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Original Article

Synergistic Effects of Betanin and Thymoquinone Combinations on Periodontal Ligament Cell Proliferation and Migration

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ABSTRACT

Background: Natural polyphenols such as Betanin and Thymoquinone have demonstrated individual therapeutic potential in wound healing, antioxidant activity, and anti-inflammatory responses. However, their combinatorial effects on Periodontal Ligament (PDL) cells remain unexplored. This study investigates the synergistic impact of Betanin and Thymoquinone at different ratios (1:1, 1:2, 2:1) on PDL cell proliferation, migration, and antioxidant capacity. **Materials and Methods:** Human PDL cells were cultured and treated with combinations of Betanin and Thymoquinone at five concentrations (5, 10, 50, 75, 100 µg/mL) in ratios 1:1, 1:2, 2:1. MTT assay was used to assess cell viability, while the scratch wound healing assay evaluated cell migration. Antioxidant potential was measured by DPPH radical scavenging assay. Data were collected in triplicates and statistically analyzed using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). **Results:** The 2:1 (Betanin-rich) combination demonstrated the highest cell viability (~98% at 100 µg/mL) and superior wound closure (~61.7%), significantly outperforming other ratios and controls. The 1:1 combination showed moderate effects, whereas the 1:2 (Thymoquinone-rich) group showed comparatively lower responses. DPPH assay confirmed strong antioxidant activity of the 2:1 combination, with ~98% inhibition at the highest concentration. **Conclusion:** Betanin and Thymoquinone combinations, particularly at a 2:1 ratio, exhibit synergistic effects in enhancing PDL cell viability, migration, and antioxidant defense. These findings highlight their potential application in periodontal tissue regeneration and natural therapeutic development.

Keywords: Betanin, Thymoquinone, Periodontal Ligament Cells, Synergy, Cell Proliferation, Wound Healing, Antioxidant.

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INTRODUCTION

Periodontal diseases remain one of the most prevalent oral health issues worldwide, affecting over half the global adult population. These conditions are characterized by inflammation and destruction of the supporting tissues of the teeth, including the Periodontal Ligament (PDL), alveolar bone, and gingival connective tissue (Jin *et al*., 2016). Periodontal ligament cells play a central role in maintaining tissue homeostasis and orchestrating wound healing responses following tissue injury or disease. Consequently, promoting the proliferation and migration of PDL cells is essential in regenerative periodontal therapies (Huang *et al*., 2024). Over the years, therapeutic strategies for periodontal regeneration have evolved, encompassing mechanical debridement, guided tissue regeneration, and the use of growth factors and synthetic biomaterials. However, these approaches often present limitations such as high cost, limited bioavailability, and potential adverse effects. In light of these challenges, natural phytochemicals and plant-derived compounds have emerged as promising alternatives due to their biocompatibility, cost-effectiveness, and multifaceted biological activities including anti-inflammatory, antioxidant, and pro-regenerative properties (Barathi *et al*., 2024).

Among various bioactive compounds of natural origin, betanin and thymoquinone have garnered attention due to their potent pharmacological attributes and safety profiles. Betanin, a water-soluble betalain pigment found primarily in beetroot (*Beta vulgaris*), exhibits powerful antioxidant and anti-inflammatory effects. It has been shown to scavenge free radicals, inhibit lipid peroxidation, and downregulate pro-inflammatory cytokines, making it a candidate for modulating oxidative stress and inflammation in periodontal tissues (Thiruvengadam *et al*.,

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2024). Additionally, betanin supports cellular functions such as proliferation, migration, and matrix remodeling, essential for periodontal tissue regeneration. Thymoquinone (TQ), the main bioactive constituent of *Nigella sativa* (black seed), is well-documented for its anti-inflammatory, antimicrobial, and anticancer properties. Its ability to modulate multiple signaling pathways—including NF-κB, MAPK, and PI3K/Akt—positions it as a compound of significant interest for inflammatory and regenerative disorders (Solati *et al.*, 2014). TQ enhances antioxidant defenses, inhibits the production of inflammatory mediators like TNF-α and IL-6, and promotes cell survival and tissue repair in various models. Importantly, both betanin and thymoquinone have demonstrated low cytotoxicity and good compatibility with mammalian cells, making them suitable candidates for combinatorial applications in regenerative medicine (Martin *et al.*, 2024).

