of 73.69 µg/mL. The calculation of the IC₅₀ 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 VNBRC1.⁵⁵

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

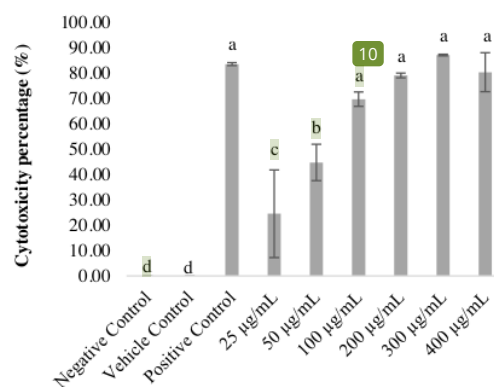


Figure 1: Effect of lemongrass leaf extract on cytotoxicity percentage of HSC-3 cell line.

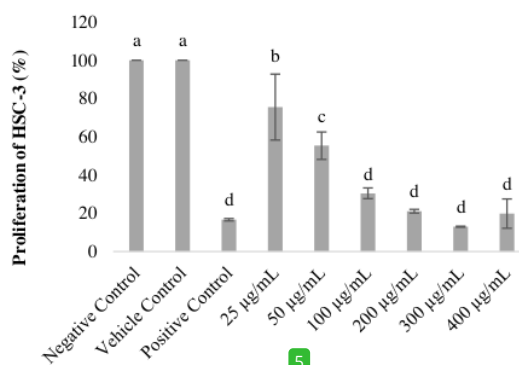


Figure 2: Effect of lemongrass leaf extract on proliferation percentage of HSC-3 cell lines.

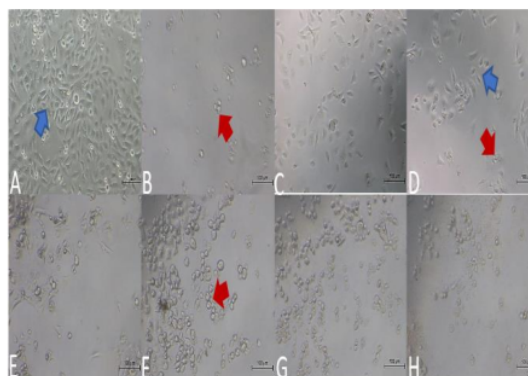


Figure 3: HSC-3 cell morphology after 24 hours of lemongrass leaf extract treatment.

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 7 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.

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 $\mu\text{g/mL}$; chronic myeloid erythroleukemia (K562) cancer cell line with an IC_{50} value of 57.9 $\mu\text{g/mL}$; and neuroblastoma cancer cell line (SH-SY5Y) with an IC_{50} value of 97.8 $\mu\text{g/mL}$.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 Caco-2 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 $\mu\text{g/mL}$, respectively); glioblastoma cancer cell lines (SF-767 and SF-763 with IC_{50} values of 45.13 and 172.05 $\mu\text{g/mL}$, 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, Phillion *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.¹³ inhibited growth of cervical cancer cell lines (HeLa and ME-180) with an IC_{50} value of 200 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ indicated that the wound area was still visible. Furthermore, at 25, 50, and 100 $\mu\text{g/mL}$, 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 $\mu\text{g/mL}$	High
21-200 $\mu\text{g/mL}$	Moderate
201-500 $\mu\text{g/mL}$	Low
>500 $\mu\text{g/mL}$	Not Toxic

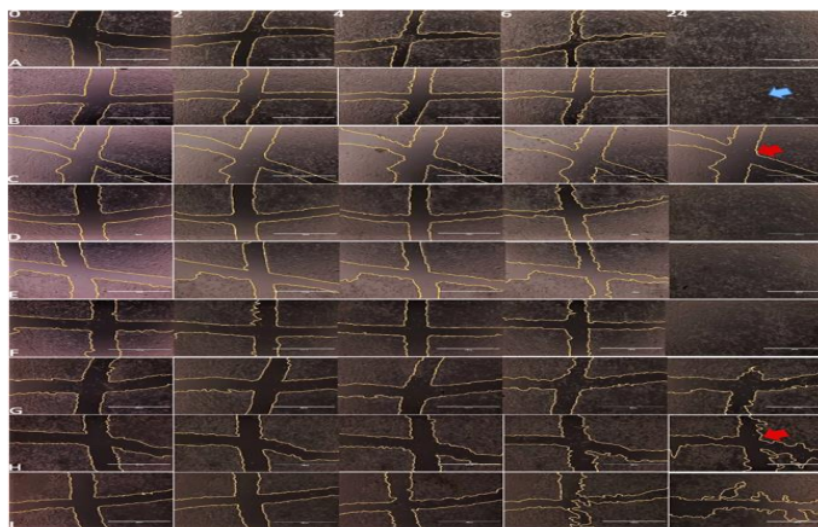


Figure 4: Wound closure in the migration test.

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 $\mu\text{g/mL}$ (D); extract 50 $\mu\text{g/mL}$ (E); extract 100 $\mu\text{g/mL}$ (F); extract 200 $\mu\text{g/mL}$ (G); extract 300 $\mu\text{g/mL}$ (H); and extract 400 $\mu\text{g/mL}$ (I). Observation was made with a magnification of 40x on a scale of 1000 μm .

