MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Muhamad *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 22 (2026) 1068–1078

RESEARCH ARTICLE

Anti-inflammatory, Antioxidant, and In Vitro Wound Healing Activities of *Parkia speciosa* Peel Extract with Application in Topical Formulation and Stability Assessment

Nisaporn Muhamad[a,*], Piyasiri Soontornnon Sinchai[b], Ubol Tansom[c], Likit Lateh[a], Habilla Chapakiya[d]

[a]Cosmetic Science, Health and Anti-Aging, Faculty of Public Health and Allied Health Sciences, Yala Rajabhat University, Yala 95000, Thailand; [b]General Science, Faculty of Science, Technology & Agriculture, Yala Rajabhat University, Yala 95000, Thailand; [c]Applied Chemistry, Faculty of Science, Technology & Agriculture, Yala Rajabhat University, Yala 95000, Thailand; [d]Department of Agricultural Extension, Bangkok 10900, Thailand

Abstract This study evaluated the anti-inflammatory, antioxidant, and in vitro wound healing activities of *Parkia speciosa* peel extract and its application in topical formulations. Peel powders were extracted separately using hexane, dichloromethane, ethyl acetate, and ethanol by maceration. Based on preliminary screening, the ethanol extract was selected for further analysis due to its higher bioactive content. Flavonoid content was determined using the aluminium chloride colorimetric method. Anti-inflammatory activity was assessed through nitric oxide (NO) inhibition in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells, while antioxidant activity was evaluated using an NO scavenging assay. The extract exhibited a flavonoid content of 34.28 ± 0.47 mg QE/g extract and showed concentration-dependent NO scavenging activity with an SC_{50} value of 0.24 ± 0.03 mg/mL, compared to 0.13 ± 0.03 mg/mL for ascorbic acid. The extract also significantly reduced NO production in LPS-stimulated macrophages. In vitro wound healing potential was evaluated using a human fibroblast scratch assay. Lower concentrations (7.81 and 15.63 µg/mL) enhanced wound closure at 48 h, whereas a higher concentration (31.25 µg/mL) promoted early cell migration. Three cream formulations (F1–F3) containing the extract were developed and evaluated for physical properties and stability. Formulation F3 exhibited improved spreadability, reduced stickiness, and maintained stability without phase separation after six freeze–thaw cycles, with pH remaining within the acceptable dermatological range. These findings indicate that *P. speciosa* peel extract possesses anti-inflammatory, antioxidant, and wound healing activities and can be incorporated into stable topical formulations. Further studies, including in vivo evaluation, are required to confirm its efficacy and safety.

Keywords: *Parkia speciosa* peel, nitric oxide inhibition, antioxidant activity, wound healing, topical formulation.

***For correspondence:**
Nisaporn.m@yru.ac.th

Received: 25 July 2025
Accepted: 30 September 2026

© Copyright Muhamad. This article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use and redistribution provided that the original author and source are credited.

Introduction

Parkia speciosa, commonly known as stink bean, is a plant widely distributed in Southeast Asia and is traditionally consumed as food. In addition to its culinary use, it has been reported to contain various phytochemicals, including phenolics, flavonoids, tannins, and alkaloids, which are associated with antioxidant, anti-inflammatory, and wound healing activities [1-4].

Wound healing is a complex biological process involving overlapping phases of hemostasis, inflammation, proliferation, and remodeling. Among the key cells involved, macrophages and fibroblasts play central roles. Macrophages regulate inflammatory responses and secrete mediators involved in tissue repair, whereas fibroblasts are responsible for extracellular matrix formation, collagen deposition, and wound contraction [5-8].

Nitric oxide (NO) is an important signaling molecule in inflammation and tissue repair. Under inflammatory conditions, excessive NO is produced by inducible nitric oxide synthase in activated macrophages, contributing to tissue damage. Therefore, inhibition of NO production is commonly used as an indicator of anti-inflammatory activity in cell-based studies [4,9]. In addition, oxidative stress may impair cell migration and tissue regeneration, highlighting the importance of antioxidant activity in wound healing.

Previous studies have shown that *P. speciosa* extracts can reduce NO production and modulate inflammatory pathways [3,4]. In addition, ethanol extracts have been reported to contain higher levels of bioactive compounds and exhibit stronger antioxidant activity compared to less polar extracts [4,10]. As the peel is commonly discarded as agricultural waste, its utilization may contribute to value-added applications.

However, limited studies have investigated the incorporation of *P. speciosa* peel extract into topical formulations. Therefore, this study evaluated the flavonoid content, anti-inflammatory activity via NO inhibition, antioxidant activity through NO scavenging, and in vitro wound healing activity of *P. speciosa* peel extract. The extract was further incorporated into topical cream formulations, and their physical properties and stability were assessed.