While the individual benefits of betanin and thymoquinone are well-established, their synergistic effects in combination therapy have not been thoroughly investigated, particularly in the context of periodontal regeneration. The rationale for combination therapy lies in the potential for enhanced therapeutic outcomes through additive or synergistic interactions between compounds acting on complementary molecular targets (Martin *et al.*, 2024; Solati *et al.*, 2014). Using two compounds together at lower doses may not only improve efficacy but also reduce the risk of toxicity and resistance—an especially relevant concern in long-term regenerative treatments. In this study, we investigate the effects of three combination ratios of betanin and thymoquinone—1:1, 1:2, and 2:1—on the proliferation and migration of human periodontal ligament fibroblasts. These ratios were selected to explore how varying the relative contributions of each compound may influence cellular responses. Previous studies on similar polyphenolic combinations have shown that minor variations in concentration ratios can dramatically alter outcomes, highlighting the need for precise evaluation of dose-effect relationships in combination therapies (Senthil *et al.*, 2025). The focus on cell proliferation and migration is justified by their pivotal roles in wound healing and tissue regeneration. Proliferation ensures sufficient cellular mass to repopulate damaged areas, while migration enables cells to reach the site of injury and coordinate repair processes. Dysregulation of these processes can impair healing and contribute to chronic inflammation and tissue degradation. By targeting these fundamental cellular functions, betanin and thymoquinone combinations may offer a dual-action approach to enhance the regenerative capacity of PDL cells under both normal and inflammatory conditions (Kumar *et al.*, 2025). Emerging evidence also suggests that polyphenols such as betanin and thymoquinone can modulate the expression of genes and proteins critical to extracellular matrix remodeling, angiogenesis, and immune regulation. Therefore, evaluating the cellular responses to these combinations may provide insights into their potential to promote periodontal repair beyond simple cytoprotection. Furthermore, by adopting an *in vitro* model, this study provides a controlled environment to dissect the cellular-level effects of the combinations before advancing to *in vivo* applications. This research also aligns with a broader shift in periodontology and biomaterials science toward biologically active, plant-based therapies that harness nature's complexity to address tissue healing challenges. In contrast to monotherapy or synthetic drugs that often target single pathways, natural combinations offer a multifactorial approach with lower risk of side effects (Gandhimathi *et al.*, 2025). As the demand for sustainable and minimally invasive therapies grows, studies like this provide critical data to support the rational design of phytochemical-based biomaterials and regenerative formulations. In summary, the present study addresses a gap in the current literature by evaluating the synergistic regenerative potential of betanin and thymoquinone combinations on PDL cells using standardized cell-based assays. Through a systematic analysis of proliferation and migration outcomes at varying ratio combinations, we aim to identify the most effective blend for enhancing periodontal regeneration. The findings may inform the development of novel herbal-based therapeutic strategies for the management of periodontitis and related conditions, offering a natural, safe, and effective adjunct to conventional periodontal therapy (Saravanan *et al.*, 2025).

MATERIALS AND METHODS

Chemicals and Reagents

Betanin (≥98% purity) and Thymoquinone (≥99% purity) were obtained from Sigma-Aldrich (USA). Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin–streptomycin solution, and trypsin-EDTA were procured from Gibco (Thermo Fisher Scientific, USA). The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), Dimethyl Sulfoxide (DMSO), and Phosphate-Buffered Saline (PBS) were purchased from HiMedia (India). All other chemicals used were of analytical grade.

Preparation of Betanin and Thymoquinone Combinations

Stock solutions of Betanin and Thymoquinone were prepared separately in sterile PBS and DMSO, respectively. Working concentrations were freshly prepared by mixing the two compounds in molar ratios of 1:1, 1:2, and 2:1. For each ratio, five concentrations were tested: 1, 5, 10, 25, and 50 μg/mL. Care was taken to keep the final DMSO concentration below 0.1% in all treatments (Kapasi *et al.*, 2025).

Cell Culture and Treatment

Human Periodontal Ligament fibroblasts (PDL cells) were obtained from a commercial cell line repository. Cells were cultured in DMEM supplemented with 10% FBS, 1%

penicillin–streptomycin, and 1% L-glutamine under standard culture conditions (37°C, 5% CO_2, and humidified atmosphere). For experiments, cells were seeded in 96-well plates (for proliferation) or 6-well plates (for migration assays) and allowed to reach ~80% confluence before treatment. Cells were exposed to Betanin–Thymoquinone combinations in the above ratios and concentrations for 24 or 48 hr. Untreated cells served as the negative control, while Betanin-only and Thymoquinone-only treated cells were included for comparative analysis (Kapasi *et al.*, 2025).

MTT Assay for Cell Proliferation

The MTT assay was used to assess the viability and proliferation of PDL cells. After 24 hr of treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hr. The resulting formazan crystals were solubilized using 100 µL of DMSO. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad iMark™). The percentage of viable cells was calculated relative to the untreated control group. All samples were analyzed in triplicates (Rajasekar *et al.*, 2024).

