RESEARCH PAPER

Effect of Vital Essence in Maintaining Periodontal Cell Viability: An In Vitro MTT Assay Study

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease of the tooth-supporting tissues and remains a major public health concern worldwide. Preservation of periodontal cell viability is essential for wound healing, repair, and regeneration of periodontal tissues. Natural essential oils and plant-derived bioactive compounds have gained attention because of their antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties.

Aim: To evaluate the effect of a natural essential oil-based formulation, "Vital Essence" on periodontal cell viability using a tetrazolium-based colorimetric MTT assay.

Materials and Methods: This in vitro experimental study evaluated periodontal cell viability after exposure to a natural oil formulation containing plant-derived oils such as rosehip oil, saffron oil, jojoba oil, almond oil, vitamin E oil, rosemary oil, pomegranate oil, and rose essential oil. Periodontal cells were seeded in 96-well plates and allowed to adhere for 24 hours under standard culture conditions. Cells were then exposed to low concentration (LC; 5 µL) and high concentration (HC; 10 µL) of the formulation. Cell viability/metabolic activity was assessed at 0, 6, and 12 hours using MTT assay. Descriptive statistics were expressed as mean ± standard deviation.

Results: MTT absorbance values increased over time in all groups. At 12 hours, the mean absorbance was highest in the HC group (0.3875), followed by LC (0.35385) and control (0.3049), suggesting enhanced cellular metabolic activity in treated groups. A second percentage-based dataset showed a time-dependent decline from baseline values.

Conclusion: Within the limitations of this in vitro study, Vital Essence demonstrated a potential beneficial effect on periodontal cell viability/metabolic activity, particularly at higher concentration and longer exposure up to 12 hours. The findings suggest that selected essential oils may support periodontal cell survival through antioxidant and cytoprotective mechanisms. However, further studies with clearly defined cell type, larger replicates, dose-response analysis, cytotoxicity profiling, and inflammatory marker assessment are required before clinical translation.

Keywords: Essential oils; periodontal cells; MTT assay; cell viability; oxidative stress; periodontal regeneration; natural compounds.

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INTRODUCTION

Periodontal diseases are chronic inflammatory conditions affecting the gingiva, periodontal ligament, cementum, and alveolar bone. In their advanced form, they result in progressive loss of periodontal attachment, alveolar bone destruction, tooth mobility, and eventual tooth loss. The global burden of periodontal disease is substantial, with severe periodontitis affecting more than one billion people worldwide and being among the most prevalent chronic diseases globally [1,2].

The pathogenesis of periodontitis involves a complex interaction between dysbiotic microbial biofilms and an exaggerated host immune-inflammatory response. Periodontal pathogens initiate tissue inflammation; however, the severity of destruction is largely mediated by host-derived inflammatory cytokines, matrix metalloproteinases, prostaglandins, and reactive oxygen species [3]. Oxidative stress plays an important role in periodontal tissue breakdown by damaging cellular proteins, lipids, nucleic acids, and extracellular matrix components [5,6]. Therefore, preservation of periodontal cell viability and control of oxidative stress are important biological requirements for periodontal wound healing and regeneration.

Periodontal fibroblasts and periodontal ligament cells are key cells involved in periodontal tissue homeostasis. These cells contribute to collagen synthesis, extracellular matrix turnover, wound contraction, tissue repair, and regeneration. Any therapeutic agent proposed for periodontal application should therefore be biocompatible and should not impair fibroblast survival, proliferation, or migration [7,8].

Plant-derived compounds, including essential oils, have attracted increasing interest in dentistry because of their antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties [9–11]. Essential oils contain multiple bioactive constituents such as terpenes, phenols, aldehydes, esters, and flavonoid-related compounds, which may exert antibacterial and cytoprotective actions. Previous studies have shown inhibitory effects of plant-derived essential oils against oral and periodontal pathogens [12–14]. Essential oil-containing mouthrinses have also demonstrated plaque- and gingivitis-reducing effects when used as adjuncts to oral hygiene [15,16].

The formulation evaluated in the present study, “Vital Essence” includes natural oils such as rosehip oil, saffron oil, jojoba oil, almond oil, vitamin E oil, rosemary oil, pomegranate oil, and rose essential oil. These oils are reported to possess biological properties that may support wound healing, reduce inflammation, and protect cells against oxidative injury [17-24]. However, the direct effect of such a combined natural oil formulation on periodontal cell viability has not been extensively studied. Hence, this in vitro study was designed to evaluate the effect of Vital Essence on periodontal cell viability using MTT assay.

