HPLC Analysis with Evaluation of Antioxidant and Antisenescence Activities of Parijoto (*Medinilla speciosa* Reinw. ex Bl.) Methanolic Extract in Vero Cells

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Abstract

Parijoto (*Medinilla speciosa* Reinw. ex Bl.) is a traditional medicinal plant rich in polyphenolic compounds that are associated with antioxidant and cytoprotective activities. The present study aims to investigate the phytochemical profile, antioxidant capacity, cytotoxicity, and anti-senescence effects of parijoto methanolic extract. The methanolic extract of parijoto (MEP) was subjected to qualitative thin-layer chromatography (TLC) for the detection of flavonoids, quantitative high-performance liquid chromatography (HPLC) for the determination of quercetin and naringin, and antioxidant activity assessment using the DPPH method. The mobile phase for quercetin analysis was water: acetonitrile (45:55), while for naringin, it was acetonitrile:water: formic acid (21:78.8:0.2). Cytotoxicity was evaluated in Vero cells using the MTT assay, while senescence in normal kidney cells was assessed by senescence-associated ß-galactosidase (SA-ß-gal) staining. Results showed that the extract contained quercetin (5.76±0.11 ppm). The extract exhibited strong antioxidant activity with an IC_{50} value of 40.46 ppm. Cytotoxicity testing of MEP on Vero cells revealed low toxicity (529±0.77 µM) at physiologically relevant concentrations, supporting its safety for normal kidney cells. Moreover, in normal kidney cells, the extract conferred cytoprotective effects by reducing oxidative stress–induced damage and attenuating senescence-associated ß-galactosidase. Collectively, these findings suggest that MEP exhibits potent antioxidant, cytoprotective, and anti-senescence activity *in vitro*, warranting further *in vivo* studies to validate its potential effects on kidney health and age-related cellular damage.

Keywords: *Parijoto, HPLC, antioxidant activity, senescence, normal kidney cell.*

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INTRODUCTION

Aging is a complex physiological phenomenon that has a profound impact on life, as it represents the onset of various degenerative diseases such as cancer, neurological disorders, diabetes mellitus, and others (Höhn, *et al*., 2017). Cellular senescence is considered one of the hallmarks of the aging process (López-Otín, *et al*., 2013). In addition to changes in gene expression and metabolic regulation, aging is also driven by the increased accumulation of reactive oxygen species (ROS) within cells. Elevated ROS levels can cause DNA damage, preventing cells from undergoing replication and regeneration. Importantly, senescent cells are characterized by impaired antioxidant defenses and reduced free radical scavenging capacity, which exacerbate oxidative stress and further promote cellular dysfunction and tissue aging (Davalli, *et al*., 2016).

Advances in science and technology have driven increasingly dynamic lifestyles, which in turn raise the risks of various diseases. Free radicals are among the major contributing factors. Environmental factors such as air pollution, radiation, and unhealthy lifestyle habits exacerbate continuous exposure to these reactive molecules (Aseervatham, *et al*., 2013). Free radicals are unstable atoms or molecules containing one or more unpaired electrons, making them highly reactive (Sundaram Sanjay & Shukla, 2021). They contribute to degenerative diseases, including liver damage, inflammation, cancer, cardiovascular disorders, and aging (Jomova, *et al*., 2023). Antioxidants play a critical role in neutralizing free radicals and protecting the body against their harmful effects (Sharma, 2014). Natural antioxidants are often derived from plants rich in secondary metabolites such as flavonoids and phenolic compounds, which are effective free radical scavengers and inhibit senescence (Domaszewska-Szostek, *et al*., 2021). One such plant is the parijoto (*Medinilla speciosa* Reinw. ex Bl.), a fruit traditionally used in Central Java for fertility enhancement, anti-inflammatory, and anticancer purposes. The fruit contains flavonoids, tannins, saponins, glycosides, and terpenoids (Vifta & Advistasari, 2018). Previous studies demonstrated the antioxidant activity of parijoto fruit extracts, with varying IC_{50} values depending on the solvent fraction used (Wijayanti, *et al*., 2020). However, studies focusing specifically on the methanolic extract of parijoto (MEP) as anti-senescence are limited. Beyond their role in scavenging free radicals, antioxidants are closely linked to the regulation of cellular senescence. Excessive oxidative stress accelerates cellular aging by inducing DNA damage, mitochondrial dysfunction, and activation of senescence pathways marked by senescence-associated β-galactosidase (SA-β-gal) (Hernandez-Segura, *et al*., 2018). Conversely, plant-derived antioxidants, especially flavonoids such as quercetin and naringin, have been reported to protect cells from oxidative stress–induced senescence by enhancing endogenous defense systems and reducing ROS accumulation (Liu, *et al*., 2023). Thus, investigating the antioxidant potential of parijoto extract is not only relevant to free radical neutralization but also to its possible role in delaying premature senescence and maintaining normal cell function. In this context, the use of vero cells, a normal kidney cell line, provides an appropriate model to assess both the cytotoxicity and cytoprotective effects of the extract. The kidney is one of the most metabolically active organs and is highly vulnerable to oxidative stress due to its rich mitochondrial content, high blood perfusion, and frequent exposure to xenobiotics Evaluating antioxidant and anti-senescence activity in vero cells provides valuable insight into the potential protective effects of parijoto against oxidative stress–induced cellular damage. However, despite its traditional use, scientific evidence regarding the phytochemical profile and the cellular antioxidant or anti-senescence effects of *Medinilla speciosa* fruit extract remains limited, particularly in kidney-derived cell models. Therefore, this study aims

