Chemical characterization, in vitro anti HSV-1 activity of the Polyphenol-enriched fractions of Cistus laurifolius L., and development of antiviral herbal lip balm

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Abstract

The cytotoxic activities of the *Cistus laurifolius* extracts and their ability to inhibit cytopathic effect were evaluated by colorimetric XTT test on Vero cells. In the first experiments, crude extracts did not show antiviral activity due to high toxicity. The solid phase extraction (SPE), purification, and polyphenolic enrichment methods (PEM) were applied for EtOH, MeOH, and dH_2O extracts to reduce the toxicity of the extracts and in this way, high antiviral results were obtained. Polyphenol-enriched fractions (PEF) of MeOH (SI: 431.17) and dH_2O (SI : >455.37), purified with SPE, showed high antiviral effects against HSV-1. In vitro cytotoxicity for PEF of EtOH, MeOH, and dH_2O from *C. laurifolius* was investigated on HDFa cells to develop herbal lip balm formulations. All fractions had no cytotoxic effect on human dermal fibroblast cells. Herbal lip balm formulations were also developed and evaluation tests were performed on the herbal lip balm in this study. Twenty-one different phenolic contents in the extracts were investigated in the HPLC-DAD system. The hyperoside (49.37 ± 0.53), isoquercitrin (48.05 ± 0.74), rutin (45.84 ± 1.02), epicatechin (14.83 ± 1.00), quercetin-3-O-glucopyranose (13.35 ± 0.16), catechin (12.80 ± 0.1), caffeic acid (3.15 ± 0.18), apigenin (6.30 ± 0.10) were determined as the highest level in CL-PEF/dH_2O than the other fraction.

The results show that the PEF extracts obtained from *C. laurifolius* have good anti-HSV-1 activity, and the herbal formulation with antiviral activity has a better option with minimum side effects though detailed clinical trials may be done to access the formulation for better efficacy.

Introduction

Viral diseases are one of the most important health problems, and new therapeutic drugs are needed to be able to treat viral infections globally (Farshadpour et al. 2014). HSV-1 (Herpes simplex virus type-1) from the Herpesviridae family is one of the most common viral diseases in the world (Fatahzadeh and Schwartz 2007). The nucleoside analogues have been used in the clinical treatment of HSV-1 infections since their development, but there is an important need for the discovery of new anti-HSV-1 drugs because of the drug-resistant strains (Xiang et al. 2008). Natural substances, which have been clinically tested and approved for health, constitute an important stage in the acquisition of new antiviral agents. Although the antiviral activities of many medicinal plants have been reported, detailed studies are needed to determine their safety and efficacy during their application as herbal antiviral drugs (Hudson et al. 2000; Helfer et al. 2014; Rebensburg et al. 2016). Turkey has a large biodiversity in terms of plant flora in Europe. In this way, many plant species are used for traditional medicine, and species belonging to the genus *Cistus* are used for this purpose (Attaguile et al. 2000; Cetin and Yanikoglu 2006; Comandini et al. 2006; Catoni et al. 2012). Researchers have stated that *Cistus* species are very rich in bioactive components such as polyphenols, terpenoids, and flavonoids (Küpeli and Yesilada 2007; Barrajon-Catalan et al. 2011; Stepien et al. 2018). These compounds obtained from *Cistus* species are effective in some diseases such as; anti-inflammatory (Demetzos et al. 2001), antimicrobial (Ehrhardt et al. 2007; Barros et al. 2013; Benali et al. 2020), analgesic (Sayah et al. 2017), and antitumoral (Dimas et al. 2000). Five *Cistus* species from the *Cistaceae* family grow naturally in Turkey. On the other hand, studies on the antiviral activity of *Cistus* species extracts are not adequate (Ustun et al. 2016).

Lip balm is a dermo-cosmetic product containing pigments, oils, waxes, preservatives, and emollients that applies colour, texture, and protection to the lips. The synthetic lip balm contains various chemical substances and heavy metals. While applying these lipsticks, some of these toxic substances could be absorbed by the lips and stomach. They also cause redness, irritation, and toxic, mutagenic, and carcinogenic effects. Herbal lip balms contain bioactive compounds, natural ingredients, and protection and moisturizing. The skin structure is preferred over synthetic lip balms (Elumalai et al. 2012; Hayati and Chabib 2016; Bornare et al. 2020; Deepika et al. 2020).

Cosmetic products are of great importance in today's lifestyle and recently, consumers are increasingly interested in derma-cosmetic products with natural ingredients. Lip balm, which is among the derma cosmetic products, contains various vitamins, antioxidant and antimicrobial components, herbal extracts and essential oils. This way, these natural ingredients protect and support the lip structure against environmental factors. Synthetic lip balm products can often contain various chemicals and heavy metals in their structure. With this content, it causes many side effects (dryness, sensitivity, sensitivity to infection, toxic effect and irritation) as well as its protective effect. Lip balms generally contain ingredients such as beeswax, vaseline, and glycerin, which provide stability and have a softening effect. New attempts are being made to create cosmetic products using plants as natural resources (Elumalai et al. 2012; Hayati and Chabib 2016; Bornare et al. 2020; Deepika et al. 2020; Amale et al. 2023; Shaikh et al. 2023).

Aloe vera, chamomile, lavender, mint and calendula are among the plants used in herbal lip balms. These plants are favoured for their soothing and moisturising properties as well as their ability to help heal and protect the skin. In addition to herbal types, typical bud balms contain a combination of oils and waxes such as olive oil, coconut oil, beeswax and shea butter. The combination of herbs, oil and wax work together to create a nourishing and protective barrier on the lips. Herbal lip balms are beneficial for dry, chapped or sensitive lip structures. In addition, many consumers prefer herbal lip balms primarily because of the pleasant scent from the natural herbs and essential oils used. Although herbal products are generally considered safe, some plants can cause irritation and allergic reactions. For this reason, the plants used should also be investigated for their safe use on the skin (Amale et al. 2023). In studies conducted to date, a limited number of works have been found on the antiviral activity of *C. laurifolius* against HSV-1 (Latiff et al. 2021). It was aimed to determine;

1. The revealing of the Cytotoxicity of *C. laurifolius* crude EtOH, MeOH, and dH_2O extracts.

2. The revealing of the cytotoxicity of *C. laurifolius* PEF extracts of EtOH, MeOH, and dH_2O

3. Defining the anti-HSV-1 effect of the extracts PEF or non-PEF of *C. laurifolius.*

4. Determining of phenolics showing antiviral activity by HPLC-DAD method.

5. Developing herbal lip balm formulation with anti-HSV-1 effect.

Materials and methods

Plant collection and Extraction

C. laurifolius samples were collected from the Sandıklı-Afyonkarahisar region (Turkey) in June 2020 (Herbarium number: AKU-10400). The fresh leaves of *C. laurifolius* were dried at 45°C in a dryer. After that, the dried plant materials were then ground into a fine powder using a mill and stored at 4°C. For the extraction, a modified Ultrasonic method was used with the solvents such as; EtOH, MeOH, and dH_2O (CL-EtOH, CL-MeOH, CL-dH_2O). Each 30 g powdered sample of *C. laurifolius* was mixed with 400 mL each of EtOH, MeOH, and dH_2O and ultrasonicated at room temperature for 1h (Latiff et al. 2021). Then, sonicated extracts were filtered through Whatman filter paper No.1. Later, solvents in the extracts were evaporated in a rotary evaporator (Heidolph) at 40°C and followed by freeze-drying.

