Trop J Nat Prod Res, October 2025; 9(10): 5004 - 5009 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

In vitro Cytoprotective Effects of *Pluchea indica* L. Leaf Extract on Vero Cells against Oxidative Stress: A Comparative Study of Preventive and Curative Treatments

Violita Syukroni[1], Nor I. M. Ismail [2], Woro A. S. Tunjung[1]*

[1]*Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia*
[2] *Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Baru Barat, 31900, Kampar, Perak, Malaysia*

ARTICLE INFO

Article history:
Received 24 July 2025
Revised 19 September 2025
Accepted 25 September 2025
Published online 01 November 2025

ABSTRACT

Pluchea indica leaf extract (PIE) has shown promise as an anti-aging agent by extending the lifespan of *Saccharomyces cerevisiae*. This study investigated the cytoprotective effects of *P. indica* in Vero cells, using cells divided into preventive and curative treatment groups. In the preventive group, PIE at concentrations of 136, 68, and 34 µg/mL was administered before the addition of 40 µM or 80 µM H_2O_2. In the curative group, the same PIE concentrations were given after the addition of 400 µM or 800 µM H_2O_2. Assays were conducted to evaluate cell viability, the production of reactive oxygen species (ROS), apoptosis, and caspase-3 activity. In the preventive treatment, H_2O_2 increased ROS formation by up to 70%, while PIE and ascorbic acid reduced ROS levels to 26.8% and 38%, respectively. In the curative treatment, H_2O_2 increased ROS production by 89.1%, with PIE and ascorbic acid reducing ROS levels by 36.6% and 52.2%, respectively. The percentages of cell death, apoptosis, and caspase-3 activity measured in cells under H_2O_2 stress and those treated with H_2O_2 combined with ascorbic acid and PIE showed consistent results, indicating a trend from highest to lowest compared to the control group. Notably, PIE demonstrated superior efficacy in maintaining cell viability compared to ascorbic acid. In conclusion, PIE exhibits cytoprotective properties in Vero cells, effectively mitigating oxidative stress in preventive treatments at lower concentrations and requiring higher concentrations for optimal repair in curative treatments

***Keywords*:** Cytoprotective, Antioxidant, *Pluchea indica* L., Vero Cells, Oxidative Stress.

Introduction

Oxidative stress poses significant risks to health and homeostasis, as it is involved in cell membrane damage, mitochondrial dysfunction, aging, neurodegenerative diseases, cardiovascular diseases, and cancer.[1] This condition arises when free radicals or reactive oxygen species (ROS) exceed the removal capacity of the antioxidant system. The sources of oxidative stress can be either endogenous or exogenous. Endogenous free radicals can result from inflammation, infection, aging, ischemia, excessive exercise, mental stress, and cancer. In contrast, exogenous free radicals originate from exposure to UV radiation, air pollution, heavy metals (Cd, Hg, Pb, Fe, As), medications, cigarette smoke, alcohol consumption, and unhealthy lifestyles.[2,3] An imbalance in ROS production can damage cellular DNA, potentially leading to cell death.[4,5] A commonly used method to induce oxidative stress in the laboratory is exposure to hydrogen peroxide (H_2O_2), which increases ROS production, triggering apoptosis and irreversible cell damage.[6-8]

*Corresponding author. Email: wanindito@ugm.ac.id
Tel: +62 (274) 580839

Citation: Syukroni V, Ismail NIM, Tunjung WAS. *In vitro* Cytoprotective Effects of *Pluchea indica* L. Leaf Extract on Vero Cells Against Oxidative Stress: A Comparative Study of Preventive and Curative Treatments. Trop J Nat Prod Res. 2025; 9(10): 5004 – 5009 https://doi.org/10.26538/tjnpr/v9i10.41

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

A cytoprotectant is a compound or agent that safeguards cells from damage caused by various stress factors, including reactive oxygen species (ROS). The primary objective of administering cytoprotectants is to minimize oxidative damage at the cellular level by enhancing antioxidant defenses and reducing the production of ROS. These agents also assist in maintaining normal cellular function, thereby preventing apoptosis.[9] Common cytoprotective agents are antioxidants, compounds that can disrupt electron chains, neutralize free radicals, and prevent the formation of ROS.[10] By maintaining normal cell function, antioxidants demonstrate significant potential as cytoprotective agents.[9] Natural antioxidants, especially those derived from plants, have shown promise in cytoprotective therapies against ROS. Silver nanoparticles synthesized from *Solanum melongena* peel extract, which contains polyphenols and anthocyanins, exhibit moderate antioxidant activity and effectively inhibit the production of free radicals[11]. *Pluchea indica* (L.) possesses several biomedical activities, including antioxidant,[12,13] anticancer,[14] and anti-inflammatory properties,[15] highlighting its potential as a cytoprotective agent. Additionally, our previous study demonstrated that the leaves of *Pluchea indica* possess enzymes with antioxidant activity, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). The leaves have demonstrated anti-aging potential by extending the lifespan of *Saccharomyces cerevisiae* and enhancing its survival under H_2O_2-induced stress. This study aims to analyze the activity of *Pluchea indica* leaf extract in protecting Vero cells from oxidative damage induced by H_2O_2. The cytoprotective effects were evaluated by administering the extract to Vero cells both before and after H_2O_2 exposure, representing respective preventive and curative treatments.

