Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Antioxidant and Anti-Inflammatory Potential of *Cymbopogon nardus* Ethanol Extract on *3T3-L1* Cells

Enny Rohmawaty[1], Hesti Lina Wiraswati[1], Tamara Aliya Zahra[2], Shabrina Nur Amalina[2], Julia Ramadhanti[1], Aziiz Mardanarian Rosdianto[1], Amila Laelalugina[3], Gita Tiara Dewi Nasution[1], Yusof Kamisah[4]

[1]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, 40161, Indonesia; [2]Undergraduate Program Medical Doctor, Faculty of Medicine, Universitas Padjadjaran, Bandung, 40161, Indonesia; [3]Oncology and Stem Cell Working Group, Universitas Padjadjaran, Bandung, 40161, Indonesia; [4]Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, 53000, Malaysia

Correspondence: Enny Rohmawaty, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jl. Ir. Soekarno KM. 21, Jatinangor, Sumedang, West Java, 45363, Indonesia, Email e.rohmawaty@unpad.ac.id

Purpose: *Cymbopogon nardus* (L). Rendle has traditionally been recognized for its medicinal properties. Recent studies have suggested that its bioactive constituents possess antioxidant and anti-inflammatory properties. However, there is limited scientific evidence of its cellular effects. Given that the pathogenesis of many diseases involves oxidative stress and inflammation, this study aimed to evaluate the potential antioxidant and anti-inflammatory effects of the plant extracts in 3T3-L1 cells.

Methods: Phytochemical screening of *C. nardus* extracts was performed to identify bioactive compounds. Antioxidant activity of the extract was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide dismutase (SOD) assays. Toxicity was evaluated using the MTT assay. Additionally, the effects of the extract on the gene expression of hypoxia-inducible factor 1α (HIF-1α) in menadione-induced 3T3-L1 cells, as well as interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) in lipopolysaccharide (LPS)-induced 3T3-L1 cells, were investigated.

Results: Phytochemical screening revealed the presence of phenolics, tannins, alkaloids, and flavonoids in the ethanolic extracts. The extract demonstrated antioxidant activity, with IC_{50} values of 178.06 ppm for DPPH and 220 ppm for SOD. It did not affect the viability of 3T3-L1 cells at concentrations of up to 500 ppm. At 100 ppm, the extract increased cell viability ($p<0.05$) and reduced HIF-1α expression in the menadione-treated cells ($p<0.05$). Additionally, it decreased the expression of IL-6 and COX-2 in LPS-induced cells ($p<0.05$).

Conclusion: The ethanol extract of *C. nardus* demonstrated promising potential as an antioxidant and anti-inflammatory agent in 3T3-L1 cells. Further analysis is recommended to confirm the potential.

Keywords: ROS, IL-6, COX-2, HIF-1α, menadione, lipopolysaccharide

Introduction

Oxidative stress and inflammation play significant roles in the pathogenesis of many diseases.[1] Therefore, treating these conditions with antioxidant and anti-inflammatory compounds may be beneficial. Several medicinal plants have been reported to possess these activities.[2–5] Historically, medicinal plants have played a crucial role in therapeutic practices by offering effective treatment for various ailments. Even today, they continue to serve as an important source for the development of novel drugs, with more than 50% of modern therapeutics derived from plants either in their natural form or as derivatives. Indonesia is known for its vast biodiversity and is home to approximately 30,000–40,000 plant species, approximately 20% of which are recognized for their medicinal properties and are either cultivated or grown in the wild.[6,7] Despite this abundance, many medicinal plants in Indonesia remain underutilized, making them a promising source for the discovery of new plant-based drugs. Herbal plants offer distinct advantages over synthetic agents, such as easier accessibility, lower cost, and fewer or no side effects. These plants are rich in phytoconstituents like flavonoids, alkaloids, terpenoids, steroids, and phenolics, which

Received: 12 November 2024
Accepted: 24 January 2025
Published: 11 February 2025

are known for their antioxidant and anti-inflammatory properties.[8] One such plant found in Indonesia, particularly in West Java, is *Cymbopogon nardus* (L). Rendle.

C. nardus, commonly known as citronella grass, is an aromatic plant belonging to the family Poaceae and is widely known for its essential oil and diverse applications. Traditionally, it has been utilized in various ways, including in the production of soaps, perfumes, and aromatherapy products. Its essential oil is particularly famous for its use as a natural insect repellent that protects against mosquito bite. Additionally, it is a common ingredient in traditional medicine and is used as a seasoning in culinary practices.[9,10] The primary components of *C. nardus* essential oil are three monoterpenes, citronellal, citronellol, and geraniol, which have distinctive fragrances and contribute to its functional properties.[11,12]

In addition to its traditional uses, *C. nardus* has significant therapeutic potential because of its rich phytochemical content. The ethanol extract of this plant contains a variety of bioactive compounds such as alkaloids, tannins, saponins, flavonoids, and phenolics. These compounds are known for their health-promoting effects, particularly their antioxidant and anti-inflammatory effects. The presence of flavonoids and phenolics in particular suggests that *C. nardus* can serve as an effective natural antioxidant, neutralizing free radicals and protecting cells from oxidative stress.[2,9] In addition to its antioxidant activity, *C. nardus* is also recognized for its potential as an anti-inflammatory agent. Studies have suggested that *C. nardus* ethanol extract can help suppress inflammation by inhibiting key inflammatory mediators, such as lipoxygenase and cyclooxygenase enzymes, as well as pro-inflammatory cytokines.[13] This makes *C. nardus* a promising candidate for the development of natural treatments aimed at managing inflammation and oxidative stress-related conditions.

Extensive in vitro and in vivo studies have explored the development of antioxidant and anti-inflammatory agents derived from herbal plants. Among the commonly used in vitro models for evaluating these properties are 3T3-L1 mouse fibroblast cells, which we used as a cell model to investigate the antioxidant capacity of plant extracts in a previous study.[14] However, to date, no study has specifically investigated the potential antioxidant and anti-inflammatory effects of *C. nardus* using the 3T3-L1 cell line. Therefore, the present study is the first to explore the effects of *C. nardus* ethanol extract on 3T3-L1 inflammatory cells stimulated with menadione and lipopolysaccharide (LPS). These findings may provide valuable insights for advancing the development of *C. nardus* as a candidate antioxidant and anti-inflammatory therapy, particularly for adipose tissue-related inflammation.