Enrichment of C. laurifolius-derived polyphenols

When investigating complex mixtures in natural sources (crude extracts), an important problem occurs some compounds in the extracts can have toxic or antagonistic effects. For this reason, the test results cannot be obtained as planned. Until nowadays, several techniques for the extraction, isolation and purification of phenolic compounds from complex mixtures are often used such as supercritical fluid extraction (SFE), semi-preparative high-performance liquid

chromatography (SP-HPLC), high-speed counter-current liquid chromatography, precipitation–adsorption and solid-phase extraction (Magalhães et al. 2010). However, these methods have some disadvantages; they are time-consuming and only allow small volumes of samples. According to (Magalhães et al. 2010), these disadvantages can be avoided by using polyvinylpyrrolidone (PVPP). PVPP can be very efficient for the adsorption of several phenolic acids (such as benzoic, p-hydroxybenzoic, protocatechuic and gallic acids, catechin, epicatechin, xanthohumol and quercetin. In our study, we used the PVPP method to eliminate crude extracts' toxicity and side effects.

2.5 g of polyvinylpyrrolidone (PVPP; Sigma-Aldrich) was added to 50 mL each of CL-EtOH, CL-MeOH, CL-dH_2O extracts in stock solution (10 mg/mL), vortexed, and polyphenols allowed to adsorb to PVPP at room temperature for 15 min before centrifugation at 4000 g. The supernatant represented the polyphenol-poor fraction (PPF) and the pellet polyphenol-enriched fraction (PEF). The pellet was washed 3 times with 25 ml dH_2O and polyphenols were eluted by washing 3 times with 25 mL 0.5 N NaOH and adjusted to pH 7. PPF and PEF were purified by solid-phase extraction (SPE) (Agilent Bond Elut C18) and these eluates were evaporated and dissolved in dH_2O to generate stock solutions with 10 mg/mL (Rebensburg et al. 2026).

Determination of phenolic compounds

Today approaches based on plant-based products are adopted for the development of new drugs. Plants have important usage areas as they contain metabolites/chemicals with various properties. In addition, new antiviral agents are needed due to the developing resistance to commercial antiviral drugs used. Due to their phytochemicals, plants are a natural resource for developing new antiviral drugs. There are some mechanisms responsible for the antiviral activity of phytochemicals. For example, phytochemicals that have the ability to bind to carbohydrates target entry into the cell. In this way, viral penetration is limited and prevents the development and growth of the virus in the cell. Some compounds inhibit viral replication by directly suppressing viral replication (Ghildiyal et al. 2020). Phenolics, alkaloids and terpenoids are important active substances among phytochemical compounds with antiviral effects (Ghildiyal et al. 2020).

Twenty-one phenolic standards viz; 4-hydroxybenzoic acid, apigenin, caffeic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, gallic acid, homogentisic acid, hyperoside, ısoquercitrin, luteolin, myricetin, naringin, quercetin, quercetin-3-o-glucopyronoside, quercetin-3-o-rutinoside 7-o-glucoside, quercitrin hydrate, rutin hydrate and vanillic acid were used for The HPLC-DAD analysis. These selected phenolic compounds were determined as the most common phenolics likely to be found in *C. laurifolius*.

HPLC analysis was performed by Shimadzu LC-2050C 3D instrument and C-18 reversed-phase column (250 mm x 4.6 µm). We adapted the protocol for the determination of phenolics with some modifications (Skendi et al. 2017; Ahmed et al. 2021). To determine the phenolic content of the plant samples, 1 gram of the powdered substances obtained after the extraction process was weighed and 10 mL of methyl alcohol was added and shaken for a while. Following this process, approximately 9 mL of dH_2O was added to the working solution and agitated again. The total volume was made up of 20 mL of distilled water. Each sample with a total volume of 20 mL was passed through 0.45 µ filters. During the study, the mobile phase flow rate was kept constant at 1 mL/min and the injection volume was 20 µL. The column was set at 40°C and the operating time was 68 minutes. Mobile phases methanol (A) and 2% acetic acid (B) were chosen. Gradient program A: B ratios 0th min 0:100, 3rd min 5:95, 18th min 20:80, 25th min 20:80, 30th min 25:75, 35th min 30:70, 40. min 40:60, 55th min 50:50, 65th min 60:40, 67th min 0:100, 68th min 0:100. Calibration curves for the standards were obtained using the peak areas of six different concentrations in triplicate experiments. Linearity was determined according to the calibration curve obtained separately for each phenolic compound. Slope, intersection, and determination coefficient (R^2) were calculated for each calibration curve (Skendi et al. 2017; Ahmed et al. 2021). Peak integration and calibrations were performed with LabSolution Lite software.

Experimental results concerning this study were mean ± SD of three parallel measurements. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Student's t-test, and p values < 0.05 were regarded as significant.

Cell line and virus

HSV-1 HF strain (ATCC VR-260) was used as a virus. The Vero cell line (African green monkey kidney cell line, ATCC-CCL81) was used for the proliferation and antiviral experiments. Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution was used to propagate Vero cells. Cells were incubated in an incubator with 5% CO_2 at 37ºC. EMEM with 2% FBS was used during virus replication and antiviral tests. $TCID_{50}$ (50% tissue culture infective dose) as a viral titer was used for the antiviral experiments as described previously by Kaerber (Kaerber 1964).