to identify and quantify quercetin in MEP and to evaluate its antioxidant and anti-senescence activity *in vitro*. In addition, the study investigates cytotoxicity in vero cells and anti-senescence effects through SA-β-gal staining, using cisplatin as a senescence-inducing agent. This approach provides a comprehensive understanding of the cytoprotective potential of parijoto, particularly in its activity as an antioxidant and anti-senescence.

MATERIALS AND METHODS

Extraction of Parijoto (*Medinilla speciosa*) Using Methanol Solvent

Fresh parijoto fruits (*Medinilla speciosa Reinw*. ex Bl.) were collected from Colo Village, Dawe District, Kudus Regency, Central Java, Indonesia. The simplicia material was subjected to botanical determination using the identification key of C.A. Backer & R.C. Bakhuizen van den Brink, Jr. (1963) to ensure the authenticity of *Medinilla speciosa* (Reinw. ex Bl.) before further processing. The plant material was determined and authenticated by a botanist at the Herbarium of Universitas Sebelas Maret, and a voucher specimen was deposited. Parijoto that have been sorted and washed using distilled water at a temperature of 90˚C for 15 minutes. Parijoto was extracted with methanol, and the filtrate was concentrated using a rotary evaporator.

Qualitative Analysis of Parijoto Extract Using Thin Layer Chromatography (TLC)

Parijoto extract was dissolved in DMSO and diluted with methanol. Thin-layer chromatography (TLC) was performed using silica GF254 plates. Spots of parijoto extract (without peel), quercetin, and naringin standards were applied with a capillary tube and developed in an appropriate mobile phase. For quercetin, the mobile phase was chloroform:acetone: formic acid (10:2:1 v/v) with $FeCl_3$ (10%) as spraying reagent.

Quantitative Analysis of Parijoto Extract Using High-Performance Liquid Chromatography (HPLC)

This study employed a validated high-performance liquid chromatography (HPLC) method, including a system suitability test, to ensure the accuracy and reliability of the quantitative analysis. Quantitative identification of quercetin in the MEP was carried out using HPLC. A total of 10 mg extract was dissolved in DMSO and methanol to a final volume of 10 mL, then sonicated for 15 minutes at 30 °C. Quercetin standard solutions were prepared at 1000 ppm and diluted to concentrations of 4–12 ppm. The mobile phase consisted of water–acetonitrile (45:55 v/v) with a flow rate of 1 mL/min, and detection was performed at 370 nm (Jain & Shaikh, 2016). Calibration curves were obtained from standard peak areas, and the quercetin content in the samples was determined after filtration through a 0.22 µm membrane. A series of five concentrations (4, 6, 8, 10, and 12 ppm) was injected into the HPLC system and analyzed at a wavelength of 370 nm with a flow rate of 1 mL/min.

Antioxidant Assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) Scavenging Assay

The antioxidant capacity of the samples was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, adapted from Siregar, *et al.* (2019). A volume of 50 µL of each sample solution (0.5–20 µM in 1% DMSO) was added into the wells of a 96-well microplate, followed by the addition of 200 µL of DPPH solution. The mixtures were incubated for 30 minutes at room temperature under dark conditions to prevent photo-degradation. Subsequently, absorbance was read at 517 nm using a microplate reader (Thermo Scientific Multiskan GO, Finland), and the percentage of scavenging activity was calculated. Each experiment was conducted in three independent replicates (n=3).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

Vero cells were obtained from Universitas Gadjah Mada and maintained under standard culture conditions at 37 °C with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Sigma) and 1% penicillin–streptomycin (Sigma). Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, vero cells (1×10^4) were seeded into 96-well plates and exposed to varying concentrations of Parijoto extract, quercetin, or cisplatin for 24 h. Following treatment, 100 µL of MTT reagent (0.5 mg/mL; Sigma-Aldrich, USA) was added and incubated for 2–4 h. The reaction was terminated by adding SDS solution containing 0.01 N HCl. Absorbance was recorded at 595 nm with an ELISA reader, and cell viability was calculated. IC_{50} values were determined by linear regression analysis of concentration versus cell viability (Blumenthal, 2005).