Materials and Methods

Collection and Preparation of *Cymbopogon nardus* Ethanol Extract

Fresh *C. nardus* stems and leaves of *C. nardus* were collected from Parongpong, West Bandung District, West Java, Indonesia. Plant identification was conducted by Budi Irawan, a taxonomist at the Jatinangor Herbarium, Biosystematics and Molecular Laboratory, Department of Biology, FMIPA, Universitas Padjadjaran (UNPAD) with reference no. 455/LBM/IT/X/2024. The samples (100 g) were thoroughly washed with tap water, sorted, and cut into small pieces before being dried at 50°C until they reached a constant weight, and macerated in 96% ethanol for 2×24 h. The resulting extract was filtered through Whatman No.1 filter paper, and the filtered extract was collected and dried using a rotary evaporator (CCA-1100, EYELA, Japan) to obtain an extract paste. The paste extract was stored at 4°C until future use.[6]

Phytochemical Test of *Cymbopogon nardus* Ethanol Extract

The samples used for phytochemical testing weighed 2 g for each test target. In this study, phenolics, tannins, flavonoids, alkaloids, and saponins were tested using specific reagents to analyze their target secondary metabolites. The phenolic test was performed using a 5% $FeCl_3$ reagent (Sigma, USA), while the tannin test utilized a 1% $FeCl_3$ reagent (Sigma, USA).[15] Flavonoid test was performed with three different reagents, namely 0.1 g of magnesium powder (Sigma, USA), and concentrated HCl (Sigma, USA), H_2SO_4 2N (Sigma, USA), and NaOH 10% (Sigma, USA).[15] Alkaloid test was performed with Wagner's reagent. The saponin test was performed with two drops of HCl 2N reagent.[16] The presence of each target is shown as follows: bluish-black color for phenolic; green, red, purple, blue, or strong black colors for tannin; orange, pink, or dark red colors, which were not lost within three minutes for flavonoids, chocolate deposition for alkaloids, and stable foam for saponin.[17,18]

Antioxidant Activity Test of *Cymbopogon nardus* Ethanol Extract

2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Assay

One milliliter of 2.2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.4 mm in 82% methanol) was added to one milliliter of samples at concentrations ranging from 0.5 to 42 ppm and incubated for 30 minutes. The absorbance of the resulting mixture was measured at 517 nm wavelength. The percentage of free radical inhibition by the sample was calculated as the percentage of absorbance difference between the sample and blank. The 50% free radical inhibitory concentration (IC_{50}) was calculated using the regression equation $y = ax + b$.[6]

SOD Activity Assay

The superoxide dismutase (SOD) activity in the extract was assessed using an established method based on the xanthine–xanthine oxidase system that generates superoxide radicals.[6]

Briefly, 40 µL of extract at concentrations ranging from 5 to 20 ppm was mixed with 200 µL of reagent mixture containing aquabidest, 1M phosphate buffer (pH 7.4), 10 mm nitro blue tetrazolium (NBT), 0.1 TEMED, and 0.5 mm riboflavin in a 96-well plate. In addition to the sample wells, three other groups were used in this test: no-inhibition groups (40 µL methanol + 200 µL reagent mixture), inhibition blanko groups (40 µL extract + 200 µL reagen without riboflavin mixture), and no-inhibition blanko groups (40 µL methanol + 200 µL reagen without riboflavin mixture). All groups were illuminated for 10 min in a lighting box containing a tungsten lamp bulb (20 W). One unit of superoxide dismutase (SOD) is defined as the amount of enzyme required to inhibit the reduction of nitro blue tetrazolium (NBT) by 50% in one minute. Inhibition of NBT reduction was measured at 560 nm using a microplate spectrophotometer (ThermoScientific ® Multiskan EX, Singapore). The SOD activity was expressed in units per milligram of protein (U/mg protein).[6]

3T3-L1 Cell Line Culture

The 3T3-L1 fibroblast cell line was obtained from the American Tissue Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in RPMI 1640 medium (Gibco, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, Waltham, MA, USA) and 1% penicillin-streptomycin (Gibco, Waltham, MA, USA). Cell culture was maintained at 37°C in a CO_2 (5%) incubator.

Toxicity Test of *C. nardus* Ethanol Extract on 3T3-L1 Cells Using MTT Assay

3T3-L1 cells (approximately 7500 cells) were incubated in a 96-well plate with various concentrations of *C. nardus* ethanol extract (1–500 ppm) prepared in dimethyl sulfoxide (DMSO, 0.1%) for 24 h in triplicate. Next, 10 µL of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Merck, USA) and 100 µL of RPMI medium were added to each well, and the microplates were incubated in a 5% CO_2 incubator at 37°C for 4 h. After incubation, 100 µL of DMSO was added to dissolve the formazan crystals, and the absorbance of the mixture was measured at 550 nm using a spectrophotometer (ThermoScientific ® Multiskan EX, Singapore).

Reactive Oxygen Species (ROS) Detection in 3T3-L1 Cells Treated with *C. nardus* Extract

To obtain the optimal concentration of *C. nardus* extract for subsequent experiments, its effect on ROS accumulation was determined. Five groups were used in this assay: a negative control group (cells and media) and four treatment groups incubated with various concentrations of *C. nardus* extract (1, 50, 100, and 300 ppm) for 24 h. Dichlorodihydrofluorescein diacetate (DCFH-DA, 10 µM) was added to each group (100 µL) and incubated in the dark for 20 min. The solution was then exposed to a light-emitting diode (LED) lamp with an optical power density of 5.43 mW/cm^2 for 20 min. Fluorescence intensity was measured using a multifunction microplate reader at excitation and emission wavelengths of 488 and 525 nm, respectively. Images were analyzed using the ImageJ software (NIH, Bethesda, MD, USA).

Effects of *C. nardus* Extract on Menadione-Induced Oxidative Stress in 3T3-L1 Cells

To assess the antioxidant properties of *C. nardus* extract, its effects on menadione-induced oxidative stress were investigated. Briefly, 3T3-L1 cells were treated with 6 µM of menadione. The administration of *C. nardus extract* was

carried out by co-treatment with menadione and incubation for 24 h in a 5% CO_2 atmosphere at 37°C. Treatment with N-acetylcysteine (NAC, 5 mm) and glutathione (GSH, 5 mm) served as positive controls.[14] Cell viability was measured using the MTT assay and Hypoxia Inducible Factor (HIF)-1α gene also measured using RT-PCR.

Effects of *C. nardus* Extract on LPS-Induced Inflammation in 3T3-L1 Cells

To investigate the potential anti-inflammatory properties of *C. nardus*, 3T3-L1 cells were treated with LPS (1 µg/mL) and the extract (100 ppm) for 24 hours.[19] Cells were also treated with NAC (5 mm) as the positive control, while the vehicle-treated group served as the control.[14] Gene expression of interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) was also examined.