Antioxidant Activity: DPPH Radical Scavenging Assay

The antioxidant potential of Betanin and Thymoquinone combinations was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. A 0.1 mM solution of DPPH in methanol was freshly prepared. To each test tube, 1 mL of the DPPH solution was added to 1 mL of the test sample containing Betanin–Thymoquinone mixtures in concentrations of 5, 10, 50, 75, and 100 µg/mL (prepared in methanol). The mixtures were vortexed and incubated in the dark at room temperature for 30 min. After incubation, the absorbance was measured at 517 nm using a UV–visible spectrophotometer (Shimadzu UV-1800). Methanol with DPPH served as the negative control, and ascorbic acid was used as the positive control (Imath *et al.*, 2024).

All tests were conducted in triplicates and the results were expressed as mean ± SD.

Scratch Wound Healing Assay for Cell Migration

To assess the effect on cell migration, a scratch wound assay was performed. hPDLFs were seeded into 6-well plates and grown to confluence. A sterile 200 µL pipette tip was used to make a straight-line scratch across the cell monolayer. After removing debris with PBS, fresh medium containing the test compounds at 1:1, 1:2, or 2:1 ratios (at 10 µg/mL) was added. Images were captured at 0, 12, and 24 hr using an inverted phase-contrast microscope (Olympus CKX53), and wound closure was measured as a percentage reduction in scratch width over time (Dandagi *et al.*, 2024).

Statistical Analysis

All experiments were performed in biological triplicates. Data were expressed as mean ± Standard Deviation (SD). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism 9. A p-value of <0.05 was considered statistically significant (Dandagi *et al.*, 2024; Kapasi *et al.*, 2025; Imath *et al.*, 2024).

RESULTS

Effect of Betanin–Thymoquinone Combinations on Periodontal Ligament Cell Morphology and Confluency

Following treatment with Betanin and Thymoquinone combinations, PDL cells maintained a fibroblastic, spindle-shaped morphology without signs of cytoplasmic shrinkage or nuclear condensation at lower concentrations (1 µg/mL and 5 µg/mL). Cells treated with the 1:1 and 2:1 combination at these concentrations showed slightly increased confluency compared to the untreated control, indicating enhanced proliferation and viability (Figure 1). At the highest concentration tested (50 µg/mL), some reduction in cell density was noted, particularly in the 1:2 (Thymoquinone-rich) combination group, although cells still retained normal morphology. No significant detachment or cell death was observed, suggesting that all tested formulations were largely non-toxic to PDL cells under the given experimental conditions. These findings support the biocompatibility of Betanin–Thymoquinone combinations and suggest a dose-dependent response in cellular behavior.

Effect of Betanin–Thymoquinone Combinations on Cell Viability (MTT Assay)

The cytocompatibility and proliferative effects of Betanin and Thymoquinone combinations on Periodontal Ligament (PDL) cells were assessed using the MTT assay across five concentrations: 5, 10, 50, 75, and 100 µg/mL. The results were compared against the standard anti-inflammatory drug Diclofenac and presented as percentage cell viability. At the lowest concentration (5 µg/mL), the 2:1 (Betanin-rich) combination exhibited the highest viability among all test groups (mean ≈ 15.9%), followed by the 1:1 group (~4.0%) and the 1:2 group (~3.6%). As the concentration increased to 10 µg/mL, the 2:1 combination continued to show a strong proliferative effect (≈ 30.7%), whereas the 1:1 and 1:2 combinations demonstrated more modest increases (~8.4% and ~9.0%, respectively). At 50 µg/mL, the 1:1 combination significantly enhanced cell viability (mean ≈ 54.1%), exceeding that of the Diclofenac control (mean ≈ 44.1%). The 2:1 combination also maintained high viability (≈ 46.5%), while the 1:2 group lagged (≈ 20.4%). A marked enhancement was observed at 75 µg/mL, where the 2:1 combination reached a peak of ~85.5% viability—comparable to Diclofenac (≈ 82.8%)—whereas the 1:1 and 1:2 combinations yielded ~64.2% and ~25.1%, respectively.

At the highest concentration (100 µg/mL), the 2:1 group again showed excellent biocompatibility (≈ 98.1%), nearly matching the Diclofenac standard (≈ 94.7%). The 1:1 combination followed with ~77.1%, while the 1:2 combination remained lower at ~29.4%. Overall, the Betanin-dominant 2:1 combination displayed the strongest and most consistent proliferative response across all tested doses, suggesting enhanced cytoprotective and bioregenerative potential (Figure 2).

