ORIGINAL ARTICLE
DOI: 10.5152/EssentDent.2025.25033

ISTANBUL UNIVERSITY CERRAHPASA

The Effect of Aqueous Okra (*Abelmoschus esculentus L.*) Extract on Human Periodontal Ligament Fibroblast Cells Viability: A Pilot Study

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Cite this article as: Tosun UU, Aydın ST, Baykara D, et al. The effect of aqueous okra (*Abelmoschus esculentus L.*) extract on human periodontal ligament fibroblast cells viability: A pilot study. *Essent Dent.* 2025, 4, 0033, doi: 10.5152/EssentDent.2025.25033.

Abstract

Background: The aim of this pilot research was to assess the cell viability activity of different concentrations of aqueous okra (*Abelmoschus esculentus* L.) extracts on human periodontal ligament fibroblast (hPDLF) cells.

Methods: Aqueous okra extract was prepared for the experiments, and the content of the extract was analyzed by high-performance liquid chromatography (HPLC). For the purpose of treating cells, the aqueous okra extract was prepared in 7 different concentrations (0.125, 0.187, 0.25, 0.375, 0.5, 0.75, and 1 mg/mL). To assess cell viability, cells were seeded in 96-well plates on 4 occasions for each concentration. The cells were treated with aqueous okra extract for a period of 24 hours. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay protocol was employed for the purpose of evaluating cell viability. The data were subjected to statistical analysis using the GraphPad Prism statistical software. A *P*-value of less than .05 was considered statistically significant.

Results: Analysis using HPLC showed the existence of rutin, 4-hydroxybenzoic acid, and syringic acid in the extract. The MTT test revealed no significant difference in the viability of hPDLF cells between the control and aqueous okra extract-treated groups ($P > .05$).

Conclusion: The outcomes of this research demonstrate that aqueous okra extract has a similar effect on the viability of hPDLF cells at different concentrations. This suggests the need for further studies in which okra is prepared at varying concentrations, dissolved in different solvents, and followed over longer time intervals.

Keywords: Cell biology, cell viability, fibroblasts, okra, periodontal ligament

What is already known on this topic?

- *Periodontal ligament fibroblasts (PDLFs) play a critical role in periodontal wound healing and regeneration due to their proliferative and remodeling capacity.*
- *The utilisation of herbal-based therapeutic agents in periodontal treatment is gaining popularity due to their biocompatibility and anti-inflammatory and antimicrobial properties.*
- *Okra (Abelmoschus esculentus L.) is a medicinal plant that is rich in phytochemicals, such as flavonoids and phenolic acids, which have demonstrated therapeutic potential in a variety of biological systems.*

What this study adds on this topic?

- *This is the first study to assess the impact of aqueous okra extract on the viability of human periodontal ligament fibroblast (hPDLF) cells using the MTT assay.*
- *The findings reveal that aqueous okra extract, across a range of concentrations, does*

INTRODUCTION

Periodontal disease is an infectious-inflammatory condition wherein the host's response to commensal organisms leads to the breakdown of tooth-supporting tissues, including the periodontal ligament and bone. The process of periodontal regeneration involves the complete restoration of the morphology and function of the missing attachment area between tissues.[1]

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Received: March 4, 2025
Revision Requested: March 27, 2025
Last Revision Received: March 30, 2025
Accepted: April 25, 2025
Publication Date: July 18, 2025

not significantly alter hPDLF cell viability, indicating a non-cytotoxic profile.
- *The study identifies rutin, 4-hydroxybenzoic acid, and syringic acid in the extract, suggesting these compounds may contribute to the bioactivity and therapeutic potential of okra in periodontal applications.*

The periodontal ligament is an essential tissue that gives the periodontium important support. Situated between the tooth root and the alveolar bone, it is mostly made up of periodontal ligament stem cells (PDLSCs), osteoblasts, cementoblasts, fibroblasts, osteoclasts, and Malassez epithelial cell remains. These cells' dynamic behavior is intimately related to homeostasis and alterations in the periodontal tissue.[2] In this regard, periodontal ligament fibroblasts (PDLFs) play a key role in wound healing and periodontal tissue regeneration.[1]

