RESEARCH ARTICLE

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Cytoprotective Properties of Citronella Oil (*Cymbopogon nardus* (L.) Rendl.) and Lemongrass Oil (*Cymbopogon citratus* (DC.) Stapf) through Attenuation of Senescent-Induced Chemotherapeutic Agent Doxorubicin on Vero and NIH-3T3 Cells

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Abstract

Objective: This study aimed to determine the cytoprotective potentials of citronella (*Cymbopogon nardus* (L.) Rendl.) essential oil (CO) and lemongrass (*Cymbopogon citratus* (DC.) Stapf) essential oil (LO). **Methods:** The essential oils from citronella and lemongrass were obtained by steam-water distillation, then analyzed using Gas Chromatography-Mass Spectrophotometry (GC-MS) to determine the chemical constituents. The antioxidant activity of CO and LO was compared using a total antioxidant capacity kit. The viability of normal kidney epithelial cells Vero and fibroblast NIH-3T3 as the cell models were tested using a trypan blue exclusion assay. The effect of cellular senescence inhibition on both cell models was measured using senescence-associated β-galactosidase (SA-β-gal) staining. The mechanism of action of CO and LO in the protection of cellular damage against doxorubicin was also confirmed through 2',7'–dichlorofluorescin diacetate (DCFDA) staining to discover the ability to decrease reactive oxygen species (ROS) levels and a gelatin zymography assay to observe the activity of matrix metalloproteinases (MMPs). **Results:** The major marker components of CO and LO were citronellal and citral, respectively. Both oils showed low cytotoxic activity against Vero and NIH-3T3 cells, with IC_{50} values of over 40 µg/mL. LO exhibited higher antioxidant capacity than CO, but there was no effect on the intracellular ROS level of both oils on Vero and NIH-3T3 cells. However, CO and LO decreased cellular senescence induced by doxorubicin exposure on both cells, as well as suppressed MMP-2 expression. **Conclusion:** Both CO and LO decrease the cellular senescence and MMP-2 expression with less cytotoxic effects on normal cells independently from their antioxidant capacities. The results were expected to support the use of CO and LO as tissue protective and anti-aging agents in maintaining the body's cellular health against chemotherapeutics or cellular damaging agents.

Keywords: Citronella and lemongrass oils- cytoprotective- cellular senescence- MMP-2- fibroblast and kidney cells

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Introduction

In modern life, humans face various risks such as a dirty environment (pollution), harmful lifestyle, damaging agents including drugs, and unhealthy food intake that affect cellular homeostasis. Therefore, finding agents that can tackle the issue of cellular damage remains a significant challenge. These risks are generally associated with an increase in cellular oxidative stress that causes macromolecular damage in cells and leads to cellular aging (Zulfin et al., 2021). This event can affect both cells on the body surface and cells in vital organs, such as the kidney, and will cause both changes in body appearance and physiological disorders such as kidney damage (Haryanti et al., 2022). Fortunately, we have agents that can overcome oxidative stress from nature due to their abundant availability and many compounds containing antioxidant properties (Meiyanto and Larasati, 2019). For example, Cymbopogon is known to have many health benefits and is promising as a cytoprotective agent that inhibits the process of cell damage (Bayala et al., 2018). The genus of Cymbopogon consists of more than one hundred species of aromatic grasses (Kumoro et al., 2021). Two of them that are most often used in the community

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are citronella (*Cymbopogon nardus* (L.) Rendl.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf). Citronella is generally used as aromatherapy and in cosmetic or skin care products (Sharma et al., 2019). Meanwhile, lemongrass is widely used as a cooking spice and directly consumed in a form of refreshing and healthy drink (Shabbir et al., 2019). Overall, citronella and lemongrass are interesting to be investigated as cytoprotective agents for both skin cells and important organs such as the kidney.

Citronella and lemongrass are two similar Cymbopogon species that possess differences that are identifiable (Kumoro et al., 2021). Citronella plant's height is greater than that of lemongrass. The color of the citronella stems is reddish, while the lemongrass stems are greenish. The smell of the two plants is also different from each other. These properties support the distinctiveness of their use in daily life. Usually, people utilize citronella as a soothing fragrance and lemongrass as a taste enrichment in meals and beverages. Citronella contains compounds that are beneficial for health, including citronellal as the marker (Bayala et al., 2020). Citronellal as the citronella's active compound has been reported to be able to capture free radical compounds (Rastuti et al., 2020) Meanwhile, lemongrass contains citral, which can increase the activity of antioxidant enzymes in the body (Bakhtiari et al., 2021). Due to the beneficial and supportive pharmacological effects of the compounds, both of these plants have the potential to be developed as anti-aging or cytoprotective agents, requiring a more in-depth investigation. Therefore, it is necessary to investigate the prospect of the above-mentioned Cymbopogon and the distinctive differences between the two species as anti-aging or tissue protective agents.

Aging is a physiological process that involves some molecular events that promote senescence and microenvironmental matrix degradation (Larasati et al., 2018) that can be induced by cellular stress and chemotherapeutic agents (cellular damaging agents), such as doxorubicin (Zulfin et al., 2021). Cellular senescence is one of the most critical processes of aging, marked by cell cycle arrest, vacualitation, and gradually leads to cell death (apoptosis) (Ahlina et al., 2020). This process is usually associated with high cellular ROS activity. Whereas, microenvironmental matrix degradation is the phenomenon accompanying cellular aging process marked by the expression of MMPs such as MMP-2 that are also associated with cellular oxidative stress (Oszajca and Szemraj, 2021). These protease enzymes digest extracellular matrices (ECMs) such as collagen, resulting in the disruption of tissue integrity (Bates et al., 2015). All these processes can occur in all parts of the body, especially in fibroblast cells (Howard et al., 2012). These two processes are interesting as targets for anti-aging agents. Since the compounds in Cymbopogon possess antioxidant properties, they are expected to inhibit senescence and MMPs activity in cellular premature aging.