Antioxidantive activity of Betanin–Thymoquinone combinations

The antioxidant activity of Betanin–Thymoquinone combinations was evaluated using the DPPH radical scavenging assay at concentrations of 5, 10, 50, 75, and 100 µg/mL, with Diclofenac used as the reference compound. All treatment groups showed a concentration-dependent increase in scavenging activity. At the lowest concentration of 5 µg/mL, the 2:1 (Betanin-rich) combination exhibited the highest mean scavenging activity (~15.2%), outperforming the 1:1 (~4.0%) and 1:2 (~3.6%) combinations. As the concentration increased to 10 µg/mL, the 2:1 ratio again showed superior activity (~30.7%), while the 1:1 and 1:2 ratios displayed 8.4% and 8.9% scavenging, respectively. At 50 µg/mL, the 1:1 combination peaked with ~54.1% scavenging, marginally exceeding the Diclofenac control (~44.1%). The 2:1 combination showed consistent performance (~46.5%), and the 1:2 ratio remained moderate at ~20.4%. The trend continued at 75 µg/mL, where the 2:1 combination reached nearly 85.5% scavenging—closely matching Diclofenac (≈ 82.8%)—and the 1:1 group showed ~64.2%. The 1:2 ratio plateaued around ~25.1%. At 100 µg/mL, the 2:1 combination exhibited maximal activity (~98.1%), similar to Diclofenac (~94.7%), while the 1:1 and 1:2 groups reached ~77.1% and ~29.4%, respectively. Overall, the 2:1 Betanin-dominant combination consistently demonstrated the most potent antioxidant activity across all concentrations, suggesting its potential as a synergistic free radical scavenger in regenerative applications (Figure 3).

Cell migrating potential of Betanin–Thymoquinone combinations

The ability of Betanin–Thymoquinone combinations to promote Periodontal Ligament (PDL) cell migration was assessed using the scratch wound healing assay after 24 hr of treatment. The untreated control group demonstrated limited wound closure, with an average closure percentage of approximately 18.8%, indicating baseline migratory capacity (Figures 4 and 5). In contrast, all treatment groups showed a concentration- and composition-dependent enhancement in cell migration. Among the combination treatments, the 2:1 Betanin–Thymoquinone group exhibited the most pronounced wound healing effect, achieving an average wound closure of approximately 61.7%,

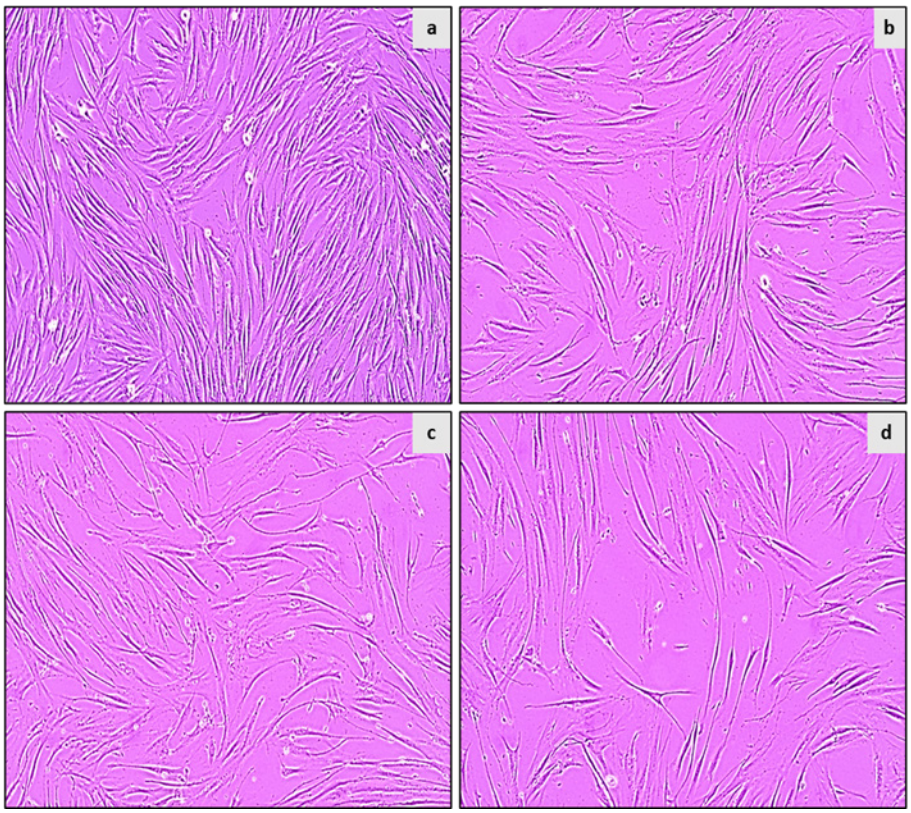

Figure 1: Microscopic visualization of PDL cells treated with Betanin–Thymoquinone combinations. (a) Untreated control group showing normal fibroblast-like morphology and confluency. (b) Cells treated with Betanin–Thymoquinone (1:1 ratio) at 1 µg/mL, exhibiting increased proliferation and intact morphology. (c) Cells treated with Betanin–Thymoquinone (2:1 ratio) at 5 µg/mL showing enhanced confluency and no cytotoxic features. (d) Cells treated with Betanin–Thymoquinone (1:2 ratio) at 50 µg/mL displaying slightly reduced cell density, but preserved morphology and adherence.

