Trop J Nat Prod Res, October 2025; 9(10): 4798 - 4802 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

Cytotoxicity, Wound Healing Activity, and Antioxidant Potential of *Peronema canescens* Leaf Extract: An *In vitro* Study on B16 Melanoma Cells

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ARTICLE INFO

Article history:
Received 11 July 2025
Revised 12 August 2025
Accepted 13 August 2025
Published online 01 November 2025

ABSTRACT

Peronema canescens Jack, commonly known as sungkai, is a medicinal plant traditionally used due to its anti-inflammatory and wound-healing properties. Therefore, this study aims to evaluate cytotoxicity, wound healing activity, antioxidant potential, and total flavonoid content of *P. canescens* leaf extract using B16 melanoma cells *in vitro*. Cytotoxicity was assessed using the MTT assay, showing that concentrations below 25 µg/mL maintained cell viability above 80%, indicating the extract is non-cytotoxic at low doses. Wound healing activity was evaluated through the scratch assay, in which extract concentrations of 6.25, 12.5, and 25 µg/mL accelerated wound closure, with the 25 µg/mL treatment showing the most significant effect (42.37% closure at 24 h). ABTS assay showed moderate antioxidant activity, with an IC_{50} value of 74.88 µg/mL, while vitamin C showed higher potency (IC_{50} of 7.04 µg/mL). The total flavonoid content of the extract was determined to be 84.08 ± 0.33 mg QE/g. These results suggest that *P. canescens* leaf extract has potential as a natural therapeutic agent for promoting wound healing and protecting cells from oxidative stress.

Keywords: *Peronema canescens*, B16 melanoma, Wound healing, Antioxidant, Cytotoxicity, scratch assay

Introduction

Wound healing is a complex biological process involving cell proliferation, migration, extracellular matrix remodeling, and tissue regeneration. [1] Impaired wound healing can result in chronic wounds, infections, and prolonged recovery periods, specifically in patients with underlying conditions such as diabetes or skin malignancies. [2] As the largest organ of the body, the skin is continually exposed to environmental stressors that generate reactive oxygen species (ROS), leading to oxidative damage and impaired wound healing. [3] Therefore, natural agents with both antioxidant and wound healing properties have garnered a growing interest in dermatological and cosmeceutical studies. [4] *Peronema canescens* Jack, (*P. canescens*) commonly known as sungkai. [5] is a traditional Indonesian medicinal plant widely used for its antipyretic, anti-inflammatory, and antidiabetic properties. [6,7] Phytochemical analyses showed that *P. canescens* contains flavonoids and other secondary metabolites known for their antioxidant and tissue-repair effects. [8] Several studies have demonstrated wound healing and antioxidant potential of plant-derived flavonoids, [9] using ABTS assays and *in vitro* scratch models. [10] These studies suggest that flavonoid-rich extracts can stimulate fibroblast migration and neutralize oxidative stress. [11]

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Citation: Putri CN, Rahmadani A, Rahardhian MRR. Cytotoxicity, Wound Healing Activity, and Antioxidant Potential of *Peronema canescens* Leaf Extract: An *In vitro* Study on B16 Melanoma Cells. Trop J Nat Prod Res. 2025; 9(10): 4798 – 4802 https://doi.org/10.26538/tjnpr/v9i10.14

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

According to results, few researchers have investigated the integrative biological activity of *P. canescens* leaf extract, particularly in terms of cytotoxicity, antioxidant potential, and wound healing using B16 melanoma cells as an *in vitro* model. There is a lack of comprehensive studies evaluating these parameters simultaneously in a controlled experimental setup. Therefore, this study aims to bridge this gap by assessing multiple bioactivities of *P. canescens* leaf extract under standardized conditions. The objectives of this study are to evaluate cytotoxicity, wound healing activity, antioxidant potential, and total flavonoid content of *P. canescens* leaf extract using B16 melanoma cells, with the aim of supporting its potential application in dermatological and cosmeceutical formulations.

Materials and Methods

Plant Collection and Identification

P. canescens leaves were harvested between May and July 2021 from trees measuring 6–7 meters in height. The plant material was collected from Padang Pariaman District, West Sumatra, Indonesia (approximately Latitude: 0°29'46.8312" S, Longitude: 100°20'7.638" E, Altitude: 100–1000 meters above sea level). Taxonomic identification was performed by a qualified botanist at the Herbarium Jatinangorriensis, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, West Java, and assigned the voucher number 05/LBM/IT/II/2021, which has been deposited at the herbarium for future reference.