RT-PCR Analysis

Treated cells were harvested, and total RNA was isolated using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA, USA). mRNA expression levels were assessed by real-time polymerase chain reaction (PCR) (AgilentTechnologies, USA) using the SensiFAST™ SYBR® No-ROX One-Step Kit (Meridian Biosciences) and sequence-specific primers on an Agilent AriaMX PCR system (Agilent Technologies, USA). Primers were synthesized by Integrated DNA Technologies (IDT, Singapore). Nucleotide sequences (5′–3′) of the forward and reverse primers are listed in Table 1. The PCR conditions were as follows: reverse transcription at 45°C for 10 min, followed by initial denaturation at 95°C for 2 min, and 40 cycles of 95°C for 5 s and 60°C for 20s. Data were analyzed using the 2-ΔΔCT method, with the housekeeping gene GAPDH serving as the internal control.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). All experiments were conducted in triplicate (n=3) with three independent biological replicates.

Statistical analysis was performed using the Shapiro–Wilk test to determine the distribution of data and Levene's test to determine the homogeneity of the data. If the data were normal and homogeneous, Post-hoc Tukey's test was used. However, if the data were normal but non-homogenous or Levene's test could not provide meaningful results, Dunnett's T3 test was used. On the other hand, if the data were abnormal, a non-parametric test, namely, the Kruskal–Wallis test, was used.

Results

Phytochemical Compounds of *C. nardus* Ethanol Extract

Table 2 shows the results of the phytochemical compound detection in *C. nardus* ethanol extract. *C. nardus* contains phenolic compounds, tannins, flavonoids and alkaloids. In the flavonoid test, the results were positive only with the 10% NaOH reagent. In the saponin test, *C. nardus* ethanol extract did not show the presence of this secondary metabolite.

Table 1 Forward and Reverse Primer for GADPH, HIF-1α, IL-6, and COX-2

Groups	Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')	References
	GADPH	CAAGATCATCAGCAATGCCTCC	GCCATCACGCCACAGTTTCC	[20]
Menadione-Induced	HIF-1α	GTAATGCTCCCCTCACCCAAC	GTGCAGGGTCAGTACTTC	
LPS-Induced	IL-6	GGAGTCACACTCCACCT	CTGATTGGAAACCTTATTAG	[21]
	COX-2	TGAGCAACTATTCCAAACCAGC	GCACGTAGTCTTCGATCACTATC	

Table 2 Phytochemical Compounds of *C. nardus* Ethanol Extract

Phytochemical	Reagent	Detection
Phenolics	$FeCl_3$ 5% reagent	+
Tannin	$FeCl_3$ 1% reagent	+
Flavonoids	HCl + Mg reagent	–
	H_2SO_4 2N reagent	–
	NaOH 10% reagent	+
Saponin	HCl 2N reagent	–
Alkaloids	Wagner reagent	+

Antioxidant Activity of *C. nardus* Ethanol Extract

The DPPH scavenging activity of *C. nardus* ethanol extract showed an IC_{50} of 178.06 ppm, placing it in the medium antioxidant category. A similar result was observed for SOD activity, with an IC_{50} of 220 ppm. The range for moderate antioxidant activity is 101–250 ppm.[22]

Cytotoxicity of *C. nardus* Ethanol Extract on 3T3-L1 Cells

Figure 1 shows the viability of 3T3-L1 cells treated with various concentrations of *C. nardus* ethanol extract. Almost all concentrations resulted in 100% cell viability, with the highest concentration tested (500 ppm) resulting in 91% viability.

The Effect of *C. nardus* Ethanol Extract Against ROS Accumulation in 3T3-L1 Cells

Table 3 and Figure 2 show the observation and luminescence intensity representing ROS levels in cells exposed to *C. nardus* ethanol extract. *C. nardus* ethanol extracts at 50, 100, and 300 ppm exhibited higher ROS accumulation than the negative control. Notably, ROS accumulation increased in a concentration-dependent manner, with 300 ppm showing a higher level of ROS than 100 ppm. Based on these findings, the extract was used at a concentration of 100 ppm for further experiments.

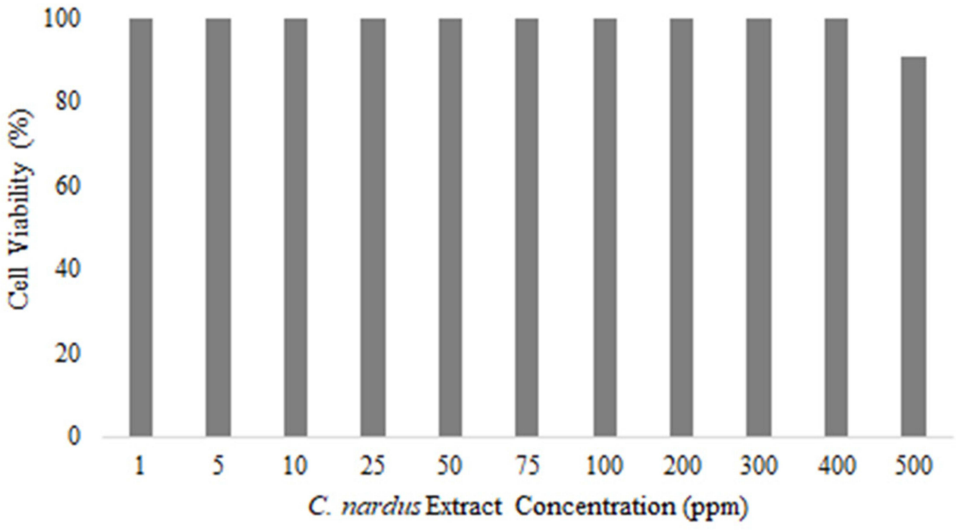

Figure 1 Viability of 3T3-L1 cells exposed to various concentrations of *C. nardus* ethanol extract.

https://doi.org/10.2147/JIR.S506189

Table 3 Fluorescence Intensity Data of ROS Accumulation in *C. nardus* (CE) Ethanol Extract-Treated 3T3-L1 Cells

Group	Treatment	Fluorescence Intensity
Negative Control	3T3-L1 Cell + Medium	9.42
Treatment	3T3-L1 Cell + CE1	7.63
	3T3-L1 Cell + CE50	10.89
	3T3-L1 Cell + CE100	18.01
	3T3-L1 Cell + CE300	37.19

Effect of *C. nardus* Ethanol Extract on the Viability of 3T3-L1 Cells Exposed to Menadione

The effects of menadione and *C. nardus* ethanol extracts on 3T3-L1 cell viability are shown in Figure 3. Menadione treatment significantly decreased cell viability compared to the control group (100% to 50.86 ± 1.56%; $p = 0.034$; $p < 0.05$). However, treatment with *C. nardus* ethanol extract significantly increased the viability of menadione-exposed cells (50.86 ± 1.56% to 100%; $p = 0.034$; $p < 0.05$). The cell viability in the *C. nardus* ethanol extract-treated group was comparable to that observed in the GSH- and NAC-treated groups (73.40 ± 4.97% and 82.70 ± 1.17%, respectively), indicating that *C. nardus* ethanol extract enhanced cell viability more effectively than the GSH and NAC treatments.