The use of herbal remedies and products for treating gingivitis and periodontitis has lately surged in popularity among dental patients and experts. Herbal medications encompass herbs, herbal substances, preparations, and products that incorporate plant parts or other botanical elements as active ingredients.[3] Phytochemicals, encompassing alkaloids, terpenes, flavonoids, lignans, phenolics, and saponins, are bioactive compounds obtained from medicinal plants that provide health benefits to humans.[4] These phytochemical constituents help inhibit inflammatory periodontal diseases by exhibiting antimicrobial, antibacterial, and wound-healing properties.[3]

Belonging to the Malvaceae family, okra (*Abelmoschus esculentus* L.) is a widely cultivated plant across tropical and subtropical regions around the globe. This vegetable crop is significant in nations like Pakistan, Southeast Asia, the USA, India, Brazil, West Africa, Australia, and Türkiye. Okra, a multifaceted commodity, possesses considerable importance due to its therapeutic and nutritional advantages for human health. Its fruits contain several beneficial substances, including carotene, polyphenols, folic acid, riboflavin, niacin, vitamin C, and thiamine.[5] These components are responsible for several significant biological processes, such as antimicrobial, antioxidant, anti-inflammatory, and wound healing properties.[6]

Given the information outlined above, this research focused on exploring the proliferative effect of aqueous okra extract on human periodontal ligament fibroblast (hPDLF) cells to investigate the healing effect of aqueous okra extract on periodontal tissues.

MATERIALS AND METHODS

Since this research was conducted on cell cultures, ethics committee approval was not required. Additionally, as the study did not include human participants, obtaining informed consent was not necessary.

Preparation of the Aqueous Okra Extract

The okra fruits used in this study were purchased at a market in Istanbul, located in Türkiye, and stored at −20 °C until the extraction process was performed. To obtain the aqueous extract, 200 g of fresh okra were homogenised in 1 L of distilled water at 3000 rpm using a homogeniser (Daihan Scientific HG-15A, a product based in Wonju, South Korea). The sample was stirred for 30 minutes at 600 rpm on a shaker set to 80 °C and subsequently filtered using coarse filtration via muslin cloth. The sample was fully lyophilised and kept at −20 °C until needed for use.[7]

High-Performance Liquid Chromatography with Diode Array Detection (HPLC-DAD) Analysis of Phenolic Compounds

The aqueous extract from the okra plant was subjected to phenolic compound analysis through an HPLC-DAD system (Agilent Technologies 1260 Infinity, Santa Clara, CA, USA). The separation of compounds in the plant was conducted using a Waters Nova-Pak C18 column (4 µm; 3.9 × 150 mm). The system's operational settings involved mobile phase (A) as a solution of 0.05% formic acid and water, while mobile phase (B) was prepared by combining 0.05% formic acid with acetonitrile. The gradient program implemented in the system was as follows: 0 min: 5% B; 1 min: 5% B; 20 min: 30% B; 25 min: 60% B; 28 min: 60% B; 33 min: 95% B; 35 min: 95% B; 40 min: 5% B. The system was operated with a flow rate of 0.5 mL/min, and 20 µL of the sample was injected. Methanol was used to dissolve the extracts, which were subsequently analysed via HPLC with the aid of a 0.45 µm microfilter containing an injector tip.[8]

Cell Culture

In this study, hPDLF cells were obtained commercially from ABMGOOD (Cat. No. T4075, Abmgood, Vancouver, Canada). Cell maintenance conditions for hPDLF cells used in the experiments: Dulbecco's Modified Eagles Medium (DMEM) containing high D-Glucose (Cat. No. 41966-029, GibcoTM, Thermo Fisher Scientific, Waltham, MA, USA), Pyruvate, 1% L-Glutamine, 1% Penicillin-Streptomycin, and 10% fetal bovine serum (Cat. No. SV30180.03, HyCloneTM, Thermo Fisher Scientific, Waltham, MA, USA). The cell viability activity experiments were conducted when the cells had reached approximately 80% confluence.