Materials and Methods

1. Plant Material and Extraction

Parkia speciosa pods were collected from Yala Province, Thailand, in August 2022. The peels were manually separated from the pods, thoroughly washed, and dried in a hot air oven at 45 °C for 3–5 days until a constant weight was achieved. The dried peels were then ground into a fine powder and stored in airtight containers at room temperature until further use. The powdered sample (500 g) was extracted separately using four solvents of different polarity, namely hexane, dichloromethane, ethyl acetate, and ethanol (5000 mL for each solvent).

Each extraction was carried out independently by maceration at room temperature in the dark for 7 days with daily agitation. Following extraction, the mixtures were filtered through filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator to obtain crude extracts. The extraction yield (%) was calculated based on the initial dry weight of the plant material. All extracts were stored at 4 °C until further analysis.

Among the obtained extracts, the ethanolic extract was selected for subsequent biological assays due to its higher ability to extract phenolic and flavonoid compounds, which are commonly associated with antioxidant and anti-inflammatory activities.

2. Flavonoid Determination

The total flavonoid content was determined using the aluminium chloride colorimetric method as described by Chang et al. [11] with slight modification. Briefly, 200 µL of the extract solution (12.5 mg/mL) was mixed with 1.8 mL of 95% ethanol, followed by the addition of 100 µL of 10% (w/v) aluminium chloride solution and 100 µL of 1 M potassium acetate. Subsequently, 2.8 mL of distilled water was added to the mixture, and the solution was incubated at room temperature for 30 min.

The absorbance was measured at 415 nm using a spectrophotometer. A calibration curve was prepared using quercetin as a standard at different concentrations. The total flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

3. Anti-inflammatory Assay

3.1 Macrophage Culture

RAW 264.7 macrophage cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Biowest, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin–streptomycin solution (100 U/mL penicillin and 100 µg/mL streptomycin).

The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. For the assay, the cells were seeded in 96-well plates at a density of 1 × 10^6 cells/mL (100 µL per well) and incubated for 24 h to allow cell attachment prior to treatment.

3.2 Nitric Oxide Inhibition Assay

The anti-inflammatory activity of the extract was evaluated by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages using the Griess reaction as previously described [12] with slight modification.

After cell attachment, the culture medium was replaced with fresh medium containing LPS (10 µg/mL) to induce NO production. The cells were then treated with the ethanolic extract at concentrations of 7.81, 15.63, and 31.25 µg/mL and incubated for 24 h under the same conditions. Untreated cells served as the negative control, while LPS-treated cells without extract were used as the positive control.

Following incubation, 85 µL of the culture supernatant was transferred to a new 96-well plate and mixed with nitrate reductase and enzyme cofactor solution, followed by incubation at room temperature for 1 h. An enhancer solution (5 µL) was then added and incubated for a further 10 min. Subsequently, 50 µL of Griess reagents (R1 and R2) were added, and the absorbance was measured at 540 nm using a microplate reader.

Nitric oxide concentration was determined using a sodium nitrite standard curve. The inhibitory effect of the extract on NO production was used as an indicator of anti-inflammatory activity.

4. Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was evaluated based on the method described by Marcocci et al. [13] with slight modification. The extract was dissolved in ethanol and prepared at various concentrations ranging from 0.001 to 10 mg/mL. Ascorbic acid was used as a positive control. Equal volumes of the extract solution and reaction mixture were combined and incubated at room temperature under light-protected conditions.

After incubation, Griess reagent was added, and the absorbance was measured at 540 nm using a microplate reader. The percentage of NO scavenging activity was calculated by comparing the absorbance of the sample with that of the control. The concentration required to scavenge 50% of nitric oxide radicals (SC_{50}) was determined from the dose–response curve.

5. In Vitro Wound Healing Assay

5.1 Fibroblast Culture

Human skin fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Biowest, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution containing penicillin (100 U/mL) and streptomycin (100 µg/mL). Culture conditions were consistent with those described for RAW 264.7 macrophages.

The cells were seeded in 24-well plates at a density of 2 × 10^5 cells per well and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h to allow cell attachment and growth to near confluence. Cell morphology and confluence were examined under an inverted phase-contrast microscope prior to performing the scratch assay.

5.2 Scratch Assay

The wound healing activity was evaluated using a scratch assay based on Cory [14]. Fibroblast cells prepared as described in Section 5.1 were allowed to reach confluence. The culture medium was removed, and the cell monolayer was gently washed with phosphate-buffered saline (PBS). A linear scratch was created across the cell monolayer using a sterile cell scraper to simulate a wound area. The detached cells were removed by washing with PBS, and fresh medium containing *P. speciosa* peel extract at concentrations of 7.81, 15.63, and 31.25 µg/mL was added.

Cell migration into the scratched area was monitored and photographed at 0, 24, 48, and 72 h using an inverted microscope. The extent of wound closure was evaluated by comparing the scratched area over time.

6. Topical Cream Formulation Development

Three oil-in-water (O/W) cream formulations (F1–F3) containing *Parkia speciosa* peel extract were

developed based on standard emulsion systems with slight modifications to accommodate extract incorporation [15-16]. The formulations differed in extract concentration (0.1%, 0.2%, and 0.3% w/w) and emollient composition to evaluate their effects on the physical properties and stability of the creams.