Effect of *C. nardus* Ethanol Extract on HIF-1α Gene Expression in Menadione-Induced 3T3-L1 Cells

Exposure to menadione increased HIF-1α gene expression compared with that in the control group ($p<0.05$) (Figure 4). This increase was prevented in cells treated with 100 ppm of *C. nardus* extract ($p<0.05$) and was almost completely diminished in the NAC-treated group.

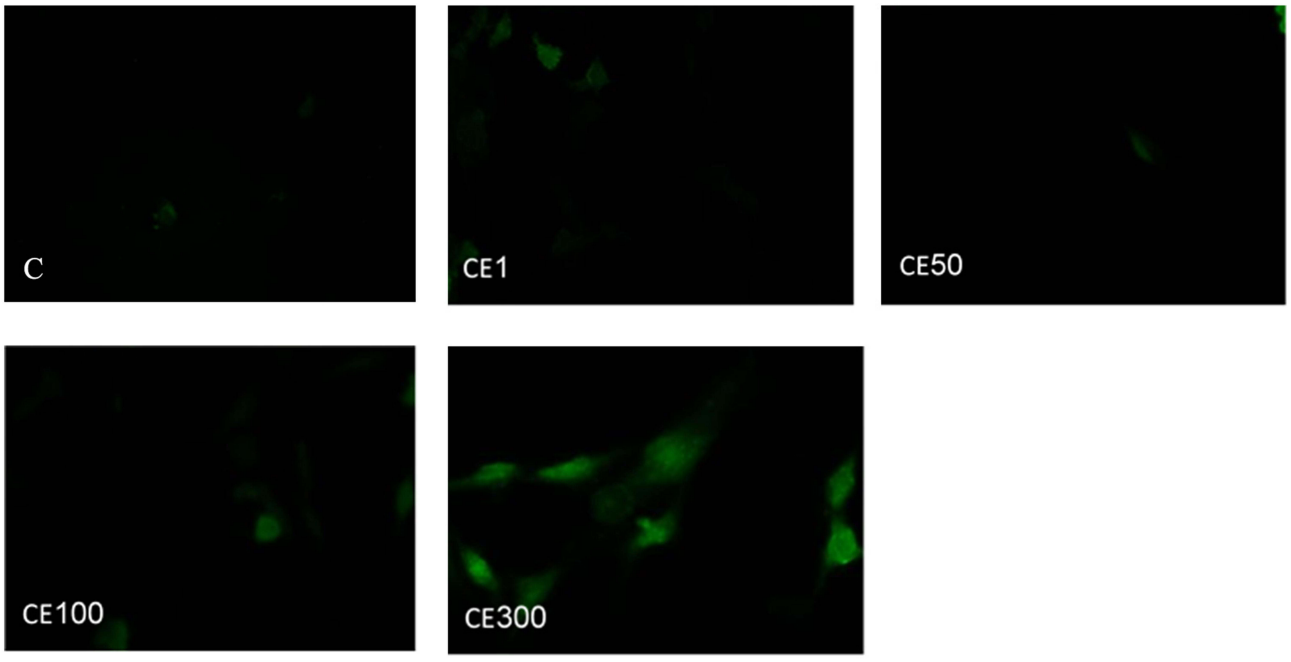

Figure 2 Observation of ROS accumulation in negative control (C) and *C. nardus* ethanol extract-treated 3T3-L1 cells (CE1 = extract at 1 ppm; CE50 = extract at 50 ppm; CE100 = extract at 100 ppm; CE300 = extract at 300 ppm).

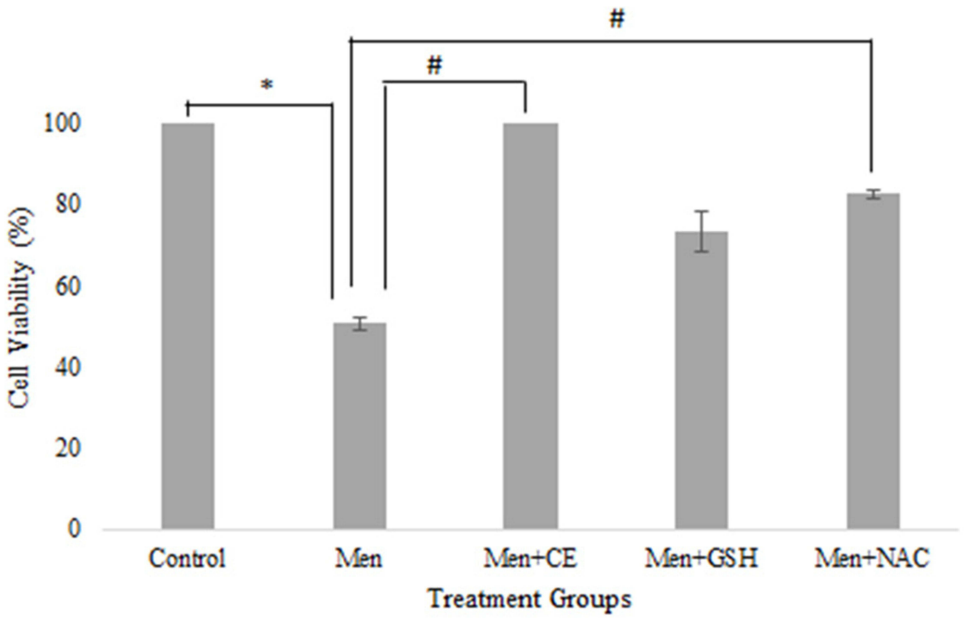

Figure 3 Viability of 3T3-L1 cells exposed to menadione (Men) and treated with 100 ppm *C. nardus* (CE) ethanol extract, glutathione (GSH) or N-acetylcysteine (NAC). *Significant different from control at p <0.05; #Significant different from menadione group at p <0.05.