Materials and Methods

Sampling and Extraction of Pluchea indica Leaves

The leaves of *Pluchea indica* were collected from a garden in Klaten, Indonesia, and their identification was confirmed by a botanist at the

Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. The selected leaves were the fifth from the apex and were characterized as mature, fresh, and green. The leaves were dried and ground into powder. A hundred grams of dried leaf powder was extracted using the maceration method with ethyl acetate as the solvent, maintaining a 1:2 ratio for 24 h at room temperature, followed by three additional maceration cycles. The extract was then filtered, and the filtrate was evaporated to yield a concentrated paste of *Pluchea indica* extract (PIE).

Vero cell treatment

The study obtained ethical approval from the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (Ref. no. KE/FK/1377/EC/2023). The cytoprotective activity of *Pluchea indica* extract (PIE) on Vero cells was assessed through both preventive and curative treatments. In the preventive treatment, the cells were pretreated with PIE at concentrations of 136, 68, and 34 µg/mL for one hour, after which they were exposed to H_2O_2 at concentrations of 40 µM and 80 µM and then further incubated in the presence of PIE for 24 hours. This approach is based on previously published methods [16,17] that involve administering H_2O_2 at low concentrations for an extended period. In the curative treatment, the Vero cells were exposed to high concentrations of H_2O_2 (400 µM and 800 µM) for two hours to induce oxidative stress.[18,6,7] The cells were subsequently washed with PBS buffer to remove H_2O_2 and treated with PIE at concentrations of 136, 68, and 34 µg/mL for 24 hours. As a positive control, Vero cells were treated with ascorbic acid at a concentration of 50 µM.[7,19]

Cell Viability Assay

The viability of Vero cells was assessed using the MTT assay (Invitrogen). Vero cells were seeded in a 96-well plate at a density of 5×10^3 cells/well and incubated for 24 hours. Following incubation, the cells were subjected to preventive and curative treatments. After treatment, 0.5 mg/mL MTT reagent was added, and the cells were incubated for an additional four hours. The reaction was terminated by adding 100 µL of 10% SDS in 0.1 N HCl as the stop reagent. The absorbance was measured at a wavelength of 595 nm. The cell viability assay was conducted to determine the optimal concentrations of H_2O_2 and the mixture of H_2O_2 and extract to be used in subsequent experiments.

Intracellular ROS Assay

The cells subjected to preventive and curative treatments were harvested, and the levels of intracellular reactive oxygen species (ROS) were assessed using a reactive oxygen species (ROS) Fluorometric Assay Kit 96T (Elabscience) according to the manufacturer's instructions. Analysis was performed using a flow cytometer (BD FACS CANTO II) and BD FACSDIVA® software.

Apoptosis Assay

The cells that received preventive and curative treatments were harvested and washed with phosphate-buffered saline (PBS). The cells were subsequently resuspended in 100 µL of 1X binding buffer. FITC Annexin V and propidium iodide (PI) were added (5 µL each) and mixed by vortexing. The cells were incubated for 15 minutes at room temperature, followed by the addition of 400 µL of PBS. Apoptosis was analyzed using a flow cytometer.

Caspase-3 Assay

Caspase-3 activity was measured using an FITC Active Caspase-3 Apoptotic Kit (BD Pharmingen™ Bioscience), following the manufacturer's protocol. The analysis was conducted using a flow cytometer.

Statistical Analysis

The cytoprotective effect of *Pluchea indica extract* (PIE) on H_2O_2-induced Vero cells was assessed using one-way ANOVA. Principal component analysis (PCA) provided additional insights by distinguishing between the control, H_2O_2-treated, and PIE-treated groups.