The composition and functional roles of the formulations are presented in Table 1. All formulations were adjusted to 100% (w/w), and deionized water was divided into separate portions for phase preparation and final adjustment during formulation.

The aqueous phase (Phase A) and oil phase (Phase C) were prepared separately and heated to 70 °C until completely dissolved. Phase B was incorporated into the aqueous phase with continuous stirring. The oil phase was then gradually added to the aqueous phase under high-shear mixing (2,000 rpm) to form an emulsion. The mixture was allowed to cool to approximately 40 °C, after which Phase D, containing the *P. speciosa* peel extract and other heat-sensitive components, was added. Stirring was continued until the formulation reached room temperature. The prepared creams were transferred into suitable containers and stored at room temperature for further evaluation.

Table 1. Composition and functions of *Parkia speciosa* peel cream formulation (F1–F3).

Phase	Ingredients	Formula (%w/w)			Function
		F1	F2	F3	
A	Deionized water	41.3	41.3	41.3	Solvent
	Disodium EDTA	0.05	0.05	0.05	Chelating agent
	Propylene Glycol	5.0	5.0	5.0	Humectant
	Allantoin	0.5	0.5	0.5	Skin conditioning agent
	Ammonium Acryloyldimethyltaurate/VP Copolymer (Aristoflex® AVC)	0.1	0.1	0.1	Thickener / Stabilizer
B	Carbomer	0.1	0.1	0.1	Thickener
C	Pentaerythrityl Distearate	2.5	-	5.0	Emollient / Consistency agent
	Cetearyl Alcohol	-	5.0	-	Emollient / Co-emulsifier
	Cetyl PEG/PPG-10/1 Dimethicone	2.5	-	-	Silicone emulsifier
	Sodium Stearoyl Glutamate	0.5	0.5	0.5	Emulsifier
	PEG-20 Glyceryl Triisostearate	1.0	1.0	1.0	Emulsifier / surfactant
	Triticum Vulgare (Wheat) Germ Oil	1.0	1.0	1.0	Emollient
	Stearic acid	3.0	3.0	3.0	Emollient / Thickener
D	Deionized water	41.2	41.1	41.0	Solvent
	Triethanolamine	0.5	0.5	0.5	Neutralizing agent
	Phenoxyethanol	0.5	0.5	0.5	Preservative
	***P. speciosa* Peel Extract**	0.1	0.2	0.3	Active (antioxidant / anti-inflammatory)

7. Topical Cream Evaluation and Stability Assessment

The prepared cream formulations were evaluated for their physical characteristics, including color, homogeneity, spreadability, and stickiness. Visual inspection was performed to assess the appearance and uniformity of the formulations. Spreadability was determined by placing a fixed amount of cream between two glass slides and measuring the diameter of spread under a specified weight. Stickiness was evaluated qualitatively based on the adhesion of the formulation to the skin surface. The pH of each formulation was measured using a calibrated pH meter to ensure compatibility with skin conditions. Stability assessment was conducted using a freeze–thaw cycling method [17-18].

The formulations were subjected to alternating temperatures of 4 °C and 45 °C for six cycles, with each cycle consisting of 24 h at each temperature. After each cycle, the formulations were evaluated for changes in physical appearance, including color, consistency, and phase separation. The formulations were also monitored for signs of instability, such as creaming, cracking, or microbial contamination, throughout the study period.

8. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), and differences were considered statistically significant at $p < 0.05$.

Results and Discussion

1. Flavonoid Content

The total flavonoid content of the ethanolic extract of *Parkia speciosa* peel was determined to be 34.28 ± 0.47 mg QE/g extract, indicating the presence of a considerable amount of phenolic compounds. Flavonoids were selected as representative phytochemicals in this study due to their well-established roles in antioxidant and anti-inflammatory activities, particularly through free radical scavenging and modulation of inflammatory pathways [11-12].

As the present study focuses on nitric oxide (NO)-related mechanisms, flavonoids are particularly relevant, as they have been widely reported to inhibit inducible nitric oxide synthase (iNOS) and reduce NO production in activated macrophages [2-4].

The flavonoid content obtained in this study is consistent with previous reports indicating that *P. speciosa* contains phenolic and flavonoid compounds as major bioactive constituents. However, variations in flavonoid content have been reported depending on the plant part, extraction solvent, and extraction conditions. In particular, ethanol is considered an effective solvent for extracting moderately polar compounds such as flavonoids, which may explain the measurable flavonoid content observed in the present study [10,19].

Compared to other studies on *Parkia* species, the flavonoid content reported here falls within a reasonable range, supporting the presence of bioactive compounds that may contribute to the observed biological activities. Differences in reported values are commonly attributed to variations in plant matrices, geographical origin, and extraction methodology.

Flavonoids exert their biological effects primarily through their ability to donate electrons or hydrogen atoms, thereby neutralizing reactive oxygen and nitrogen species. In addition, they have been shown to regulate inflammatory mediators and promote tissue repair processes, including fibroblast proliferation and collagen synthesis [20]. Therefore, the flavonoid content of *P. speciosa* peel extract is likely a key contributing factor to the nitric oxide inhibition and wound healing activities observed in this study.