Table 4: Wound closure of migration assay

Group	Repetition (n)	Migration \pm SD (million μm^2)				
		0 Hour	2 Hour	4 Hour	6 Hour	24 Hour
Negative control	3	1.70 \pm 0.13 ^{ab}	1.33 \pm 0.13 ^{bc}	1.09 \pm 0.17 ^c	0.91 \pm 0.14 ^d	0.00 \pm 0.00 ^c
Vehicle control	3	1.49 \pm 0.06 ^{ab}	1.33 \pm 0.10 ^{bc}	1.22 \pm 0.12 ^{bc}	1.07 \pm 0.14 ^d	0.00 \pm 0.00 ^c
Positive control	3	1.76 \pm 0.03 ^a	1.76 \pm 0.03 ^a	1.82 \pm 0.08 ^a	1.85 \pm 0.11 ^a	1.87 \pm 0.10 ^a
25 $\mu\text{g/mL}$	3	1.56 \pm 0.10 ^{ab}	1.39 \pm 0.07 ^{bc}	1.25 \pm 0.08 ^{bc}	1.12 \pm 0.07 ^{bcd}	0.00 \pm 0.00 ^c
50 $\mu\text{g/mL}$	3	1.45 \pm 0.09 ^b	1.30 \pm 0.14 ^c	1.20 \pm 0.20 ^{bc}	1.09 \pm 0.15 ^{cd}	0.00 \pm 0.00 ^c
100 $\mu\text{g/mL}$	3	1.51 \pm 0.11 ^{ab}	1.43 \pm 0.17 ^{bc}	1.28 \pm 0.11 ^{bc}	1.09 \pm 0.15 ^{cd}	0.04 \pm 0.01 ^c
200 $\mu\text{g/mL}$	3	1.74 \pm 0.09 ^a	1.64 \pm 0.09 ^{ab}	1.49 \pm 0.02 ^{ab}	1.44 \pm 0.00 ^b	1.36 \pm 0.13 ^b
300 $\mu\text{g/mL}$	3	1.63 \pm 0.06 ^{ab}	1.55 \pm 0.09 ^{abc}	1.48 \pm 0.05 ^{ab}	1.42 \pm 0.02 ^{bc}	1.36 \pm 0.14 ^b
400 $\mu\text{g/mL}$	3	1.69 \pm 0.14 ^{ab}	1.61 \pm 0.13 ^{abc}	1.42 \pm 0.15 ^{bc}	1.21 \pm 0.02 ^{bcd}	1.19 \pm 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 (saikosaponin, ginsenosides, etc.)	Anticancer	Induce apoptosis and immunomodulatory effects	79
Tannins (corilagin, ellagitannin, 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, xanthenes, 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 \cdot) 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	0.905	<0.001
	17.16	41.03		
	20.21	25.71		
50	39.53	45.33		
	37.26	37.18		
	15.03	51.43		
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 IC₅₀ 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.

4

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.

Acknowledgments

The authors wish to express their deepest gratitude to Professor Rahmi Amtha (Department of Oral Medicine), Professor Melanie Sadono Djamil (Department of Biochemistry and Molecular Biology), Doctor Monica Dewi Ranggani (Department of Physiology), and Doctor

5

Moehamad Orliando Roeslan (Department of Oral Biology), at the Trisakti University, for all the support and advice. We are also grateful to the Faculty of Dentistry at Trisakti University for their assistance, as well as the Cell Culture Laboratory at YARSI University and BALITRO for the materials.

References

- Rahayuwati L, Rizal IQ, Pahria T, Lukman M, Juniarti N. Pendidikan kesehatan tentang pencegahan penyakit kanker dan menjaga kualitas kesehatan. Media Karya Kesehatan. 2020; 3(1):59-69.
- Wijaya CA and Muchtaridi M. Pengobatan kanker melalui metode gen terapi. Farmaka. 2017; 15(1):53-68.
- Lan Z, Jia Z, Guo H, Yang Z, Yang Z, Pan X. Model of human tongue squamous cell lines stably transfected with human papillomavirus (HPV)16 E6 and E7 genes and biological characteristic analysis. Biomed Res Int. 2021; 2021:9968691.
- Xie S, Zhang XY, Shan XF, Yau V, Zhang JY, Wang W, Yan YP, Cai ZG. Hyperion image analysis depicts a preliminary landscape of tumor immune microenvironment in OSCC with lymph node metastasis. J Immunol Res. 2021; 2021:9975423.
- Langlais RP, Miller CS, Gehrig JS. Atlas berwarna lesi mulut yang sering ditemukan. 5th ed. Translator: Gunardi I. Jakarta: EGC; 2020; 162p.
- Yamamoto N and Shibahara T. Epidemiology of oral cancer. In: Kirita T and Omura K (editors). Oral cancer diagnosis and therapy. Tokyo: Springer; 2015. 3p.
- Hamdy R and Halim A. Squamous cell carcinoma of the oral tongue: a single institution retrospective cohort study from Mansoura university hospital. Indones J Cancer. 2019; 12(4):102-108.
- Ribeiro IP, Rodrigues JM, Mascarenhas A, Kosyakova N, Caramelo F, Liehr T, Melo JB, Carreira IM. Cytogenetic, genomic, and epigenetic characterization of the HSC-3 tongue cell line with lymph node metastasis. J Oral Sci. 2018; 60(1):70-81.
- Komariah K, Ageng A, Kusuma I. Efek kombinasi asam valproat dan nano kitosan kumbang tanduk (*Xylotrupes gideon*) terhadap viabilitas dan sitotoksitas sel kanker lidah (HSC-3). Proceedings of the 2nd national expert seminars; 2019 Mar 2; Jakarta: Lembaga Penelitian Universitas Trisakti; 2019.
- Vilen ST, Salo T, Sorsa T, Nyberg P. Fluctuating roles of matrix metalloproteinase-9 in oral squamous cell carcinoma. Sci World J. 2013; 2013:920595.
- Kurihara-Shimomura M, Sasahira T, Nakashima C, Kuniyasu H, Shimomura H, Kirita T. The multifarious functions of pyruvate kinase M2 in oral cancer cells. Int J Mol Sci. 2018; 19(10):2907.
- Rahmawati D, Anggraini W, Djamil M. Cytotoxicity of red fruit ethyl acetate extract (*Pandanus conoideus* lam.) on squamous cell carcinoma cell line (HSC-3). Sci Dent J. 2021; 5(1):42-46.
- Ohnishi S and Takeda H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. Front Pharmacol. 2015; 6:14.
- Shah G, Shri R, Panchal V, Sharma N, Singh B, Mann AS. Scientific basis for the therapeutic use of *Cymbopogon citratus*, stapf (lemongrass). J Adv Pharm Technol Res. 2011; 2(1):3-8.
- Chen Z, Ye X, Qingkui G, Wenliang Q, Wen Z, Ning W, Min Z. Anticancer activity of green synthesized AgNPs from *Cymbopogon citratus* (LG) against lung carcinoma cell line A549. IET Nanobiotechnol. 2019; 13(2):178-182.
- Li M, Liu B, Bernigaud C, Fischer K, Guillot J, Fang F. Lemongrass (*Cymbopogon citratus*) oil: a promising mitocidal and ovidical agent against *Sarcoptes scabiei*. PLoS Negl Trop Dis. 2020; 14(4):e0008225.