Assessment of Cell Viability

To assess the survival rate of hPDLF cells, the CyQUANT MTT Cell Viability Assay (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) was used to test the aqueous okra extract. For cell attachment, hPDLF cells were seeded at 1×10^4 cells per well in 96-well plates and incubated for 24 hours. At the end of the 24-hour incubation, the culture liquid in each well was discarded, and fresh medium supplemented with different concentrations of the extract (0.125, 0.187, 0.25, 0.375, 0.5, 0.75, and 1 mg/mL) was applied, with cells incubated for another 24 hours. Dimethyl sulfoxide (DMSO) was used to treat the cells as a control. Upon completion of the 24-hour incubation, each well was supplemented with 5 mg/mL MTT solution, and the incubation continued for an extra 3 hours. After the termination of the specified incubation period, the culture medium was discarded, and each well was treated with 100 µL of DMSO. To break down the formazan crystals synthesised via the MTT reaction, the plate was kept at 37 °C in a CO_2 incubator for 10 minutes. The optical density (OD) at 590 nm was quantified using a plate reader (Perkin Elmer, Waltham, MA, USA). % viability values were calculated using the following formula from the absorbance values observed in the reader.

$$[(OD\ sample - OD\ blank)/(OD\ control - OD\ blank)] \times 100$$

Statistical Analysis

The statistical analysis of the data was executed using GraphPad Prism v8.0.1 software. The Kruskal-Wallis test was employed for comparisons involving many groups. Comparisons between pairs were analysed using Dunn's multiple comparisons test, with statistical significance defined as $P < .05$.

RESULTS

HPLC-DAD Analysis

The HPLC-DAD analysis demonstrated the presence of rutin, 4-OH benzoic acid, and syringic acid in the aqueous okra extract (Figure 1).

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

To explore how aqueous okra extract influences cell viability, hPDLF cells were subjected to different extract concentrations