2. Anti-inflammatory

The anti-inflammatory activity of *Parkia speciosa* peel ethanolic extract was assessed by measuring nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages (Figure 1). The extract significantly reduced NO levels in a concentration-dependent manner compared to the LPS-treated control ($p < 0.05$).

Nitric oxide concentration (µM) was quantified using a sodium nitrite standard curve. A reduction in NO levels indicates suppression of inflammatory mediator production. LPS stimulation induces the expression of inducible nitric oxide synthase (iNOS) in macrophages, resulting in increased NO production associated with inflammatory responses [11-12]. Therefore, inhibition of NO production is commonly used as an indicator of anti-inflammatory activity.

The observed inhibitory effect is consistent with previous studies reporting that *P. speciosa* extracts reduce NO production and modulate inflammatory responses in cellular models [2-4]. This activity may be associated with the presence of flavonoids and other phenolic compounds, which have been reported to regulate inflammatory pathways and suppress iNOS expression [4,10,19].

The extract showed a stronger effect in the cellular NO inhibition assay than in the chemical NO scavenging assay. This suggests that the anti-inflammatory activity is more likely related to modulation of intracellular pathways rather than direct radical scavenging. Similar observations have been reported for plant-derived compounds, where cellular effects are more pronounced than chemical antioxidant activity [21-22]. These results demonstrate that *P. speciosa* peel extract exhibits anti-inflammatory activity by inhibiting nitric oxide production in activated macrophages.

The inhibitory effect of the extract on nitric oxide production may be associated with the modulation of intracellular inflammatory signaling pathways. Flavonoids and phenolic compounds have been widely reported to regulate nuclear factor-kappa B (NF-κB), a key transcription factor involved in the expression of pro-inflammatory mediators, including inducible nitric oxide synthase (iNOS). Suppression of NF-κB activation may therefore contribute to the reduced nitric oxide levels observed in this study. In addition to NF-κB, mitogen-activated protein kinase (MAPK) pathways are also involved in inflammatory responses and cellular signaling. Previous studies have demonstrated that plant-derived polyphenols

can modulate MAPK signaling, leading to decreased production of inflammatory mediators [23]. This mechanism may further explain the significant inhibition of nitric oxide production observed in LPS-stimulated macrophages.

The stronger inhibitory effect observed in the cellular assay compared to the chemical scavenging assay suggests that the extract exerts its anti-inflammatory activity primarily through biological regulation rather than direct radical neutralization. This is consistent with the role of reactive nitrogen species and intracellular signaling pathways in inflammation [24-25], highlighting the importance of cellular models in evaluating the anti-inflammatory potential of natural products.

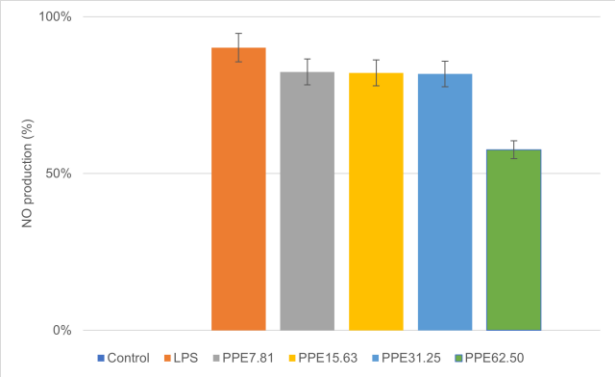


Figure 1. Inhibitory effect of *P. speciosa* peel extracts on NO production in LPS-stimulated macrophage RAW 264.7 cells.

3. Nitric Oxide Scavenging Activity

The nitric oxide (NO) scavenging activity of *Parkia speciosa* peel ethanolic extract is shown in Figure 2. The extract exhibited a concentration-dependent increase in scavenging activity, with an SC_{50} value of 0.24 ± 0.03 mg/mL, while ascorbic acid demonstrated higher activity with an SC_{50} value of 0.13 ± 0.03 mg/mL.

In the microwell assay, each well represents a specific concentration of the extract or standard. The intensity of the pink coloration reflects the amount of nitrite formed via the Griess reaction, where darker color indicates higher nitric oxide levels and lighter color indicates greater scavenging activity. The gradual decrease in color intensity with increasing extract concentration confirms its ability to neutralize nitric oxide radicals.

Although the extract showed lower activity than the standard, it exhibited moderate antioxidant capacity. This is consistent with previous studies attributing the antioxidant activity of *P. speciosa* to its phenolic and flavonoid constituents, which can donate electrons or hydrogen atoms to stabilize reactive nitrogen species and reduce oxidative stress [2,4,10].