17. Rajesvari R and Lakshmi T. Lemon grass oil for improvement of oral health. Dent Hypotheses. 2013; 4(4):115-117.
18. Rubin CCB, Horn RC, Hirsch G, Gellatti GT, Tissiani AC, Calçada JPC, Heringer T, Cunha A. Redox alterations in pregnant women: antioxidant effect of lemongrass (*Cymbopogon citratus* (DC.) Stapf). Braz J Dev. 2020; 6(9):67171-67187.
19. Nguyen C, Mehaidli A, Baskaran K, Grewal S, Pupulin A, Ruvinov I, Scaria B, Parashar K, Vegh C, Pandey S. Dandelion root and lemongrass extracts induce apoptosis, enhance chemotherapeutic efficacy, and reduce tumour xenograft growth *in vivo* in prostate cancer. Evid-Based Compl Altern Med. 2019; 2019:2951428.
20. Devi RC, Sim SM, Ismail R. Effect of *Cymbopogon citratus* and citral on vascular smooth muscle of the isolated thoracic rat aorta. J Evid-Based Compl Altern Med. 2012; 2012:539475.
21. Umukoro S, Ben-Azu B, Ajayi AM, Adebisin A, Emokpae O. *Cymbopogon citratus* aqueous leaf extract attenuates neurobehavioral and biochemical changes induced by social defeat stress in mice. Chin Herb Med. 2020; 12(3):303-309.
22. Al Sears MD. Healing Herbs of Paradise: Discover useful, practical cures and treatments from a rich herbal tradition almost unknown in the western world. Florida: Wellness Research & Consulting; 2016. 218p.
23. Dudhgaonkar S, Mahajan M, Deshmukh S, Admane P, Khan H. Evaluation of anti-depressant effect of lemon grass (*Cymbopogon citratus*) in albino mice. Int J Basic Clin Pharmacol. 2014; 3(4):656-660.
24. Kruk J, Aboul-Enein BS, Bernstein J, Gronostaj M. Psychological stress and cellular aging in cancer: a meta-analysis. Oxid Med Cell Longev. 2019; 2019:1270397.
25. Kim YK, Na KS, Myint AM, Leonard BE. The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. Prog Neuropsychopharmacol Biol Psychiatry. 2016; 64:277-284.
26. Nuryadin Y, Naid T, Dahlia AA, Dali S. Kadar flavonoid total ekstrak etanol daun serai dapur dan daun alang-alang menggunakan spektrofotometri UV-VIS. Window of Health: J Kesehatan. 2018; 1(4):337-345.
27. Basera P, Lavania M, Agnihotri A, Lal B. Analytical investigation of *Cymbopogon citratus* and exploiting the potential of developed silver nanoparticle against the dominating species of pathogenic bacteria. Front Microbiol. 2019; 10:282.
28. Majewska E, Kozłowska M, Gruczyńska-Sekowska E, Kowalska D, Tamowska K. Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation – a review. Pol J Food Nutr Sci. 2019; 69(4):327-341.
29. Ranitha M, Nour AH, Sulaiman ZA, Nour AH, Thana Raj S. Comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) method. Int J Chem Eng Appl. 2014; 5(2):104-108.
30. Lu JJ, Bao JL, Chen XP, Huang M, Wang YT. Alkaloids isolated from natural herbs as the anticancer agents. Evid-Based Compl Altern Med. 2012; 2012:485042.
31. Rayan A, Raiyn J, Falah M. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. PLoS ONE. 2017; 12(11):e0187925.
32. El-Nabi SH, El-Garawani IE, El-Berry A. The anticancer potential of lemongrass and cinnamon extracts on human hepatocellular carcinoma cell line (HepG2): the possible effectiveness of combined treatment. Egypt J Exp Biol (Zoo). 2017; 13(2):139-148.
33. Sarangarajan R, Meera S, Rukkumani R, Sankar P, Anuradha G. Antioxidants: friend or foe? Asian Pac J Trop Med. 2017; 10(12):1111-1116.
34. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017; 18(1):96.
35. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015; 30(1):11-26.
36. Chusri S, Singthong P, Kaewmanee T. Antioxidant, anticancer, and cytotoxic effects of Thai traditional herbal preparations consumed as rejuvenators. CyTA J Food. 2015; 13(1):40-48.
37. Wang J, Luo B, Li X, Lu W, Yang J, Hu Y, Huang P, Wen S. Inhibition of cancer growth *in vitro* and *in vivo* by a novel ROS-modulating agent with ability to eliminate stem-like cancer cells. Cell Death Dis. 2017; 8(6):e2887.
38. Kawamura T, Tanisawa K, Kawakami R, Usui C, Ito T, Tabata H, Nakamura N, Kurosawa S, Choi W, Ma S, Radak Z, Sawada SS, Suzuki K, Ishii K, Sakamoto S, Oka K, Higuchi M, Muraoka I. Determinants of resting oxidative stress in middle-aged and elderly men and women: WASEDA'S health study. Oxid Med Cell Longev. 2021; 2021:1-11.
39. Wang S, Meckling KA, Marccone MF, Kakuda Y, Tsao R. Can phytochemical antioxidant rich foods act as anti-cancer agents? Food Res Int. 2011; 44(9):2545-2554.
40. Maesroh K, Kurnia D, Al-Anshori J. Perbandingan metode uji aktivitas antioksidan DPPH, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. Chimica et Natura Acta. 2018; 6(2):93-100.
41. Marvibaigi M, Amini N, Supriyanto E, Abdul Majid FA, Kumar Jaganathan S, Jamil S, Hamzehalipour Almaki J, Nasiri R. Antioxidant activity and ROS-dependent apoptotic effect of *Scurrula ferruginea* (Jack) Danser methanol extract in human breast cancer cell MDA-MB-231. PLoS ONE. 2016; 11(7):e0158942.
42. Wang WH, Hsuan KY, Chu LY, Lee CY, Tyan YC, Chen ZS, Tsai WC. Anticancer effects of *Salvia miltiorrhiza* alcohol extract on oral squamous carcinoma cells. Evid-Based Compl Altern Med. 2017; 2017:1-9.
43. Wilujeng S, Laksitarahmi IP, Suharnanik, Tiaransa P. Antioxidant maja fruit (*Aegle marmelos* (L) Carrea) lowering blood sugar mus musculus. Bir Ex J. 2020; 2(3):362-367.
44. Dona R, Frimayanti N, Ikhtiarudin I, Iskandar B, Maulana F, Silalahi NT. Studi *in silico*, sintesis, dan uji sitotoksik senyawa p-metoksi kalkon terhadap sel kanker payudara MCF-7. J Sains Farm Klin. 2019; 6(3):243-249.
45. Cai H, Li J, Zhang Y, Liao Y, Zhu Y, Wang C, Hou J. LDHA promotes oral squamous cell carcinoma progression through facilitating glycolysis and epithelial-mesenchymal transition. Front Oncol. 2019; 9:1446.
46. Wahjuningrum DA, Elizabeth ME, Puteri FH, Mardiyah AA, Subiyanto A. Cytotoxicity assay of sodium hypochlorite and Qmix on cultured human periodontal ligament fibroblast cells. J Int Oral Health. 2019; 11:204-207.
47. Orellana EA and Kasinski AL. Sulforhodamine B (SRB) assay in cell culture to investigate cell proliferation. Bio Protoc. 2016; 6(21):1-9.
48. Nicklisch SC and Waite JH. Optimized DPPH assay in a detergent based buffer system for measuring antioxidant activity of proteins. MethodsX. 2014; 1:233-238.
49. Hasim H, Nasution SP, Kurniawati SO, Rachmawati I. Aktivitas sitotoksik sitral serai sebagai antikanker payudara MCM-B2. Curr Biochem. 2020; 7(1):29-36.
50. Rabima R, Pangaman SD. Formulasi dan uji aktivitas antioksidan dari sediaan masker *peel-off* ekstrak umbi wortel varietas chantenay (*Daucus carota* L.). Indones Nat Res Pharm J. 2020; 5(2):135-148.

