

IN VITRO EVALUATION OF THE CYTOTOXICITY OF THE PLANT EXTRACT OBTAINED FROM *EPILOBIUM PARVIFLORUM* SCHREB

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ABSTRACT

Aim of the study: This study investigates the antibacterial efficacy and cytotoxicity of a hydroalcoholic extract derived from *Epilobium parviflorum* Schreb for potential use in endodontic therapy. **Materials and methods:** The extract was prepared through dehydration, yielding a concentration of 10 mg/mL, which was subsequently assessed for its bioactive compounds, revealing a significant increase in polyphenol concentration. Cytotoxicity was evaluated using the MTT assay on L929 fibroblast cell cultures, where concentrations below 0.2 mg/mL demonstrated no cytotoxic effects. In contrast, higher concentrations resulted in decreased cell viability, highlighting the extract's selective toxicity profile. **Results** The absence of cytotoxicity at lower concentrations supports its potential application as an alternative to traditional endodontic irrigants, which often exhibit significant cytotoxic effects. Recent studies underscore the importance of biocompatibility in dental treatments, reinforcing the notion that plant-derived solutions can effectively balance antimicrobial efficacy and cellular safety. This research aligns with growing evidence advocating for the integration of herbal extracts in dental practice, promoting safer treatment alternatives that enhance patient outcomes without compromising tissue integrity. **Conclusions** Overall, this study contributes to the understanding of herbal applications in dentistry, emphasizing the need for further exploration of natural compounds in therapeutic settings.

Key words: *Epilobium parviflorum* Schreb; endodontic therapy; cytotoxicity; natural extracts

INTRODUCTION

The assessment of the cytotoxicity of chemical substances is crucial for understanding their effects on cells and tissues in contexts such as drug testing, pesticides, cosmetics, and other industrial substances. According to research, cytotoxicity assays are essential to predict the toxicological profile of chemicals and to identify their harmful effects on human health". In vitro tests are particularly valuable because they offer a reliable, scalable approach to evaluate the risks posed by these compounds. For pesticides, in vitro evaluations using different cellular models demonstrated that toxicity is influenced by properties such as water solubility and the

octanol-water partition coefficient (log P). These tests allow for examining effects on gastrointestinal and skin cells, employing cell lines like Caco-2 and HepG2 to simulate human exposure. Studies have found that glyphosate exhibits relatively low toxicity, while others like imazalil are more harmful, significantly impacting cell viability depending on concentration and exposure time [1].

In vitro cytotoxicity assays are also employed to explore the effects of plant-derived antioxidant compounds, like polyphenols. For instance, MTT assays can present challenges due to interference from

some plant compounds, while alternative methods such as CellTiter-Glo "provide more accurate and reliable results in these cases" [2].

Moreover, these methods have become vital tools in occupational risk assessments and in evaluating the impact of exposure to multiple pollutants in contaminated environments, supporting the characterization of long-term exposure risks [3].

The success of endodontic treatments is closely tied to the effective eradication of bacteria within the root canal system and the prevention of reinfection. The process of chemo-mechanical preparation, combined with three-dimensional obturation using appropriate sealers, is critical for long-term endodontic success. Persistent or secondary infections often lead to treatment failure, whether in inadequately treated or correctly managed canals [4].

A key factor in ensuring treatment success is irrigation of the root canal space. An ideal irrigation solution should possess a broad-spectrum antibacterial effect, be non-toxic, and have the ability to dissolve necrotic pulp tissue and endotoxins. It should also prevent or dissolve the smear layer that forms during the cleaning process. Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are widely accepted as the gold standard combination in endodontic irrigation due to their combined ability to disinfect and remove organic and inorganic debris from the canal [5].

Studies have shown that NaOCl is highly effective in dissolving organic matter and has a strong antimicrobial effect, while EDTA is efficient in removing the inorganic portion of the smear layer, enhancing the overall cleaning process. This combination has become a

cornerstone of modern endodontic protocols [4,5].