Notably, the scavenging activity observed in this chemical assay was less pronounced than the nitric oxide inhibition observed in the cellular model. This suggests that the extract may exert its biological effects not only through direct radical scavenging but also through modulation of intracellular inflammatory pathways. Similar trends have been reported for plant-derived extracts, where cellular anti-inflammatory effects are often more prominent than chemical antioxidant activity. The *P. speciosa* peel extract showed moderate nitric oxide scavenging activity, which may contribute to its anti-inflammatory and wound healing effects by reducing oxidative stress associated with inflammation. The nitric oxide scavenging activity observed in this study is primarily associated with the ability of phenolic and flavonoid compounds to donate electrons or hydrogen atoms, thereby stabilizing reactive nitrogen species. These compounds can interact directly with nitric oxide radicals, leading to the formation of more stable and less reactive products [19,23-27].

However, the moderate scavenging activity observed compared to the standard suggests that direct radical neutralization may not be the dominant mechanism. Instead, the stronger inhibitory effect observed in the cellular model indicates that the extract may exert its biological activity through modulation of intracellular signaling pathways rather than purely chemical antioxidant activity.

This distinction between chemical scavenging and cellular inhibition has been widely reported in plant-

derived extracts, where the biological response is often more pronounced due to interactions with multiple molecular targets. Therefore, the nitric oxide scavenging activity observed in this study may contribute partially to the overall anti-inflammatory effect, while intracellular regulatory mechanisms likely play a more significant role.

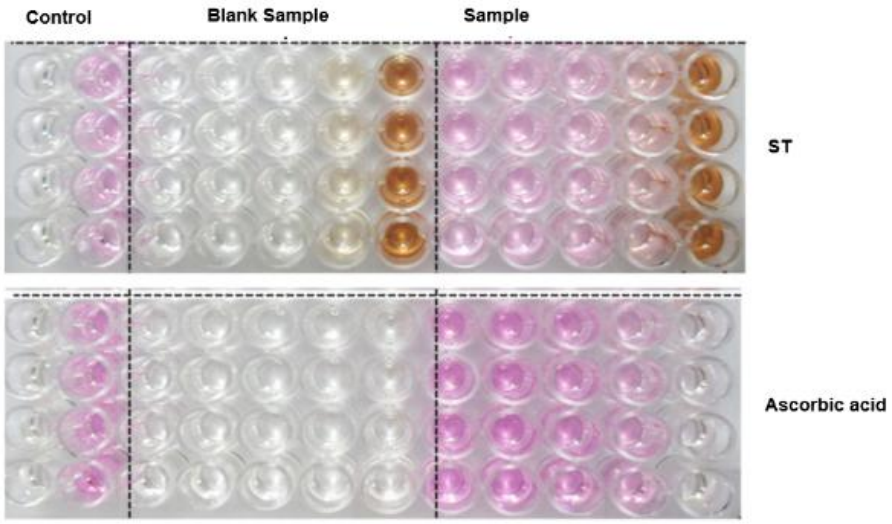


Figure 2. Nitric oxide (NO) scavenging activity of *P. speciosa* peel ethanolic extract (ST) and ascorbic acid.

4. In Vitro Wound Healing Activity

The wound healing activity of *Parkia speciosa* peel ethanolic extract was evaluated using a scratch assay in human fibroblast cells. Quantitative results are presented in Figure 3, and representative images are shown in Figure 4, illustrating progressive fibroblast migration and reduction of the wound area over time, with faster closure observed in treated groups. Figure 3A shows the percentage of wound closure, while Figure 3B presents the corresponding cell migration rate. The extract enhanced wound closure compared to the untreated control. At 48 h, treatment with 7.81 and 15.63 µg/mL resulted in wound closure of approximately 52% and 64%, respectively, whereas the control showed 28% closure. The highest concentration (31.25 µg/mL) exhibited lower wound closure at this time point.

At 24 h, the highest concentration showed greater wound closure and migration rate, indicating enhanced early cell migration. At later time points, lower concentrations resulted in improved wound closure. A similar trend was observed in the migration rate, with increased migration at early stages followed by a decline as wound closure progressed.

These results indicate a concentration-dependent effect, where higher concentrations promote early cell migration, while lower concentrations support sustained wound closure. The reduced effect at the highest concentration at later time points may be associated with inhibitory effects at higher doses, consistent with previous reports describing biphasic responses of plant extracts on fibroblast activity [28].

The observed wound healing activity is consistent with studies on other *Parkia* species. For example, *Parkia clappertoniana* extract has been reported to enhance wound contraction, collagen deposition, and epithelialization in vivo, supporting its role in tissue repair [28,30]. Similarly, extracts from *Parkia* species have been shown to promote fibroblast migration and tissue regeneration in wound models.

These effects may be attributed to the presence of phenolic and flavonoid compounds, which have been reported to stimulate fibroblast activity, collagen synthesis, and angiogenesis, which are essential for wound healing processes, including tissue remodeling and extracellular matrix formation [27]. In addition, reduction of oxidative stress and inflammatory mediators further supports the wound healing process by promoting cell migration and tissue remodeling [28-30].