51. Purwanto D, Bahri S, Ridhay A. Uji aktivitas antioksidan ekstrak buah purnajawa (*Kopsia arborea* Blume.) dengan berbagai pelarut. Kovalen. 2017; 3(1):24-32.
52. Galvao J, Davis B, Tilley M, Normando E, Duchon MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. FASEB J. 2014; 28(3):1317-1330.
53. Chen JL, Lai CY, Ying TH, Lin CW, Wang PH, Yu FJ, Liu CJ, Hsieh YH. Modulating the ERK1/2-MMP1 axis through corosolic acid inhibits metastasis of human oral squamous cell carcinoma cells. Int J Mol Sci. 2021; 22(16):8641.
54. Kim LH, Khadka S, Shin JA, Jung JY, Ryu MH, Yu HJ, Lee HN, Jang B, Yang IH, Won DH, Kwon HJ, Jeong JH, Hong SD, Cho NP, Cho SD. Nitidine chloride acts as an apoptosis inducer in human oral cancer cells and a nude mouse xenograft model via inhibition of STAT3. Oncotarget. 2017; 8(53):91306-91315.
55. Nguyen S, Nguyen H, Truong K. Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. BMRAT. 2020; 7(7):3855-3859.
56. Capriotti K and Capriotti JA. Dimethyl sulfoxide: history, chemistry, and clinical utility in dermatology. J Clin Aesthet Dermatol. 2012; 5(9):24-26.
57. Yuan C, Gao J, Guo J, Bai L, Marshall C, Cai Z, Wang L, Xiao M. Dimethyl sulfoxide damages mitochondrial integrity and membrane potential in cultured astrocytes. PLoS ONE. 2014; 9(9):e107447.
58. Verheijen M, Lienhard M, Schrooders Y, Clayton O, Nudischer R, Boerno S, Timmermann B, Selevsek N, Schlapbach R, Gmuender H, Gotta S, Geraedts J, Herwig R, Kleinjans J, Caiment F. DMSO induces drastic changes in human cellular processes and epigenetic landscape *in vitro*. Sci Rep. 2019; 9(1):4641.
59. Damasuri AR, Sholikhah EN, Mustofa M. Cytotoxicity of ((E)-1-(4-aminophenyl)-3-phenylprop-2-en-1-one)) on hela cell line. Indon J Pharmacol Ther. 2020; 1(2):54-59.
60. Thangam R, Sathuvan M, Poongodi A, Suresh V, Pazhanichamy K, Sivasubramanian S. Activation of intrinsic apoptotic signaling pathway in cancer cells by *Cymbopogon citratus* polysaccharide fractions. Carbohydr Polym. 2014; 107:138-150.
61. Ruvinov I, Nguyen C, Scaria B, Vegh C, Zaitoon O, Baskaran K, Mehaidli A, Nunes M, Pandey S. Lemongrass extract possesses potent anticancer activity against human colon cancers, inhibits tumorigenesis, enhances efficacy of FOLFOX, and reduces its adverse effects. Integr Cancer Ther. 2019; 18:1-13.
62. Bayala B, Bassole IHN, Maqdas S, Baron S, Simporé J, Lobaccaro JA. *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule. Biochimie. 2018; 153:162-170.
63. Maruoka T, Kitanaka A, Kubota Y, Yamaoka G, Kameda T, Imataki O, Dobashi H, Bandoh S, Kadowaki N, Tanaka T. Lemongrass essential oil and citral inhibit Src/Stat3 activity and suppress the proliferation/survival of small cell lung cancer cells, alone or in combination with chemotherapeutic agents. Int J Oncol. 2018; 52(5):1738-1748.
64. Trang DT, Hoang TKV, Nguyen TTM, Van Cuong P, Dang NH, Dang HD, Nguyen Quang T, Dat NT. Essential oils of lemongrass (*Cymbopogon citratus* Stapf) induces apoptosis and cell cycle arrest in A549 lung cancer cells. Biomed Res Int. 2020; 2020:5924856.
65. Phillion C, Ma D, Ruvinov I, Mansour F, Pignatelli C, Noel M, Saleem A, Arnason J, Rodrigues M, Singh I, Ropat J, Pandey S. *Cymbopogon citratus* and *Camellia sinensis* extracts selectively induce apoptosis in cancer cells and reduce growth of lymphoma xenografts *in vivo*. Oncotarget. 2017; 8(67):110756-110773.
66. Halabi MF and Sheikh BY. Anti-proliferative effect and phytochemical analysis of *Cymbopogon citratus* extract. Biomed Res Int. 2014; 2014:906239.
67. Rojas-Armas JP, Arroyo-Acevedo JL, Palomino-Pacheco M, Herrera-Calderón O, Ortiz-Sánchez JM, Rojas-Armas A, Calva J, Castro-Luna A, Hilario-Vargas J. The essential oil of *Cymbopogon citratus* Stapf and carvicol: an approach of the antitumor effect on 7,12-Dimethylbenz-[a]-anthracene (DMBA)-induced breast cancer in female rats. Molecules. 2020; 25(14):3284.
68. Ghosh K. Anticancer effect of lemongrass oil and citral on cervical cancer cell lines. Pharmacogn Commun. 2013; 3(4):41-48.
69. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA, Karaman R. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins (Basel). 2019; 11(11):656.
70. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutr. 2020; 12(2):457.
71. Dey P, Kundu A, Chakraborty HJ, Kar B, Choi WS, Lee BM, Bhakta T, Atanasov AG, Kim HS. Therapeutic value of steroidal alkaloids in cancer: current trends and future perspectives. Int J Cancer. 2019; 145(7):1731-1744.
72. Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Sci Afr. 2019; 6:e00137.
73. Andikoputri SF, Komariah K, Roeslan MO, Ranggani D, Bustami DA. Nano chitosan encapsulation of *Cymbopogon citratus* leaf extract promotes ROS induction leading to apoptosis in human squamous cells (HSC-3). Curr Issues Pharm Med Sci. 2021; 34(3):134-137.
74. Veronica G, Komariah K, Gabriella Clara Maria L, editors. Microencapsulation of lemongrass leaves effects on reactive oxygen species (ROS) fibroblasts. 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce); 2021 July 14-16; 2021.
75. Shendurse AM, Sangwan RB, Amit K, Ramesh V, Patel AC, Gopikrishna G, Roy SK. Phytochemical screening and antibacterial activity of lemongrass (*Cymbopogon citratus*) leaves essential oil. J Pharmacogn Phytochem. 2021; 10(2):445-449.
76. Madivoli ES, Gita L, Gumba E. Isolation and identification of essential oils from *Cymbopogon citratus* (Stapf) Dc using Ge-MS and Ft-Ir. Chem Mater Res. 2012; 2(4):13-22.
77. Kiseleva LN, Kartashev AV, Vartanyan NL, Pinevich AA, Samoilovich MP. A172 and T98G cell lines characteristics. Cell Tissue Biol. 2016; 10(5):341-348.
78. Macáková K, Afonso R, Saso L, Mladěnka P. The influence of alkaloids on oxidative stress and related signaling pathways. Free Rad Biol. 2019; 134:429-444.
79. Elekofehinti OO, Iwaloye O, Olawale F, Ariyo EO. Saponins in cancer treatment: current progress and future prospects. Pathophysiol. 2021; 28(2):250-272.
80. Sieniawska E. Activities of tannins – from *in vitro* studies to clinical trials. Nat Prod Commun. 2015; 10(11):1877-1884.
81. Ghante MH and Jamkhande PG. Role of pentacyclic triterpenoids in chemoprevention and anticancer treatment: an overview on targets and underlying mechanisms. J Pharmacopunct. 2019; 22(2):55-67.
82. Bouriche H, Kada S, Senator A, Demirtas I, Ozen T, Toptanci BÇ, Kizil G, Kizil M. Phenolic content and biomolecule oxidation protective activity of *Globularia alypum* extracts. Braz Arch Biol Technol. 2017; 60:e17160409.
83. Anantharaju PG, Gowda PC, Vimalabike MG, Madhunapantula SV. An overview on the role of dietary phenolics for the treatment of cancers. Nutr J. 2016; 15(1):99.
84. Zeng S, Kapur A, Patankar MS, Xiong MP. Formulation, characterization, and antitumor properties of trans- and cis-citral in the 4T1 breast cancer xenograft mouse model.