Cytotoxicity assay

XTT based cell proliferation kit (Biological Industries, Israel) was used to test of cytotoxic effects of CL-EtOH, CL-MeOH, CL-dH_2O crude extracts, a polyphenol-poor fraction (CL-PPF/EtOH, CL-PPF/MeOH, CL-PPF/dH_2O), and a polyphenol-enriched fraction (CL-PEF/EtOH, CL-PEF/MeOH, CL-PEF/dH_2O). Acyclovir (ACV), was used as a positive control for HSV-1. Firstly, Vero cells were seeded in 96 well plates at a density of 1×10^5 per mL per well and incubated at 37°C with 5% of CO_2 for 24 h. Then prepared serial dilutions starting from 75000 µg/mL to146.484 µg/mL for CL-EtOH, CL-MeOH, CL-dH_2O, and 1000 µg/mL-1.96 µg/mL for ACV were seeded, separately. The microplates were incubated for 72 h. 50 µL of a suspension from a mixture of 5 mL commercially available XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxamide) reagent and 0,1 mL PMS (N-methyl dibenzo pyrazine methyl sulfate) were dispensed onto each well of the microplate. The microplates were incubated for a further 3 h. ELISA reader (Multiskan EX, Labsystems) was used to identify Optical densities (ODs) at a reference wavelength of 490 nm and a reference wavelength of 630 nm. Experiments were done in triplicate and the results were shown as mean cytotoxicity ratio according to cytotoxic concentration.

Cytotoxicity percentages were calculated as [(A-B)/A x 100], where A and B are the absorbances of control and treated cells, respectively (Duman et al. 2019).

The percentages of cytotoxic effects of different concentrations of the extracts or ACV were determined. CC_{50} (50% Cytotoxic Concentration values) and MNTC of the extracts or ACV were determined by using the optical densities. MNTC values of the extracts and ACV were used as initial concentrations in antiviral activity assays.

Antiviral activity assay

The extracts and ACV were prepared to determine MNTC values. Then, dilutions prepared on a log2 base starting from these concentrations were used in antiviral experiments. The concentration of the Vero cells was used at 1×10^5 cells/mL and the HSV-1 titer was at 100 $DCID_{50}$. Medium control, cell control, and virus control were used in this assay. Serial dilutions of the extracts and cell suspension (1×10^5 cells/mL) were seeded onto 96-well culture plates and incubated at 37ºC in 5% CO_2 for 4 h. Then, 20 µL of HSV-1 suspensions and 20 µL maintenance medium were added, and the plate was incubated for a further 2 h. 2-fold dilutions from the extract solutions in 10×MNTC were prepared using a 2% FBS medium. Each 10 µL extract solution was dispensed to wells and serial dilutions were done. The plates were incubated for 72 h. 50 µL XTT reagent was added to all wells. Plates were incubated for 3 h. ODs were measured at 490 nm and 630 nm reference wavelengths.

% protection rates of the extracts and ACV were calculated following the formula;

% protection: [(A-B)/(C-B) x 100]

A: the absorbances of the extracts or ACV

B: the absorbances of the virus

C: the absorbances of cell control

The 50% effective concentration (EC_{50}) value is the concentration of the extract or ACV that protects 50% of the infected cells. The selectivity index (SI) of the extracts and ACV indicates the CC_{50}/EC_{50} ratio. Experiments were done in triplicates. GraphPad Prism Version 5.03 statistical software with nonlinear regression analysis was used to calculate cytotoxicity and antiviral test results.

In vitro cytotoxicity studies-MTT assay

According to the antiviral activity data obtained, polyphenols should be separated to reduce the cytotoxic feature. Hence, CL-PEF/EtOH, CL-PEF/MeOH, and CL-PEF/dH_2O fractions showed significant antiviral effects, they were investigated on HDFa to evaluate the potential usage in the biomedical and pharmaceutical areas. In vitro, cell viability of polyphenol-enriched fraction was determined on HDFa cell line by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay (Kumar et al. 2018).

The HDFa cells (ATCC, PCS-201-012) were cultured using Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, and 1% penicillin-streptomycin solution. HDFa cells were first seeded in 96 well plates at a density of 2×10^4 cells per well, treated with different concentrations of fractions (46.87–1500 µg/mL), and incubated at 37°C with 5% of CO_2 and 95% of humidity for 24, 48 and 72 h. Experiments were repeated 12 times for each concentration. After the incubation period, 20 µL of MTT solution was added to each well and the plates were incubated for 3 h at 37°C. Then, the formed formazan crystals were dissolved in DMSO (0.5% v/v) and the optical density was measured with a microplate reader (Gen5 Biotech®) at the 570 nm wavelength. Fraction-free complete medium was used as the control (blank) and was treated in the same way as the extract-containing media.

The percentage cell viability (CV) was calculated using the formula below:

CV (%): [Absorbance of test sample/Absorbance of control] x100

All data were expressed as mean ± SD, statistical analysis was performed with Graph Pad Prism 9 (GraphPad Software, Inc., USA), and One Way Anova with 95% confidential interval (CI) was used together with Tukey's multiple comparisons tests. The P-value below 0.05 was considered statistically significant.

Development of antiviral herbal lip balm formulation

Herbal lip balm was prepared by mixing beeswax, cacao butter, coconut oil, castor oil, jojoba oil, orange oil, lemon oil, and CL-PEF/dH_2O fraction that high antiviral effect (Aher et al. 2012; Kadu et al. 2015).

Cacao butter and beeswax were used as the cream base and orange oil and lemon oil were used as fragrance agents. Also, these essential oils were used to obtain the soothing and preservative properties of the lip balm. The usage of Coconut oil in the formulation of this herbal lip balm is exploited for its blending properties with the waxes, and imparting the lip balm to coat in the thin film. The beeswax could retain the moisture necessary for healing the lips (Kasparaviciene et al. 2016). CL-PEF/dH_2O extracts were used at 1500 µg/mL in herbal lip balm formulations and thus, impart antiviral properties. All ingredients were natural in herbal lip balm formulations and chemical substances were not used.

Evaluation of herbal lip balm

Formulated herbal lip balm formulations were checked for colour and texture, pH, solubility, melting point, softening point, surface anomalies, ageing stability, and fragrance stability. The solubility of herbal lip balm was tested using a different solvent. Determination of melting point, which is an indication of the limit of safe storage, is an important parameter for herbal lip balm formulation; it was determined by the capillary tube method. The softening point of lip balm was determined by the Ring and Ball method. Surface anomalies such as the formation of crystals on the surface, contamination, formation of wrinkles, etc. were examined. Herbal lip balm was stored at three different temperatures i.e. refrigerator (4^{o}C), room (22^{o}C) (Ozçelik et al. 2016; Latiff et al 2021), and high temperature (30–40 ^{o}C) for 1h to determine ageing stability. After an incubation period, parameters such as bleeding, streaking, catering, and blooming were observed. The prepared herbal lip balm formulation was tested after 30 days, to evaluate fragrance stability.