Results and Discussion

This study analyzed the cytoprotective activity of *Pluchea indica* extract (PIE) on Vero cells subjected to H_2O_2 stress. Vero cells were used because they are commonly employed in vaccine development, drug metabolism testing, cytotoxicity assays, antibody production, and studies of gene function.[20] The use of Vero cells can manifest responses in MTT assays that are similar to those observed with other cell types, making them a suitable alternative model for toxicology and cytoprotective studies. [21]

Morphology of Vero Cells

H_2O_2 induced oxidative stress and decreased the viability of Vero cells. The representative morphology of normal Vero cells (control group) and those experiencing oxidative stress is shown in Figure 1. The untreated Vero cells exhibited normal morphology, characterized by elongated, polygonal, and flat shapes (Figure 1A). In contrast, Vero cells treated with H_2O_2 appeared round and irregular, and shriveled, and displayed signs of detachment (Figure 1B). Vero cells treated with H_2O_2 and either the extract or ascorbic acid exhibited morphology similar to that of the control group, appearing elongated and polygonal (Figures 1C and 1D).

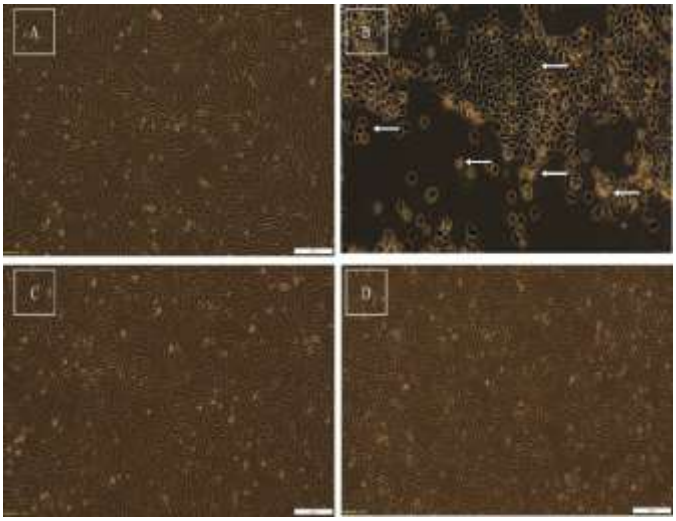

Figure 1: Morphology of Vero Cells. Normal Vero cells (A), after exposure to H_2O_2 stress (B), after exposure to H_2O_2 stress and PIE (C), and after exposure to H_2O_2 stress and Asc (D). Observations were conducted using an inverted microscope at a magnification of 20x. PIE: *Pluchea indica* extract; Asc: Ascorbic acid.

Vero cells normally have an elongated or polygonal shape. Cultured Vero cells form a monolayer, adhere to the well's surface, and display an epithelial-like structure.[22,18] Oxidative stress caused by H_2O_2 led to changes in the morphology of Vero cells, including irregular elongation, alterations in cell shape (becoming rounded or shriveled) with uneven cell contours (Figure 1). These findings were consistent with previous studies.[7] In this study, the preventive treatment involved applying PIE before exposure to low H_2O_2 concentrations, whereas the curative treatment involved applying PIE after exposure to high H_2O_2 concentrations to induce oxidative stress. In the preventive model, low H_2O_2 concentrations (40 and 80 µM) simulated mild oxidative stress, similar to sub-toxic levels of reactive oxygen species under normal conditions. Exposure to such low H_2O_2 concentrations increases ROS production, causes DNA damage, reduces cell viability, and activates aging-associated genes, including P53.[16,17] This condition reflects the continuous exposure to free radicals that occurs in daily life, allowing the assessment of PIE's ability to prevent the progression of oxidative stress. In the curative model, the higher H_2O_2 concentrations (400 and 800 µM) generated severe oxidative stress, with 800 µM representing the IC_{50} value from preliminary cytotoxicity assays. This design

evaluated the capacity of PIE to restore cellular function after substantial oxidative damage, thereby providing a framework to investigate both the preventive and curative effects of the extract.

The Effects of PIE on Cell Viability

Based on our preliminary study, exposure to H_2O_2 at a concentration of 800 µM for two hours reduced the viability of Vero cells by 56.4% (data not shown). Therefore, H_2O_2 concentrations of 800 µM and 400 µM were employed in the assay of the cytoprotective effects of PIE on Vero cells. The viability of Vero cells after receiving preventive and curative treatments with PIE is illustrated in Figure 2. The treatment with 50 µM ascorbic acid resulted in higher cell viability compared to H_2O_2 only, indicating that ascorbic acid serves as an effective positive control. In the preventive treatment, PIE resulted in a higher percentage of Vero cell viability compared to ascorbic acid under both 80 µM and 40 µM H_2O_2.