- Pharm Res. 2015; 32(8):2548-2558.
85. Zhao Y, Meng X, Zeng Y, Wang C, Chen J, She Z. Linalool inhibits MCF-7 tumor growth in a xenograft model by apoptosis induction and immune modulation. Nat Prod Commun. 2021; 16(5):1-4.
 86. Chang MY, Shieh DE, Chen CC, Yeh CS, Dong HP. Linalool induces cell cycle arrest and apoptosis in leukemia cells and cervical cancer cells through CDKs. Int J Mol Sci. 2015; 16(12):28169-28179.
 87. Iwasaki K, Zheng YW, Murata S, Ito H, Nakayama K, Kurokawa T, Sano N, Nowatari T, Villareal MO, Nagano YN, Isoda H, Matsui H, Ohkohchi N. Anticancer effect of linalool via cancer-specific hydroxyl radical generation in human colon cancer. World J Gastroenterol. 2016; 22(44):9765-9774.
 88. Grigalius I and Petrikaite V. Relationship between antioxidant and anticancer activity of thihydroflavones. Molecules. 2017; 22(12):2169.
 89. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015; 20(12):21138-21156.
 90. Rauf A, Imrah M, Khan IA, Ur-Rehman M, Gilani SA, Mehmood Z, Mubarak MS. Anticancer potential of quercetin: a comprehensive review. Phytother Res. 2018; 32(11):2109-2130.
 91. Hermi K, Subarnas A, Diantini A, Iskandar Y. Cytotoxicity of quercetin and quercetin-3-O-rhamnoside of *Ertlingera elatior* (Jack) R.M.Sm. leaves against HeLa cervical cancer cells. J Appl Pharm Sci. 2021; 11(05):085-090.
 92. Eghbaliferiz S and Iranshahi M. Prooxidant activity of polyphenols, flavonoids, anthocyanins and carotenoids: updated review of mechanisms and catalyzing metals. Phytother Res. 2016; 30(9):1379-1391.
 93. Shin J, Song MH, Oh JW, Keum YS, Saini RK. Pro-oxidant actions of carotenoids in triggering apoptosis of cancer cells: a review of emerging evidence. Antioxid (Basel). 2020; 9(6):532.
 94. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoter. 2011; 82(4):513-523.
 95. Koçyiğit A and Selek Ş. Exogenous antioxidants are double-edged swords. Bezmialem Science. 2016; 2:70-75.