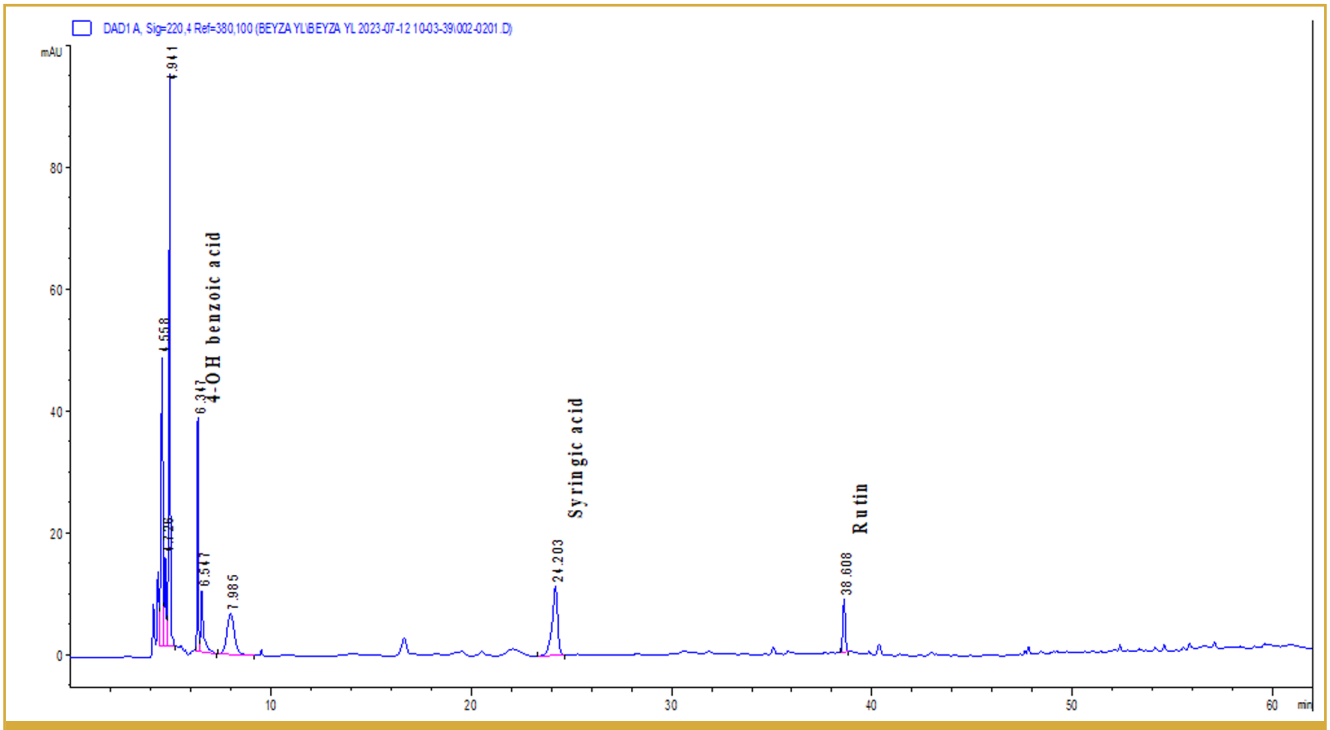

Figure 1. Okra extract high-performance liquid chromatography chromatogram.

Table 1. Viability Values (%) in Human Periodontal Ligament Fibroblast Cells

	Mean ± SD	Minimum-Maximum	Median
Control[a] (DMSO)	100.00 ± 0.00	100.00-100.00	100.00
0.125 mg/mL[b]	92.92 ± 7.47	83.17-98.76	94.87
0.187 mg/mL[c]	108.88 ± 14.49	89.96-120.23	112.66
0.25 mg/mL[d]	119.29 ± 6.14	111.27-124.09	120.89
0.375 mg/mL[e]	120.89 ± 2.51	117.61-122.86	121.54
0.5 mg/mL[f]	116.58 ± 6.43	108.19-121.62	118.26
0.75 mg/mL[g]	151.85 ± 6.95	142.78-157.30	153.67
1 mg/mL[h]	93.21 ± 0.00	93.21-93.21	93.21
P value*	< **.001**		

Mean ± SD values for 4 replicates are shown here.
*Kruskal Wallis test, $P < .05$.

Table 2. Pairwise Comparisons

P#a-b	*P*#a-c	*P*#a-d	*P*#a-e	*P*#a-f	*P*#a-g	*P*#a-h
> .999	> .999	> .999	> .999	> .999	= .114	> .999
P#b-c	*P*#b-d	*P*#b-e	*P*#b-f	*P*#b-g	*P*#b-h	*P*#c-d
> .999	= .255	= .255	= .795	= **.004**	> .999	> .999
P#c-e	*P*#c-f	*P*#c-g	*P*#c-h	*P*#d-e	*P*#d-f	*P*#d-g
> .999	> .999	= .436	> .999	> .999	> .999	> .999
P#d-h	*P*#e-f	*P*#e-g	*P*#e-h	*P*#f-g	*P*#f-h	*P*#g-h
= .285	> .999	> .999	= .285	> .999	= .875	= **.004**

#Dunn's multiple comparisons test, $P < .05$.

(0.125, 0.187, 0.25, 0.375, 0.5, 0.75, and 1 mg/mL). After 24 hours of treatment, the groups treated with aqueous okra extract and the control group did not vary statistically significantly, according to the viability data in hPDLF cells ($P > .05$) (Tables 1 and 2).

DISCUSSION

Periodontal diseases are chronic inflammatory polymicrobial conditions associated with biofilm that damage the supporting tissues of the teeth, leading to tooth loss and associated bone lesions.[9] Numerous treatment modalities exist for periodontal therapy, encompassing both surgical and non-surgical methods, as well as solid dosage forms, hydrogels, dental devices, films, and an array of additional technologies and treatment protocols.[10] Periodontal ligament fibroblasts, the predominant cell of the periodontal ligament, are essential for periodontal healing owing to their rapid turnover, significant remodeling capacity, and potential to differentiate into osteoblasts and cementoblasts, thereby promoting tissue regeneration and repair in response to mechanical stresses and injuries.[11] Phytotherapy, defined as treatment utilizing herbal medicines, is frequently favored over traditional chemical medications due to its extensive biological benefits, enhanced safety profiles, and reduced costs.[12] As a key component in periodontal wound healing, the periodontal ligament consists of various cell populations and is marked by its accelerated turnover rate and remarkable regenerative and remodeling abilities.[13] So, this investigation's goal was to look into the viability impact of various doses of aqueous okra extracts on human periodontal ligament fibroblast cells.