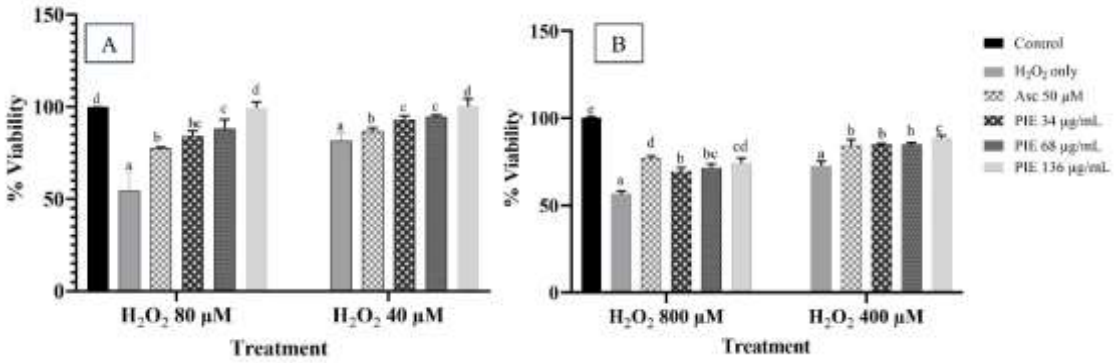

Figure 2: Viability of Vero Cells Exposed to hydrogen peroxide (H_2O_2)-induced Stress and PIE. Preventive effects (A) and curative effects (B). PIE: *Pluchea indica* extract; Asc: Ascorbic acid. Note: *P < 0.05 indicates significant differences as determined by Duncan's tests at the 95% confidence level.

In the curative treatment, PIE at concentrations of 34, 68, and 136 µg/mL maintained Vero cell viability at 69%, 71%, and 74% in the 800 µM H_2O_2 treatment group, and at 84%, 84%, and 88% in the 400 µM H_2O_2 treatment group, respectively. These results demonstrate that although Vero cells experienced oxidative damage, treatment with PIE had a concentration-dependent effect and reversed the damage to H_2O_2-treated cells. In contrast, the effects of PIE on Vero cells exhibited a different trend. In the preventive treatment, the lowest concentration of PIE (34 µg/ml) maintained cell viability comparable to that of ascorbic acid. However, in the curative treatment, a higher concentration of PIE (136 µg/ml) was required to sustain Vero cell viability equivalent to that of ascorbic acid. The concentrations of H_2O_2 used for subsequent testing were based on the lowest viability conditions. Therefore, H_2O_2 at a concentration of 80 µM and PIE at 34 µg/ml were used for the preventive treatment, while for the curative treatment, H_2O_2 was administered at 800 µM and PIE at 136 µg/ml for subsequent experiments.

Effects of PIE on ROS Production

This experimental system confirmed that H_2O_2 effectively triggered ROS production, as indicated by the lowest ROS level in the control (untreated cells). Figure 3 illustrates the similar trends for both sets of treatments. In the preventive treatment, the highest ROS level occurred in the H_2O_2-only group (80 µM), followed by the ascorbic acid group and the PIE group (34 µg/mL), while the lowest ROS level was observed in the control group. In the curative treatment, the highest ROS production was observed in the group administered H_2O_2 only at a concentration of 800 µM. In the curative treatment, the highest production of ROS occurred in the cells treated with 800 µM of H_2O_2 alone. The addition of ascorbic acid reduced ROS levels compared to the H_2O_2-only group, while treatment with PIE at 136 µg/mL further decreased ROS production. Accordingly, ROS levels were highest in the H_2O_2-only group, lower in the ascorbic acid group, and lowest in the PIE-treated group. These findings show that higher concentrations of H_2O_2 were associated with increased ROS production. In both the preventive and curative treatments, PIE reduced ROS levels to 26.8% and 36.6%, respectively, lower than those observed with ascorbic acid (38.0% and 52.2%). This trend indicates that PIE led to a stronger reduction of ROS compared to the positive control.

The Effect of PIE on H_2O_2-Induced Apoptosis

Under normal conditions, Vero cells exhibit 90.6% viability, with 6.4% undergoing apoptosis and 2.8% undergoing necrosis. Administration of 80 µM H_2O_2 only (the preventive treatment) reduced cell viability to 22.6%, with apoptosis and necrosis occurring in 69.2% and 7.7% of the cells, respectively. Treatment with PIE maintained cell viability at 90.9%, comparable to normal conditions, and was superior to ascorbic acid, which only resulted in 81.1% viability. In the curative treatment, administration of 800 µM H_2O_2 decreased cell viability to 12.6%, with 83.0% of cells undergoing apoptosis and 4.2% undergoing necrosis. Treatment with PIE preserved cell viability at 87.2%, comparable to normal conditions, and was significantly more effective than ascorbic acid, which only achieved 36.6% viability.