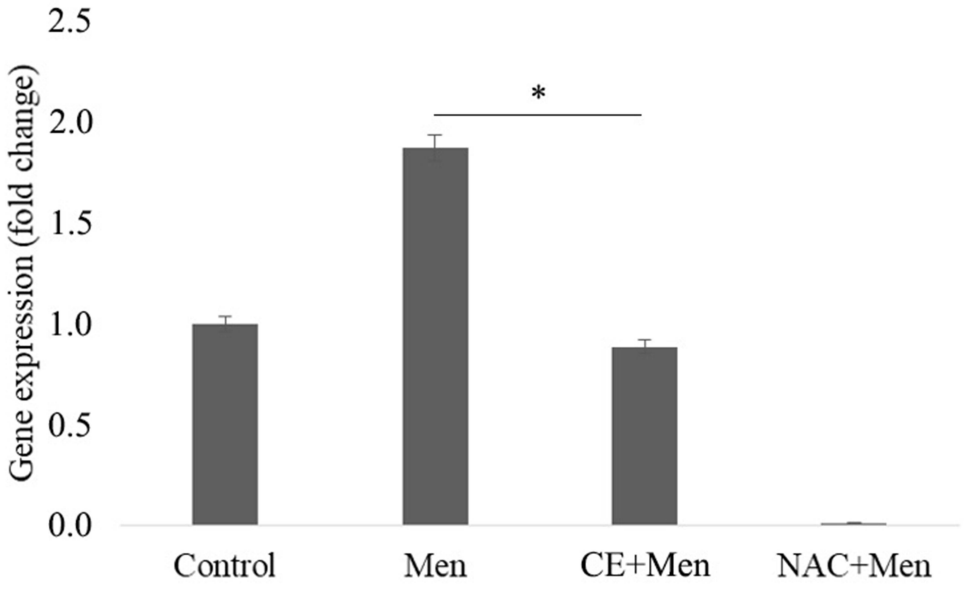

Figure 4 The effect of *C. nardus* ethanol extract (CE) and N-acetylcysteine (NAC) on HIF-1α gene expression in menadione (Men)-induced 3T3-L1 cells. *Significant different at p <0.05.

Effect of *C. nardus* Ethanol Extract on IL-6 and COX-2 Gene Expression in LPS-Induced 3T3-L1 Cells

Exposure to LPS resulted in a more than two-fold increase in IL-6 gene expression in 3T3-L1 cells compared to the control group (Figure 5A). Treatment with *C. nardus* ethanol extract and NAC similarly decreased the expression compared to the LPS group. IL-6 expression in the treated groups was similar to that in the control group. However, based on statistical analysis, there is no significant different between all groups.

Exposure to LPS also led to a significant increase in COX-2 gene expression by nearly five-fold compared to the control ($p=0.023$; $p<0.05$) (Figure 5B). Interestingly, *C. nardus* ethanol extract and NAC similarly mitigated this

https://doi.org/10.2147/JIR.S506189

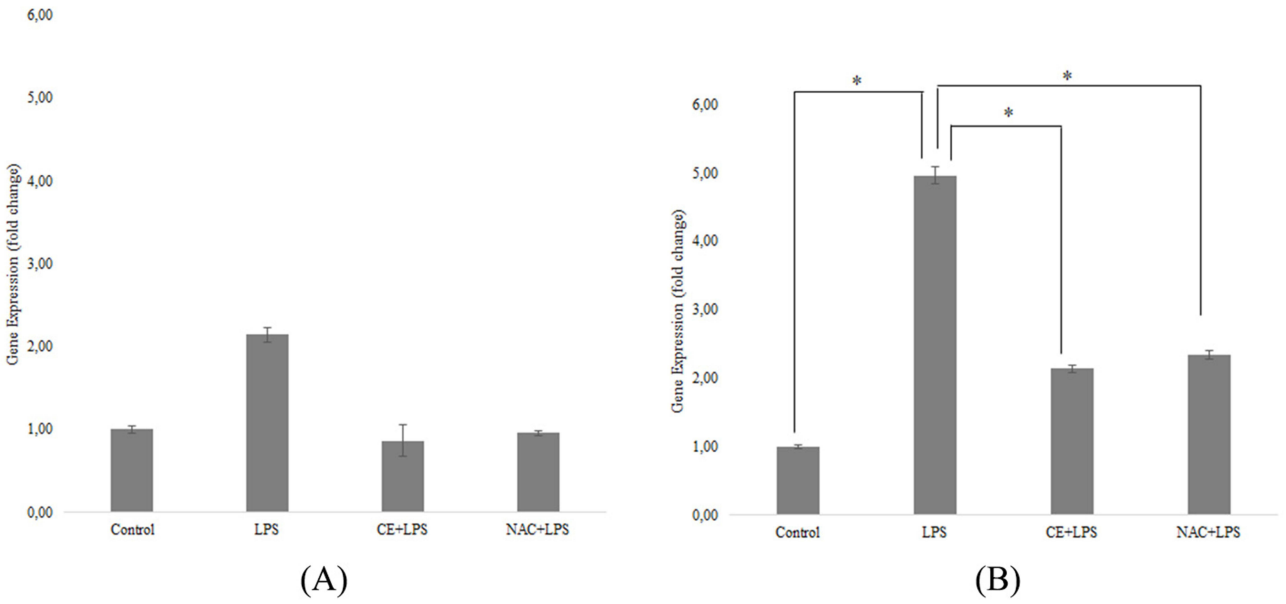

Figure 5 The Effect of *C. nardus* ethanol extract (CE, 100 ppm) and N-acetylcysteine (NAC) on (**A**) IL-6 and (**B**) COX-2 gene expression in LPS-induced 3T3-L1 cells. *Significant different at p <0.05.

elevation compared to the LPS group (p=0.021; p=0.016; p<0.05). However, COX expression in the *C. nardus* ethanol extract- and NAC-treated groups was higher than that in the control group.

Discussion

Oxidative stress, primarily caused by the overproduction of reactive oxygen species (ROS), plays a pivotal role in the pathogenesis of various inflammatory diseases.[23] Excessive ROS production leads to cellular damage by oxidizing proteins, lipids, and nucleic acids, thereby triggering inflammatory responses.[24] In this context, antioxidants play a critical role by neutralizing ROS, thus protecting cells from oxidative damage and reducing inflammation.[25] One of the natural sources of antioxidants is plants, and one that is known to have a rich antioxidant content is *Cymbopogon nardus*.[26]

In our phytochemical screening, *C. nardus* ethanol extract was found to contain phenolic compounds, tannins, flavonoids, and alkaloids. Previous studies have reported similar findings, and some studies detected the presence of saponins in *C. nardus* ethanol extracts.[13,27] Ethanol, being a moderately polar solvent, effectively extracts a range of polar to semi-polar compounds, which likely accounts for the detection of both phenolic compounds and alkaloids.[28] In different types of extracts, *C. nardus* methanol extract contains flavonoids and triterpenoid phytochemicals, whereas *C. nardus* water extract contains alkaloids, flavonoids, saponins, and triterpenoid phytochemicals.[29] Methanol's polarity can enhance the solubility of certain triterpenoids that might be less soluble in ethanol, while water extract reflects the high polarity of water and its capacity to extract highly polar compounds such as saponins more effectively than ethanol or methanol.[28,30] This variation in compound detection across solvents suggests that the choice of solvent plays a crucial role in determining the phytochemical profile of *C. nardus*, with ethanol being particularly suitable for isolating a broad range of semi-polar compounds.