Extraction of Plant Material

The dried *P. canescens* leaves (1000 g) were extracted by maceration using 96% ethanol (20 L) for 72 hours at room temperature (25 ± 2°C). The macerate was filtered, and the solvent was removed under reduced pressure using a rotary evaporator, and the resulting thick extract was stored at 4°C until further use.[12] A stock solution was prepared in dimethyl sulfoxide (DMSO) and diluted in complete medium prior to application in biological assays.

Cell Line and Culture Conditions

B16 melanoma cells were obtained from a certified cell bank and cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Biowest, France) and 1% penicillin–streptomycin.[13] The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Culture medium was replaced every 2–3 days, and cells were subcultured upon reaching 70–80% confluency. [5]

MTT Cytotoxicity Assay

Cytotoxicity of *P. canescens* leaf extract was evaluated using the MTT assay, as previously described by [13] with slight modifications. B16 melanoma cells were seeded into 96-well plates at a density of 1×10^4 cells/well in 50 µL of complete DMEM. After 24 hours of incubation, cells were treated with 50 µL of the extract at various concentrations, specifically 400.00, 25.00, 12.50, 6.25, and 3.13 µg/mL, each prepared in DMSO (≤1% final concentration) and diluted in medium, resulting in a final volume of 100 µL per well. Untreated cells, vehicle (DMSO), and doxorubicin (as positive control) were included for comparison. After another 24-hour incubation, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates were incubated for 4 hours. Formazan crystals formed by metabolically active cells were solubilized with 150 µL of DMSO, and absorbance was measured at 492 nm using a BIOBASE microplate reader. Cell viability was calculated as a percentage relative to the control group, and IC_{50} values were determined by linear regression of the dose–response curve.

Scratch Wound Healing Assay

Wound healing potential of *P. canescens* extract was investigated using an *in vitro* scratch assay based on the method described by [14]. B16 melanoma cells were seeded at a density of 2×10^5 cells/well in 24-well plates and incubated with complete DMEM containing 10% FBS for 24 hours. When confluency reached approximately 80%, a straight scratch was made in the cell monolayer using a sterile 200 µL pipette tip. The wells were then gently rinsed twice with DPBS 1X to remove detached cells. Cells were then treated with 500 µL of extract solution at concentrations of 6.25, 12.5, and 25 µg/mL. Negative controls (DMEM 3% and EtOH 0.1%) and a positive control (doxorubicin 0.15 µg/mL) were included.

ABTS Antioxidant Assay

Antioxidant capacity of the extract was measured using the ABTS radical scavenging assay according to. [15,16] $ABTS^+$ radicals were generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for 12–16 hours. Prior to testing, the solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.020 at 734 nm, measured using a UV-Vis spectrophotometer (Model T1800, Hizamitsu, Japan).

Extracts with concentrations of 20, 40, 60, 80, and 100 µg/mL were mixed with ABTS solution, and after 6 minutes of reaction, the reduction in absorbance was measured at 734 nm. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC), and results were compared to a standard curve generated using Trolox.

Total Flavonoid Content (TFC)

Total flavonoid content of the extract was determined using a colorimetric method adapted from.[17] Briefly, the extract solution was reacted with 10% aluminum chloride and potassium acetate, and following incubation, the absorbance was measured at 415 nm using a microplate reader.[18] Quercetin was used to construct the standard calibration curve, and the results were expressed as mg quercetin equivalent per gram of extract (mg QE/g). [19]

Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). [20] One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to evaluate statistical significance among treatment groups. Differences were considered statistically significant at $p < 0.05$. IC_{50} values were determined from dose–response curves using linear regression analysis.

Results and Discussion

Cytotoxicity of P. canescens Leaf Extract on B16 Melanoma Cells

The cytotoxic effect of *P. canescens* leaf extract was evaluated using the MTT assay. The extract demonstrated dose-dependent cytotoxicity, with a significant reduction in cell viability at higher concentrations. [21] At 400 µg/mL, cell viability decreased to 12.12 ± 1.24%, indicating strong cytotoxicity. In contrast, the concentrations below 25 µg/mL maintained viability above 80%, suggesting safety for further biological applications such as wound healing.[22] IC_{50} value reflected moderate cytotoxicity, beneficial for selective inhibition of cancer cell proliferation while preserving normal cell function. [23] These results are consistent with prior investigations on polyphenolic and flavonoid compounds inducing apoptosis and oxidative stress in melanoma cells. [24] The percentage of cell viability following treatment with various concentrations of *P. canescens* leaf extract is presented in Table 1, showing a clear dose-dependent cytotoxic effect on B16 melanoma cells. The graphical representation of cell viability in response to increasing extract concentrations is shown in Figure 1, illustrating the inverse relationship between extract dose and cell survival.