Senescence-associated ß-galactosidase (SA-ß-Gal) Senescence-based Assay

SA-βGal activity was assessed using the SA-βGal staining assay. Briefly, cells (2×10^4 per well) were seeded into 24-well plates and allowed to adhere for 24 h. The cells were then washed twice with 1× PBS, fixed with a fixative solution, and rinsed again with 1× PBS. Subsequently, 500 µl of X-Gal staining solution was added, and the plates were incubated at 37 °C. After 72 h, the cells were examined under a microscope (Olympus CKX-41) at 200× magnification. β-galactosidase–positive cells were quantified by counting green-stained cells in at least five randomly selected microscopic fields. The percentage of senescent cells was calculated as the number of β-gal–positive cells divided by the total number of cells in each field. The appearance of green-stained cells indicated β-galactosidase-positive cells, characteristic of cellular senescence (Artanti, *et al*., 2023).

Data Analysis

Data are expressed as mean±standard deviation (SD) and were analyzed using SPSS 21.0 software. One-way ANOVA was employed to evaluate statistical differences between the untreated and treatment groups, followed by a post hoc Tukey's test to determine pairwise differences. Significance levels are indicated in each figure. (*p<0.05; **p<0.01).

RESULTS

Qualitative and Quantitative Analysis of Parijoto Extract

The extraction yield was 2.94% (w/w), obtained from 13.24 g of dried extract from 450 g of dried plant material. Qualitative identification of secondary metabolites was performed to determine the presence of quercetin in MEP. The analysis was carried out using TLC. Extract samples and quercetin standards were spotted on silica GF254 plates, developed in an appropriate mobile phase, and subsequently visualized under UV light at 254/366 nm. Quercetin was selected as the reference standard because it has been consistently reported as a major flavonoid constituent in *Medinilla speciosa* and related species. TLC analysis revealed the presence of quercetin in MEP, as indicated by a spot with an Rf value and fluorescence pattern identical to those of the quercetin standard (Figure 1).

Visualization of TLC spots under UV254 (Figure 1) revealed three distinct spots in the MEP, with Rf values of 0.21, 0.56, and 0.70 (Table 3). The quercetin standard exhibited an Rf value of 0.70, which is consistent with previous reports using silica gel GF254 and a mobile phase of chloroform–acetone–formic acid (10:2:1 v/v), where quercetin was detected at Rf 0.7. Detection with a 10% FeCl3 reagent, which typically produces a blue–black coloration for quercetin (Nurmalasari, *et al*., 2019), showed only faint spots in the parijato extract. This suggests that quercetin may be present in very low

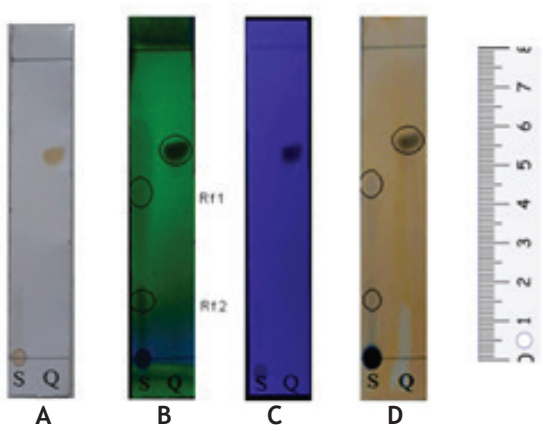

Figure 1. TLC profile of MEP (S) compared with quercetin standard (Q) observed under (A) visible light, (B) UV254, (C) UV366, and (D) after spraying with 10% FeCl3 reagent.

concentrations in the MEP, making it difficult to visualize clearly through TLC. While TLC provides a preliminary qualitative indication of quercetin in the parijoto extract, its sensitivity and resolution are limited, particularly when the compound is present in low concentrations. Therefore, HPLC was employed as a more advanced analytical technique to confirm and quantify the presence of quercetin. HPLC offers higher sensitivity, specificity, and reproducibility compared to TLC, allowing for more accurate detection even at trace levels. In addition, HPLC enables precise determination of retention times and peak areas, which are essential for validating the presence of quercetin and assessing its relative abundance within the extract.