In a previous study, the ethanol (80%) extract of *C. nardus* obtained through dynamic maceration was reported to contain 17.51 ± 0.04 EP/g (pyrogallol equivalent mg/g extract) of total polyphenols, 11.58 ± 0.06 EP/g of total tannins, and 12.98 ± 0.02 EQ/g (quercetin equivalent mg/g extract) (Chiamenti et al, 2019).[31] Geraniol and 1.2-dimethoxy-4-(1-propenyl)-benzene were identified as the major compounds in the ethanol extract of *C. nardus* leaves (Wahyuni et al, 2024).[32] Meanwhile, the essential oil derived from *C. nardus* leaves was shown to contain citronellal (33.06%), geraniol (28.40%), nerol (10.94%), elemol (5.25%), and delta-elemene (4.09%) (Bayala et al, 2020).[33]

In the current study, two types of antioxidant activities were evaluated: DPPH radical-scavenging activity and SOD activity, each with distinct mechanisms and implications for combating oxidative stress. The DPPH assay was conducted to assess the antioxidant capacity of the compounds in directly neutralizing free radicals. It is widely used as a quick

measure of a compound's potential to reduce oxidative damage through direct electron or hydrogen donation to stabilize free radicals.[34] The SOD activity indicates the compound's potential to stimulate or enhance the activity of SOD, an enzyme critical for endogenous antioxidant defense. By promoting SOD activity, compounds can contribute to the body's natural ability to convert superoxide radicals into less reactive species, supporting long-term cellular protection against oxidative damage.[35] Therefore, while DPPH-scavenging suggests immediate antioxidant action, SOD activity implies a sustained defense by bolstering the body's internal antioxidant systems.

C. nardus ethanol extract exhibited moderate antioxidant activity in terms of DPPH scavenging and SOD activity. A previous study also reported that *C. nardus* essential oil fell into a moderate antioxidant category for DPPH scavenging with IC_{50} at 102.19 ± 4.2 ppm,[33] which was lower than the IC_{50} observed in our study. This indicates that the compounds in the essential oil may possess better antioxidant properties than those in the ethanol extract. In that study, it was also shown that *C. nardus* essential oil obtained from its leaves has modest antioxidant compared to the standard gallic acid with DPPH IC_{50} value 0.11 ± 0.04 μg for gallic acid and the antioxidant activity by the ABTS+• method value of 0.009 ± 0.0004 μM TE/g against 2.66 ± 0.31 μM TE/g for the gallic acid. The antioxidant activity of *C. nardus* extract could be explained by its high content of monoterpene aldehyde and monoterpene alcohol like citronellal, citronellol, and geraniol (Bayala et al, 2020).[33]

Based on the viability percentage of over 90% in the current study, it can be concluded that *C. nardus* ethanol extract up to 500 ppm is relatively less toxic to 3T3-L1 cells than the other reported plant extracts. For instance, a water extract from the seeds of *Phoenix dactylifera* L. at concentrations higher than 2.5 mg/mL (equivalent to 2500 ppm) resulted in a decrease in cell viability to less than 60%.[36] The relatively lower toxicity of *C. nardus* extract implies a higher potential for safe application in formulations that require minimal cytotoxicity. This suggests that it could be well tolerated in contexts where cell viability is crucial, such as in therapeutic products.

Based on the fluorescence intensity produced by DCFH oxidation by ROS, 3T3-L1 cells treated with 300 ppm *C. nardus* ethanol extract produced the highest level of ROS in the cell line. The extract at this concentration led to the highest ROS accumulation in the cell line, indicating that *C. nardus* ethanol extract induced oxidative stress in cells. High concentrations of citronella and citronellol have been reported to induce the production of reactive oxygen species (ROS) in cells. This phenomenon is likely due to their interaction with cellular membranes, leading to oxidative stress and disruption of the redox balance (Lins et al, 2019).[37] While citronellol and citronella are known for their antioxidant properties at lower concentrations, excessive levels can trigger pro-oxidant effects, stimulating ROS generation and potentially causing cellular damage in certain biological systems.

In this study, it was shown that moderate ROS accumulation was observed at 100 ppm and was selected for further experiments. Moderate accumulation of ROS is often desired in studies exploring the potential therapeutic effects of plant extracts, as ROS can trigger beneficial cellular responses such as apoptosis or autophagy, which are particularly relevant in cancer therapy. The desired level of ROS accumulation should be sufficient to activate these cellular pathways without overwhelming the cells or causing extensive damage. High ROS accumulation, which is beneficial in inducing cell death in diseased cells, can also lead to cytotoxicity if levels become too elevated.[38] Therefore, a concentration of 100 ppm was chosen as it provided balanced ROS induction, ensuring more controlled and safer experimental conditions for further study.

Menadione is commonly used as an inducer of oxidative stress.[39] By generating reactive oxygen species (ROS), menadione can cause cells to become dysfunctional or die.[40,41] *C. nardus* ethanol extract restored the viability of 3T3-L1 cells exposed to menadione, possibly through its ability of *C. nardus* ethanol extract to downregulate HIF-1α gene expression. HIF-1α is one of several target genes activated by reactive oxygen species (ROS), plays a crucial role in the cellular response to hypoxic conditions, and is linked to oxidative stress and inflammation.[14,42] The phytochemical compounds in the extract likely suppress oxidative stress-related pathways, thereby mitigating cellular damage. These compounds have been reported to possess good antioxidant property.[43] The antioxidant property of the extract is evidenced by their ROS-scavenging capacity and their ability to increase superoxide dismutase activity.

LPS is frequently employed to induce inflammation.[44] It effectively inducing a strong inflammatory response by enhancing COX-2 activity, which then triggers the production of pro-inflammatory cytokines such as IL-6.[45] The anti-inflammatory effects of *C. nardus* ethanol extract were evidenced by the downregulation of COX-2 and IL-6 gene expression in LPS-induced cells. In line with other in vitro studies, the methanol extract of lemongrass from the *Cymbopogon* genus has

been shown to reduce IL-6 levels in macrophage cells,[46] whereas lemongrass oil decreased *COX-2* mRNA expression in human macrophage-like U937 cells.[47]

COX-2 plays a key role in the inflammatory pathway, leading to the production of prostaglandin E2 (PGE2), an important mediator of inflammation. In contrast, IL-6 expression is regulated by multiple pathways, some of which are not directly involved in COX-2 expression. For instance, its expression can be modulated via ROS-dependent mechanisms through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation, which can occur independently of COX-2.[48,49] The extract of *C. nardus* may contain bioactive compounds that specifically inhibit the COX-2 signaling pathway, resulting in a decrease in PGE2 production, while certain compounds may also inhibit IL-6 expression. These findings suggest that *C. nardus* extract modulates inflammatory responses through multiple mechanisms.