In vitro biocompatibility test of herbal lip balm formulations

The biocompatibility of herbal lip balm formulations was evaluated by modifying the method (Demirci et al. 2020). First, herbal lip balm extracts were plated into 24-well cell culture plates and sterilized by UV light at 2 h. For stabilization, samples were left in a cell culture medium at 37^{o}C overnight before cell seeding. HDFa cell lines were seeded onto the herbal lip balm extract at a density of 5x10^{4} cells/well for each sample. Experiments were repeated 12 times for each formulation. The culture plates were incubated in a CO_2 incubator (5%) at 37°C for 24 and 48 h. After the incubation period, 20 µL of MTT solution was added to each well and incubated for 3 h. The formed formazan crystals were dissolved in (0.5% v/v) DMSO. The absorbance was measured at 570 nm by Gen5 Biotech® Microplate Reader. The biocompatibility of the herbal lip balm formulations was expressed as % cell viability, which was calculated from the ratio between the number of cells treated with the herbal lip balm extract and that of nontreated cells (control).

Results and discussion

Chemical composition

Phenolics are divided into several derivates such as phenolic acids, flavonoids, stilbenes, coumarins and tannins. It is possible to classify flavonoids as anthocyanidins, flavanones, flavanols, flavones and isoflavonoids (Ghildiyal et al. 2020). Phenolic compounds such as phenolic acids and flavonoids have been reported to have many biological effects, including antibacterial, antiallergic, antiviral, antioxidant, anti-inflammatory and anti-ageing activities (Čanadanović-Brunet et al. 2006). Salicylic acid, gallic acid, ferulic acid, caffeic acid, chlorogenic acid, and rosmarinic acid are antiviral phenolic acids; apigenin, luteolin, quercetin, isoquercitrin, rutin, myricetin, quercitrin, catechin, epicatechin and naringin are antiviral flavonoids; diterpenoid, sesquiterpenoid, β-caryophyllene, caryophyllene oxide, germacrene D are terpenoids and neo-claredones with antiviral effects (Bezić et al 2011; Bekhit and Bekhit 2014; Hudson et al. 2018; Vuko et al. 2020). Myricetin is one of the natural compounds identified against SARS-CoV enzymes. Quercetin has been reported to have Anti-DENV (Dang virus) activity. Gallic acid obtained from *Woodfordia fruticosa* flowers has been observed to show anti-EV71 (Enterovirus 71) activity. It has been observed that bioactive compounds such as linalool and apigenin obtained from *Ocimum basilicum* have antiviral activity against CVB1 (Coxsackievirus B). Caffeic acid has been observed to have antiviral activity against a novel type of influenza NA (Lin et al. 2014). In the studies carried out, it has been reported that catechin, myricetin and quercetin inhibit protein synthesis and viral protein synthesis; quercetin inhibits the life cycle of the virus; caffeic acid inhibits RNA and DNA replication cycle; quercetin and luteolin inhibit viral transcription and translation for HSV-1. It is stated that ferulic acid, gallic, caffeic acid inhibit the replication of HIV virus, and apigenin prevents the activation of HIV virus. Chlorogenic acid significantly inhibited HSV-1 replication without any cytotoxicity. The effects of various naturally occurring dietary flavonoids, including quercetin, on the infectivity and replication of herpes simplex virus type 1 (HSV-1), polio virus type 1, Para influenza virus type 3, and respiratory syncytial virus (RSV)

were studied, and each was determined that it caused a concentration-dependent decrease in the infectivity of the virus. Myricetin has shown excellent antiviral activity against hepatitis B virus, influenza virus and/or coronavirus (Naithani et al. 2010). In a study, the antiviral activity of quercetin, galangin, catechin hydrate, epigattocatechin, epigallocatechin gallate, epicatechin, epicatechin gallate, naringenin, naringin, apigenin, luteolin, chrysin, rutin, genistein, baicalin, fisetin, myricetin and kaempferol against HSV-1 and HSV-2 was investigated, and it was determined that the compounds except chrysin and kaempferol showed a higher selectivity index than acyclovir, especially against HSV-1 (Lyu et al. 2005). In addition, it is stated in other studies that quercetin, catechin hydrate and naringin show antiviral activity on different viruses (Loaiza-Cano et al. 2020).

Twenty-one phenolic compounds were used to identify compounds with possible antiviral activity in the extracts (Fig. 1a and b). The presence of different amounts of phenolic compounds for each extract is shown in Table 1. The most abundant compounds for mg/g extract were determined as 16.01 ± 0.31 chlorogenic acid in CL-PPF/EtOH, 17.06 ± 1.18 catechin in CL-PEF/EtOH, and 10.51 ± 0.03 gallic acid in CL-PPF/MeOH, 18.01 ± 0.18 hyperoside in CL-PEF/MeOH, and 14.95 ± 0.03 rutin in CL-PPF/dH_2O, 49.37 ± 0.53 hyperoside in CL-PEF/dH_2O.