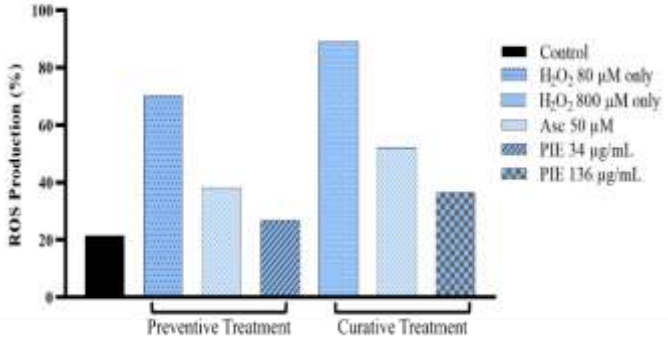

Figure 3: Percentage of ROS Production in Vero Cells Subjected to Preventive and Curative Treatments. PIE: *Pluchea indica* extract; Asc: Ascorbic acid.

H_2O_2 can induce both apoptosis and necrosis, depending on the concentration used and the type of cells involved. In our study, H_2O_2 primarily induced cell death through apoptosis, while higher dosages were associated with necrosis. These findings are consistent with several previous studies, showing that H_2O_2 at low concentrations, specifically < 0.4 mM, can induce apoptosis and cause cell necrosis at higher concentrations.[23]

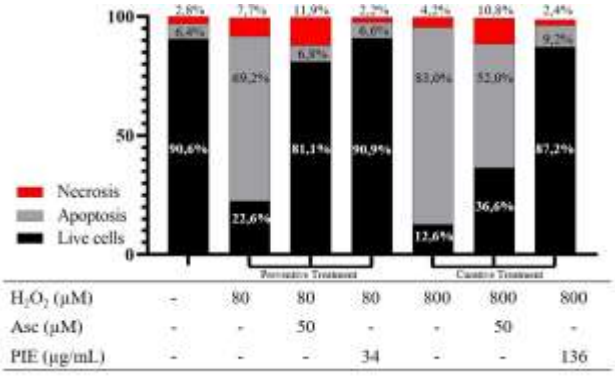

Figure 4: Percentage of Live and Apoptotic Vero Cells Subjected to Preventive and Curative Treatments. PIE: *Pluchea indica* extract; Asc: Ascorbic acid.

Effect of PIE on Caspase-3 Activity

Caspase-3 is a key enzyme involved in apoptosis. In this study, caspase-3 activity was lowest at baseline (Figure 5). The highest activity was observed in the treatment with H_2O_2 only, followed by the ascorbic acid and PIE treatments in both the preventive and curative groups (Figure 4). These findings confirm that apoptosis in this system is mediated through the activation of caspase-3.

H_2O_2 caused significant cell death and triggered an increase in ROS production (Figure 6). In contrast, the addition of PIE notably enhanced cell viability compared to other treatments in both the preventive and curative groups. In both treatments, the addition of H_2O_2 induced oxidative stress, as evidenced by the increased production of ROS (Figure 3). H_2O_2 exposure reduced cell viability compared to the control groups (Figure 2); based on the apoptosis assay using flow cytometry, H_2O_2 resulted in cell death through both necrosis and apoptosis (Figure 4). Apoptosis, a form of programmed cell death, involves mechanisms such as the caspase-3 pathway, which leads to mitochondrial dysfunction and ultimately cell death.[24,25] Notably, apoptosis was more prominent than necrosis in both the preventive and curative treatments. While H_2O_2 is typically associated with necrosis at higher concentrations,[26] low concentrations of H_2O_2 (up to 1 mM) predominantly trigger apoptosis. This supports the conclusion that the H_2O_2 concentrations used in both treatments were relatively low, as indicated by the induction of apoptosis rather than necrosis, despite the reduction in cell viability.

In this study, the addition of ascorbic acid and PIE demonstrated protective effects against H_2O_2-induced damage in Vero cells, in both the preventive and curative treatments. The addition of ascorbic acid and PIE to H_2O_2-exposed Vero cells resulted in a reduction in the production of ROS, indicating protection against oxidative stress. Furthermore, the addition of ascorbic acid and PIE after H_2O_2 exposure resulted in Vero cell morphology similar to that of normal cells in the control group (Figure 1). Additionally, both ascorbic acid and PIE maintained cell viability following exposure to H_2O_2 (Figure 4).