Conclusion

Cymbopogon nardus exerts antioxidant and anti-inflammatory activities by suppressing HIF-1α expression in menadione-induced cells and reducing COX-2 and IL-6 gene expression in LPS-induced cells. The protective effects of the extract are likely attributable to the presence of various phytochemicals including phenolics, tannins, flavonoids, and alkaloids. Therefore, *C. nardus* has the potential to serve as a natural antioxidant through DPPH and SOD activity and an anti-inflammatory agent through HIF-1α, IL-6, and COX-2 pathway. Further analysis using additional antioxidant and anti-inflammatory parameters, as well as in vivo studies, is recommended to confirm the potential, mechanism of action, and potential physiological implications of *Cymbopogon nardus* extract.

Acknowledgments

We gratefully acknowledge Dr. Achadiyani from Universitas Padjadjaran for her essential contributions and support in this study.

Funding

This study was financed by the Directorate of Research, Community Service, and Innovation at Universitas Padjadjaran under Grant 1822/UN6.3.1/PT.00/2024.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ahmad A, Ahsan H. Biomarkers of inflammation and oxidative stress in ophthalmic disorders. *J Immunoassay Immunochem*. 2020;41(3):257–271. doi:10.1080/15321819.2020.1726774
2. Allegra M. Antioxidant and anti-inflammatory properties of plants extract. *Antioxidants*. 2019;8(11):549. doi:10.3390/antiox8110549
3. Zammel N, Saeed M, Bouali N, et al. Antioxidant and anti-inflammatory effects of *Zingiber officinale* roscoe and *Allium subhirsutum*: in silico, biochemical and histological study. *Foods*. 2021;10(6):1383. doi:10.3390/foods10061383
4. Li W, Zhang X, Chen R, et al. HPLC fingerprint analysis of *Phyllanthus emblica* ethanol extract and their antioxidant and anti-inflammatory properties. *J Ethnopharmacol*. 2020;254:112740. doi:10.1016/j.jep.2020.112740
5. Mousavi SM, Hashemi SA, Behbudi G, et al. A review on health benefits of *Malva sylvestris* L. nutritional compounds for metabolites, antioxidants, and anti-inflammatory, anticancer, and antimicrobial applications. *Evid Based Complement Alternat Med*. 2021;2021(1):5548404. doi:10.1155/2021/5548404
6. Wiraswati HL, Fauziah N, Pradini GW, et al. Breynia cernua: chemical profiling of volatile compounds in the stem extract and its antioxidant, antibacterial, antiplasmodial and anticancer activity in vitro and in silico. *Metabolites*. 2023;13(2):281. doi:10.3390/metabo13020281
7. Wiraswati HL, Pradini GW, Fauziah N, et al. Biological potential of eight medicinal plants collected in the restored landscape after mining in South Kalimantan. *Discov Appl Sci*. 2024;6(6):1–5. doi:10.1007/s42452-024-05824-2
8. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus*. 2022;65 (3):399–411. doi:10.1007/s13237-022-00405-3
9. Toledo LG, Ramos MA, Spósito L, et al. Profiling the Cymbopogon nardus ethanol extract and its antifungal potential against Candida species with different patterns of resistance. *J Braz Chem Soc*. 2020;31:1926–1938.
10. Ranasinghe MS, Arambewela L, Samarasinghe S. Development of herbal mosquito repellent formulations. *Int J Pharm Sci Res*. 2016;7 (9):3643–3648.
11. Bimantio MP, Wardoyo AD. Sensitivity and feasibility analysis of citronella oil business. *SOCA*. 2020;14(2):313–324. doi:10.24843/SOCA.2020.v14.i02.p11