ORIGINALITY REPORT

16%

SIMILARITY INDEX

12%

INTERNET SOURCES

8%

PUBLICATIONS

14%

STUDENT PAPERS

PRIMARY SOURCES

1

Submitted to Fakultas Kedokteran Gigi
Universitas Trisakti

Student Paper

4%

2

Submitted to Universitas Airlangga

Student Paper

3%

3

Submitted to Badan PPSDM Kesehatan
Kementerian Kesehatan

Student Paper

2%

4

Submitted to Syiah Kuala University

Student Paper

1%

5

e-journal.unair.ac.id

Internet Source

1%

6

www.sciendo.com

Internet Source

1%

7

Submitted to Universiti Sains Malaysia

Student Paper

1%

8

mzuir.inflibnet.ac.in

Internet Source

1%

9

researchid.co

Internet Source

1 %

10

www.hindawi.com

Internet Source

1 %

11

bmccomplementalternmed.biomedcentral.com

Internet Source

1 %

12

vital.seals.ac.za:8080

Internet Source

1 %

13

Andrea M. Tomko, Erin G. Whynot, Lee D. Ellis, Denis J. Dupré. "Anti-Cancer Potential of Cannabinoids, Terpenes, and Flavonoids Present in Cannabis", Cancers, 2020

Publication

1 %

Exclude quotes On

Exclude matches < 1%

Exclude bibliography On

Dear Felicia,
Thank you for your submission to 'The Tropical Journal of Natural Product Research' (www.tjnpr.net) (<https://www.scopus.com/sourceid/211100937270>).
SCOPUS, published by the University of Borneo and Natural Product Research Series.



The peer-review process will commence immediately, as the manuscript will be passed to an editor for initial assessment as soon as possible.

Title: **Antioxidant from Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract as Anticancer Towards HSC-3 Cancer Cell Line**

Best regards,

Wendy

Professor Abiodun Fabodun, PhD

Editor-in-Chief
Tropical Journal of Natural Product Research (TJNPR)
Nat. Natural Product Research Series, University of Borneo
Email: abiodun.fabodun@uniborneo.edu.my
www.tjnpr.org SCOPUS, SCOPUS ID: 24 9117
https://www.scopus.com/sourceid/tjnpr.pdf

Professor of Pharmaceutical Chemistry
Fakulti Farmasi (UKF)
Universiti Kebangsaan Malaysia (UKM) 43600
Faculty of Pharmacy
University of Borneo
Phone: +654-857-3116-4488
Email: abiodun.fabodun@uniborneo.edu.my
Google Scholar: <https://scholar.google.com/citations?hl=en&user=abiodun.fabodun>
SCOPUS: <https://www.scopus.com/sourceid/tjnpr.pdf>

https://doi.org/10.1002/2023.1100937270

10/10/2023 10:10:00 AM



Show quoted text

OS1- Felicia 10/10/2023 🔍 ⋮
to Editor-in-Chief...

Good evening,

When I responded, Prof. Abiodun Fabodun

for some I just read one email because the notification is off. 🙄

We would like to suggest following reviewers:
1. Karim Arifin (Department of Natural Products, Faculty of Dentistry, Trusmi University) Email: karim.arifin@trusmi.ac.id

2. Nurul Hafidza (Department of Biochemistry and Molecular Biology, Faculty of Dentistry, Trusmi University) Email: nurulhafidza@trusmi.ac.id

3. Ignat (Department of Drug Chemistry, Faculty of Pharmacy, Universiti Kebangsaan Malaysia) Email: ignat@pharmacy.ukm.edu.my

All authors email address:
1. Felicia felicia.fabodun@uniborneo.edu.my
2. Karim Arifin karim.arifin@trusmi.ac.id
3. Nurul Hafidza nurulhafidza@trusmi.ac.id

The roles in this study:
1. Felicia (1st author) development of main idea, literature research, write manuscript, funding
2. Karim Arifin (2nd author) and corresponding author concept of idea, data analysis, check manuscript
3. Nurul Hafidza (3rd author) laboratory works, funding

Best regards,

Felicia

Show quoted text

Manuscript Under Peer-Review Process

Inbox



Editor-in-Chief... 2/19/2022

to me, komariah, indra.kusuma



The manuscript submitted to the Tropical Journal of Natural Product Research <https://www.scopus.com/sourceid/21100933230> SCOPUS by the corresponding author is undergoing the peer-review process.

Title: Antioxidant from Lemongrass (*Cymbopogon citratus*) Leaf Ethanol Extract as Anticancer Towards HSC-3 Cancer Cell Line

Journal: Tropical Journal of Natural Product Research www.tjnpr.org

Corresponding Author: **Komariah,**

Co-authors: **Felicia , Indra Kusuma**

Manuscript No: TJNPR OC249ARN

If you have any objections, please contact the editorial office as soon as possible. If we do not hear from you, we will assume you agree with your co-authorship.

If you did not co-author this submission, please contact the corresponding author directly

Thank you very much.