Table 1: Cell Viability of *P. canescens* Leaf Extract on B16 Melanoma Cells (MTT Assay)

Concentration (µg/mL)	log Concentration	% Viability
400.00	2.6021	12.12 ± 1.24
25.00	1.3979	88.81 ± 6.11
12.50	1.0969	90.64 ± 6.50
6.25	0.7959	84.89 ± 3.80
3.13	0.4949	81.62 ± 2.26

Cytotoxicity of *P. canescens* leaf extract was evaluated on B16 melanoma cells using the MTT assay, and the results were expressed as percentage cell viability at various concentrations (3.13–400 µg/mL). Figure 1 shows the cell viability in relation to the logarithmic concentration of the extract.

A significant decrease in cell viability was observed at the highest concentration (400 µg/mL), with an average viability of only 12.12%, indicating strong cytotoxic effects. In contrast, at concentrations below 25 µg/mL, the extract maintained cell viability above 80%, suggesting that these concentrations are considered non-cytotoxic and suitable for further biological studies, such as wound healing assays.

The shaded region around the viability curve in the graph represents the standard deviation (SD), indicating the degree of variability between replicates. The relatively small SD at lower concentrations supports the consistency and reliability of the results in the non-toxic range. These results indicate that *P. canescens* leaf extract exhibits a dose-dependent cytotoxic effect on B16 melanoma cells, potentially attributable to its phenolic and flavonoid constituents known for their bioactive properties. However, the ability of the extract to retain high cell viability at lower concentrations highlights its safety profile for topical or regenerative applications.

Consequently, extract concentrations of 6.25, 12.5, and 25 µg/mL were selected for the scratch assay based on their non-cytotoxic profile. This approach ensures that the observed wound-healing activity can be attributed to enhanced cell migration and proliferation, rather than to cytotoxic stress.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

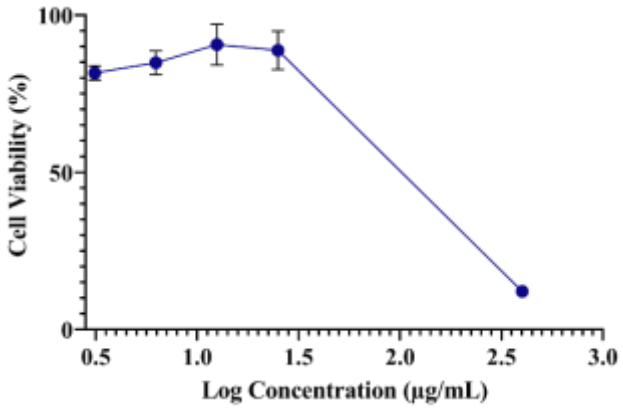

Figure 1: MTT Assay: Cell Viability vs. log Concentration

In addition, a chloroform extract of Moricandia arvensis significantly inhibited the growth of B16-F0 melanoma cells, indicating the potential of lipophilic compounds in exerting antiproliferative effects. [27] These studies support the role of bioactive plant compounds in targeting melanoma cells through cytotoxic mechanisms.

Wound Healing Activity in Scratch Assay

Wound healing was assessed through a scratch assay at 0, 6, and 24 hours post-treatment. Extract concentrations of 6.25, 12.5, and 25 µg/mL enhanced fibroblast migration, with 25 µg/mL yielding the highest closure (42.37%), followed by 6.25 µg/mL (41.30%) and 12.5 µg/mL (39.05%). Doxorubicin showed the lowest healing effect (9.67%), while DMEM 3% and EtOH 0.1% showed moderate activity (both 34.77%). Quantitative analysis of wound area and corresponding closure percentage after treatment with the extract is summarized in Table 2, showing improved fibroblast migration over time compared to controls. The trend of wound area reduction over 24 hours post-treatment is shown in Figure 2, demonstrating the extract's ability to accelerate wound closure in a dose-dependent manner.