Ascorbic acid is an antioxidant compound capable of protecting cells from oxidative stress, based on its ability to neutralize reactive oxygen species (ROS) that cause oxidative stress. Ascorbic acid at concentrations of 40-80 µM exhibits antioxidant activity.[27] Ascorbic acid reacts with species such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, thereby preventing their interaction with biomolecules and maintaining normal cell function.[19]

Vero cells treated with various PIE concentrations maintained greater viability compared to cells treated with ascorbic acid in both the preventive and curative groups (Figure 6). The use of PIE was more effective in protecting Vero cells from the effects of oxidative stress. This was evidenced by the fact that low concentrations of PIE were sufficient to preserve cell viability, reduce intracellular ROS production, and reduce cell death more effectively than ascorbic acid, which served as the positive control. In the curative treatments, however, the highest concentration of PIE was required to maintain cell viability at levels comparable to those achieved with ascorbic acid.

The cytoprotective activity of PIE is influenced by the presence of antioxidant compounds. The constituents of *Pluchea indica* include alkaloids, sesquiterpenes, tannins, flavonoids, saponins, thiophenes, sterols,[28] lignans, phenylpropanoids, benzenoids, alkanes, phenolic hydroquinones, and flavonols (e.g., quercetin, kaempferol, and myricetin).[29] Our previous study demonstrated that PIE contains compounds such as n-hexadecanoic acid and neophytadiene that possess antioxidant properties.[12] These compounds play a crucial role in protecting cells from oxidative stress induced by ROS and free radicals.[30] The antioxidant activity of phenolic compounds in PIE employs several mechanisms to neutralize free radicals, including radical scavenging, hydrogen donation, metal ion chelation, and serving as substrates for radicals such as superoxide and hydroxyl radicals.[31]

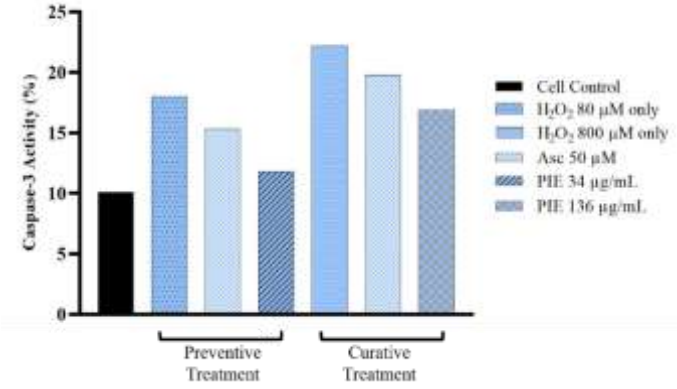

Figure 5: Percentage of caspase-3 activity in Vero cells following preventive and curative treatments. PIE: *Pluchea indica* extract; Asc: Ascorbic acid.

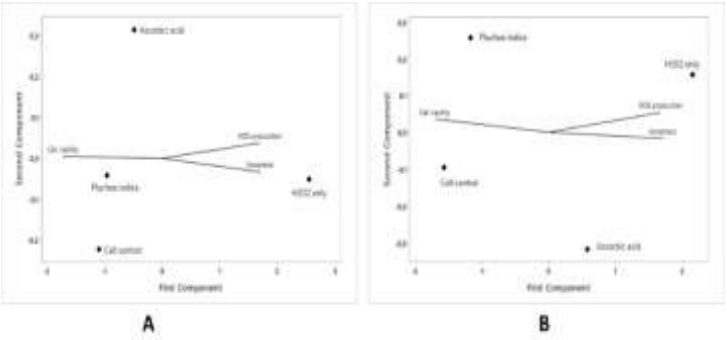

Figure 6: Principal Component Analysis of all parameters. (a) Preventive treatment, (b) Curative treatment.

Conclusion

PIE exhibited cytoprotective properties in Vero cells in both preventive and curative treatments. In the preventive treatment, the lowest concentration of PIE effectively protected Vero cells from oxidative stress, while in the curative treatment, a higher concentration was required for optimal repair of oxidative damage.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgment

This research was funded by a grant from the BIMA Program of the Indonesian Ministry of Education and Culture under the master's thesis research scheme (PTM), with Decree Number 0459/E5/PG.02.00/2024 and Agreement/Contract Numbers 048/E5/PG.02.00.PL/2024 and 2837/UN1/DITLIT/PT.01.03/2024 (to W.A.S.T).