In vitro cytotoxicity tests on these substances have shown that their concentration and exposure time can influence their toxicity to human tissues. Proper use of these irrigants is thus crucial to minimize adverse effects while maximizing their benefits in endodontic therapy [6].

MATERIALS AND METHODS

Preparation of Plant Extract from *Epilobium parviflorum* Schreb

For this study, the hydroalcoholic extract was selected due to its demonstrated antibacterial efficacy against strains isolated from pathological samples collected from patients requiring endodontic therapy [7]. Additionally, the hydroalcoholic solution derived from *Epilobium parviflorum* Schreb was chosen for its ease of use during the medicinal phase of endodontic treatment. To achieve this, the extract was first dehydrated using a rotary evaporator with a vertical condenser, resulting in a dry plant extract with a concentration of 10 mg/mL, selected for cytotoxicity testing.

The total polyphenol content of the dehydrated plant extract was determined using the Folin-Ciocalteu method as part of the study on biologically active compounds. Dehydration resulted in an increased polyphenol concentration, from 17.44 mg EqPir/g of plant powder in the hydroalcoholic extract to 46.78 mg EqPir/g of plant powder in the dehydrated extract. However, the total flavonoid content could not be determined as these compounds were denatured during the low-pressure evaporation process.

Processing of Plant Extract from *Epilobium parviflorum* Schreb: Cell Cultures

To determine the potential biological

reactivity of mammalian cell cultures in response to treatment with the test sample, the dried plant extract was incorporated into the complete culture medium used for cell cultivation, thereby obtaining a liquid extract from the test sample with a concentration of 0.2 mg/mL, which is equivalent to the dried plant extract concentration of 10 mg/mL. The extraction in liquid medium was conducted by incubating the mixture for 24 hours at 37 °C. The final extract was sterilized by vacuum filtration using a Millipore filter (0.22 micrometers, as specified in the Romanian Pharmacopoeia – Sterility Testing) [8].

In the cytotoxicity tests, a cell line obtained from the ATCC cell bank, specifically L929 with fibroblast morphology (ATCC® CCL-1™), was utilized. For the experiments, cells were subcultured from passages 20-30. L929 cells were cultured in MEM (Biochrom GmbH) supplemented with 10% fetal bovine serum (FBS) and antibiotic (100 U/mL penicillin and 100 µg/mL streptomycin). The cultures were maintained in an incubator at 37 °C in a humid atmosphere with 5% CO₂ and were examined daily using an inverted phase-contrast microscope to determine the degree of confluence and to verify normal cellular morphology and culture sterility. Cells were passaged upon reaching a confluence of 70-80%, detaching them with trypsin/EDTA and re-seeding them at a lower density in new culture vessels [9,10]

MTT Testing Method. Microscopic Evaluation

The cytotoxicity testing method was conducted in accordance with SR EN ISO 10993-5 and SR EN ISO 10993-12. The extraction ratio used for the dried plant powder was 0.2 mg/mL (mass/volume), as specified in Table 1 of the ISO 10993-12 standard (2012) [11,12].

To evaluate the cytotoxicity of the plant extract, L929 fibroblast cells were cultivated

in 96-well plates at a density of 12,000 cells per well (in 100 microliters of culture medium per well) and incubated for 24 hours to allow attachment. Following this incubation, the growth media of the L929 cells were replaced with dilutions of the liquid extract to be tested (three replicates for each condition) and then incubated again for another 24 hours.

Cell viability after 24 hours of treatment with the extract at various concentrations was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay. This analysis is based on the reduction of the yellow tetrazolium salt MTT to blue formazan, carried out by intracellular enzymes, particularly those in the mitochondria. This reduction occurs only in viable cells, making the MTT method an indicator of cellular/mitochondrial integrity. Thus, after 24 hours of treatment, the extracts were removed from the cell layer, and MTT solution was added to each well, followed by incubation for an additional 3-4 hours to allow for formazan crystal formation.