In this study, quercetin content in the MEP was determined using a quercetin standard as a reference. The mobile phase consisted of acetonitrile–water (55:45, v/v), while the stationary phase was an octadecylsilane (C-18) column, representing a reversed-phase chromatography system where the mobile phase is more polar than the stationary phase. Before use, the mobile phase was filtered to remove particulate matter that could clog the column and subsequently sonicated to eliminate air bubbles, which may interfere with pump pressure. Standard solutions of quercetin were prepared in the same solvent system as the mobile phase to avoid differences in solvent strength (Snyder, *et al*., 2011). Quercetin was detected at a retention time of 3.537 min (5.76±0.11 ppm).

The HPLC analysis of quercetin showed consistent chromatographic performance with sharp peaks appearing at retention times around 3.5 minutes. The retention times of the three replications were highly reproducible, ranging from 3.537 to 3.576 minutes, indicating good stability of the chromatographic system (Figure 2). The quantitative analysis yielded an average quercetin concentration of 5.76 ppm, with a standard deviation of 0.11 ppm and a coefficient of variation of 1.95%, as presented in Table 1. This value is below the generally accepted threshold of 2% for analytical repeatability. These results confirm that the HPLC method employed in this study provides precise and reliable quantification of quercetin in the MEP.

Table 1. Quercetin concentration on the MEP.

Replication	Peak Area (unit)	Retention Time (min)	Concentration (ppm)	Average Concentration (ppm)	SD
1	13828168	3.537	5.79		
2	13448160	3.537	5.64	5.76	0.11
3	13996178	3.576	5.86		

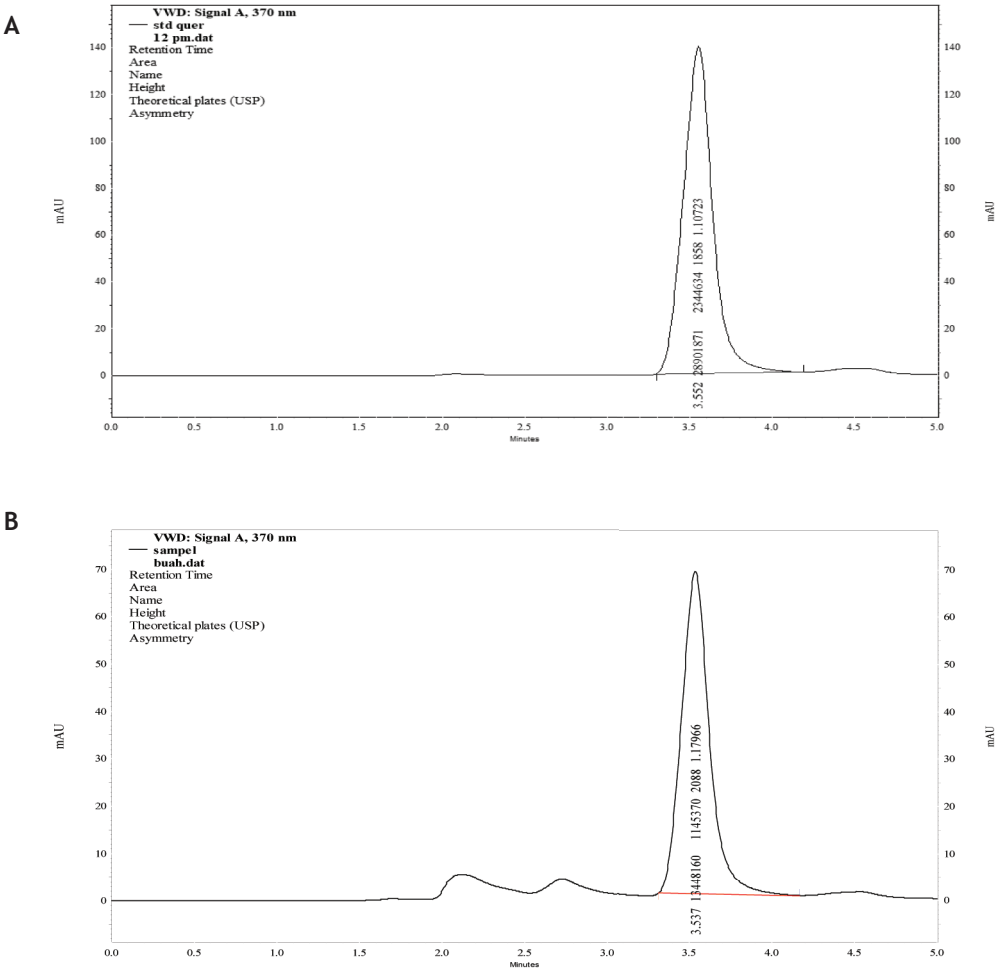

Figure 2. Chromatograms of quercetin analysis: (A) standard quercetin and (B) MEP, obtained using a reversed-phase HPLC system with a C-18 column, acetonitrile-water (55:45, v/v) as the mobile phase, a flow rate of 1.0 mL/min, and detection at 370 nm.