References

1. Reddy VP. Oxidative Stress in Health and Disease. Biomedicines. 2023; 11(11): 2925, 1 - 17. doi: 10.3390/biomedicines11112925.
2. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and benefits for human health. Oxid Med Cell Longev. 2017; 2017:8416763, 1 - 13. doi: 10.1155/2017/8416763
3. Trojahn C, Dobos G, Lichterfeld A, Blume-Peytavi U, Kottner J. Characterizing facial skin ageing in humans: disentangling extrinsic from intrinsic biological phenomena. BioMed Res Int. 2015; 2015:318586, 1 - 9. doi: 10.1155/2015/318586
4. Pillai S, Oresajo C, Hayward J. Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation - a review. Int J Cosmet Sci. 2005; 27(1):17–34. doi: 10.1111/j.1467-2494.2004.00241.x
5. Qin D, Lee WH, Gao Z, Zhang W, Peng M, Sun T, Gao Y. Protective effects of antioxidin-RL from *Odorrana livida* against ultraviolet B-irradiated skin photoaging. Peptides. 2018; 101:124–134. doi: 10.1016/j.peptides.2018.01.009
6. Fernando IPS, Kirindage KGIS, Jayasinghe AMK, Han EJ, Dias MKHM, Kang KP, Moon SI, Shin TS, Ma A, Jung K, Ahn G. Makino & Shibata Abate Hydrogen Peroxide-Induced Oxidative Stress and Apoptosis in Kidney Epithelial Cells. Antioxid. 2022; 11(5):1013, 1 - 14. doi: 10.3390/antiox11051013
7. Kirindage KGIS, Fernando IPS, Jayasinghe AMK, Han EJ, Dias MKHM, Kang KP, Moon SI, Shin TS, Ma A, Ahn G. *Moringa oleifera* hot water extract protects vero cells from hydrogen peroxide-induced oxidative stress by regulating mitochondria-mediated apoptotic pathway and Nrf2/HO-1 signaling. Foods. 2022; 11(3):420, 1 – 16. doi: 10.3390/foods11030420
8. Lee HG, Jayawardena TU, Liyanage NM, Song KM, Choi YS, Jeon YJ, Kang MC. Antioxidant potential of low molecular weight fucoidans from *Sargassum autumnale* against H_2O_2-induced oxidative stress in vitro and in zebrafish models based on molecular weight changes. Food Chem. 2022; 384:132591. doi: 10.1016/j.foodchem.2022.132591
9. Dare RG, Nakamura CV, Ximenes VF, Lautenschlager SOS. Tannic acid, a promising anti-photoaging agent: Evidences of its antioxidant and anti-wrinkle potentials, and its ability to prevent photodamage and MMP-1 expression in L929 fibroblasts exposed to UVB. Free Radic Biol Med. 2020; 160:342–355. doi: 10.1016/j.freeradbiomed.2020.08.019
10. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010 Jul;4(8):118–126. doi: 10.4103/0973-7847.70902
11. Retnaningtyas Y, Dewi N, Kristiningrum N, Kuswandi B. Antioxidant activity and H2O2 sensing ability of silver nanoparticles synthesized using *Solanum melongena* L. peel extract. Trop J Nat Prod Res. 2025; 9(6):2587–2594. doi: 10.26538/tjnpr/v9i6.34
12. Perdani L, Azasi NM, Sofyantoro F, Nugroho RA, Ismail NIM, Tunjung WAS. Analysis of anti-aging activity of Chinese perfume (*Aglaia odorata*) and Indian camphorweed (*Pluchea indica*) leaves using *Saccharomyces cerevisiae* model system. J Res Pharm. 2025; 29(1):396-406. doi: 10.29228/jrp.2022.00
13. Vongsak B, Kongkiatpaiboon S, Jaisamut S, Konsap K. Comparison of active constituents, antioxidant capacity, and α-glucosidase inhibition in *Pluchea indica* leaf extracts at different maturity stages. Food Biosci. 2018; 25:68–73. doi: 10.1016/j.fbio.2018.08.006
14. Cho JJ, Cho CL, Kao CL, Chen CM, Tseng CN, Lee YZ, Liao LJ, Hong YR. Crude aqueous extracts of *Pluchea indica* (L.) Less. inhibit proliferation and migration of cancer cells through induction of P53-dependent cell death. BMC Complement Altern Med. 2012; 12:265, 1 - 11. doi: 10.1186/1472-6882-12-265
15. Fitriansyah MI, Indradi RB. Review: Phytochemical Profile and Pharmacological Activities of Beluntas (*Pluchea indica* L.). Farmaka. 2018; 16(2):337-346. Retrieved from https://jurnal.unpad.ac.id/farmaka/article/view/17554
16. Kiyoshima T, Enoki N, Kobayashi I, Sakai T, Nagata K, Wada H, Fujiwara H, Ookuma Y, Sakai H. Oxidative stress caused by a low concentration of hydrogen peroxide induces senescence-like changes in mouse gingival fibroblasts. Int J Mol Med. 2012; 30(5):1007–1012. doi: 10.3892/ijmm.2012.1102
17. Wang L, Jayawardena TU, Yang HW, Lee HG, Kang MC, Sanjeewa KKA, Oh JY, Jeon YJ. Isolation, characterization, and antioxidant activity evaluation of a fucoidan from an enzymatic digest of the edible seaweed, *Hizikia fusiforme*. Antioxidants. 2020; 9(5):363,1 - 14. doi: 10.3390/antiox9050363
18. Haryanti S, Budiarti M, Farida S, Dewi APK, Supriyati N, Jokopriyambodo W, Wahyono S, Widowati L. The palm oil-based chlorophyll removal and the evaluation of antiaging properties on *Centella asiatica* ethanolic extract. IOP Conf Ser Earth Environ Sci. 2024; 1312(1):012041. doi: 10.1088/1755-1315/1312/1/012041
19. Reang J, Sharma PC, Thakur VK, Majeed J. Understanding the therapeutic potential of ascorbic acid in the battle to overcome cancer. Biomolecules. 2021; 11(8):1130, 1 - 24. doi: 10.3390/biom11081130
20. Kaur G, Dufour JM. Cell lines: Valuable tools or useless artifacts. Spermatogenesis. 2012; 2(1):1–5. doi: 10.4161/spmg.19885
21. Borges GDSC, Gonzaga LV, Jardini FA, Mancini Filho J, Heller M, Micke G, Costa ACO, Fett R. Protective effect of *Euterpe edulis* M. on vero cell culture and antioxidant evaluation based on phenolic composition using HPLC−ESI-MS/MS. Food Res Int. 2013; 51(1):363–369. doi: 10.1016/j.foodres.2012.12.035
22. Ammerman NC, Beier-Sexton M, Azad AF. Growth and maintenance of Vero cell lines. Curr Protoc Microbiol. 2008; 11(1): A-4E. doi: 10.1002/9780471729259.mca04es11
23. Xiang J, Wan C, Guo R, Guo D. Is hydrogen peroxide a suitable apoptosis inducer for all cell types? BioMed Res Int. 2016; 2016:7343965, 1 - 6. doi: 10.1155/2016/7343965
24. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, Pramodh S, Alsulimani A, Alkhanani MF, Harakeh S, Hussain A. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. Cells. 2022; 11(3):552, 1 - 27. doi: 10.3390/cells11030552
25. Hong Y, Boiti A, Vallone D, Foulkes NS. Reactive oxygen species signaling and oxidative stress: Transcriptional regulation and evolution. Antioxidants. 2024; 13(3):312, 1 - 24. doi: 10.3390/antiox13030312
26. Ali MAM, Kandasamy AD, Fan X, Schulz R. Hydrogen peroxide-induced necrotic cell death in cardiomyocytes is independent of matrix metalloproteinase-2. Toxicol Vitro Int J Publ Assoc BIBRA. 2013; 27(6):1686–1692. doi: 10.1016/j.tiv.2013.04.013.
27. Muchtaridi M, Az-Zahra F, Wongso H, Setyawati LU,