These crystals were then dissolved by adding dimethyl sulfoxide (DMSO). Following solubilization, the formazan dye was quantified using an ELISA plate reader (Mithras, Berthold Technologies) by measuring the absorbance in each well, including blanks, at 590 nm with a reference wavelength of 690 nm. These values directly correlate with the number of viable cells in the measured wells. Viability was calculated by normalizing the obtained values from the treated cells against the same number of untreated control cells [13].

Cell viability (%) was calculated using the formula: Viability (%) = $\frac{[\text{Abs } 590\text{nm (corrected)} (\text{treated sample}) \times 100]}{[\text{Abs } 590\text{nm (corrected)} (\text{untreated sample})]}$.

For qualitative assessment of cellular viability, 25,000 cells were cultivated on glass slides with a diameter of 10 mm, positioned in 24-

well plates. After incubating for 24 hours, the growth media in the wells were replaced with dilutions of the liquid extract to be tested. At the end of the treatment, the slides were prepared for fluorescence microscopy using an acridine orange (AO) staining method applied to the cells. The samples marked in

this way were visualized using a fluorescence microscope (LX71, Olympus, Shinjuku, Tokyo, Japan), employing appropriate filters. This micro-copy method highlights the induction of morphological changes in the treated cells [14].

RESULTS AND DISCUSSIONS

As shown in Table 1, the strong cytotoxic effect begins to manifest at concentrations greater than 0.4 mg/mL of the solution to be tested.

Table 1 Viability of the plant extract at different concentrations.

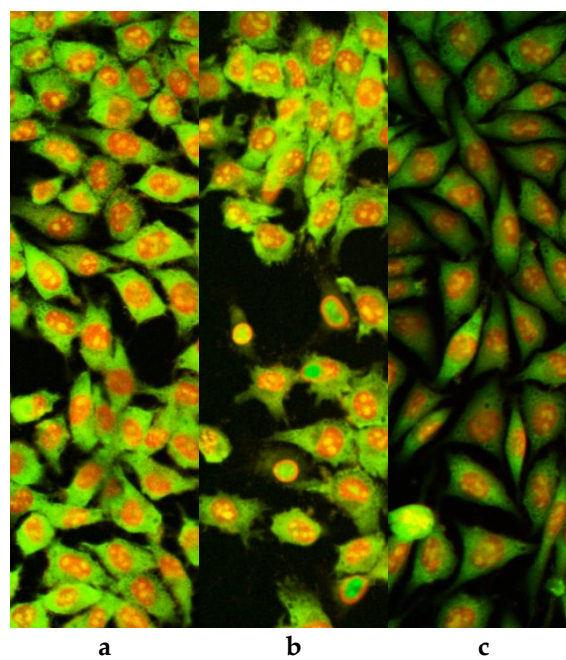
Concentration of Sample (mg/mL)	MTT Method Viability	Conclusions
0.05	106.7%	Non-cytotoxic
0.2	119%	Non-cytotoxic
0.4	50.1%	Strongly cytotoxic
0.67	28.1%	Severely cytotoxic
2	19.7%	Severely cytotoxic

Table 2 presents the scale of cytotoxicity in relation to cellular viability. Both the visualization of cellular morphology through microscopy (Figure 3.1) and the colorimetric MTT method for testing cytotoxicity (Graph 3.1) highlighted the emergence of a strong cytotoxic effect induced by the liquid extract in the culture medium at concentrations greater than 0.4 mg/mL.

Table 2. Value Scale for Toxicity/Cytotoxicity.