This study explores the potential anti-cellular senescence properties of essential oils from CO and LO, including the chemical constituents of the essential oil, antioxidant activities, and senescent cellular inhibition. In addition, we defined the ability of these oils to inhibit the expression of MMP-2 as a marker of cellular aging. As we assumed that the aging process can occur due to cellular oxidative stress either in surface cells such as skin cells or vital organs such as the kidney (Abotorabi et al., 2020), in this experiment we used NIH-3T3 fibroblast cells to represent the skin tissue and Vero epithelial cells to represent kidney tissue. All this data was used to compare the potential cytoprotective agents of the two essential oils against cellular damaging agents or senescence inducing agents.

Materials and Methods

Sample preparation and phytochemical component identification

Citronella (*Cymbopogon nardus* (L.) Rendl.) and lemongrass (*Cymbopogon citratus* (DC.) Stapf) were obtained from local farmers in Yogyakarta, Indonesia, and have been identified by the Faculty of Pharmacy, Universitas Gadjah Mada (UGM) (Salsabila, 2022). Citronella and lemongrass were steam-water distilled for 4 h. The phytochemical compounds of the oils (CO and LO) were characterized using GC-MS (Shimadzu) with a 30 meter long HP-5MS UI column, helium UHP as carrier gas, and a maximum column temperature of 325-350 °C in the Integrated Research and Testing Laboratory (LPPT) UGM.

Cell culture

The kidney epithelial cells Vero or NIH-3T3 skin fibroblasts (collection of the Laboratory of Parasitology, Faculty of Medicine, Nursing, and Public Health, UGM) were maintained and seeded in a high glucose Dulbecco's Modified Eagle Medium (DMEM). Antibiotics streptomycin (150 IU/mL), penicillin (150 IU/mL), and 10% (v/v) fetal bovine serum (FBS) were added for supplementing the culture medium. The cells were maintained in an incubator with 5% CO_2 at 37°C as previously described (Zulfin et al, 2021; Salsabila, 2022).

Trypan blue exclusion assay

Vero and NIH-3T3 cells were seeded at a density of 50,000 cells/well onto 3.5 cm-tissue culture dishes. After 24 h of incubation, the cells were treated with either CO or LO at a serial concentration (1-500 µg/mL). Both CO or LO were first diluted in dimethyl sulfoxide (DMSO) and then in complete medium with a final volume of 1 mL, were added to the cells, followed by a 24 h incubation. After receiving treatment for 24 h, the cell morphology was documented, and viable cells were calculated with the trypan blue exclusion assay (Lestari et al., 2019). Briefly, the cells were harvested from each dish. After that, 10 µL cell suspension was sampled and mixed well with 10 µL of 0.4% trypan blue. Then, using a microscope (CKX-41 Olympus), the cells were directly counted with a hemocytometer.

SA-β-galactosidase assay

CO or LO at concentrations lower than IC_{50} were added to the cultured Vero or NIH-3T3 (100,000 cells/well) in 6-well plates and incubated for 24 h. The following

day, cells were fixed using 4% paraformaldehyde after rinsing in phosphate-buffered saline (PBS). After adding the X-gal staining solution (Zulfin et al., 2021), the cells were once again rinsed in PBS before being placed for incubation at 37 °C. A microscope (CKX-41 Olympus) at 400x magnification was used to observe the senescent cells after 72 h of incubation.

Total antioxidant capacity assay

The total antioxidant capacities of oils (CO or LO) were measured according to the procedure from the Total Antioxidant Capacity Assay Kit (Sigma-Aldrich MAK187). Briefly, the Cu^{2+} reagent, protein mask, assay diluent, and Trolox as the standard were prepared first. A series of concentrations of Trolox standard was added into 96-well plates and diluted with water to reach the final volume of 100 µL. The samples were added after being diluted at a 1:1 ratio using a protein mask and adding water to the final volume of 100 µL. The Cu^{2+} working solution (100 µL) was added to all of the standard and sample wells, followed by incubation at room temperature, shielded from light, for 90 min. The broad absorbance peak at 570 nm, which is proportional to the overall antioxidant capacity in Trolox equivalent, was measured by a microplate reader.

DCFDA staining flow cytometry assay

Cultured Vero or NIH-3T3 cells (50,000 cells/well) in a 24-well plate were harvested in 500 µL PBS supplemented with 10% FBS, following 30 min of staining with DCFDA 25 mM and incubation at 37 °C, 5% CO_2 atmosphere. The essential oils and Doxorubicin (Dox) were added to the cell suspension at a selected concentration for 4 h after the staining incubation. The percentage of fluorescein representing the ROS level in 20,000 cells was determined using the Becton Dickinson (BD) Accuri C6TM flow cytometer (Zulfin et al., 2021).

Gelatin zymography test

The cells (500,000/well) were cultured in a 6-well plate overnight, then treated with 100 nM Dox to induce the cells' senescence. The essential oils below the IC_{50} value, either alone or in combination with Dox, were prepared in a low serum medium and were given to the cells for 24 h. Next, the culture medium was harvested and centrifuged to obtain a supernatant containing a secreted protein extract. Twenty microliters of total protein extract, which had been added with a sample buffer, were resolved in SDS polyacrylamide gel electrophoresis with 0.1% gelatin for each well. After electrophoresis, Triton X-100 2.5% was used to rinse the gel for 24 h. Then, the gel was stained with a solution containing Coomassie Brilliant Blue for 30 min. The gel was destained with 80% methanol and 10% acetic acid to allow the visibility of clear bands produced by protein degradation (Wulandari et al., 2021). The bands on the gel that have been documented were analyzed quantitatively with ImageJ version 1.53k.