Wound healing potential of *P. canescens* leaf extract was evaluated using the scratch assay on B16 melanoma cells. The progression of wound closure was monitored by measuring the average wound area (µm²) at 0, 6, and 24 hours after treatment with various extract concentrations (6.25, 12.5, and 25 µg/mL), as well as with control treatments (DMEM 3%, EtOH 0.1%, and Doxorubicin 0.15 µg/mL), and the results are presented in Figure 2.

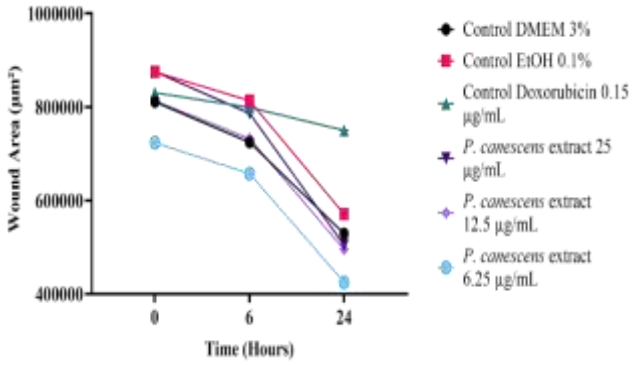

Figure 2: Wound Area Over Time (Scratch Assay)

The extract-treated groups showed a significant reduction in wound area over time compared to the controls. At 24 hours, the most prominent wound closure was observed in the 25 µg/mL group (504,454.63 µm²), followed by 6.25 µg/mL (424,748.41 µm²) and 12.5 µg/mL (495,441.90 µm²). The Doxorubicin-treated group exhibited minimal wound closure (750,018.78 µm²), consistent with its known antiproliferative effect that inhibits fibroblast migration. Both DMEM 3% and 0.1% ethanol controls showed moderate levels of wound closure.

The results suggest that *P. canescens* extract enhances fibroblast migration and accelerates wound closure in a dose-responsive manner, with the 25 µg/mL concentration demonstrating the highest wound healing efficiency. The extract's effectiveness may be attributed to the presence of flavonoids and antioxidants, which have been reported to modulate signaling pathways involved in tissue regeneration, including MAPK and PI3K/Akt.[28] Wound healing activity of the extract, supported by the non-cytotoxic nature at selected concentrations (as demonstrated by MTT assay), highlights its potential for development as a plant-based therapeutic agent in dermal regeneration or cosmeceutical formulations.

Table 2: Wound Area and Closure Percentage of B16 Melanoma Cells Treated with *P. canescens* Extract

Time (hrs)	DMEM 3%	EtOH 0.1%	Doxorubicin 0.15 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL
Wound Area (µm²)						
0	810941.78	874682.70	830269.28	875285.19	812852.70	723645.92
6	724260.43	813423.14	798930.19	786422.04	732272.40	657242.03
24	528967.38	570567.10	750018.78	504454.63	495441.90	424748.41
Wound Closure (%)						
6	10.69	7.00	3.77	10.15	9.91	9.18
24	34.77	34.77	9.67	42.37	39.05	41.30

Several medicinal plants have been reported to promote fibroblast proliferation and migration, essential for wound healing. *Calendula officinalis* has shown stimulatory effects on fibroblast proliferation and migration at low concentrations, with 10 µg/mL increasing cell numbers by 64.35% and 70.53%, respectively. Interestingly, inhibition studies showed that the observed effect was primarily due to enhanced cell migration rather than proliferation. [29] Similarly, *Achillea eriophora* D.C extract stimulated human fibroblast proliferation at very low concentrations (0.1–0.8 µg/mL), with the highest proliferation rate at 0.1 µg/mL. Cell migration was promoted at intermediate concentrations

(1–30 µg/mL), with 1 µg/mL being the most effective dose.[30] However, *Calendula officinalis* extracts only marginally influenced keratinocyte migration in a scratch assay, suggesting cell-type-specific effects during tissue regeneration.[31] In another study, *Scrophularia striata* extract showed significant proliferative and migratory effects on fibroblast cells, further supporting the role of plant-derived compounds in enhancing wound healing. [32]

Antioxidant Activity by ABTS Assay

The extract exhibited concentration-dependent radical scavenging activity against $ABTS^+$ radicals, as expressed in Trolox Equivalent Antioxidant Capacity (TEAC). [15] While vitamin C exhibited a low IC_{50} of 7.04 µg/mL, *P. canescens* extract showed moderate antioxidant capacity with an IC_{50} of 74.88 µg/mL, suggesting its flavonoid content contributed significantly to this effect.