Table 1
Concentration (mg/g extract) of selected phenolic compounds in extracts

Compounds	CL-PPF/EtOH	CL-PEF/EtOH	CL-PPF/MeOH	CL-PEF/MeOH	CL-PPF/dH_2O	CL-PEF/dH_2O
Gallic Acid	2.99 ± 0.04 *x,b*	4.01 ± 0.03 *y, C*	10.51 ± 0.03 *y,c*	0.69 ± 0.01 *x, B*	0.06 ± 0.001 *x,A*	0.24 ± 0.01 *y, a*
Homogentisic Acid	2.92 ± 0.15 *x,b*	2.96 ± 0.26 *x,B*	1.05 ± 0.01 *x,a*	15.32 ± 0.07 *y, C*	0.34 ± 0.01 *x,A*	12.75 ± 0.07 *y,c*
3.4 Dihidroxybenzoic Acid	9.07 ± 0.07 *x,c*	11.89 ± 0.05 *y, C*	3.64 ± 0.02 *y,b*	2.00 ± 0.02 *x,B*	0.29 ± 0.01 *x,A*	1.85 ± 0.03 *y, a*
Chlorogenic Acid	16.01 ± 0.31 *y,c*	10.09 ± 0.07 *x,C*	4.45 ± 0.02 *y, a*	4.23 ± 0.01 *x,B*	0.23 ± 0.01 *x,A*	11.11 ± 0.04 *y,b*
Quercetin-3-O-Rutinoside 7-O-Glucoside	12.78 ± 0.20 *y,c*	11.86 ± 0.19 *x,B*	5.09 ± 0.20 *x,a*	15.28 ± 2.06 *y,C*	1.41 ± 0.02 *x,A*	6.29 ± 0.04 *y,b*
Catechin	7.48 ± 0.08 *x,b*	17.06 ± 1.18 *y, B*	3.16 ± 0.02 *y, a*	1.57 ± 0.12 *x,A*	2.11 ± 0.04 *x,A*	12.80 ± 0.13 *y,c*
4-Hydroxybenzoic Acid	3.35 ± 0.09 *x,b*	4.08 ± 0.28 *y, B*	1.91 ± 0.01 *y, a*	0.82 ± 0.31 *x,A*	0.21 ± 0.05 *x,A*	1.96 ± 0.04 *y, a*
Vanillic Acid	4.22 ± 0.50 *x,b*	3.50 ± 0.48 *x,C*	2.32 ± 0.01 *y, a*	1.51 ± 0.12 *x,B*	0.10 ± 0.16 *x,A*	1.56 ± 0.17 *y, a*
Epicatechin	5.11 ± 0.28 *y,a*	3.13 ± 0.39 *x,B*	6.03 ± 0.02 *y,a*	1.09 ± 0.01 *x,A*	0.80 ± 0.01 *x,A*	14.83 ± 1.00 *y,b*
Rutin	7.96 ± 0.42 *y,b*	6.83 ± 0.14 *x,A*	2.15 ± 0.02 *x,a*	17.50 ± 0.15 *y, C*	14.95 ± 0.03 *x,B*	45.84 ± 1.02 *y,c*
Ferulic Acid	4.78 ± 0.08 *x,c*	4.82 ± 0.01 *x,C*	1.68 ± 0.01 *y,b*	0.34 ± 0.01 *x,B*	0.21 ± 0.01 *x,A*	0.66 ± 0.09 *y,a*
Isoquercitrin	9.12 ± 0.14 *y,b*	6.30 ± 0.01 *x,B*	2.32 ± 0.01 *x,a*	17.69 ± 0.04 *y,C*	1.18 ± 0.01 *x,A*	48.05 ± 0.74 *y,c*
Naringin	2.54 ± 0.04 *y,c*	1.69 ± 0.01 *x,C*	0.47 ± 0.01 *x,a*	1.03 ± 0.01 *y,B*	0.18 ± 0.01 *x,A*	2.21 ± 0.02 *y,b*
Quercitrin	7.70 ± 0.13 *y,b*	2.45 ± 0.02 *x,C*	1.94 ± 0.10 *y,a*	1.50 ± 0.01 *x,B*	1.18 ± 0.02 *x,A*	1.77 ± 0.01 *y,a*

The data indicated by the same superscripts (x and y) within the same row for each extract are not different from each other according to the Student's t-test at a 5% significance level

The data indicated by the same superscripts (a, b, and c) within the same row for different PPF extracts are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level.

The data indicated by the same superscripts (A, B, and C) within the same row for different PEF-extracts are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level

Compounds	CL-PPF/EtOH	CL-PEF/EtOH	CL-PPF/MeOH	CL-PEF/MeOH	CL-PPF/dH_2O	CL-PEF/dH_2O
Myricetin	3.15 ± 0.04 *y,c*	1.96 ± 0.20 *x,C*	1.79 ± 0.02 *y,b*	1.21 ± 0.03 *x,B*	0.37 ± 0.01 *x,A*	1.15 ± 0.02 *y, a*
Quercetin	7.21 ± 0.12 *x,c*	7.52 ± 0.15 *x,C*	1.65 ± 0.11 *x,a*	5.83 ± 0.15 *y,B*	0.41 ± 0.10 *x,A*	2.11 ± 0.08 *y,b*
Apigenin	0.85 ± 0.12 *x,b*	1.01 ± 0.07 *x,A*	0.44 ± 0.09 *x,a*	2.34 ± 0.15 *y,B*	0.77 ± 0.08 *x,A*	6.30 ± 0.10 *y,c*
Caffeic Acid	2.51 ± 0.05 *y,b*	0.68 ± 0.08 *x,B*	1.27 ± 0.01 *y, a*	0.87 ± 0.07 *x,C*	0.23 ± 0.01 *x,A*	3.15 ± 0.18 *y,c*
Hyperoside	9.36 ± 0.16 *y,b*	6.48 ± 0.02 *x,B*	2.41 ± 0.01 *x,a*	18.01 ± 0.18 *y,C*	1.32 ± 0.01 *x,A*	49.37 ± 0.53 *y,c*
Quercetin-3-O-Glucopyronoside	2.19 ± 0.06 *y,b*	1.70 ± 0.07 *x,B*	0.89 ± 0.06 *x,a*	6.00 ± 0.05 *y,C*	0.57 ± 0.01 *x,A*	13.35 ± 0.16 *y,c*
Luteolin	5.22 ± 0.10 *x,c*	4.93 ± 0.87 *x,B*	1.19 ± 0.14 *x,a*	3.54 ± 0.21 *y,C*	0.35 ± 0.02 *x,A*	1.57 ± 0.06 *y,b*

The data indicated by the same superscripts (x and y) within the same row for each extract are not different from each other according to the Student's t-test at a 5% significance level

The data indicated by the same superscripts (a, b, and c) within the same row for different PPF extracts are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level.

The data indicated by the same superscripts (A, B, and C) within the same row for different PEF-extracts are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level

Generally, CL-PEF/dH2O is very rich in phenolic content. In this extract, hyperoside 49.37 and isoquercitrin 48.05 were determined as the highest phenolic compounds. These are followed by rutin 45.84, epicatechin 14.83, catechin 12.80 and homogentisic acid 12.75. The high rate of phenolic compounds in CL-PEF/dH2O also had a positive effect on its antiviral activity against HSV-1 (Table 2). The SI index of CL-dH2O was 1.08, while the SI index of CL-PEF/dH2O was > 455.37. According to this result, enrichment of the raw extracts with polyphenol creates a positive factor in terms of antiviral activity. Likewise, although the amount of phenolics obtained from CL-PEF/MeOH was less than CL-PEF/dH2O, it was determined that it had richer phenolic contents than other untreated extracts. Some of the highest phenolics identified in CL-PEF/MeOH are as follows; hyperoside 18.01, isoquercitrin 17.69, rutin 17.50, homogentisic acid 15.32 ve quercetin-3-O-rutinoside 7-O-glucoside 15.28. In parallel with these results, the phenolics obtained from CL-PEF/EtOH are higher than the others.

Table 2
Cytotoxicity and antiviral activity of crude extracts and a polyphenol-poor fraction, a polyphenol-enriched fraction of *C. laurifolius*.