12. Ben Ammar R. Potential effects of geraniol on cancer and inflammation-related diseases: a review of the recent research findings. *Molecules*. 2023;28(9):3669. doi:10.3390/molecules28093669
13. Najmah N, Fitria R, Kurniawati E. Skrining fitokimia, total flavonoid dan fenolik daun sereh wangi (*Cymbopogon nardus* (L.) Rendle). *Jurnal Crystal*. 2023;5(1):62–70. Indonesian. doi:10.36526/jc.v5i1.2642
14. Wiraswati HL, Ekawardhani S, Rohmawaty E, et al. Antioxidant, antiinflammation, and antifibrotic activity of ciplukan (*Physalis angulata* L). extract. *J Inflamm Res*. 2024;Volume 17:6297–6306. doi:10.2147/JIR.S470318
15. Manongko PS, Sangi MS, Momuat LI. Uji senyawa fitokimia dan aktivitas antioksidan tanaman patah tulang (*Euphorbia tirucalli* L.). *Jurnal Mipa*. 2020;9(2):64–69. Indonesian. doi:10.35799/jmuo.9.2.2020.28725
16. Sulistyarini I, Sari DA, Wicaksono TA. Skrining fitokimia senyawa metabolit sekunder batang buah naga (Hylocereus polyrhizus). *Cendekia Eksakta*. Indonesian. 2020;5(1).
17. Nortjie E, Basitere M, Moyo D, et al. Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: a review. *Plants*. 2022;11(15):2011. doi:10.3390/plants11152011
18. Fadri RA, Roza I, Tazar N, et al. Phytochemical screening and antioxidant test of arabica roasted coffee bean extract (*Coffea arabica* L.) from Agam Regency. *IOP Conf Ser Earth Environ Sci*. 2022;1097(1):012028. doi:10.1088/1755-1315/1097/1/012028
19. Sharma PP, Chonche MJ, Mudhol S, et al. Anti-inflammatory efficacy of brown seaweed (*Padina tetrastromatica*) in 3T3-L1 adipocytes and low-dose LPS induced inflammation in C57BL6 mice. *Algal Res*. 2023;71:103027. doi:10.1016/j.algal.2023.103027
20. Aslan C, Maralbashi S, Kahroba H, et al. Docosahexaenoic acid (DHA) inhibits pro-angiogenic effects of breast cancer cells via down-regulating cellular and exosomal expression of angiogenic genes and microRNAs. *Life Sci*. 2020;258:118094. doi:10.1016/j.lfs.2020.118094
21. Kiselova-Kaneva Y, Tasinov O, Vankova D, et al. Ethanol induces IL-6 and TNF-α cytokine and iNOS and COX-2 enzyme gene expression in 3T3-L1 preadipocytes. *Scr Sci Med*. 2012;44(2):31–35.
22. Nurdyansyah F, Widyastuti DA. Comparison of antioxidant activity of ethanolic, methanolic, n-hexan, and aqueous extract of *Parkia speciosa* peel based on half-maximal inhibitory concentration through free radical inhibition. *Adv Sustain Sci Eng Tech*. 2020;2(2):343559.
23. Li P, Chang M. Roles of PRR-mediated signaling pathways in the regulation of oxidative stress and inflammatory diseases. *Int J mol Sci*. 2021;22 (14):7688. doi:10.3390/ijms22147688
24. Checa J, Aran JM. Reactive oxygen species: drivers of physiological and pathological processes. *J Inflamm Res*. 2020;13:1057–1073. doi:10.2147/JIR.S275595
25. Ali SS, Ahsan H, Zia MK, et al. Understanding oxidants and antioxidants: classical team with new players. *J Food Biochem*. 2020;44(3):e13145. doi:10.1111/jfbc.13145
26. Solekha R, Purnobasuki H, Puspaningsih NN, et al. Secondary metabolites and antioxidants activity from citronella grass extract (*Cymbopogon nardus* L.). *Indian J Pharm Educ Res*. 2024;58(1):298–305. doi:10.5530/ijper.58.1s.32
27. Dewi PS, Rachmawan RL, Prasayundari FS, et al. Ethanol extract of lemongrass leaves (*Cymbopogon nardus* (L.) Rendle) as an inhibitor to enhancement of cholesterol levels in wistar rats. *IOP Conf Ser Earth Environ Sci*. 2022;1104(1):012020. doi:10.1088/1755-1315/1104/1/012020
28. Peña-Portillo GC, Acuña-Nelson SM, Bastías-Montes JM. From waste to wealth: exploring the bioactive potential of wine by-products—A review. *Antioxidants*. 2024;13(8):992. doi:10.3390/antiox13080992
29. Shin SA, Joo BJ, Lee JS, et al. Phytochemicals as anti-inflammatory agents in animal models of prevalent inflammatory diseases. *Molecules*. 2020;25(24):5932. doi:10.3390/molecules25245932
30. Ng ZX, Samsuri SN, Yong PH. The antioxidant index and chemometric analysis of tannin, flavonoid, and total phenolic extracted from medicinal plant foods with the solvents of different polarities. *J Food Process Preserv*. 2020;44(9):e14680. doi:10.1111/jfpp.14680
31. Chiamenti L, Silva FP, Schallemberger K, et al. Cytotoxicity and antiviral activity evaluation of *Cymbopogon* spp hydroethanolic extracts. *Braz J Pharm Sci*. 2019;55:e18063. doi:10.1590/s2175-97902019000118063
32. Wahyuni DK, Kharisma VD, Murtadlo AA, et al. The antioxidant and antimicrobial activity of ethanolic extract in roots, stems, and leaves of three commercial *Cymbopogon* species. *BMC Complement Med Ther*. 2024;24(1):272. doi:10.1186/s12906-024-04573-4
33. Bayala B, Coulibaly AY, Djigma FW, et al. Chemical composition, antioxidant, anti-inflammatory and antiproliferative activities of the essential oil of *Cymbopogon nardus*, a plant used in traditional medicine. *Biomol Concepts*. 2020;11(1):86–96. doi:10.1515/bmc-2020-0007
34. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. *Processes*. 2023;11(8):2248. doi:10.3390/pr11082248
35. Islam MN, Rauf A, Fahad FI, et al. Superoxide dismutase: an updated review on its health benefits and industrial applications. *Crit Rev Food Sci Nutr*. 2022;62(26):7282–7300. doi:10.1080/10408398.2021.1913400
36. Christodoulou MC, Orellana Palacios JC, Hesami G, et al. Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. *Antioxidants*. 2022;11(11):2213. doi:10.3390/antiox11112213
37. Lins L, Dal Maso S, Foncoux B, et al. Insights into the relationships between herbicide activities, molecular structure and membrane interaction of cinnamon and citronella essential oils components. *Int J mol Sci*. 2019;20(16):4007. doi:10.3390/ijms20164007
38. An X, Yu W, Liu J, et al. Oxidative cell death in cancer: mechanisms and therapeutic opportunities. *Cell Death Dis*. 2024;15(8):556. doi:10.1038/s41419-024-06939-5
39. Marinovska PG, Todorova TI, Boyadzhiev KP, et al. Cellular susceptibility and oxidative stress response to menadione of logarithmic, quiescent, and nonquiescent *Saccharomyces cerevisiae* cell populations. *BioRisk*. 2022;17:127–138. doi:10.3897/biorisk.17.77320
40. Kesari KK, Dhasmana A, Shandilya S, et al. Plant-derived natural biomolecule picein attenuates menadione induced oxidative stress on neuroblastoma cell mitochondria. *Antioxidants*. 2020;9(6):552. doi:10.3390/antiox9060552
41. Wiraswati HL, Hangen E, Sanz AB, et al. Apoptosis inducing factor (AIF) mediates lethal redox stress induced by menadione. *Oncotarget*. 2016;7 (47):76496. doi:10.18632/oncotarget.12562
42. Korbecki J, Simińska D, Gąssowska-Dobrowolska M, et al. Chronic and cycling hypoxia: drivers of cancer chronic inflammation through HIF-1 and NF-κB activation: a review of the molecular mechanisms. *Int J mol Sci*. 2021;22(19):10701. doi:10.3390/ijms221910701
43. Angwa LM, Jiang Y, Pei J, Sun D. Antioxidant phytochemicals for the prevention of fluoride-induced oxidative stress and apoptosis: a review. *Biol Trace Elem Res*. 2022;200(3):1418–1441. doi:10.1007/s12011-021-02729-8
44. Skrzypczak-Wiercioch A, Sałat K. Lipopolysaccharide-induced model of neuroinflammation: mechanisms of action, research application and future directions for its use. *Molecules*. 2022;27(17):5481. doi:10.3390/molecules27175481

45. Saanin SN, Wahyudianingsih R, Afni M, et al. Suppression of pro-inflammatory cytokines and mediators production by ginger (*Zingiber officinale*) ethanolic extract and gingerol in lipopolysaccharide-induced RAW264.7 murine macrophage cells. *Indian J Nat Prod Resour*. 2021;11(4):260–266.
46. Bachiega TF, Sforcin JM. Lemongrass and citral effect on cytokines production by murine macrophages. *J Ethnopharmacol*. 2011;137(1):909–913. doi:10.1016/j.jep.2011.07.021
47. Katsukawa M, Nakata R, Takizawa Y, et al. Citral, a component of lemongrass oil, activates PPARα and γ and suppresses COX-2 expression. *Biochim Biophys Acta mol Cell Biol Lipids*. 2010;1801(11):1214–1220. doi:10.1016/j.bbalip.2010.07.004
48. Kang JK, Chung YC, Hyun CG. Anti-inflammatory effects of 6-methylcoumarin in LPS-stimulated RAW 264.7 macrophages via regulation of MAPK and NF-κB signaling pathways. *Molecules*. 2021;26(17):5351. doi:10.3390/molecules26175351
49. Zhao Y, Usatyuk PV, Gorshkova IA, et al. Regulation of COX-2 expression and IL-6 release by particulate matter in airway epithelial cells. *Am J Respir Cell mol Biol*. 2009;40(1):19–30. doi:10.1165/rcmb.2008-0105OC