The cellular viability of the aqueous okra extract was analyzed in this study via the MTT assay method. The MTT assay, a colorimetric detection pioneered by Mosmann in 1983, is predominantly utilized to assess cell growth and viability.[14,15] This approach is grounded in the principle that MTT, an indicator of mitochondrial function, is transformed into formazan crystals by viable cells.[16] The MTT test offers several advantages over alternative viability assessment methods, including ease of use, cost-effectiveness, lack of necessity for specialized equipment, brevity of testing duration, and reproducibility of results.[16,17] The utilization of a 96-well plate in the experiment facilitates the employment of minimal quantities of cells and active substances while simultaneously enabling the examination of various concentrations of the same active substance or other active substances.[17]

In cell culture studies, cellular responses are assessed based on study protocols with varying time intervals. The research assessing the viability of PDLF cells utilized durations ranging from 1 to 72 hours and 4 to 7 days, followed by assessments.[18-22] Adeli et al[18] evaluated the effects of green tea extract on the viability of PDLF cells at time points of 1, 2, 4, and 24 hours using the MTT assay. Their results demonstrated that the extract provided a significantly greater protective effect at the 2, 4, and 24-hour marks in comparison to the control group.[18] Bijlani and Shanbhog explored the influence of ice apple water and ice apple fruit pulp extract on the viability of hPDLF cells at multiple time intervals (1, 3, and 24 hours) through the MTT assay. Their findings indicated that a 10% concentration of ice apple fruit pulp extract exhibited the highest capacity to sustain hPDLF cell viability across all evaluated time points.[19] Coaguila-Llerena et al[20] assessed the cytotoxicity of 3 root-end filling materials on hPDLF cells at 1, 3, and 7 days using the MTT assay. Their findings revealed that while significant differences were observed at high dilutions, no noticeable variation was detected at lower dilutions.[20] de Souza et al[21] investigated the impact of milk renewal on hPDLF cell viability at 24, 48, 72, 96, and 120-hour intervals using the MTT assay. Their results demonstrated that, irrespective of temperature, replacing milk with fresh milk had no discernible effect on the ability of hPDLF cells to sustain viability.[21] Kavuncu et al[22] examined the cytotoxic effects of various nanocomposites on hPDLF cells at 24-hour and 7-day intervals using the MTT assay. Their findings indicated that the cytotoxic impact of composite resins on hPDLF cells varied depending on the type of nanocomposite and the duration of exposure.[22] Based on existing studies, a 24-hour treatment period was chosen for cell viability assessment in the research. This duration is commonly employed in similar research and has demonstrated consistency and reproducibility in yielding dependable results. It also ensures adequate interaction between the cells and the compound being tested.

This research identified rutin, 4-OH benzoic acid, and syringic acid in the aqueous okra extract through HPLC-DAD analysis. High-performance liquid chromatography and Nuclear Magnetic Resonance (NMR) spectroscopy revealed that okra extract contains rutin, while NMR spectroscopy identified 4-hydroxybenzoic acid.[23,24] No research demonstrates that okra extract contains syringic acid. Rutin exhibits antioxidant, anti-inflammatory, antibacterial, cytoprotective, and antiviral activities.[25,26] 4-hydroxybenzoic acid is recognized for its potent antimicrobial, anti-inflammatory, and antioxidant characteristics.[27] Syringic acid exhibits several effects, including antioxidant, anti-inflammatory, and antimicrobial properties.[28] Given these findings, it can be inferred that the bioactivity exhibited by the plant is primarily due to these substances.