Crude extracts	Cytotoxicity		Anti-HSV-1 Activity		Fractions	Cytotoxicity		Anti-HSV-1 Activity	
	MNTC (µg/mL)	CC_{50} (µg/mL)	EC_{50} (µg/mL)	SI		MNTC (µg/mL)	CC_{50} (µg/mL)	EC_{50} (µg/mL)	SI
CL-EtOH	15.63	184.40	71.09	2.59	CL-PPF/EtOH	375	1008.00	281.10	3.59
CL-MeOH	31.25	113.80	139.50	0.82	CL-PEF/EtOH	187.5	591.00	2.091	282.64
CL-dH_2O	7.81	149.50	138.80	1.08	CL-PPF/MeOH	187.5	888.40	3.150	282.03
					CL-PEF/MeOH	375	4094.00	9.495	431.17
					CL-PPF/dH_2O	375	913.10	4.754	192.07
					CL-PEF/dH_2O	1500	1500*	3.294	> 455.37
ACV	31.25	1948	1.909	1020.43	ACV	31.25	1948	1.909	1020.43

As a result, it was determined that the phenolic contents of the extracts enriched in terms of phenolic were higher than the extracts without phenolic enrichment.

Cytotoxicity on Vero cells and Antiviral activity

HSV-1 titer on Vero cells was determined as $DCID_{50}$: 10^{-5}/0.1 mL at the end of 72 h. The appearance of uninfected healthy Vero cells and the CPE (cytopathic effects) caused by the virus on Vero cells.

In the first cytotoxicity experiments on Vero cells, the extracts showed a highly toxic effect (Fig. 2 and Table 2). MNTC and CC_{50} of these extracts are as follows; CL-EtOH: 15.63 µg/mL and 184.40 µg/mL, CL-MeOH: 31.25 µg/mL and CC_{50}: 113.80 µg/mL, CL-dH_2O: 7.81 µg/mL and 149.50 µg/mL and ACV: 31.25 µg/mL and 1948 µg/mL). The cytotoxic effects on Vero cells for CL-PPF/EtOH, CL-PPF/MeOH, CL-PPF/dH_2O and CL-PEF/EtOH, CL-PEF/MeOH, CL-PEF/dH_2O were evaluated. Extracts that were not enriched with polyphenols showed high toxicity. The toxicity values are as follows (Table 2, Fig. 3); CL-PPF/EtOH (MNTC: 375.00 µg/mL, CC_{50}: 1008.00 µg/mL). CL-PEF/EtOH (MNTC: 187.50 µg/mL, CC_{50}: 591.00 µg/mL), CL-PPF/MeOH (MNTC: 187.50 µg/mL, CC_{50}: 888.40 µg/mL), CL-PEF/MeOH (MNTC: 375.00 µg/mL, CC_{50}: 4094.00 µg/mL), CL-PPF/dH_2O (MNTC: 375.00 µg/µL, CC_{50}: 913.10 µg/mL), CL-PEF/dH_2O (MNTC: 1500 µg/mL, CC_{50}: 1500 µg/mL).

The percentage protection rates and Selectivity index (SI) values of crude CL-EtOH, CL-MeOH, and CL-dH_2O extracts prepared from *C. laurifolius*, and ACV against HSV-1 are as follows; CL-EtOH (EC_{50}: 71.09 µg/mL, SI: 2.59), CL-MeOH (EC_{50}: 139.50 µg/mL, SI:0.82), CL-dH_2O (EC_{50}:138.80 µg/mL, SI:1.08) and ACV (EC_{50}:1.909, SI:1020.43) (Table 2, Fig. 4).

The antiviral activity results of the polyphenol-enriched extracts are shown in Table 2, Fig. 5. CL-PPF/EtOH (EC_{50}: 281.10 µg/mL, SI: 3.59), CL-PEF/EtOH (EC_{50}: 2.091 µg/mL, SI: 282.64), CL-PPF/MeOH (EC_{50}: 3.150 µg/mL, SI: 282.03), CL-

PEF/MeOH (EC_{50}: 9.495 µg/mL, SI: 431.17), CL-PPF/dH_2O (EC_{50}: 4.754 µg/mL, SI: 192.07); CL-PEF/dH_2O (EC_{50}: 3.294 µg/mL, SI: >455.37).

In order to be able to say about antiviral effectiveness, the SI value must be 10 and above. According to Table 2, the most effective antiviral extract is CL-PEF/dH2O with a SI value of > 455.37. This is followed by CL-PEF/MeOH with SI 282.03 and CL-PEF/EtOH with SI 431.17. As can be seen, a very high antiviral effect has been obtained due to the phenolic enrichment. We can attribute this result to the removal of other toxic or cell growth-influencing ingredients in the extracts. In addition, most of the phenolic substances are known as natural antiviral agents.

Cytotoxicity of CL-PEF of EtOH, MeOH, and dH_2O fractions on HDFa cells determined by MTT test. Although there was a decrease in cell viability parallel with increased concentration of fractions, they did not show a cytotoxic effect on HDFa cells for 24, 48, and 72 h (Fig. 6–8). Cell viability of the highest concentration (1500 µg/mL) of CL-PEF/dH_2O extracted on HDFa cells for 24, and 48, 72 h were determined as 104.07%, 100.19%, and 94.59%, respectively.

The crude extracts of CL-EtOH, CL-MeOH, and CL-dH_2O obtained from the leaves of CL-PEF/dH_2O extracted with high antiviral activity and low cytotoxicity and increasing cell viability to be used in lip balm development studies were determined considering the results of antiviral assay and MTT test. The tested formula of herbal lip balm is shown in Table 3. Among these formulas, number 1 determined the most suitable content for the development of herbal lip balm formulation for colour and texture. The structures of the other formulas tested were not found suitable for lip balm development. For this reason, formula number one content is used for the evaluation of product tests.

Table 3
The tested formulas of herbal lip balm.

Formula No	Coconut oil	Beeswax	Cocoa oil	Castor oil	Jojoba oil	Orange oil	Lemon oil	*C. laurifolius* (µg/mL)
1	%10	%15	%40	%10	%10	%15	%0	1500
2	%10	%15	%40	%10	%10	%0	%15	1500
3	%10	%15	%40	%10	%10	%10	%5	1500
4	%10	%20	%35	%10	%10	%15	%0	1500
5	%10	%20	%30	%15	%15	%10	%0	1500
6	%10	%20	%30	%15	%15	%0	%10	1500
7	%15	%20	%30	%10	%10	%15	%0	1500
8	%15	%25	%35	%10	%10	%5	%0	1500
9	%10	%15	%40	%10	%10	%15	%0	1500
10	%10	%30	%25	%10	%10	%15	%0	1500

Herbal lip balm formulation

Biocompatibility of antiviral herbal lip balm formula number one tested by MTT assay. Three formulas (F1, F2, and F3) were prepared by adding 1500 µg/mL, 750 µg/mL, and 375 µg/mL CL-PEF/dH_2O extracts and tested cytotoxicity.