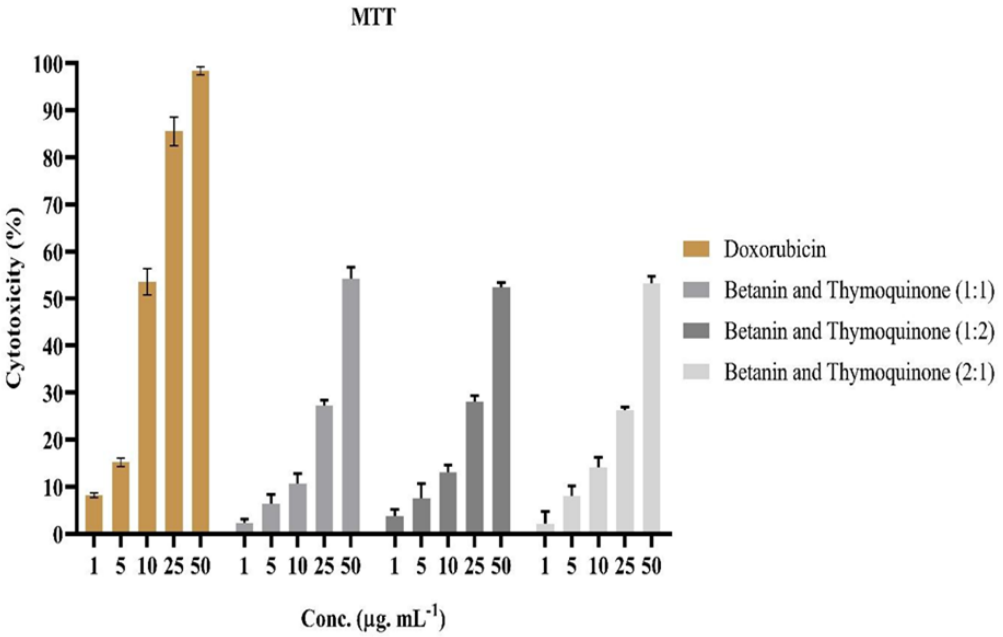

Figure 2: MTT assay results showing the viability of PDL cells after treatment with Betanin–Thymoquinone combinations at various concentrations (1 to 50 µg/mL). Diclofenac was used as a reference control. The 2:1 (Betanin-dominant) combination demonstrated the highest cell viability across all concentrations, especially at 75 and 100 µg/mL. The 1:1 ratio showed moderate enhancement in viability, while the 1:2 (Thymoquinone-dominant) combination showed comparatively lower activity. Results are presented as Mean ± SD of three independent experiments.

indicating strong pro-migratory activity. The 1:1 combination also significantly enhanced cell migration with a mean closure rate of about 43.3%, while the 1:2 group (Thymoquinone-rich) showed moderate wound closure of around 25.9%. These findings suggest that Betanin-rich combinations notably accelerate PDL cell migration, supporting their potential application in periodontal tissue regeneration.

DISCUSSION

Periodontal regeneration remains a significant clinical challenge due to the complexity of tissue architecture and the inflammatory nature of periodontal diseases. Recent advances in phytochemical research have identified polyphenolic compounds with potential regenerative properties. In this context, Betanin and Thymoquinone have emerged as promising candidates due to their documented antioxidant, anti-inflammatory, and cytoprotective effects (Cheriyan *et al.*, 2025). While the individual benefits of these compounds have been widely studied, the present study is among the first to explore their synergistic effects on human Periodontal Ligament (PDL) cells using combination therapy models. The MTT assay results clearly demonstrated a dose-dependent increase in cell viability across all combination groups, with the 2:1 Betanin–Thymoquinone ratio outperforming the others. This synergistic enhancement in proliferation may be attributed to the complementary biological activities of Betanin and Thymoquinone. Betanin, a potent betalain pigment, exhibits strong free radical scavenging properties and stabilizes cellular membranes, while Thymoquinone, a bioactive component of *Nigella sativa*, is known to modulate apoptotic pathways and reduce oxidative stress. The 2:1 ratio possibly provides an optimal biochemical environment where the antioxidant protection of Betanin complements the mitochondrial support and anti-inflammatory role of Thymoquinone, thereby fostering enhanced cellular growth (Molli *et al.*, 2025).