Data analysis

The triplicate data was analyzed by One-way ANOVA using the Bonferroni Post-hoc test with SPSS v.23. The mean standard deviation (SD) from three independent experiments was provided. To identify statistically significant differences, $p<0.05$ was applied. The data were visualized using Prism 9 v.9.1.1.

Results

Phytochemical constituents of CO and LO

CO and LO obtained by steam-water distillation were analyzed using GC-MS for their phytochemical constituents. We found 73 peaks of CO and 86 peaks of LO which were commonly terpenoids. We took seven major compounds from each that may contribute to the proposed mechanism of antioxidant activity (Figure 1). Citral was found to be the highest compound found in both oils, with different percentages for CO (15.61%) and LO (42.42%). It is confirmed from the common evidence that citral is a marker of LO (Sharma et al., 2021), while a marker of CO is citronellal (Anwar et al., 2020) which is the second most abundant compound after citral with 14.92%. These two markers are responsible for the different scents of CO and LO. The phytochemical constituents of both oils possess antioxidant activity, which reduces oxidative stress; citral is one example (Safaeian et al., 2020). Thus, the safety of both oils as antioxidant agents needs to be explored further to support their use.

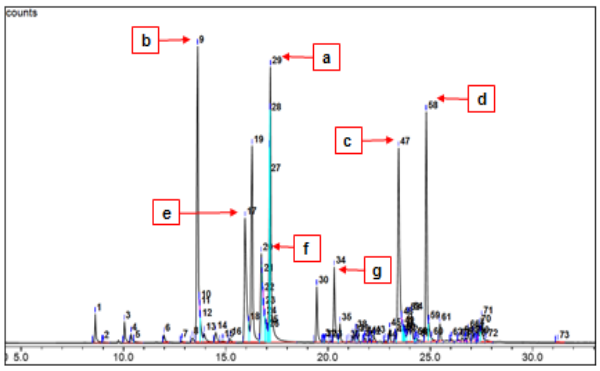
Effect of CO and LO on normal cell viability

Since CO and LO are expected to act as anti-senescent agents when applied to normal cells, their safety is important to consider. We used Vero and NIH-3T3 cell lines as representative models for kidney epithelial cells and skin fibroblast cells, respectively (Figure 2), to evaluate the cytotoxicity of CO and LO. We tested a series of concentrations with a range of 1-500 µg/mL. We confirmed that CO was less toxic on both cell lines compared to LO with the IC_{50} of more than 100 µg/mL. Meanwhile, LO showed a weak cytotoxic effect on both cell lines, with IC_{50} values of >40 µg/mL. Therefore, CO and LO are weakly cytotoxic to Vero and NIH-3T3 cells. But, for further examination, it is necessary to apply the concentration below the IC_{50} values in order to ensure that the effect is not due to the cytotoxic activity of the two essential oils.

Cellular senescence inhibition of CO and LO on normal cells

Senescence is physiological evidence where a cell goes through a permanent cell cycle arrest in response to stress conditions that are common in the aging process. One of the markers of cellular senescence is an increase in the activity of the SA-β-galactosidase enzyme which produces a bluish green color when reacted with X-gal solution (Itahana et al., 2013). We examined the effect of CO and LO in decreasing senescent cells by SA-β-gal assay using Dox 100 nM as a senescence inducer (Sun et al., 2022) (Figure 3). We kept the concentrations of CO and LO below the IC_{50} value to maintain cell viability. We could see that Dox significantly increased senescent cells on Vero ($p<0.001$) and NIH-3T3 cells ($p<0.001$), shown

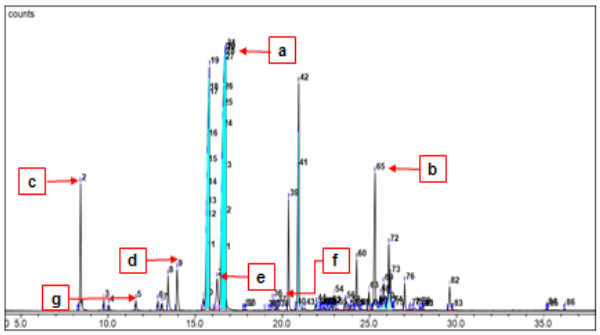
A



B

	Compound	Structure	% Area	Retention time (min)
a	Citral		15.61	17.18
b	Citronellal		14.92	13.63
c	Methyleugenol		11.90	23.45
d	Hedycaryol		10.10	24.80
e	Citronellol		8.44	15.94
f	Geraniol		6.67	16.78
g	Geranyl acetate		3.27	20.30

C



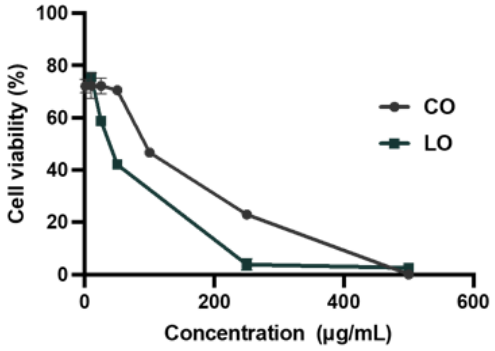
D

	Compound	Structure	% Area	Retention time (min)
a	Citral		42.42	16.68
b	Selin-6-en-4α-ol		9.99	25.33
c	β-Myrcene		3.63	8.42
d	Isogeranial		3.09	13.96
e	Geraniol		1.58	16.26
f	Geranyl acetate		0.49	19.44
g	Linalool		0.36	11.59