Antioxidant Activity of Parijoto Extract

The antioxidant activity of the MEP was evaluated using the DPPH method, which is the most widely applied assay for determining antioxidant capacity due to its simplicity, rapidity, and minimal requirement of reagents and sample. The assay was performed under dark conditions, as DPPH is highly sensitive to light exposure. The principle of the DPPH assay is based on the reduction of DPPH radical, which is initially purple due to the presence of an unpaired electron. Upon reaction with a hydrogen-donating antioxidant, the radical is reduced to diphenylpicrylhydrazine, leading to a color change from purple to yellow. This decrease in purple intensity is proportional to the concentration of the antioxidant present. The change in absorbance is measured at the maximum wavelength of DPPH using a UV-Vis spectrophotometer, and the antioxidant capacity is expressed as the inhibitory concentration (IC_{50}) value (Molyneux, 2004). Quercetin was used as a reference compound or positive control, as it is a standard with strong antioxidant activity (Pratama, *et al*., 2015). The function of a positive control is to serve as a benchmark to determine whether the tested compound exhibits comparable effects to the standard. Moreover, quercetin is one of the bioactive compounds naturally present in *Medinilla speciosa*

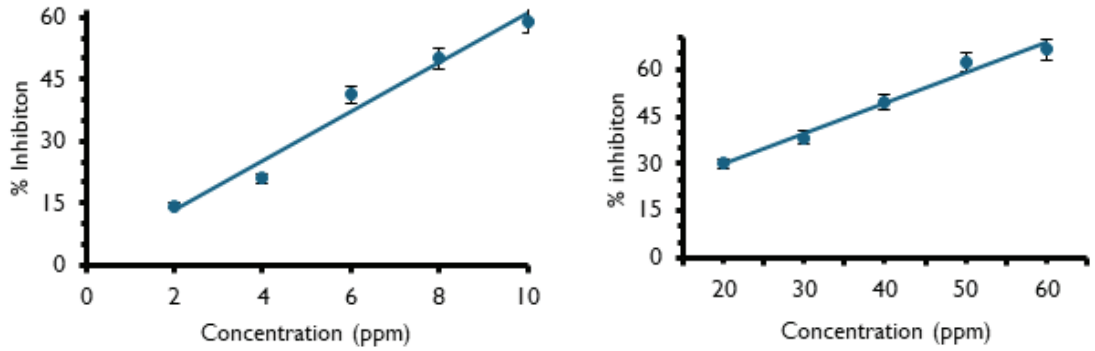

Figure 3. Antioxidant activity expressed as percentage inhibition determined by the DPPH assay: (A) standard quercetin and (B) MEP. The decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and the antioxidant capacity was expressed as % inhibition relative to control. Values are presented as mean ±SD (n=3). Error bars represent standard deviation.

Table 2. Antioxidant activity of quercetin and the MEP.

Sample	Concentration (ppm)	Average of % inhibition ±SD	IC_{50} (ppm)
quercetin	2	14.270 ± 0.9908	8.169
	4	21.009 ± 1.3517	
	6	41.333 ± 0.7820	
	8	50.018 ± 0.5441	
	10	59.063 ± 0.5331	
MEP	20	30.018 ± 0.6516	40.67
	30	38.306 ± 0.5954	
	40	49.441 ± 0.3475	
	50	62.126 ± 0.3796	
	60	66.450 ± 0.5441	

fruit (Artanti, *et al*., 2022). The regression equation obtained from the antioxidant activity curve of quercetin was y=5.9297x+1.5604 with an R^2 value of 0.9718 (Figure 3). The R^2 value represents the linearity between concentration and percentage of antioxidant activity, where an R^2 value close to 1 indicates good linearity. Meanwhile, the % antioxidant activity curve of the MEP is presented in Figure 3.

The parameter used to evaluate antioxidant activity is the inhibitory concentration (IC_{50}). The determination of IC_{50} for the MEP was conducted to establish the extract concentration required to reduce the absorbance of the DPPH radical solution by 50% compared to the negative control, calculated using linear regression analysis. The negative control consisted of DPPH solution mixed with methanol. Based on the regression equation obtained, the IC_{50} value reflects the concentration at which the tested sample is able to scavenge 50% of DPPH radicals. According to (Noviyanty & Salingkat, 2019), an antioxidant is categorized as very strong when the IC_{50} value is below 50 ppm, strong at 50–100 ppm, moderate at 101–150 ppm, and weak when higher than 150 ppm.