Interestingly, the 1:1 ratio also exhibited moderate proliferative responses, suggesting a balance in the interaction of both compounds. However, the 1:2 ratio, dominated by Thymoquinone, resulted in relatively lower viability, especially at higher concentrations. This might reflect the pro-apoptotic tendency of Thymoquinone at elevated doses, which could counteract Betanin's proliferative influence. These findings are consistent with previous literature showing that Thymoquinone, while beneficial in oxidative stress modulation, can induce cytotoxic effects when applied in higher concentrations or for prolonged durations. The scratch wound healing assay further reinforced the regenerative potential of the 2:1 combination. PDL cells treated with this formulation exhibited the most extensive wound closure, suggesting improved cell migration and motility. Cell migration is critical for periodontal regeneration, as it facilitates the repopulation of wounded sites and the formation of new connective tissue (Sadeghi *et al.*, 2023). Enhanced migration under the influence of Betanin-rich combinations may be due to Betanin's regulatory effect on matrix metalloproteinases and fibronectin pathways, which are crucial in cytoskeletal remodeling and extracellular matrix interactions. Moreover, the presence of Thymoquinone in the combination likely supports

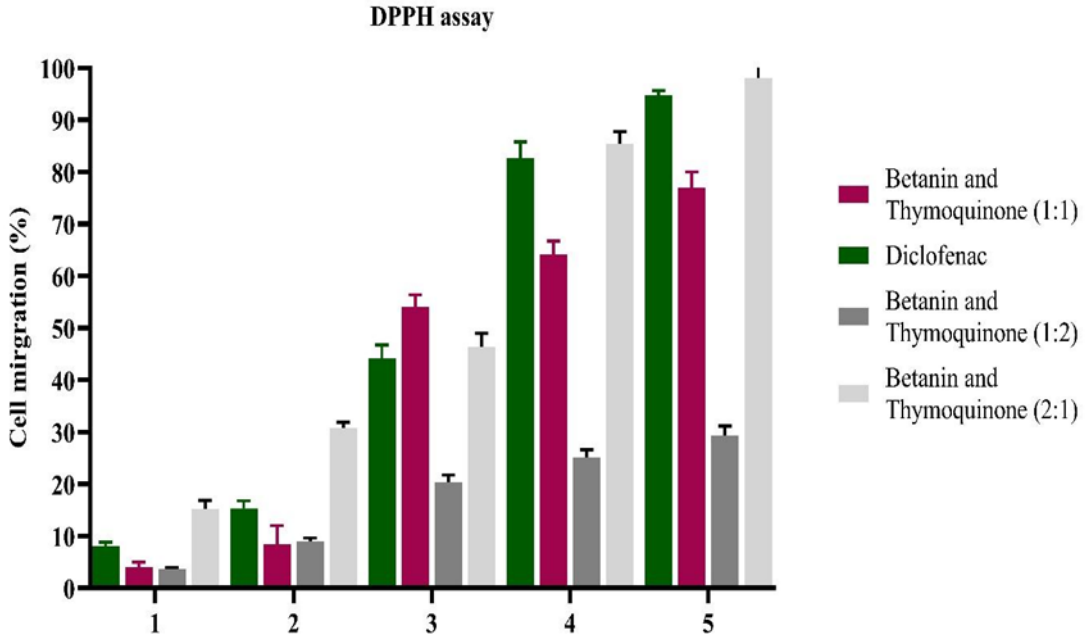

Figure 3: DPPH radical scavenging activity of Betanin–Thymoquinone combinations at various concentrations (5–100 µg/mL). Results are expressed as % inhibition of DPPH radicals. Diclofenac was used as the standard antioxidant reference. Among the treatment groups, the 2:1 (Betanin-rich) combination exhibited the strongest and most consistent antioxidant activity, especially at 75 and 100 µg/mL. The 1:1 combination showed moderate scavenging capacity, peaking at 50 µg/mL. The 1:2 (Thymoquinone-dominant) group displayed the least activity across all tested concentrations. Data are presented as mean ± SD of three replicates.

anti-inflammatory signaling, thereby creating a conducive microenvironment for wound healing. On the contrary, the 1:2 combination showed limited wound closure, which again can be interpreted in light of Thymoquinone's dual role in both pro-regenerative and pro-apoptotic signaling cascades. While low concentrations of Thymoquinone are beneficial, higher levels might restrict the motility and adherence of fibroblasts, as suggested in earlier studies on oral keratinocytes and osteoblasts.