Rationale

Although natural essential oils are widely studied for antimicrobial and anti-inflammatory properties, their direct influence on periodontal cell viability and proliferation remains insufficiently explored. Since periodontal regeneration depends not only on microbial control but also on survival and function of resident periodontal cells, it is important to assess whether such formulations are cytocompatible.

The present study attempts to address this gap by evaluating the effect of a novel essential oil-based formulation on periodontal cell viability using a standard MTT colorimetric assay.

Aim

To assess the effectiveness of natural compounds in promoting periodontal cellular viability and proliferation using the tetrazolium-based colorimetric MTT assay.

Objective

To evaluate the effect of natural essential oils on periodontal cell viability using an MTT assay.

MATERIALS AND METHODS

Study design

This was an in vitro experimental study using an MTT colorimetric assay to assess periodontal cell viability after exposure to different concentrations of a natural essential oil-based formulation.

Test formulation

The test formulation, Vital Essence, consisted of selected natural oils with reported antioxidant, anti-inflammatory, antimicrobial, and wound-healing properties. The formulation included:

Rosehip oil (Rosa canina), saffron oil (Crocus sativus), jojoba oil (Simmondsia chinensis), almond oil (Prunus amygdalus dulcis), vitamin E oil/tocopherol, rosemary oil (Rosmarinus officinalis), pomegranate oil (Punica granatum), and rose essential oil (Rosa damascena).

Cell preparation

Periodontal cells were introduced into a 96-well culture plate at an appropriate density. The cells were allowed to

adhere and grow for 24 hours under standard culture conditions before treatment.

Figure 1: Representative image of the prepared Vital Essence essential oil formulation used for the in vitro cell viability study

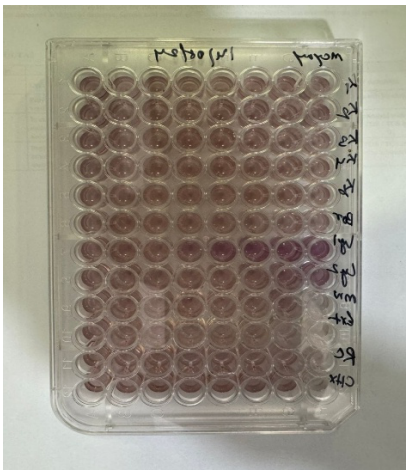


Figure 2: The 96-well plate showing the MTT assay performed to evaluate periodontal cell viability after treatment with Vital Essence formulation at different concentrations and time intervals.

Treatment groups

The cells were divided into the following groups:

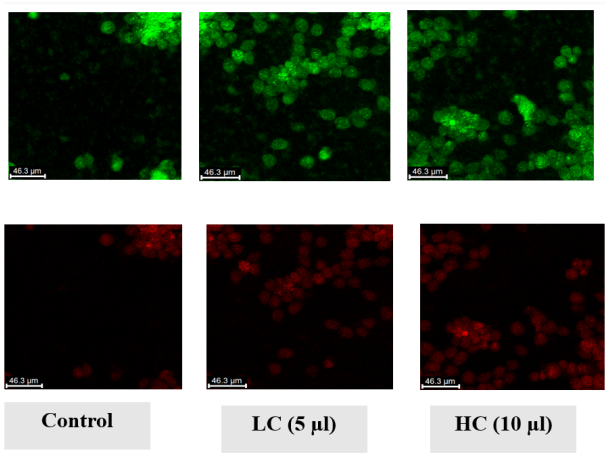
Group	Description
Control	Untreated cells
LC	Low concentration, 5 µL of test formulation
HC	High concentration, 10 µL of test formulation

The essential oils were obtained from certified suppliers and diluted before use. Suggested concentration ranges include 0.1%, 0.5%, 1%, and 5%.

Figure 3: Instrument setup used for measuring absorbance values in the MTT assay.

MTT assay

Cell viability was evaluated using the MTT assay. The MTT assay is based on the ability of metabolically active viable cells to reduce yellow tetrazolium salt into purple formazan crystals through mitochondrial dehydrogenase activity [25,26]. After treatment, MTT reagent was added to each well and incubated. Formazan crystals were then solubilized, and absorbance was measured using a microplate reader.



Statistical analysis:

Data were summarized using descriptive statistics and expressed as mean ± standard deviation. Normality of the data was assessed using the Shapiro–Wilk test. For normally distributed data, intergroup comparisons were performed using one-way ANOVA followed by post hoc analysis. For non-normally distributed data, the Kruskal–Wallis test was used followed by pairwise comparison.