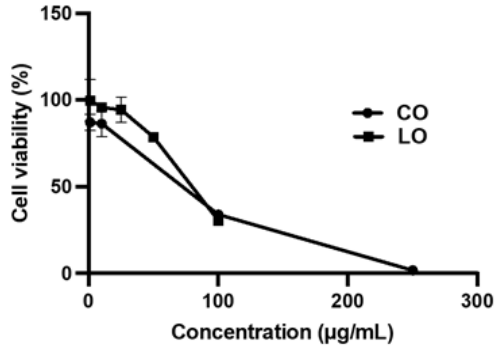
Figure 1. Phytochemical Constituents of CO and LO. Chromatograms of CO (A) and LO (C), run on a Shimadzu GCMS-QP2010S with helium carrier gas. Seven major phytochemical constituents of CO (B) and LO (D) were shown by red arrows in the chromatograms.

by the green color of the cells' appearance, which conveys that treatment with Dox is appropriate for inducing senescence in normal cells. Then, we treated CO and LO on normal cells alone or in combination with Dox. We found that CO (50 µg/mL) and LO (40 µg/mL) decreased the senescent cells in Vero cells significantly ($p<0.001$ and $p<0.05$, respectively). Furthermore, in NIH-3T3 cells, we obtained that CO (12.5 µg/mL) and LO (20 µg/mL) were also able to decrease the senescent cells significantly ($p<0.01$ and $p<0.001$, respectively). Both oils managed to

A



B



C

Cell line	Treatment	IC_{50} (µg/mL)
Vero	CO	140
Vero	LO	40
NIH3T3	CO	100
NIH3T3	LO	80

Figure 2. The Effects of CO and LO on Normal Cell Viability. Vero and NIH-3T3 cells (50,000 cells/well) were seeded in 3.5 cm dishes and treated with CO and LO for 24 h. The viability of Vero (A) and NIH-3T3 cells (B) was tested using trypan blue exclusion and counted directly. The data were presented as IC_{50} values for both Vero and NIH-3T3 cells under CO or LO treatments (C).

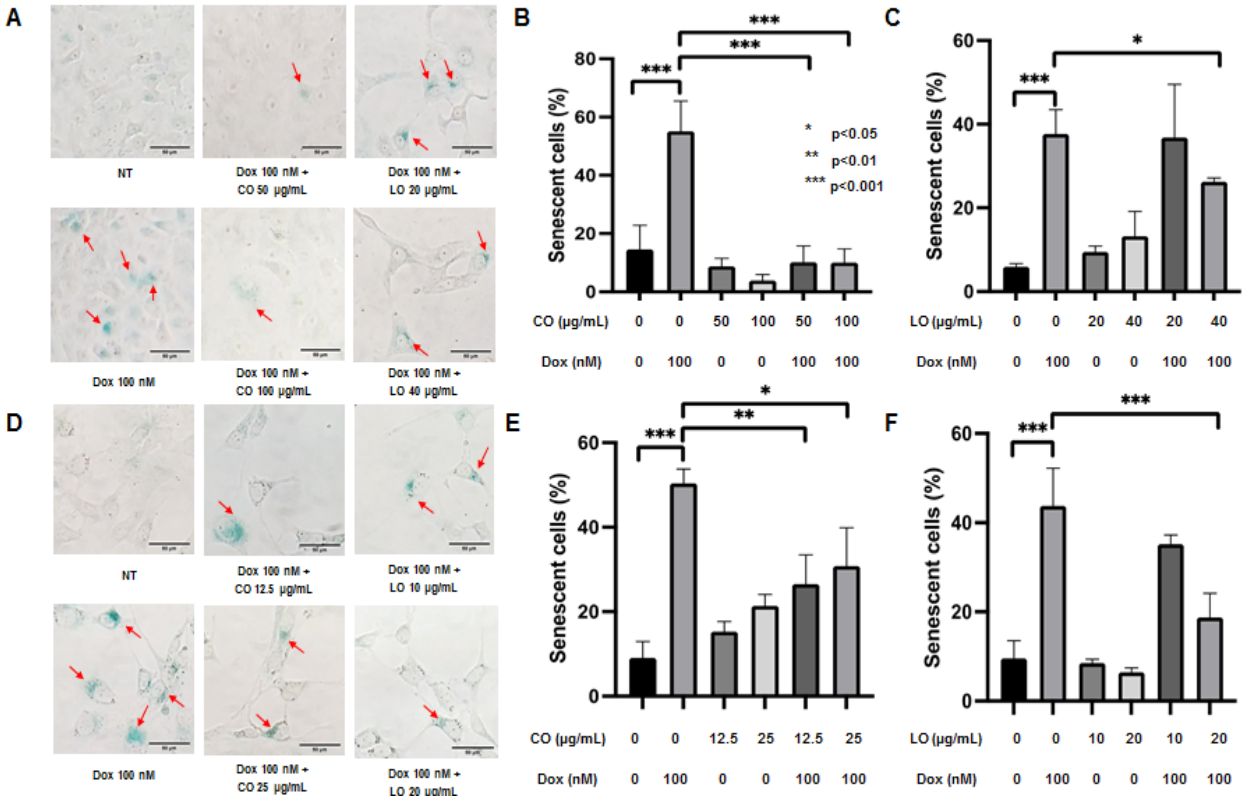


Figure 3. Cellular Senescence Inhibition of CO and LO in Normal Cells. The assay was performed using SA-β-gal staining with Dox as a positive control. Cells that undergo cellular senescence would produce a bluish green color, as indicated by the red arrows in the cell morphology of Vero (A) and NIH-3T3 (D). The decreased percentage of cellular senescence incidence in Vero cells (B, C) and NIH-3T3 cells (E, F) treated with CO or LO was quantified and presented, respectively. The data is presented as an average ± SD (n=3). *, p<0.05; **, p<0.01; ***, p<0.001.

significantly reduce cellular senescence in normal cells, with CO being more powerful than LO. However, it is necessary to explore more deeply related to the antioxidant activity and the molecular mechanism of CO and LO in acting as cytoprotective agents.