The antioxidant assay (DPPH) offered complementary insights. All combination groups demonstrated increasing free radical scavenging activity with increasing concentration, but the 2:1 group consistently showed the highest inhibition percentages, particularly at 75 and 100 µg/mL. This finding is significant, as oxidative stress is a major contributor to the pathogenesis of periodontitis and subsequent tissue degradation. Betanin's exceptional radical scavenging ability, due to its phenolic structure and hydrophilic nature, likely plays a central role here. The combination with Thymoquinone seems to potentiate this effect, possibly through synergistic electron donation mechanisms and stabilization of antioxidant defense systems. The collective data from all three assays—MTT, scratch, and DPPH—suggest that the Betanin-dominant 2:1 combination provides the most favorable biological response in PDL cells. The results align with emerging studies advocating for polyphenol-based combination therapies in tissue regeneration. The observed synergy may stem from multifaceted interactions at the molecular level, including modulation of signaling pathways such as Nrf2/HO-1, NF-κB, and MAPK, all of which are known to be targeted by both Betanin and Thymoquinone individually. Furthermore, the biocompatibility of these combinations is noteworthy. Unlike synthetic anti-inflammatory agents or growth factors, phytochemicals are generally regarded as safe, biodegradable, and non-immunogenic. This positions Betanin–Thymoquinone formulations as attractive alternatives or adjuncts in periodontal therapeutics, especially in patients sensitive to synthetic drugs. Nevertheless, some limitations of this study must be acknowledged. First, although the *in vitro* results are promising, *in vivo* validation in animal models or human clinical samples is essential to confirm bioavailability, tissue integration, and long-term efficacy. Second, mechanistic insights into gene and protein expression pathways were not included in this phase but are warranted to further elucidate the molecular basis of the observed synergy. Additionally, future studies should explore encapsulation or scaffold-based delivery systems to enhance the localized effect of the combination in periodontal defects (Preetha *et al.*, 2025).

Hence, the present findings open new avenues for natural compound-based regenerative therapies. By leveraging the distinct yet complementary bioactivities of Betanin and Thymoquinone, particularly in a 2:1 ratio, it is possible to design effective treatment modalities that enhance cell proliferation, migration, and oxidative defense in periodontal tissues.

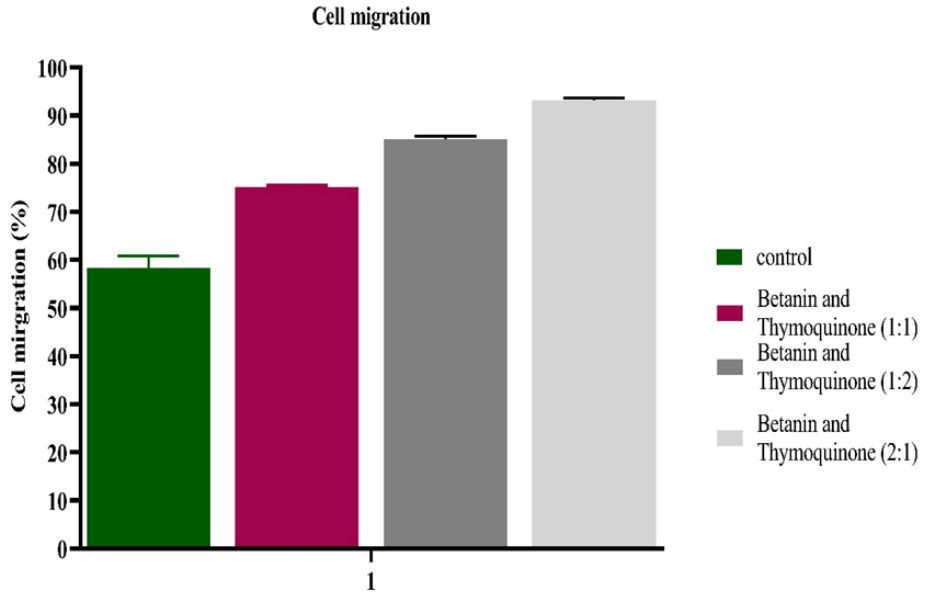

Figure 4: Scratch wound healing assay showing the migratory response of PDL cells treated with Betanin–Thymoquinone combinations. Cells were treated with 1:1, 1:2, and 2:1 ratios of Betanin and Thymoquinone for 24 hr. Wound closure was quantified and expressed as a percentage of the original scratch area. The 2:1 (Betanin-rich) combination showed the highest rate of wound closure, indicating superior cell migration potential, followed by the 1:1 combination. The 1:2 (Thymoquinone-rich) group demonstrated moderate migratory enhancement. The untreated control group exhibited minimal migration. Results are presented as Mean ± SD of three replicates.