Viability	Cytotoxicity Scale	Description
90-100 %	0	Non-cytotoxic

80-90 %	1	Weakly cytotoxic
60-80 %	2	Moderately cytotoxic
30-60 %	3	Strongly cytotoxic
0-30 %	4	Severely cytotoxic



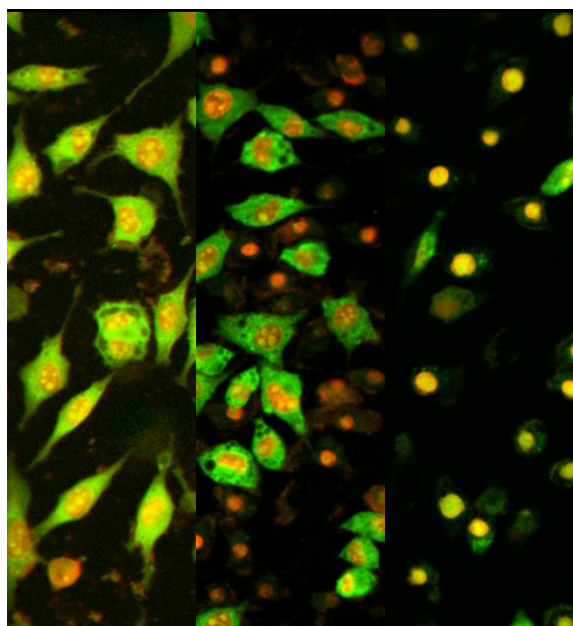
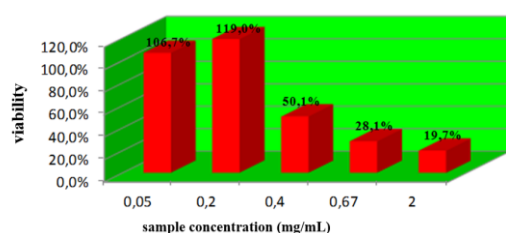


Figure 1. Representative fluorescence images showing morphological changes in L929 fibroblast cultures treated with the liquid extract (cells labeled with AO) a- Negative Control – MEM; b- Sample (0.05 mg/mL); c- Sample (0.2 mg/mL); d- Sample (0.4 mg/mL); f- Sample (0.67 mg/mL)

As shown in Graph 3.1, starting from 0.4 mg/mL of liquid extract, morphological changes appear in L929 cells treated for 24 hours with the plant extract, changes correlated with a decrease in cell viability.



Graph 1. MTT Test on L929 fibroblast cells grown for 24 hours in the presence of different concentrations of the extract in liquid medium

Complete debridement of the root canal system using appropriate irrigation substances reduces the number of

microorganisms, increasing the chances of a successful endodontic treatment. The toxicity of materials used in endodontic therapy remains a constant concern, as their extrusion into periapical tissue could cause irritation or de-generation and delay periodontal healing. The ideal endodontic irrigation solution should be selectively toxic, acting as an antibacterial agent while simultaneously exhibiting low toxicity to periradicular tissue. The MTT method assesses the cytotoxicity of dental materials based on changes in the number of viable cells, cellular metabolism, and morphology; this method evaluates cell death in the apoptotic phase due to severe cellular homeostasis disruption [15, 16].

Similar recent studies have been conducted by Karkehabadi H et al. using the same MTT method to determine the cytotoxicity of commonly used endodontic irrigants such as NaOCl, CHX, and EDTA; all solutions induced cytotoxicity in human periodontal ligament cells, which was time-dependent [15].

Other studies similar to those in this thesis were conducted by Evren Ok and collaborators, focusing on the cytotoxicity of an extract obtained from *Origanum* compared to conventional endodontic antiseptics such as NaOCl and CHX. The study was performed on cell cultures obtained from human periodontal ligament and concluded that the cytotoxicity of the solutions is interdependent with the concentrations used. The *Origanum* extract at a concentration of 0.5% exhibited lower cytotoxic effects than the other lavage solutions; the extract at a concentration of 1% and 2% CHX showed similar antibacterial effects, being less cytotoxic than 5.25% NaOCl [17].

In this study, the extract obtained from *Epilobium parviflorum* Schreb is not cytotoxic up to a concentration of 0.2 mg/mL.

CONCLUSIONS

1. The absence of cytotoxicity in the herbal extract suggests its potential use in en-

dodontic therapy as an alternative to traditional irrigation solutions.

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