Novitasari D, Ikram EHK. Molecular mechanism of natural food antioxidants to regulate ros in treating cancer: A review. Antioxidants. 2024; 13(2):207, 1 - 18. doi: 10.3390/antiox13020207

28. Ruan J, Li Z, Yan J, Huang P, Yu H, Han L, Zhang Y, Wang T. Bioactive constituents from the aerial parts of *Pluchea indica* Less. Molecules. 2018; 23(9): 2104, 1 - 11. doi: 10.3390/molecules23092104
29. Widyawati PS, Wijaya CH, Hardjosworo PS, Sajuthi D. Volatile compounds of *Pluchea indica* Less and *Ocimum basillicum* Linn essential oil and potency as antioxidant. HAYATI J Biosci. 2013 Sep 1;20(3):117–126. doi: 10.4308/hjb.20.3.117
30. Pekkoh J, Phinyo K, Thurakit T, Lomakool S, Duangjan K, Ruangrit K, Pumas C, Jiranusornkul S, Yooin W, Cheirsilp B, Pathom-Aree W. Lipid profile, antioxidant and antihypertensive activity, and computational molecular docking of diatom fatty acids as ACE inhibitors. Antioxidants. 2022. 11(2); 186, 1 - 16. doi: 10.3390/antiox11020186
31. Alsawaf S, Alnuaimi F, Afzal S, Thomas RM, Chelakkot AL, Ramadan WS, Hodeify R, Matar R, Merheb M, Siddiqui SS, Vazhappilly CG. Plant flavonoids on oxidative stress-mediated kidney inflammation. Biology. 2022; 11(12):1717, 1 - 27. doi: 10.3390/biology11121717