Antioxidant activities of CO and LO

To assess the underlying mechanism of CO and LO

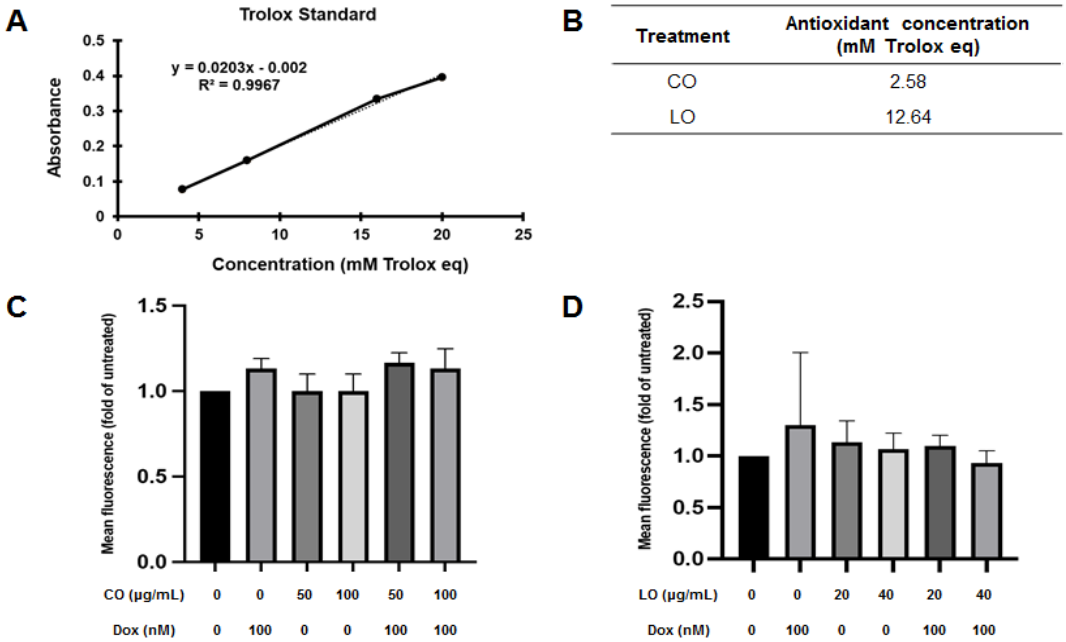


Figure 4. Antioxidant Activities of CO and LO. We compared the antioxidant activities of CO and LO using the commercial total antioxidant capacity kit (Sigma) with Trolox as an antioxidant standard (A). The results from the kit are shown as Trolox equivalents (B). We also performed DCFDA staining to define the effect of CO and LO in decreasing ROS amounts in Vero (C) and NIH-3T3 (D) cells. The data in C and D are presented as average ± SD (n=3).

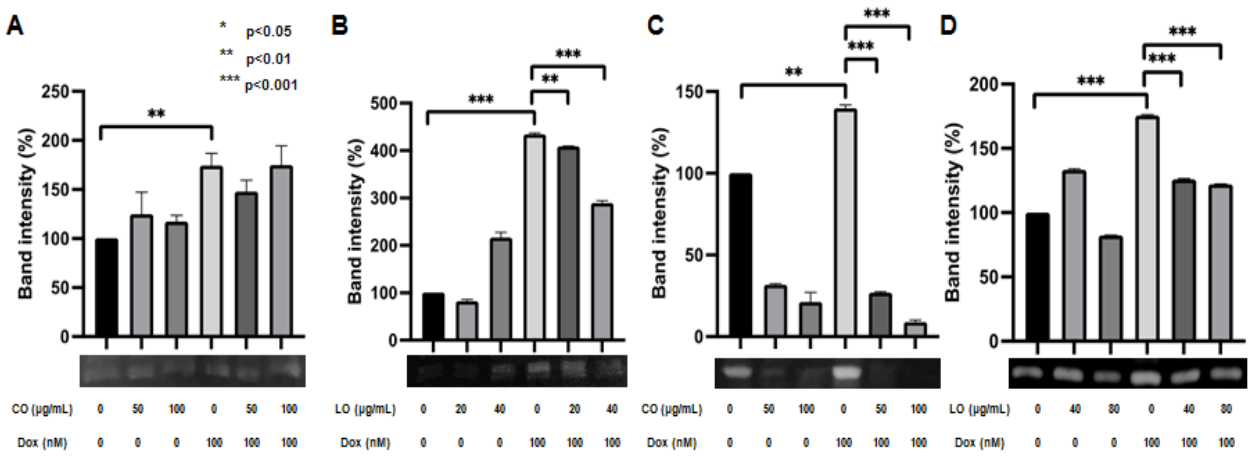


Figure 5. Inhibition of MMP-2 Activity by CO and LO in Normal Cells. We examined the effect of CO and LO on MMP-2 activity using a gelatin zymography assay. MMP-2 activity was indicated by the white band after staining the gel with Coomassie Brilliant Blue. The color intensity of the bands of Vero cells that had been treated with CO (A) or LO (B) and NIH-3T3 cells that had been treated with CO (C) or LO (D) was quantified by ImageJ. The data are shown as an average ± SD (n=3). **, p<0.01; ***, p<0.001.

in inhibiting cellular senescence in normal cells, we compared the antioxidant activities of both oils. Based on our preliminary DPPH assay, CO and LO did not show any radical scavenging activity (data not shown). Therefore, it probably indicates that CO and LO may have antioxidant activity but do not act as radical scavengers. Then, we tested the antioxidant activities of CO and LO using a commercial total antioxidant capacity kit from Sigma. The kit measures the ability of the sample to act as a reducing agent and expresses its antioxidant concentration as Trolox equivalent (Figure 4A). We found that CO and LO performed antioxidant activities by reducing Cu^{2+} to Cu^{+}. LO showed a concentration of antioxidants five times greater than CO (Figure 4B). Furthermore, to support the molecular mechanism in normal cells, we performed DCFDA staining to detect intracellular ROS levels. We found evidence that CO and LO did not significantly decrease ROS levels induced by Dox in Vero and NIH-3T3 cells. These results showed that further investigation is needed on the molecular pathways and mechanisms of CO and LO as antioxidant agents in inhibiting cellular senescence.