In vitro studies provide insights into fundamental mechanisms of periodontal healing, including cell proliferation, attachment, and differentiation.[29] In this investigation, the MTT assay indicated no significant difference in cell viability between the control and treatment groups. According to the current literature, there are no reports examining the impact of aqueous okra extract on hPDLF cell viability. Patwardhan and Bhatt carried out ethyl acetate okra extract (30 µg/mL) on human dermal fibroblast cells for 24 hours and saw no variation compared to the control group.[30] Guebebia et al[31] administered different doses (0.01, 0.1, 0.5, and 1 mg/mL) of ethanolic extracts of okra fruit to kidney leukocytes of European sea bass for 24 hours, observing no significant differences between the control and extract-treated groups.[31] Sipahi et al[7] explored the effects of aqueous okra extracts from 2 varieties on human dermal fibroblast cell viability at concentrations of 0.5, 1, 2, and 5 mg/mL over 24 hours, demonstrating that the 5 mg/mL extract decreased cell viability.[7] The discrepancies in the studies may arise from variations in okra species, extract concentration, cell type, collection location, and temporal factors. More investigation is necessary to assess the impact of aqueous okra extract on the viability of hPDLF cells.

This study has several key strengths that enhance its significance. Notably, it is the first to explore the effect of aqueous okra extract on the viability of hPDLF cells, offering new insights into an unexplored area and providing a foundation for future research. The study's credibility is further reinforced by using the reliable and widely recognized MTT assay, known for its ease of use and reproducibility when evaluating cell viability. Additionally, the chemical analysis through HPLC-DAD has revealed valuable bioactive compounds in the aqueous okra extract, including rutin, 4-hydroxybenzoic acid, and syringic acid, known for their antioxidant, anti-inflammatory, and antimicrobial properties, potentially contributing to periodontal healing. The 24-hour treatment period, consistent with similar studies, ensures reliable and comparable results.

Despite the strengths, there are several limitations to this study. Firstly, the research was conducted in vitro, which may not entirely replicate the intricate in vivo conditions of periodontal tissues. Therefore, further in vivo and clinical studies are necessary to validate these findings. Additionally, the MTT assay was solely used to assess cell viability, which primarily evaluates mitochondrial function. To get a fuller picture of how cells react, using extra methods like live/dead staining or flow cytometry might be helpful. The MTT assay only lasts for a short time, which means it cannot really show long-lasting effects on cells, like morphological changes or apoptosis. Furthermore, variations in the extraction methodology and plant origin could influence the bioactive composition and resulting cellular responses, underscoring the need for standard protocols in future investigations.

This research aimed to analyze the impact of aqueous okra extract on the viability of hPDLF cells. To this end, the MTT test was employed to evaluate cell proliferation. Notwithstanding the presence of bioactive components in aqueous okra extract, including rutin, 4-hydroxybenzoic acid, and syringic acid, recognized for their antioxidant and anti-inflammatory characteristics, the findings showed no notable difference in cell viability between the control and the groups treated with aqueous okra. This finding supports previous studies that similarly revealed no observable effects of okra extracts on several cell types. The lack of significant results may be attributed to differences in extract concentration, species of okra, and experimental conditions. As one of the first studies to investigate the impact of aqueous okra extract on hPDLF cells, this research paves the way for further exploration in this area. Ongoing investigations are needed to assess the possible effects of aqueous okra extract on hPDLF cells and its contribution to the process of periodontal healing.

Data Availability Statement: All data generated or analyzed during this study are included in this published article.

Ethics Committee Approval: N/A.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception – Ö.B.A.; Design –T.T., C.E.D., O.G. Ö.B.A.; Supervision – Ö.B.A.; Materials – U.U.T., S.T.A., A.T., D.B., T.T., C.E.D., O.G.; Data Collection and/or Processing – U.U.T., S.T.A., T.T., Ö.B.A.; Analysis and/or Interpretation – U.U.T., T.T., Ö.B.A.; Literature Search – U.U.T., Ö.B.A.; Writing Manuscript – U.U.T.; Critical Review – T.T., C.E.D., O.G., Ö.B.A.

Acknowledgements: The results of this study were presented as an oral presentation at the Turkish Dental Association 27th International Dental Congress (October 26-29, 2023).

Declaration of Interests: The authors declare that they have no competing interests.

Funding: The authors declared that this study has received no financial support.

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