A p-value of <0.05 was considered statistically significant.

RESULTS

MTT absorbance values

The absorbance values increased over time in all groups. At baseline, the HC group showed higher absorbance compared with LC and control. A similar trend was observed at 6 and 12 hours.

TABLE 1:

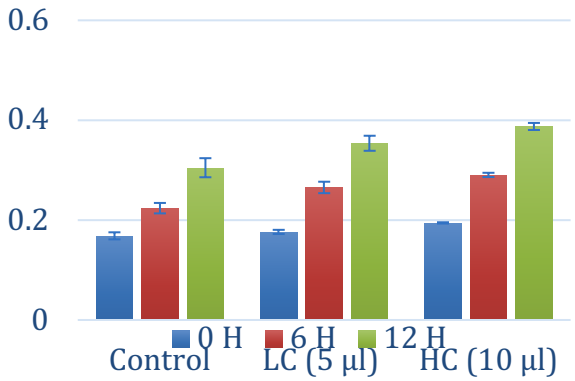
Time point	Control	LC , 5 µL	HC, 10 µL
0 hours	0.1685	0.17635	0.1944
6 hours	0.2239	0.2654	0.2905
12 hours	0.3049	0.35385	0.3875

At 12 hours, the HC group showed the highest absorbance value, followed by LC and control. This suggests that exposure to the formulation may have enhanced cellular metabolic activity in a concentration-dependent manner.

TABLE 2 :

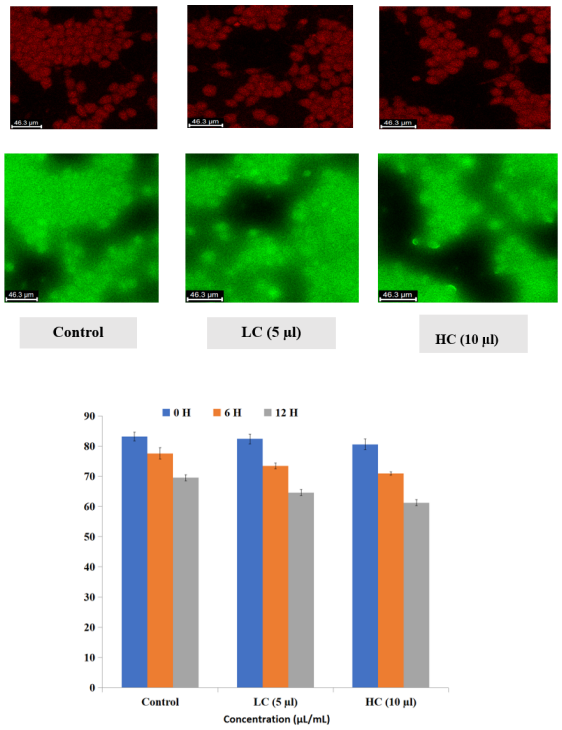
Percentage-based values

Time point	Control	LC, 5 µL	HC, 10 µL
0 hours	83.15	82.365	80.56
6 hours	77.61	73.46	70.95
12 hours	69.51	64.615	61.25



GRAPH 1: Effect of Vital Essence formulation on periodontal cell viability assessed by MTT assay. The optical density values increased from 0 to 12 hours in all groups, indicating increased metabolic activity of viable cells. Both low concentration, 5 µL, and high concentration, 10 µL, showed higher absorbance values than the control group, with the highest value observed in the high-concentration group at 12 hours

The percentage cell viability showed a gradual reduction over time in all groups. Compared to the control, both low concentration and high concentration groups showed lower cell viability, with the high concentration group showing the maximum reduction at 12 hours. This indicates a possible time-dependent and concentration-dependent cytotoxic effect of the formulation.



GRAPH 2: Percentage cell viability of periodontal cells after treatment with Vital Essence formulation at different time intervals. Cell viability decreased gradually from 0 to 12 hours in all groups. The reduction was more evident in the low-concentration and high-concentration groups compared with the control, with the maximum decrease observed in the high-concentration group at 12 hours.

Effect of Vital Essence formulation on periodontal cell viability at different time intervals. Cell viability decreased progressively from 0 to 12 hours in all groups. Compared with the control group, both low concentration, 5 µL, and high concentration, 10 µL, showed reduced cell viability, with the greatest reduction observed in the high-concentration group at 12 hours. This suggests a time-dependent and concentration-dependent reduction in periodontal cell viability.