Best regards

Abiodun

Professor Abiodun Falodun, PhD

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@uniben.edu; editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q4 0.13
<https://www.scopus.com/sourceid/uniben>

Professor of Pharmaceutical Chemistry
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy
University of Benin
Phone: +234-807-318-4488;
email: falodabi@uniben.edu; abiodun.falodun@fulbrightmail.org
Google Scholar Citations
SCOPUS [https://www.scopus.com/authid/detail.uri?](https://www.scopus.com/authid/detail.uri?https://orcid.org/0000-0003-2929-3305)

<https://orcid.org/0000-0003-2929-3305>
[https://www.scopus.com/authid/detail.uri?](https://www.scopus.com/authid/detail.uri?https://orcid.org/0000-0003-2929-3305)



University of Benin www.uniben.edu TJNPR SCOPUS Q4 www.tjnpr.org

11:55

Revised manuscript (398K) ☆

 **O51- Felicia** 3/14/2022
to Editor-in-Chief w

Good morning,

When I respectfully, Prof. Abiodun Fabodun

Thank you for your kindness for giving me more time to revise the manuscript, today I want to send you the revised manuscript with an English professional editing certificate and an iThenticate plagiarism report.

Thank you so much, Prof. 🙏🙏


Best regards,

Felicia

 Responses to comments.docx

 Potential of...te result.pdf

 Copy of Certif...editing.pdf

 Manuscript_for TAPAC.docx

 **Editor-in-Chief Tjnpjr** 3/14/2022
Thank you, recommended regards Abiodun

 **O51- Felicia** 3/14/2022
Thank you for letting me know Prof. 🙏🙏 Best regards, Felicia

 **O51- Felicia** 3/17/2022
to Editor-in-Chief w

Good morning,

When I respectfully, Prof. Abiodun Fabodun

Prof, I am I add about 8 words "natural antioxidants potentially as antioxidant" at the start of abstract and the conclusion!

Because I'm afraid that the conclusion not enough. 🙏🙏

Here's the file that I put that about six words in Prof. (the green highlights)

I'm really really sorry, my deepest apologies, Prof. 🙏🙏

Thank you so much, Prof. 🙏🙏

Best regards,

Felicia

On Sat, Mar 26, 2022, 20:04 Editor-in-Chief Tjnpjr <EditorTjnpjr@gmail.com> wrote:

She quoted text

 Abstract and...revision.docx

 **Editor-in-Chief Tjnpjr** 3/17/2022
Recommended regards Abiodun

 **O51- Felicia** 3/17/2022
to Editor-in-Chief w

Thank you so much for your kindness, Prof. 🙏🙏

Best regards,

Felicia

She quoted text

↩ Reply

↩ Reply all

➡ Forward

11:57

📶 🔋 🔍

🔍 📧 📁 📧 📧



D51- Felicia

to Editor-in-Chief

Good afternoon,

When I respectfully, Prof. Abiodun Falodun

The manuscript already revised, Prof. 🙏🙏 The manuscript, responds to managing editor reviews, and the certificate will be in attachment, Prof. 🙏🙏
I'm so sorry for my delay, because of the unexpected, 🙏🙏

Thank you for your kindness, Prof. 🙏🙏

Best regards,

Felicia

Show quoted text

 Edited Manus... Felicia.docx

 Responses to...ments.docx

 Proofreading... Felicia.pdf



Editor-in-Chief Tynpr

Thank you best regards Abiodun



D51- Felicia

Thank you, Prof. 🙏🙏 Best regards, Felicia



Draft

to Editor-in-Chief

Good afternoon,

When I respectfully, Prof. Abiodun Falodun

I have already tested the Libriatike app for the second revision, Prof., the result will be on the attachment... I think I have to send you the result, just in case needed, 🙏🙏

Best regards,

Felicia

On Mon, Apr 4, 2022 at 8:17 AM D51- Felicia <felicia.falodun@gmail.com> wrote:
Thank you, Prof. 🙏🙏

Best regards,

Felicia

On Mon, Apr 4, 2022, 06:11 Editor-in-Chief Tynpr <tynpr@tynpr.com> wrote:
Thank you

Best regards

Abiodun

Professor Abiodun Falodun, PhD

Editor-in-Chief

Topical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email address: lynpr@uniben.edu tynpr@gmail.com

www.tynpr.org www.tynpr.org www.tynpr.org www.tynpr.org

www.tynpr.org www.tynpr.org www.tynpr.org www.tynpr.org

Professor of Pharmaceutical Chemistry

Fellow, Publishers USA

Deputy Vice-Chancellor (Academic) 2014-2018

Faculty of Pharmacy

University of Benin

Phone: +234-807-51284466

email: falodun@uniben.edu

abiodun@uniben.edu

Google Scholar: <https://www.google.com/citations?user=falodun>

Scopus: [https://orcid.org/0000-0002-7129-7129](https://www.scopus.com/authid/detail.uri?https://orcid.org/0000-0002-7129-7129)

<https://orcid.org/0000-0002-7129-7129>



University of Benin

www.uniben.edu



Topical Journal of Natural Product Research

www.tynpr.org

On Sat, 3 Apr 2022 at 22:55, D51- Felicia <felicia.falodun@gmail.com> wrote:
Good afternoon,

When I respectfully, Prof. Abiodun Falodun

The manuscript already revised, Prof. 🙏🙏 The manuscript, responds to managing editor reviews, and the certificate will be in attachment, Prof. 🙏🙏
I'm so sorry for my delay, because of the unexpected, 🙏🙏

Thank you for your kindness, Prof. 🙏🙏

Best regards,

Felicia

On Fri, Apr 1, 2022 at 11:38 PM D51- Felicia <felicia.falodun@gmail.com> wrote:
Good evening,

When I respectfully, Prof. Abiodun Falodun

Prof. the manuscript with today still in progress by Prof. Quodon Shetta, Prof. 🙏🙏

Can you give me time to send you the revised manuscript tomorrow or day after tomorrow (if the manuscript hasn't done tomorrow, Prof)? 🙏🙏

I'm sorry for the delay, Prof. 🙏🙏 (the deadline of revision on the email is before 2nd April)

Because, first delay from my professor send the email towards me and second from the person I request help to transfer the charge of editing, 🙏🙏

I'm so sorry Prof. 🙏🙏

Really really sorry, Prof. 🙏🙏



Edited Manuscript of Felicia



Inbox



OLALEKAN SHITTU 4/2/2022

to me



Hello,

Please, find attached the edited version of your manuscript and the Certificate of Proofreading.

We tried to restrict our service to the English editing of our clients' manuscripts and tried not to act as a reviewer. Some of our clients are not comfortable informing them of the shortcomings in their manuscripts. However, here are some comments to consider for making some improvements to the manuscript.