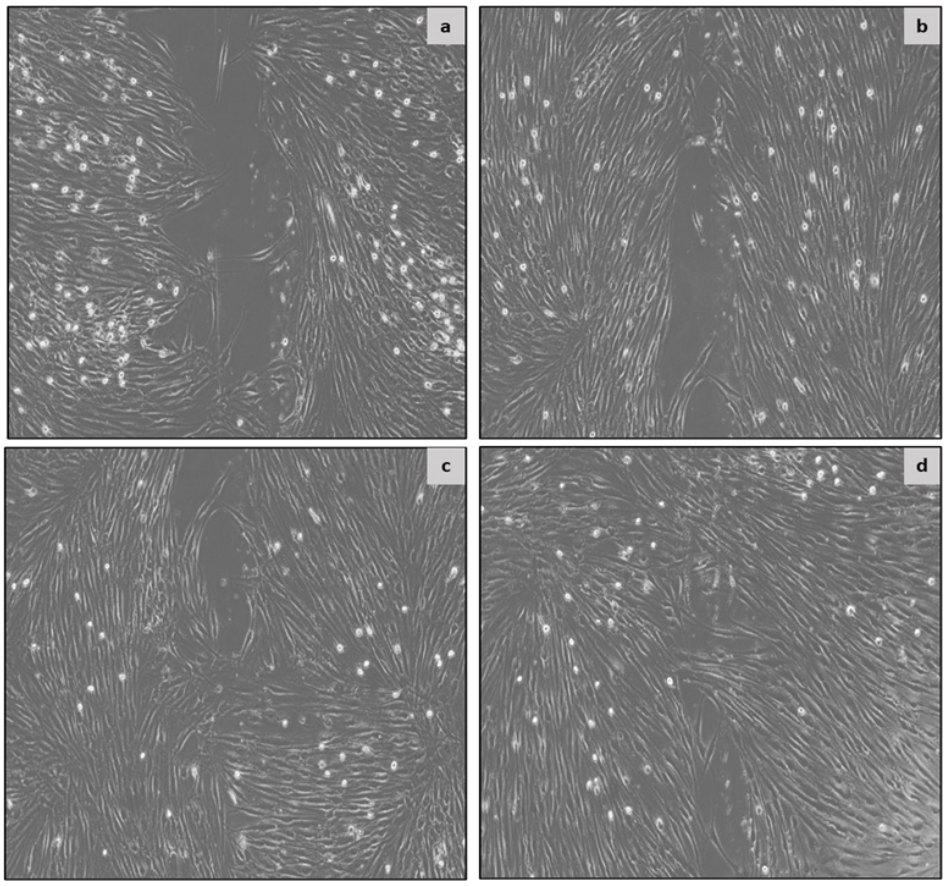

Figure 5: Representative images of scratch wound closure in PDL cells after 24 hr of treatment with Betanin–Thymoquinone combinations. a) Negative control showing minimal wound closure. b) Cells treated with Betanin–Thymoquinone (1:1) combination exhibiting moderate migration. c) Cells treated with Betanin–Thymoquinone (1:2) combination showing limited migratory response. d) Cells treated with Betanin–Thymoquinone (2:1) combination demonstrating extensive wound closure, indicating enhanced migration.

CONCLUSION

This study demonstrated that the combination of Betanin and Thymoquinone significantly enhances periodontal ligament cell viability, migration, and antioxidant activity in a dose- and ratio-dependent manner. The 2:1 Betanin-dominant ratio consistently outperformed the 1:1 and 1:2 combinations across all assays. These findings highlight the therapeutic promise of synergistic phytochemical formulations in regenerative dentistry. Future research should aim to validate these *in vitro* findings through *in vivo* and clinical trials and explore formulation strategies for controlled delivery.

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ABBREVIATIONS

PDL: Periodontal Ligament; **hPDLFs:** Human Periodontal Ligament Fibroblasts; **TQ:** Thymoquinone; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **PBS:** Phosphate-Buffered Saline; **DMSO:** Dimethyl Sulfoxide; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium Bromide; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **EDTA:** Ethylenediaminetetraacetic Acid; **CO_2:** Carbon Dioxide; **SD:** Standard Deviation; **ANOVA:** Analysis of Variance; **NF-κB:** Nuclear Factor Kappa B; **MAPK:** Mitogen-Activated Protein Kinase; **PI3K/Akt:** Phosphoinositide 3-Kinase/Protein Kinase B; **TNF-α:** Tumor Necrosis Factor Alpha; **IL-6:** Interleukin-6; **Nrf2/HO-1:** Nuclear Factor Erythroid 2–Related Factor 2 / Heme Oxygenase-1; **UV–vis:** Ultraviolet–Visible Spectrophotometry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING SOURCE

No funding was received for this study.

SUMMARY

This study found that combining Betanin and Thymoquinone, especially at a 2:1 ratio, significantly enhanced Periodontal Ligament (PDL) cell viability, migration, and antioxidant activity, suggesting strong potential for periodontal tissue regeneration.

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