DISCUSSION

The present in vitro study evaluated the effect of a natural essential oil-based formulation on periodontal cell viability using MTT assay. The findings showed increased absorbance values in treated groups compared with the control group, particularly at 6 and 12 hours. Since MTT absorbance reflects mitochondrial metabolic activity of viable cells, this suggests that the formulation may support periodontal cell viability or proliferation under the tested conditions [25,26].

Periodontal wound healing requires survival and functional activity of gingival fibroblasts and periodontal ligament cells. These cells are responsible for collagen synthesis, extracellular matrix remodeling, and repair of periodontal tissues. Therefore, therapeutic formulations intended for periodontal use should not be cytotoxic to host cells. Tsourounakis et al. reported that diluted essential oil mouthrinses showed no detectable detrimental effect on human gingival and periodontal ligament fibroblasts, whereas chlorhexidine at certain concentrations reduced fibroblast migration and survival [7]. This supports the possibility that properly diluted essential oil-based preparations may be biologically compatible with periodontal cells.

The beneficial effect observed in the present study may be attributed to the antioxidant-rich nature of the oils included in the formulation. Oxidative stress is a key mechanism in periodontal tissue destruction, and antioxidants can counteract reactive oxygen species-mediated cellular injury [5,6]. Vitamin E, a lipid-soluble antioxidant, protects cell membranes from lipid peroxidation and may contribute to cellular protection [22]. Similarly, rosehip oil has been reported to support wound healing and tissue repair, possibly through modulation of inflammation and collagen remodeling [17,18].

Pomegranate-derived compounds have been investigated in periodontal therapy because of their antimicrobial, anti-inflammatory, and antioxidant potential. Pomegranate preparations have shown beneficial effects as adjuncts in plaque control, gingivitis, and periodontitis-related clinical parameters [19–21]. The presence of pomegranate oil in the present formulation may therefore contribute to the observed cytoprotective effect.

Jojoba oil has demonstrated wound-healing and anti-inflammatory properties in experimental studies. Ranzato et al. reported that jojoba liquid wax accelerated wound closure in keratinocytes and fibroblasts in vitro [23]. Rosemary oil and its bioactive constituents have also shown antioxidant, antimicrobial, and anti-inflammatory effects, which may be useful in reducing oxidative damage and supporting tissue repair [24].

Saffron and its active constituents, including crocin, crocetin, and safranal, have been reported to possess antioxidant and anti-inflammatory properties [27,28]. Almond oil is also known for emollient, anti-inflammatory, and wound-healing applications [29]. Rose essential oil has been reported to possess antimicrobial and anti-inflammatory effects, although its direct effect on periodontal cells requires further investigation.

However, the concentration and exposure time of essential oils are critical. Essential oils are concentrated bioactive substances and may become cytotoxic at higher concentrations or prolonged exposure. Prashar et al. demonstrated cytotoxicity of lavender oil and its major components in human skin cells [30]. Similarly, in vitro studies have reported that certain essential oil components may affect fibroblast viability depending on concentration and exposure time [31]. Therefore, although the present findings are promising, dose optimization and safety profiling are essential before clinical use.

Overall, the present study suggests that Vital Essence may have a favorable effect on periodontal cell metabolic activity under short-term in vitro conditions. The findings support further investigation of natural oil-based formulations as potential adjuncts for periodontal wound healing and regeneration.

Limitations

This study has several limitations. First, it was an in vitro study and cannot fully reproduce the complex oral environment, only short-term exposure up to 12 hours was evaluated; chronic exposure was not assessed, the formulation contains multiple oils, so the individual contribution of each oil cannot be determined, only MTT assay was used; additional assays such as live/dead staining, LDH release assay, scratch wound assay, ROS assay, and inflammatory cytokine analysis would strengthen the findings. Finally, possible allergic reactions, irritation, and clinical safety concerns were not evaluated.

CONCLUSION

Within the limitations of this in vitro study, Vital Essence, a natural essential oil-based formulation, demonstrated a potential positive effect on periodontal cell viability/metabolic activity as assessed by MTT assay. The increase in absorbance values in LC and HC groups suggests that selected essential oils may support periodontal cell survival and proliferation, possibly through antioxidant and anti-inflammatory mechanisms. However, careful standardization of dosage, exposure time, and formulation composition is necessary to avoid

cytotoxic effects. Further laboratory, animal, and clinical studies are required before recommending clinical use in periodontal therapy.

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