Inhibition of MMP-2 expression by CO and LO in normal cells

It was not known with certainty the mechanism

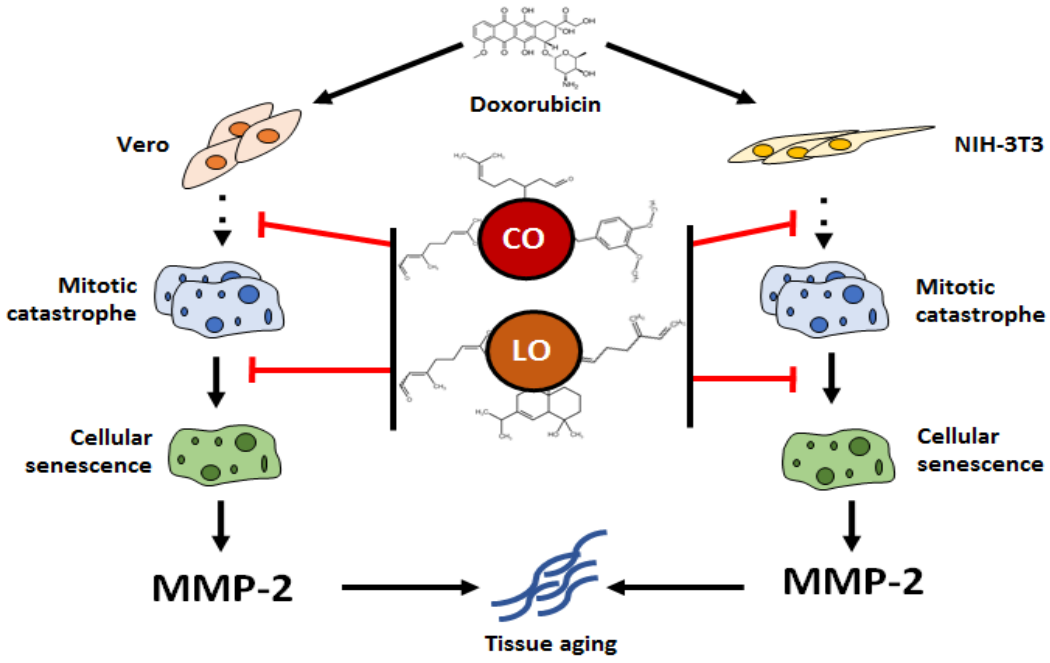


Figure 6. Graphical Abstract. Doxorubicin was used as a senescent inducing compound on both Vero and NIH-3T3 cells that may pass through mitotic catastrophe. Compounds in CO and LO may interact and inhibit doxorubicin-induced mitotic catastrophe and cellular senescence, leading to decreased MMP-2 expression. These mechanisms give valuable meaning to the development of CO and LO as cytoprotective agents.

by which CO and LO inhibit cellular senescence in normal cells, apart from acting as antioxidants. Thus, we wanted to know the effect of CO and LO treatments on the expression of matrix metalloproteinase-2 (MMP-2) enzymes in Vero and NIH-3T3 cells. MMP-2 is crucial for the breakdown of the ECM, which damages various tissues of the body, including the skin and kidneys, to undergo premature aging so that their function is abrogated (Cheng et al., 2017). MMP-2 expression is known to increase with increasing cellular senescence activity (Hassona et al., 2014). We used Dox 100 nM as an inducer agent for cellular senescence that increased MMP-2 expression and as a chemotherapeutics model. The white band on the gel indicated that MMP-2 with a size of 72 kDa was able to degrade gelatin in the gel as a representation of the ECM model (Figure 5). CO and LO at concentrations of 50 µg/mL and 20 µg/mL were able to reduce MMP-2 activity from Vero-induced Dox cells (Figure 5A,C). Similar effects were also found with CO and LO at concentrations of 50 µg/mL and 40 µg/mL, respectively, in NIH-3T3 cells (Figure 5B, D). Hence, the inhibition of MMP-2 activity may support the mechanism by which CO and LO act as cytoprotective agents by inhibiting cellular senescence.

Discussion

So far, CO and LO have been identified and widely used by the people for a variety of purposes. CO, for example, is used in aromatherapy and skin care products, while LO is used for foods and beverages. Although both are frequently used in a mixture to improve health through herbal medicine, the GC-MS chemical content results reveal that these two herbs have unique chemical signatures (Figure 1). CO detected citronella as its marker compound, but LO identified citral as its major component. Although they contain nearly the same components with different compositions, our study shows that these oils can prevent cellular aging in both skin normal fibroblasts and kidney epithelial cells (Figure 3). However, they did not appear to have radical scavenging properties, as evidenced by the findings of the ROS assay (Figure 4C, D), which revealed no significant effects.