Several medicinal plants have demonstrated antioxidant activity [16] through ABTS radical scavenging assays. *Torilis leptophylla* exhibited notable free radical scavenging activity, with an ABTS IC_{50} value of 10.0 ± 0.9 µg/mL.[33] Similarly, the crude methanol extract of *Pongamia timoriana* seeds showed ABTS radical scavenging activity with an IC_{50} value of 45.39 µg/mL. [34] In another study, *Tridax procumbens* demonstrated moderate antioxidant activity with ABTS IC_{50} values ranging from 0.51 to 1.04 mg/mL. [35] These results indicate that plant-derived phenolic compounds, particularly flavonoids, play a key role in scavenging reactive oxygen species and reducing oxidative stress. [36] Antioxidant activity supports wound healing by reducing oxidative stress and maintaining cellular homeostasis during regeneration. Antioxidant activity of the extract and vitamin C as a standard is compared in Figure 3, where ABTS radical scavenging efficiency increases with concentration.

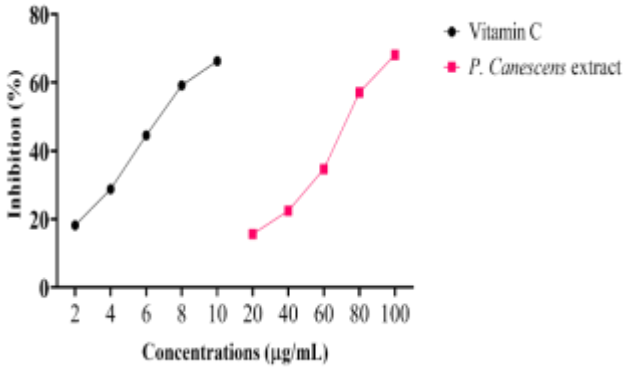

Figure 3: ABTS Radical Scavenging Activity of *P. canescens* Extract Compared to Vitamin C

Total Flavonoid Content (TFC)

Total flavonoid content analysis using a colorimetric method showed that the extract contains 84.08 ± 0.33 mg QE/g extract. This high flavonoid concentration corroborates the antioxidant and regenerative effects observed in ABTS and scratch assays. Total flavonoid content was determined using a standard calibration curve constructed with quercetin as the reference compound, as shown in Figure 4. Flavonoids likely contribute to both ROS scavenging and stimulation of cellular migration. [37] These results reinforce the potential application of *P. canescens* extract as a multifunctional agent for skin therapy.

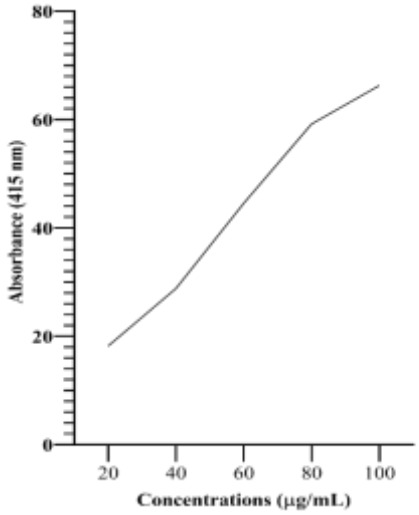

Figure 4: Total Flavonoid Content of *P. canescens* Extract

Conclusion

In conclusion, this study showed the potential of *P. canescens* leaf extract as a natural therapeutic candidate for dermatological applications. By addressing cytotoxicity, antioxidant activity, and wound healing efficacy in a single *in vitro* model, this study advances current understanding of *P. canescens* as a multifunctional bioactive agent. These results suggest that, at low concentrations, the extract is safe and, due to its flavonoid-rich composition, may contribute to cellular protection and enhanced tissue repair. The results justify further exploration of *P. canescens* in topical formulations. Future studies should investigate the mechanisms of action at the molecular level and validate their efficacy in *in vivo* wound healing and clinical settings.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

Acknowledgements

The authors are grateful to Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPPM), Universitas Islam Sultan Agung (UNISSULA), for providing funding under Grant No. 69/B.1/SA/LPPM/VII/2023.

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