The enhanced wound healing activity observed in this study may be attributed to multiple biological mechanisms associated with the phytochemical constituents of the extract. Flavonoids and phenolic compounds have been reported to promote fibroblast proliferation and migration, which are essential processes in the proliferative phase of wound healing. These compounds may also stimulate collagen

synthesis and extracellular matrix formation, contributing to improved tissue remodeling [19].

In addition, the modulation of inflammatory responses plays a crucial role in wound healing. Excessive production of nitric oxide and other inflammatory mediators can delay tissue repair by causing oxidative damage. The inhibitory effect of the extract on nitric oxide production, as demonstrated in this study, may help create a favorable microenvironment for cell migration and tissue regeneration [24].

Furthermore, antioxidant activity may reduce oxidative stress at the wound site, thereby protecting cells from damage and supporting the healing process. The combined effects of anti-inflammatory and antioxidant activities may therefore contribute synergistically to the observed wound closure and enhanced fibroblast migration.

The concentration-dependent behavior observed in this study, where higher concentrations promoted early migration while lower concentrations supported sustained wound closure, may reflect a balance between stimulatory and inhibitory effects of bioactive compounds. Such biphasic responses have been reported in previous studies and highlight the importance of optimizing extract concentration for wound healing applications [31-34].

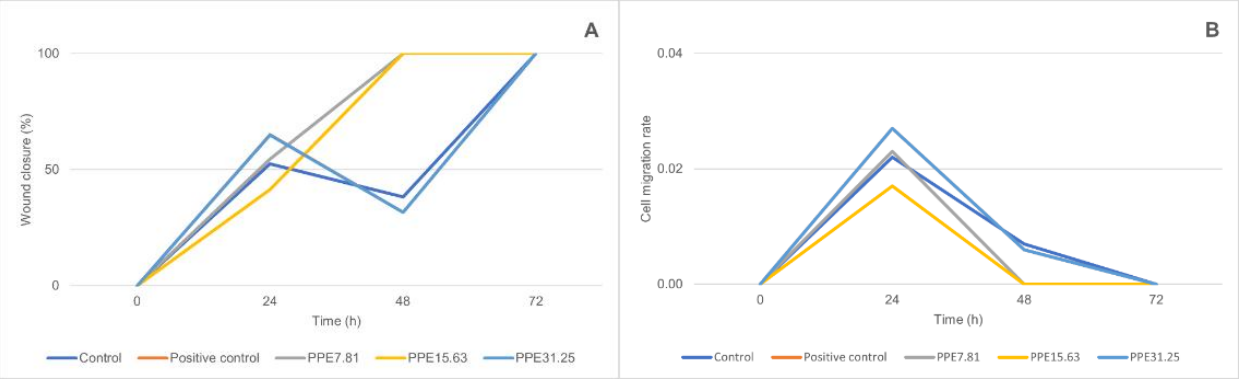


Figure 3. (A) Wound closure (%) and (B) cell migration rate of human fibroblasts treated with *P. speciosa* peel ethanolic extract over 0–72 h. Values represent the mean of three independent experiments.

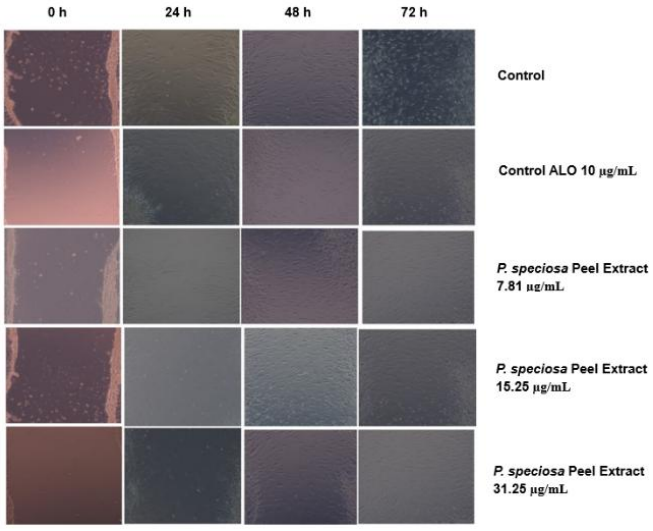


Figure 4. Wound healing activity of *P. speciosa* peel ethanolic extract in human fibroblast cells at 0, 24, 48, and 72 h. Progressive wound closure indicates enhanced cell migration compared to the control.

5. Formulation Development and Physical Properties

Three cream formulations (F1–F3) containing *Parkia speciosa* peel ethanolic extract were developed with varying extract concentrations and emollient compositions. The compositions are presented in Table 1, and their physical properties are summarized in Table 2. All formulations produced homogeneous

creams with smooth texture and no visible phase separation. Based on visual and sensory observation, differences in physical characteristics were observed among the formulations. F1 and F3 exhibited a translucent white appearance with uniform consistency, whereas F2 appeared more opaque and showed slight clumping, indicating reduced homogeneity.