The MTT assay results of antiviral herbal lip balm formulas are shown in Fig. 9. According to the MTT assay, F1, F2, and F3 showed high degrees of cell viability on HDFa cells. The highest cell viability was determined in F3, which contained 375 µg/mL CL-PEF/dH_2O extracts, as 154.13% for 24 h and 143.38% for 48 h. The lowest cell viability % was determined

in F1 which contained 1500 µg/mL CL-PEF/dH_2O extracts, as 135.41% for 24 h and 123.89% for 48 h. F1 was determined as a final product because 1500 µg/mL CL-PEF/dH_2O extracts showed higher anti-HSV activity although cell viability was % lower than other formulations.

F1 antiviral herbal lip balm formula has checked some parameters like colour and texture, pH, solubility, melting point, softening point, surface anomalies, ageing stability, and fragrance stability and results are shown in Table 4. It has been determined that the antiviral herbal lip balm formula has desired balm structure, easy to apply, and does not have any surface anomalies. The product dissolves better in alcohol-based solvents than in other solvents.

Table 4
Evaluation of herbal lip balm (F1)

Evaluation Parameter	**F1**
Color and texture	Yellow, smooth
Melting point	52 ^oC
Softening point	50 ^oC
pH	6,4
Solubility	Ethanol (++), Methanol (++) Isopropanol (++), DMSO (++) dH_2O (+), Aceton (-)
Surface anomalies	Not detected (Bacteria, fungi, and surface anomalies)
Aging stability	No crystallization Easy to apply No fluidity
Fragrance stability	Good
++: good solubility, +: low solubility, -: no solubility	

Treatment of viral diseases is a great challenge even today because of the virus's adaptation, the emergence of resistant viral pathogens, new viral strains, the high cost and side effects of medicine, and host resistance to antiviral drugs (Zalegh et al. 2021). Plant products and their bioactive compounds are promising sources for obtaining new therapeutic agents, especially as a complement to traditional drug regimens or for the development of alternative drugs. Due to the inadequacy of current treatments against HSV-1 infection, the requirement for the development of drugs with new viral targets and/or mechanisms of action and specific treatments increases. Most modern medicines (about 40%) come from compounds derived from natural sources. This research was conducted to identify potential natural products that can be used in the treatment of HSV-1.

Shrub species growing in the Mediterranean region, such as *C. laurifolius*, are naturally extremely rich in polyphenols. Owing to these properties, they can be used as a source of bioactive compounds needed in the development of new drugs. In Turkish traditional medicine, *C. laurifolius* is a species that has been widely used against some different traditional ailments. In this study, we demonstrate that cytotoxicity and antiviral activity of crude extract of *C. laurifolius* (CL-EtOH, CL-MeOH, CL-dH_2O) and a polyphenol-poor fraction (CL-PPF/EtOH, CL-PPF/MeOH, CL-PPF/dH_2O) and a

polyphenol-enriched fraction of these extracts (CL-PEF/EtOH, CL-PEF/MeOH, CL-PEF/dH_2O). As seen in Table 1, the CC_{50} values of *C. laurifolius* extract that is not enriched with polyphenols is 113.80-184.40 µg/mL, whereas the CC_{50} value of the extract fractions enriched in polyphenols is 750.00-4094.00 µg/mL. Thus, polyphenol-enriched extract fractions were found to have lower cytotoxicity. Rebensburg et al. (2016), supporting our findings, determined that a polyphenol-enriched fraction of *Cistus incanus* L. aqueous extract had lower cytotoxicity than non-polyphenol-enriched aqueous extract, and attributed this to the fact that enrichment of polyphenols removes the cytotoxic components of the extract (Ehrhardt et al. 2007). According to Chattopadhyay et al. (2009) to observe antiviral activity, the SI value should be 10 or greater and this value is an indicator of positive antiviral activity.

According to these criteria, as seen in Table 2. We can say that extracts not enriched with polyphenols have anti-HSV-1 activity (SI: 0.82–2.59) that can be considered insignificant, whereas extract fractions enriched with polyphenols have very high anti-HSV-1 activity (SI: 33.94-455.37). In addition, significant differences were found between the antiviral activities of polyphenol-reduced (SI: 3.59-362.93) and polyphenol-enriched (SI: 33.94-455.37) extract fractions (Table 2). The reason for these differences can be interpreted as the enrichment of polyphenols, the removal of cytotoxic components in the extracts, and the increase in antiviral activity (Rebensburg et al. 2016).

C. laurifolius have in different rates of anti-HSV-1 activities that can be considered negligible (SI: 0.82–2.59), as stated above, this may be due to the low amounts of polyphenolic compounds, especially flavonoids, that were stated to be responsible for the anti-HSV-1 activity. On the other hand, the extract fractions enriched with polyphenols have high anti-HSV-1 activity (Table-2 reveals the importance of enrichment with polyphenols in increasing the antiviral effects of the extracts). It has been reported that the *Cistus* species are rich in polyphenolic and that the antiviral effect of this genus is due to these secondary metabolites (Rebaya et al. 2016; Waed et al. 2016). Thus, the inhibition of HSV-1 replication seen in the present study may probably be due to the action of these secondary metabolites of *C. laurifolius*. Also, recent research has demonstrated that some phenolic compounds in plant extracts can stimulate cell proliferation in fibroblast cells (Alkan et al. 2014; Dinda et al. 2015; Filho et al. 2019). The cell culture studies results obtained in the present study demonstrated that all tested concentrations of CL-PEF showed significant effects on the proliferation of the HDFa cell line but there was a decrease in cell proliferation depending on the increasing concentration. Researchers revealed that *C. laurifolius* has antioxidant (Liu et al. 2019), antimicrobial (Ustun et al. 2016), analgesic (Kupeli et al. 2012), anti-inflammatory and antinociceptive activity (Kupeli Akkol and Yesilada 2007). However, studies on whether *C. laurifolius* can promote fibroblast proliferation have not been found.