In this study, the IC_{50} value of standard quercetin was 8.169 ppm, which indicates very strong antioxidant activity. The IC_{50} value of the MEP was 40.76 ppm (Table 2), approximately five times higher than that of quercetin, yet still classified as very strong antioxidant activity. The difference in IC_{50} values between the standard quercetin and the MEP may be attributed to the distinct capacities of the compounds to donate electrons to DPPH radicals.

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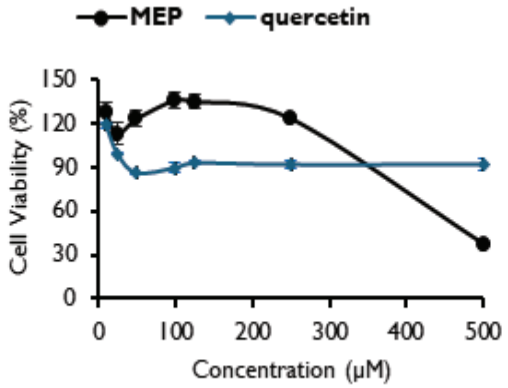
A greater electron-donating capacity results in a more pronounced reduction in absorbance, thereby increasing the percentage inhibition and lowering the IC_{50} value (Pertiwi, *et al*., 2016). It is important to note that IC_{50} values are inversely proportional to antioxidant activity; the smaller the IC_{50} value, the stronger the antioxidant potential. Both quercetin and MEP demonstrated very strong antioxidant activity. The potent antioxidant activity of the extract is closely related to its secondary metabolite content, particularly flavonoids. Quercetin, a flavonoid compound, is known as an exogenous antioxidant containing phenolic groups and has been reported to prevent oxidative stress-induced cellular damage. The antioxidant mechanism of flavonoids involves direct hydrogen atom donation, which stabilizes reactive free radicals and neutralizes them effectively (Jabbar, *et al*., 2019).

Cytotoxic Effect of Quercetin, Doxorubicin, and MEP on Vero Cell Line

Cell viability testing was performed to evaluate the effects of MEP in comparison with quercetin and doxorubicin. The assay was conducted using the MTT method on vero cells, a normal kidney cell line commonly used to assess the safety of bioactive compounds. Testing on vero cells was intended to determine whether the extract exhibits toxicity toward normal cells, thereby indicating its safety for further development. Quercetin was included as a natural flavonoid reference compound, whereas doxorubicin served as a reference cytotoxic agent. Although primarily used as an anticancer drug, doxorubicin is also known to exert cytotoxic effects on normal cells, which makes it suitable as a comparative control. This experimental design allowed a direct comparison of cytotoxic responses between the extract, a natural antioxidant compound, and a chemotherapy agent.

A



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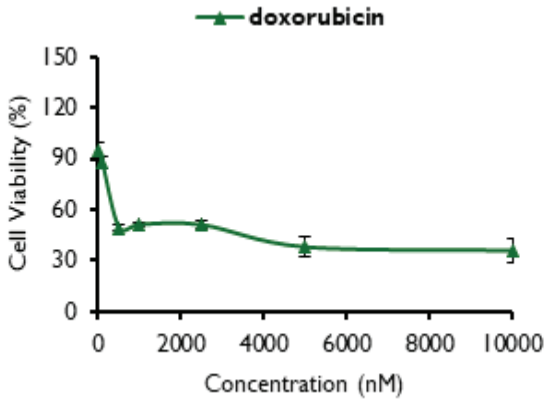

C

Cell Line	Sample	IC_{50} value
Vero	MEP	529±0.77 (µM)
	Quercetin	2200±0.23(µM)
	Doxorubicin	4420±1.21(nM) ≈ 4.4±1.21 (µM)

Figure 4. Cytotoxic Effect of quercetin, doxorubicin, and MEP on vero cells. Vero cells (1×10^4 cells/mL) were exposed to MEP and quercetin concentrations (10 to 500 µM) and Dox concentration (10-104 nM) for 24 h and subsequently assessed using the MTT assay. The figure consists of the following components: (A) Depicts the influence of MEP and quercetin treatment on the growth profiles of Vero cells. (B) Depicts the influence of Dox treatment on the growth profiles of vero cells (C). The IC_{50} value of quercetin, doxorubicin, and MEP on vero cells. Data are presented as mean ±SD (n=3). Error bars indicate standard deviation.

This study evaluated the effects of the MEP, quercetin, and doxorubicin on vero cells. Doxorubicin, a chemotherapeutic drug, is known to be toxic to both cancerous and normal kidney cells (Sheibani, *et al*., 2022). MEP exhibited an IC_{50} of 529±0.77 µM in vero cells. Since the assay was intended to establish a non-toxic concentration for the SA-β-gal evaluation, the viability data were interpreted cautiously and not as a cytotoxicity profile. Quercetin also exhibited low cytotoxicity with an IC_{50} value of 2,200±0.23 µM. In contrast, Dox was highly cytotoxic to vero cells, with an IC_{50} value of 4.5±1.21 µM (Figure 4). These findings suggest that both MEP and quercetin are relatively safe toward normal vero cells compared to the strong cytotoxic effects of doxorubicin.