Variations in spreadability and texture were also observed. F3 demonstrated the highest spreadability with low stickiness, while F1 showed moderate spreading and stickiness. In contrast, F2 exhibited lower spreadability and relatively higher stickiness. These differences are likely attributed to variations in emollient composition, which influence viscosity, spreadability, and overall texture of topical formulations [15,17]. The absence of pentaerythrityl distearate in F2 may have contributed to its reduced uniformity and suboptimal texture, as emollients and structuring agents play a key role in stabilizing emulsion systems and improving sensory properties [17]. Among the formulations, F3 showed the most favorable characteristics, indicating its suitability for topical application.

The differences in physical characteristics among the formulations can be further explained by the interactions between emulsifiers, emollients, and thickening agents within the oil-in-water system. Emulsifiers play a critical role in reducing interfacial tension and stabilizing dispersed oil droplets, while structuring agents contribute to the formation of a three-dimensional network that enhances viscosity and physical stability [15-17].

In particular, the presence of fatty alcohols and ester-based emollients can influence the rheological behavior of the formulation, resulting in improved spreadability and sensory attributes. These components contribute to the formation of a semi-solid structure that supports uniform distribution of the active ingredient on the skin surface. In contrast, the absence or imbalance of such components, as observed in F2, may lead to reduced structural integrity and lower homogeneity.

Furthermore, the incorporation of *P. speciosa* peel extract into the emulsion system may also affect the physicochemical properties of the formulation. Plant extracts containing phenolic compounds can interact with the aqueous phase and polymeric thickeners, potentially influencing viscosity, stability, and appearance. Despite this, all formulations remained physically stable, indicating good compatibility between the extract and the formulation components.

The superior performance of F3 suggests that an optimal balance between emulsifier concentration, emollient composition, and extract loading is essential for achieving desirable physical properties. This highlights the importance of formulation optimization in the development of stable and effective topical delivery systems.

Table 2. Physical properties of skincare cream formulations containing *P. speciosa* peel extract.

Formulation	Appearance	Spreadability	Odor	Stickiness
F1	Translucent white	Moderate	Low	Moderate
F2	Opaque white	Low	Low	Moderate
F3	Translucent white	High	Low	Low

6. Topical Cream Stability Assessment

The stability of the cream formulations was evaluated under accelerated conditions using a freeze–thaw cycling method (Table 3). All formulations remained physically stable throughout the test period, with no evidence of phase separation, cracking, or significant changes in texture. The absence of phase separation indicates good emulsion stability and structural integrity [16].

A slight color change to a light brown tint was observed in all formulations during the stability study. This change may be attributed to mild oxidation of phenolic compounds present in the *Parkia speciosa* peel extract. Despite this, the overall appearance of the formulations remained acceptable.

The pH values increased gradually from 5.55 ± 0.02 to 6.12 ± 0.02 after six cycles. However, the pH remained within the acceptable range for topical applications (5.5–6.5), indicating compatibility with skin conditions. No microbial or fungal contamination was detected in any formulation throughout the study period, confirming the effectiveness of the preservative system [18]. In addition, all formulations maintained structural integrity without signs of instability.

These results indicate that the developed cream formulations exhibit good physical stability, an appropriate pH range, and effective microbial control under accelerated conditions, supporting their

suitability for topical application.

Despite the promising results, several limitations should be considered. The biological activities in this study were evaluated using in vitro models, which may not fully represent the complexity of in vivo wound healing processes. Interactions among multiple cell types, extracellular matrix components, and systemic physiological factors may influence the overall therapeutic outcome.

In addition, while nitric oxide inhibition and scavenging assays provide important insights into anti-inflammatory and antioxidant activities, these mechanisms represent only part of the wound healing process. Other relevant pathways, such as cytokine regulation, angiogenesis, and collagen deposition, were not directly investigated and may contribute to the overall effect.

From a formulation perspective, the stability assessment was conducted under accelerated conditions for a limited duration. Although the formulations demonstrated acceptable physical stability, further evaluation under long-term storage conditions is necessary. Additional studies on rheological properties, release behavior of active compounds, and skin permeation would also provide a more comprehensive understanding of formulation performance.

Future work should therefore focus on in vivo evaluation to confirm wound healing efficacy, as well as the identification and quantification of specific bioactive compounds responsible for the observed activities. Optimization of formulation parameters and investigation of delivery characteristics are also recommended to support potential clinical and commercial applications.

Table 3. Stability evaluation of *P. speciosa* peel cream formulations under accelerated conditions

Parameter	Initial	2 Cycles	4 Cycles	6 Cycles
Physical stability	Uniform	Uniform	Uniform	Uniform
Color	White	Slightly yellowish	Light brown	Light brown
pH	5.55 ± 0.02	5.73 ± 0.01	5.89 ± 0.01	6.12 ± 0.02
Microbial contamination	ND	ND	ND	ND
Cracking	ND	ND	ND	ND

ND = Not Detected

Conclusions

This study demonstrates that *Parkia speciosa* peel ethanolic extract exhibits anti-inflammatory and wound healing activities, as shown by its ability to reduce nitric oxide levels and enhance fibroblast migration in vitro. The extract showed concentration-dependent effects, with higher concentrations promoting early cell migration and lower concentrations supporting sustained wound closure.