Meanwhile, the antioxidant assay revealed that the two oils performed different antioxidant capacities. The antioxidant capacity of CO was lower than that of LO (Figure 4B). The antioxidant capacity of LO was reported to be equivalent to the range of Trolox antioxidant capacity used as the reference (4-20 nmol/mL). These findings revealed that the antioxidant capabilities of LO were produced by direct interactions with oxidant species rather than its ability to scavenge free radicals. The efficacy of the two oils to prevent cellular senescence in Vero and NIH3T3 cells appeared to be unrelated to their unique antioxidant capacities. Nevertheless, the LO cellular senescence assay used lower concentrations than the CO assay. Overall, additional studies are required to conclude that LO's ability was superior to CO's.

One interesting finding in this study is that both CO and LO were able to reduce MMP-2 activity (Figure 5). MMP-2 is an enzyme that is crucial in the degradation of ECM in the tissue aging process (Yu et al., 2013). This enzyme is usually expressed by cells that are damaged and are undergoing senescence, especially in fibroblast cells (Hawwa et al., 2011). The study's findings supported the idea that increased MMP-2 expression or activity occurs during senescence events as a result of the oxidative stress caused by Dox (Wulandari et al., 2021). In the meantime, treatment of both CO and LO can inhibit senescence-induced Dox while concurrently lowering MMP-2 activity. Although LO's antioxidant capacities appeared to be superior to those of CO's, both oils had similar impacts on preventing cell damage (aging) and lowering MMP-2 expression. This evidence demonstrates a direct connection between the two oils' ability to lower MMP-2 expression, which was probably caused by their senescence inhibition. The mechanism of senescence inhibition by these oils requires further study because it appears to be unrelated to the antioxidant properties of the compounds contained in these two oils (Figure 6).

In this study, we also discovered that Dox administration in Vero and NIH-3T3 cells did not significantly raise ROS levels, leading us to hypothesize that the effect generated by Dox is caused by another mechanism that has to be investigated further. Cell cycle arrest, for example, is caused by irreversible DNA damage, which causes cells to be unable to repair themselves and undergo catastrophic cell cycle arrest. This occurrence is typical in cells that have been damaged by the administration of physical or chemical substances (Endah et al., 2022). The catastrophic cell cycle arrest mechanism can occur in the G1, S, and mitosis phases (Lombardi and Lasagni, 2016). After connecting it to the chemical mechanisms of the active constituents in CO and LO, we believe that there is a chance that the two oils enable cells to avoid catastrophic cell cycle arrest, which can be further investigated.

Overall, the findings of this study offer valuable information on CO and LO usage in relation to cellular damage to the skin and other organs caused by stress or damaging agents such as chemotherapeutics agents. LO is better than CO in terms of antioxidant properties. However, both oils can prevent cellular senescence due to the administration of agents that can cause mitotic catastrophe. As a result of this senescent cellular inhibition, both of them can reduce the activity or expression of MMP-2 which plays a significant role in cellular senescence and tissue damage. These two oils are projected to be developed into products that can be utilized to preserve tissue or cell damage caused by cellular stress due to exposure to a chemical or physical agent including chemotherapeutic agents that induces permanent cell cycle arrest as CO and LO showed properties to prevent cellular senescence. Therefore, in-depth research on the mechanism of action, in vivo studies, and formulation can be established to better understand the molecular targets and their applications.

Author Contribution Statement

EM designed the study, prepared the manuscript, and secured the funding. DUS conducted almost all the experiments and wrote the first draft of the manuscript. UMZ and MI checked over the laboratory work and the

manuscript. RKW, NUH, and AST assisted with laboratory work, data analysis, and figure preparation.

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Ethical issue

Not applicable

Conflict of Interest

We declare that we have no conflict of interest.

References

Abotorabi Z, Khorashadizadeh M, Arab M, Hassanpour Fard M, Zarban A (2020). Jujube and green tea extracts protect human fibroblast cells against UVB-mediated photo damage and MMP-2 and MMP-9 production. *Avicenna J Phytomed*, **10**, 287–96.

Ahlina FN, Nugraheni N, Salsabila IA, et al (2020). Revealing the Reversal Effect of Galangal (Alpinia galanga L.) Extract Against Oxidative Stress in Metastatic Breast Cancer Cells and Normal Fibroblast Cells Intended as a Co- Chemotherapeutic and Anti-Ageing Agent. *Asian Pac J Cancer Prev*, **21**, 107–17.

Anwar Y, Ningtiyas NA, Simanjuntak P (2020). Isolation of citronellal from Cymbopogon nardus (L) Rendle and its activity test as a burn healing in mice. *Curr Res Biosciences Biotechnol*, **2**, 105–8.

Bakhtiari KM, Sharifiyazdi H, Nazifi S, Ghaemi M, Hadadipour Zarandi M (2021). Effects of citral on serum antioxidant status and liver genes expressions of paraoxonase 1 and nitric oxide synthase in a rat model of streptozotocin-induced diabetes mellitus. *Iran J Vet Res*, **22**, 195–202.

Bates AL, Pickup MW, Hallett MA, et al (2015). Stromal matrix metalloproteinase 2 regulates collagen expression and promotes the outgrowth of experimental metastases. *J Pathol*, **235**, 773–83.

Bayala B, Bassole IHN, Maqdasy S, et al (2018). 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*, **153**, 162–70.

Bayala B, Coulibaly AY, Djigma FW, et al (2020). Chemical composition, antioxidant, anti-inflammatory and antiproliferative activities of the essential oil of Cymbopogon nardus, a plant used in traditional medicine. *Biomol Concepts*, **11**, 86–96.

Cheng Z, Limbu MH, Wang Z, et al (2017). MMP-2 and 9 in Chronic Kidney Disease. *Int J Mol Sci*, **18**, 776.