Cellular Senescence in Vero Cells after Treatment with Quercetin, Doxorubicin, and MEP

The cytotoxicity assay demonstrated that doxorubicin was highly toxic to vero cells, consistent with its well-documented side effects on normal kidney cells. In contrast, both quercetin and MEP exhibited relatively low cytotoxicity, as reflected by their high IC_{50} values. This result is important because, in normal cells, the desired effect is the inhibition rather than the induction of senescence. Accordingly, the absence of senescence induction by quercetin and MEP suggests that these compounds are not only safe for normal kidney cells but may also exert protective, anti-senescence effects. To evaluate the senescence profile of quercetin and MEP, their effects were tested on vero cells. Quercetin at 50 and 100 µM, as well as MEP at the same concentrations, did not increase the percentage of β-galactosidase-positive cells, indicating that they are safe for normal kidney cells. In contrast, doxorubicin significantly increased β-galactosidase-positive cells, confirming its ability to induce senescence (Figure 5). These findings suggest that both quercetin and MEP possess anti-senescence properties and are nontoxic to normal kidney cells.

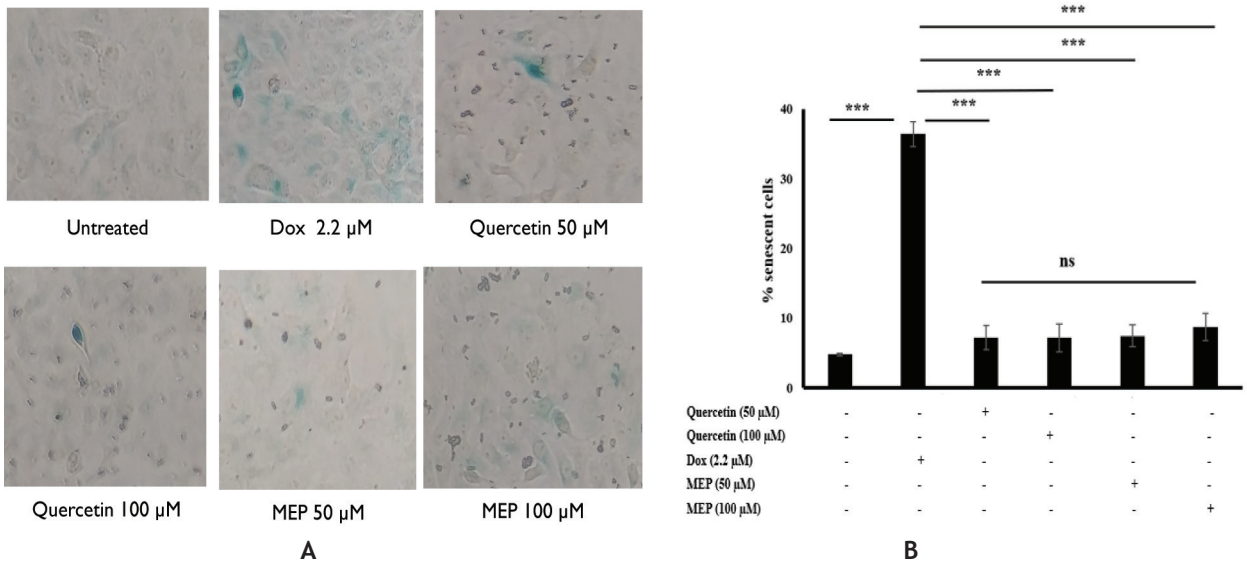

Figure 5. Induction of senescence in vero cells following treatment with quercetin, doxorubicin, and MEP was analyzed using the SA-β-galactosidase staining assay. Vero cells (2×10^4 cells/mL) were treated with quercetin and MEP at concentrations of 50 µM and 100 µM for 24 h. After treatment, the cells were stained for β-galactosidase activity. Dox (2.2 µM) was used as a positive control with a 24-h exposure. The percentages of senescent cells (β-galactosidase-positive cells) were calculated (n=3). (A) Shows the cellular morphology of vero cells after 72 h of staining, observed under an inverted microscope at 200x magnification. (B) Displays the percentage of senescent vero cells following the treatments. ***, $p<0.005$.