The extract was successfully incorporated into cream formulations, yielding physically stable products with acceptable texture and spreadability. Among the formulations, F3 demonstrated the most favorable physical characteristics and stability under accelerated conditions.

These findings suggest that *P. speciosa* peel extract has potential as a natural bioactive ingredient for topical applications. In addition, the use of agricultural waste materials supports its relevance for sustainable product development. Further studies are required to evaluate in vivo efficacy, long-term stability, and safety to support future application and development.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

This work is part of a research project, FRGS19-090-0699, supported by the Ministry of Higher Education, Malaysia, and the International Islamic University Malaysia.

References

[1] K. Yusoff, S.F.Z. Japar, H.J. Abdul, J. Kamsiah, Biomed. Pharmacother. 96 (2017) 291–298.
[2] N.H. Mustafa, A. Ugusman, J. Jalil, J. Appl. Pharm. Sci. 8 (2018) 152–158.
[3] J.S. Gui, J. Jalil, Z. Jubri, Cytotechnology 71 (2019) 79–89.
[4] K. Azemi, M.L. Nordin, K.A. Hambali, Antioxidants 11 (2022) 431.
[5] P. Krzyszczyk, R. Schloss, A. Palmer, F. Berthiaume, Front. Physiol. 9 (2018) 419.
[6] A. Joorabloo, T. Liu, J. Nanobiotechnol. 20 (2022) 407.
[7] S.Y. Kim, M.G. Nair, Immunol. Cell Biol. 97 (2019) 450–460.
[8] A. Hassanshahi, M. Moradzad, S. Ghalamkari, Cells 11 (2022) 2953.
[9] L. Sautebin, Fitoterapia 71 (2000) 48–57.
[10] N. Muhamad, P.S. Sinchai, U. Tansom, J. Adv. Dev. Eng. Sci. 14 (2024) 101–110.
[11] C. Chang, M.Yang, H. Wen, J. Chern, J. Food Drug Anal. 10 (2002) 178–182.
[12] P. Griess, Ann. Chem. Pharm. 106 (1858) 123–125.
[13] L. Marcocci, J.J. Maguire, M.T. Droy-Lefaix, L. Packer, Biochem. Biophys. Res. Commun. 201 (1994) 748–755.
[14] G. Cory, in: C. Wells, M. Parsons (Eds.), Cell Migration, Methods Mol. Biol. 769, Humana Press, Totowa, 2011, p. 25–30.
[15] A.O. Barel, M. Paye, H.I. Maibach, Handbook of Cosmetic Science and Technology, 4th ed., CRC Press, Boca Raton, 2014.
[16] M.E. Aulton, K.M.G. Taylor, Aulton's Pharmaceutics: The Design and Manufacture of Medicines, 5th ed., Elsevier, Edinburgh, 2018.
[17] W. Siegert, Cosmetics 6 (2019) 25.
[18] S.M. Jafari, Adv. Colloid Interface Sci. 254 (2018) 1–17.
[19] M.S.M. Saleh, Int. J. Mol. Sci. 22 (2021) 618.
[20] F.H. Builders, Phytomedicine 20 (2021) 521–530.
[21] S. Guo, L.A. DiPietro, J. Dent. Res. 89 (2010) 219–229.
[22] H.A. Wallace, B.M. Basehore, P.M. Zito, Wound Healing Phases, StatPearls Publishing, Treasure Island, 2023.
[23] J. Dai, R.J. Mumper, Molecules 15 (2010) 7313–7352.
[24] J.S. Beckman, W.H. Koppenol, Am. J. Physiol. 271 (1996) C1424–C1437.
[25] S.N. Siti, J. Jalil, A.Y. Asmadi, Y. Kamisah, Front. Pharmacol. 12 (2021) 741623.
[26] J. Mlcek, Molecules 21 (2016) 623.
[27] N.I. Ahmad, J. Food Sci. Nutr. Res. 2 (2019) 151–173.
[28] D.N. Kuma, A. Boye, G. Kwakye-Nuako, Biomed. Res. Int. 2022 (2022) 9709365.
[29] G. Ayman, O.V. Mart, P.C. Francisc, J. Invest. Dermatol. 137 (2017) e11–e16.
[30] A. Boye, V.A. Boampong, N. Takyi, O. Martey, J. Ethnopharmacol. 185 (2016) 155–161.
[31] C. Wang, S.M.S. Shahriar, Y. Su, F. Hayati, S.M. Andrabi, Y. Xiao, M.E. Busquets, N.S. Sharma, J. Xie, ACS Nano 19 (2025) 21411–21425.
[32] N. Zulkefli, Int. J. Mol. Sci. 24 (2023) 2563.
[33] A.H. Adnan, Pharmacogn. Mag. 14 (2018) S586–S593.
[34] I.M. Builder, Afr. J. Pharm. Pharmacol. 13 (2019) 84–89.