Endah E, Wulandari F, Putri Y, Jenie RI, Meiyanto E (2022). Piperine Increases Pentagamavunon-1 Anti-cancer Activity on 4T1 Breast Cancer Through Mitotic Catastrophe Mechanism and Senescence with Sharing Targeting on Mitotic Regulatory Proteins. *Iran J Pharm Res*, **21**.

Haryanti S, Zulfin U, Salsabila I, Wulandari F, Meiyanto E (2022). The Cytotoxic and Anti-Migratory Properties of Caesalpinia sappan and Ficus septica, in Combination with Doxorubicin on 4T1 TNBC Cells with Nephroprotective Potential. *Asian Pac J Cancer Prev*, **23**, 743–52.

Hassona Y, Cirillo N, Heesom K, Parkinson EK, Prime SS (2014). Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte dis-cohesion and invasion. *Br J Cancer*, **111**, 1230–7.

Hawwa RL, Hokenson MA, Wang Y, et al (2011). Differential expression of MMP-2 and -9 and their inhibitors in fetal lung cells exposed to mechanical stretch: regulation by IL-10. *Lung*, **189**, 341–9.

Howard EW, Crider BJ, Updike DL, et al (2012). MMP-2 expression by fibroblasts is suppressed by the myofibroblast phenotype. *Exp Cell Res*, **318**, 1542–53.

Itahana K, Itahana Y, Dimri GP (2013). Colorimetric detection of senescence-associated β galactosidase. *Methods Mol Biol*, **965**, 143–56.

Kumoro AC, Wardhani DH, Retnowati DS, Haryani K (2021). A brief review on the characteristics, extraction and potential industrial applications of citronella grass (Cymbopogon nardus) lemongrass (Cymbopogon citratus) essential oils. *IOP Conf Ser Mater Sci Eng*, **1053**, 012118.

Larasati YA, Yoneda-Kato N, Nakamae I, et al (2018). Curcumin targets multiple enzymes involved in the ROS metabolic pathway to suppress tumor cell growth. *Sci Rep*, **8**, 2039.

Lestari B, Nakamae I, Yoneda-Kato N, et al (2019). Pentagamavunon-1 (PGV-1) inhibits ROS metabolic enzymes and suppresses tumor cell growth by inducing M phase (prometaphase) arrest and cell senescence. *Sci Rep*, **9**, 14867.

Lombardi D, Lasagni L (2016). Cell-cycle Alterations in Post-mitotic Cells and Cell Death by Mitotic Catastrophe, in: Najman, S. (Ed.). Cell Biology - New Insights, London.

Meiyanto E, Larasati YA (2019). The Chemopreventive Activity of Indonesia Medicinal Plants Targeting on Hallmarks of Cancer. *Adv Pharm Bull*, **9**, 219–30.

Oszajca K, Szemraj J (2021). Assessment of the correlation between oxidative stress and expression of MMP-2, TIMP-1 and COX-2 in human aortic smooth muscle cells. *Arch Med Sci Atheroscler Dis*, **6**, 158–65.

Rastuti U, Diastuti H, Chasani M, Purwati, Hidayatullah R (2020). Chemical composition and antioxidant activities of citronella essential oil Cymbopogon nardus (L.) rendle fractions. *AIP Conf Proc*, **2237**, 020035.

Safaeian L, Sajjadi SE, Montazeri H, Ohadi F, Javanmard S (2020). Citral Protects Human Endothelial Cells Against Hydrogen Peroxide-induced Oxidative Stress. *Turk J Pharm Sci*, **17**, 549–54.

Salsabila DU (2022). Minyak Serai Wangi (Cymbopogon nardus (L.) Rendl.) Sebagai Agen Nefroprotektif: Efek Anti-Senescence pada Sel Vero dan Pelacakan Mekanisme Molekuler dengan Bioinformatika, Undergraduate Thesis, Faculty of Pharmacy Universitas Gadjah Mada Yogyakarta, Indonesia (in Indonesian).

Shabbir A, Khan MMA, Ahmad B, et al (2019). Vetiveria zizanioides (L.) Nash: A Magic Bullet to Attenuate the Prevailing Health Hazards, in: Ozturk, M., Hakeem, K.R. (Eds.), Plant and Human Health, 2, pp 99–120.

Sharma R, Rao R, Kumar S, Mahant S, Khatkar S (2019). Therapeutic Potential of Citronella Essential Oil: A Review. *Curr Drug Discov Technol*, **16**, 330–9.

Sharma S, Habib S, Sahu D, Gupta J (2021). Chemical Properties and Therapeutic Potential of Citral, a Monoterpene Isolated from Lemongrass. *Med Chem*, **17**, 2–12.

Sun T, Zhang L, Feng J, et al (2022). Characterization of cellular senescence in doxorubicin-induced aging mice. *Exp Gerontol*, **163**, 111800.

Wulandari F, Ikawati M, Kirihata M, Kato JY, Meiyanto E (2021). A new curcumin analog, CCA-1.1, induces cell death and cell cycle arrest in WiDr colon cancer cells via ROS generation. *J Appl Pharm Sci*, **11**, 99–105.

Wulandari F, Utomo R, Novitasari D, et al (2021). The anti-migratory activity of a new curcumin analog, CCA-1.1, against T47D breast cancer cells. *Int J Pharm Res*, **13**, 2877-87.

Yu TY, Pang JHS, Wu KPH, et al (2013). Aging is associated with increased activities of matrix metalloproteinase-2 and -9 in tenocytes. *BMC Musculoskelet Di*, **14**, 2.

Zulfin UM, Rahman A, Hanifa M, et al (2021). Reactive oxygen species and senescence modulatory effects of rice bran extract on 4T1 and NIH-3T3 cells co-treatment with doxorubicin. *Asian Pac J Trop Biomed*, **11**, 174.