DISCUSSION

The present study demonstrates that the MEP contains bioactive compounds with significant antioxidant and cytoprotective activities. Future studies should include *in vivo* validation, evaluation of molecular markers associated with oxidative stress and senescence, and the use of additional kidney or human-derived cell models to further elucidate the therapeutic potential of MEP. HPLC analysis confirmed the presence of quercetin, a flavonoid known for its strong antioxidant and anti-aging properties. HPLC enables compound separation, identification, and quantification by comparing chromatographic peak areas with standard references (Ali, 2022). This method offers several advantages, including rapid analysis, high specificity in separating complex mixtures into individual components, high accuracy, and the capability to analyze unstable samples (Ahmed, 2024). The detection of quercetin in MEP represents novel phytochemical information, given that earlier studies on *Medinilla speciosa* were limited to reporting total phenolic content without detailed constituent profiling, where flavonoids contribute to free radical scavenging and cytoprotective effects. The antioxidant activity of MEP, as reflected by its IC_{50} value of 40.46 ppm, falls within the category of very strong antioxidants, highlighting its ability to neutralize free radicals and mitigate oxidative stress. Importantly, the cytotoxicity evaluation using the MTT assay indicated that MEP exhibits low toxicity in vero cells, with an IC_{50} value exceeding 500 µM. These results suggest that MEP is not cytotoxic to normal kidney cells within the tested concentration range, although further studies are needed to confirm its safety profile, distinguishing it from chemotherapeutic agents such as doxorubicin, which demonstrated marked cytotoxicity in this study. The low cytotoxicity of MEP aligns with the expected safety profile of natural polyphenols, which are often cytoprotective in non-cancerous cells while exerting selective toxicity in malignant cells (Hołota & Posmyk, 2025). MEP, as a crude extract, contains a broader range of phytochemicals, some of which may exhibit mild cytotoxicity at higher concentrations. In contrast, quercetin represents a purified single compound with well-characterized safety at similar doses. These compositional differences may explain the slightly higher cytotoxicity observed for MEP.

The senescence assay further supports the cytoprotective role of MEP, potentially through mechanisms related to its antioxidant constituents, such as quercetin, which are known to reduce oxidative stress, modulate cellular redox balance, and suppress senescence-associated pathways. MEP did not increase senescence-associated β-galactosidase activity in normal kidney cells, unlike doxorubicin, indicating a potential anti-senescence effect. This activity may be attributed to its flavonoid constituents, particularly quercetin, known for its senolytic and senomorphic properties (Mbara, *et al*., 2022). Similar senescence-inhibiting effects have also been reported for other flavonoids such as naringenin, kaempferol, and apigenin, which modulate oxidative stress, reduce pro-inflammatory SASP factors, and regulate pathways such as p53/p21 and p16INK4a. This suggests that the collective action of these flavonoids may contribute to the anti-senescence activity observed in MEP. Flavonoid-rich plants, including blueberries, pomegranates, and green tea, have shown similar antioxidant and anti-senescence effects attributed to quercetin and related polyphenols (Stiller, *et al*., 2021). Berry extracts have been shown to prevent senescence in fibroblast models by enhancing antioxidant enzymes and reducing pro-inflammatory mediators. Likewise, citrus-derived flavonoids such as hesperetin and naringin have demonstrated antioxidant and anti-senescence effects *in vitro* (Mbara, *et al*., 2022). These parallels strengthen the evidence that MEP may exert similar health-promoting benefits through its polyphenolic constituents. This study has several limitations. First, the findings are based solely

on *in vitro* experiments, which may not fully represent physiological conditions *in vivo*. Second, the use of a single cell line (vero cells) limits the generalizability of the results to other kidney or human-derived cell models. Third, molecular markers related to oxidative stress and senescence (such as p16, p21, or SIRT1) were not assessed.

Collectively, these findings highlight the therapeutic potential of MEP as a natural antioxidant and cytoprotective agent. Its strong free radical–scavenging capacity, low cytotoxicity in normal kidney cells, and anti-senescence effects suggest potential applications in preventing oxidative stress–related kidney damage and delaying age-associated cellular decline. Future studies are warranted to investigate the molecular pathways underlying these protective effects, as well as to explore the *in vivo* efficacy and safety of MEP.

CONCLUSION

The MEP was found to contain quercetin and exhibited strong antioxidant activity, low cytotoxicity, and protective effects against senescence in normal kidney cells. These findings highlight the potential of parijoto as a natural source of bioactive compounds with antioxidant and cytoprotective properties. MEP demonstrates potential antioxidant and cytoprotective properties, suggesting its possible role in mitigating oxidative stress–related cellular changes. However, these findings are preliminary and based on *in vitro* data, therefore, further *in vivo* studies and mechanistic investigations are required before any conclusions regarding its potential biological or health-related applications can be made.

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