- 1) Page 5: ppm is not an SI unit. Please, convert and replace all values expressed in ppm with a SI unit.
- 2) Page 8: Include the version of SPSS used for the analysis.
- 3) Page 8: Please double check, is it hydrazyl or hydroxyl?

Also, please take note of the following:

- 1) This manuscript has been properly formatted according to the style of the TJNPR.
- 2) We only did the English editing and formatting, as the reviewers may or may not raise some technical issues in the manuscript.
- 3) The proofreading process was done in a way that did not alter the author's intended meaning, but it is highly recommended to go through the entire manuscript to double-check for accuracy.

Thank you so much and we wish you success in your publication endeavor. .

Best regards,

Prof. Hakeem Shittu
Director,
SBL Proofreading Services,
University of Benin,
Benin City,
Nigeria

"Disclaimer"

This communication is intended for the above named person and is confidential and / or legally privileged. Any opinion(s) expressed in this communication are not necessarily those of UniBen (University of Benin). If it has come to you in error you must take no action based upon it, nor must you print it, copy it, forward it, or show it to anyone. Please delete and destroy the e-mail and any attachments and inform the sender immediately. Thank you.

UniBen is not responsible for the political, religious, racial or partisan opinion in any correspondence conducted by its domain users. Therefore, any such opinion expressed, whether explicitly or implicitly, in any said correspondence is not to be interpreted as that of UniBen.

UniBen may monitor all incoming and outgoing e-mails in line with UniBen business practice. Although UniBen has taken steps to ensure that e-mails and attachments are free from any virus, we advise that, in keeping with best business practice, the recipient must ensure they are actually virus free.



Proofreading...e (Felicia).pdf



Edited Manusc...f Felicia.docx



051- Felicia 4/3/2022

Good evening, Whom I respect fully, Prof. Hakeem

**051- Felicia** 4/4/2022

to Editor-in-Chief ▾



Good afternoon,

Whom i respectfully, Prof. Abiodun Falodun

The manuscript already revised, Prof.. 🙏 The manuscript, responds to managing editor reviews, and the certificate will be on attachment, Prof.. 🙏

I'm so sorry for my delay, because of the unexpected.. 🙏

Thank you for your kindness, Prof.. 🙏

Best regards,

Felicia

[Show quoted text](#)



Edited Manusc...f Felicia.docx



Responses to...mments.docx



Proofreading...e (Felicia).pdf

**Editor-in-Chief Tjnpr** 4/4/2022

Thank you Best regards Abiodun

**051- Felicia** 4/4/2022

Thank you, Prof.. 🙏🙏 Best regards, Felicia

**Draft** 4/6/2022

I have already tested the ithenticate again for the second revision, Prof.. the result will be on th

Payment proof of manuscript

OC249ARN Inbox



051- Felicia 3/11/2022
to Editor-in-Chief ▾



Good evening,

Whom I Respectfully, Prof. Abiodun Falodun

Thank you for accepting our manuscript Prof,
I would like to send the payment proof of manuscript TJNPR
OC249ARN, with title : Antioxidant from Lemongrass
(Cymbopogon citratus) Leaf Ethanol Extract as Anticancer
Towards HSC-3 Cancer Cell Line. with authors: Felicia,
komariah,* Indra kusuma.

The bank said that will arrive at Guaranty Trust Bank Plc
around 2 until 5 days.

Thank you so much Prof..

Best Regards,

Felicia



Transfer for...249ARN.jpeg



Draft 3/11/2022

On Fri, Mar 11, 2022 at 10:55 AM 051- Felicia
wrote: Good evening, Whom I Respectfully, Prof.



Editor-in-Chief... 3/11/2022
to me ▾



The review comments will be sent on Monday next week

Best regards

Abiodun

Professor Abiodun Falodun, PhD

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@uniben.edu; editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q4 0.13
<https://www.scopus.com/sources.uri>

Professor of Pharmaceutical Chemistry

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016

Faculty of Pharmacy

University of Benin

Phone: +234-807-3184488;

email: faloabi@uniben.edu; abiodun.falodun@fulbrightmail.org

Google Scholar Citations

SCOPUS <https://www.scopus.com/authid/detail.uri?>

<https://orcid.org/0000-0003-2929-3305>
authid=12794326500#top



University of Benin
www.uniben.edu

TJNPR SCOPUS Q4
www.tjnpr.org

Galley proof revision Inbox**051- Felicia** 4/26/2022

to p.editor.tjnpr, Editor-in-Chief ▾



Good evening,

Whom i respectfully, Prof. Abiodun Falodun and Managing Editor

Thank you for the email, i will attached the galley proof revision in this email..🙏

Thank you...🙏

Best Regards,
Felicia



TJNPR-2022 ...-revision.docx

**Managing Editor...** 4/30/2022

to me ▾



Received.

[Show quoted text](#)

**051- Felicia** 4/30/2022

to Managing ▾



Good morning,

Whom i respectfully, Managing editor

Thank you for letting me know..🙏

Best regards,
Felicia

[Show quoted text](#)

Reply

Reply all

Forward

**051- Felicia** 4/3/2022

to OLALEKAN ▾



Good evening,

Whom I respectfully, Prof. Hakeem Shittu

I have received the email, Thank you so much Prof..🙏

Prof, may I ask? the reviewer suggested to include the limitation of the research.. could I write this Prof?

"Limitations

The limitations in this research are observation of proliferation only observed in 24 hours and doesn't perform fractionation in extraction to determine anticancer and antioxidant activity of each active compound on HSC-3 cell line."

Are the grammar are correct, Prof?

Thank you so much..🙏

Best regards,

Felicia

[Show quoted text](#)

**OLALEKAN SHITTU** 4/3/2022

to me ▾



Hello,

Thank you so much for acknowledging the receipt of the edited manuscript.

No problem, if the reviewer asked you to include the limitations of the study. I can include that and send it back to you later in the day.

Best regards,

Prof. Hakeem Shittu
Director,
SBL Proofreading Services,
University of Benin,
Benin City,
Nigeria

[Show quoted text](#)