In the present day, the use of cosmetics is increasing tremendously. Various chemicals used in the production of these cosmetics can be hazardous to the health of the user (Deepika et al. 2020). Among the various cosmetic products, different formulations of lip balms are used to enhance the beauty of the lips. Lip balms offer a natural opportunity to protect and develop healthy lips. Current cosmetic lip products consist of enormous chemical ingredients with various side effects. Herpes labialis, the most conspicuous form of recurrent mucocutaneous herpes, often occurs in association with stress (Ark et al. 2004; Waggoner and Grossman 2004). Thus, we aimed to determine the antiviral properties of *C. laurifolius* L. and formulate a herbal lip balm that had antiviral activity against HSV-1. *C. laurifolius* L. polyphenol-enriched fractions of H_2O extract (1500 µg/mL) were determined according to antiviral activity assay and in vitro cytotoxicity results and were incorporated into the lip balm formula. Also, hyperoside (49.37 ± 0.53), isoquercitrin (48.05 ± 0.74), rutin (45.84 ± 1.02), epicatechin (14.83 ± 1.00), quercetin-3-O-glucopyranose (13.35 ± 0.16), catechin (12.80 ± 0.1), caffeic acid (3.15 ± 0.18), apigenin (6.30 ± 0.10) were highest level in CL-PEF/dH_2O than the other fraction. Therefore, the antiviral activity of CL-PEF/dH_2O may be high. Similar to our study, Yadav et al. (2020), formulated and evaluated herbal lip balm from Amaranth leaf colour pigment. It was concluded that lip balm formulation provided a better option for anyone applying lip balm with minimal side effects and has antioxidant properties.

Conclusion

The present study showed that CL-PEF of EtOH, MeOH, and dH_2O extracts could be effective in herpes infections. Crude extracts of *C. laurifolius* L. had a high cytotoxic effect on Vero cells and did not have antiviral activity. However, CL-PEF of EtOH, MeOH, and dH_2O extracts did not show a cytotoxic effect on Vero cells and they had remarkable antiviral effects against HSV-1. Thus, polyphenols should be separated to reduce the cytotoxic feature of *C. laurifolius* L. This work is the first report on the antiviral properties of CL-PEF of EtOH, MeOH, and dH_2O extracts. In addition, the cytotoxicity of fractions on HDFa cells, which showed antiviral activity, was determined by the MTT test. Fractions had no cytotoxic effect on HDFa and stimulate cell viability. According to the results of the antiviral activity and cytotoxicity test, antiviral herbal lip balm formulations were developed and evaluation tests were performed on the antiviral herbal lip balm. The results obtained in the present investigation show that antiviral herbal lip balm could be successfully formulated using different natural ingredients. Thus, the antiviral herbal lip balm formulation can take safe and effective after thorough clinical trials.

Authors' contributions n.O., S.F.E., S.Ç. and Ö.E.A. contributed to the study design, data collection, performing of field experiments, statistical analysis, and writing-original draft preparation of the manuscript. H.H.D., R.D. and Ü.Ü. contributed to antiviral experiments, HPLC and statistical analysis, reviewing and editing the manuscript. All authors read and approved the final manuscript.

Declarations

Authors' contributions n.O., S.F.E., S.Ç. and Ö.E.A. contributed to the study design, data collection, performing of field experiments, statistical analysis, and writing-original draft preparation of the manuscript. H.H.D., R.D. and Ü.Ü. contributed to antiviral experiments, HPLC and statistical analysis, reviewing and editing the manuscript. All authors read and approved the final manuscript.

Conflict of interest The authors declare no conflict of interest.

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Figures

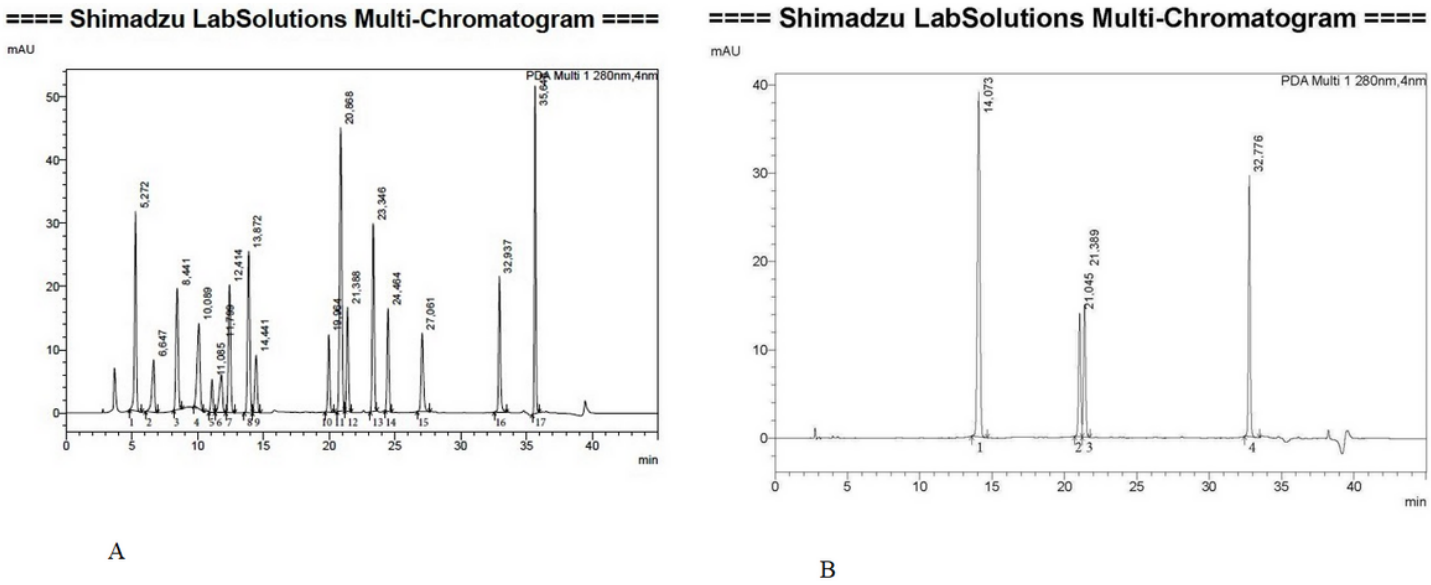

Figure 1

a.Chromatograms of 17 phenolics; 1-Gallic Acid, 2-Homogentisic Acid, 3-3,4 Dihidroxybenzoic Acid, 4-Chlorogenic Acid, 5-Quercetin-3-O-Rutinoside 7-O-Glucoside, 6-Catechin Hydrate, 7-4-Hydroxybenzoic Acid, 8-Vanillic Acid, 9-Epicatechin, 10-Rutin Hydrate, 11-Ferulic Acid, 12-Isoquercitrin, 13-Naringin, 14-Quercitrin Hydrate, 15-Myricetin, 16-Quercetin, 17-Apigenin

b.Chromatograms of 4 phenolics; 1-Caffeic Acid, 2- Hyperoside, 3- Quercetin-3-O-Glucopyronoside, 4-Luteolin.

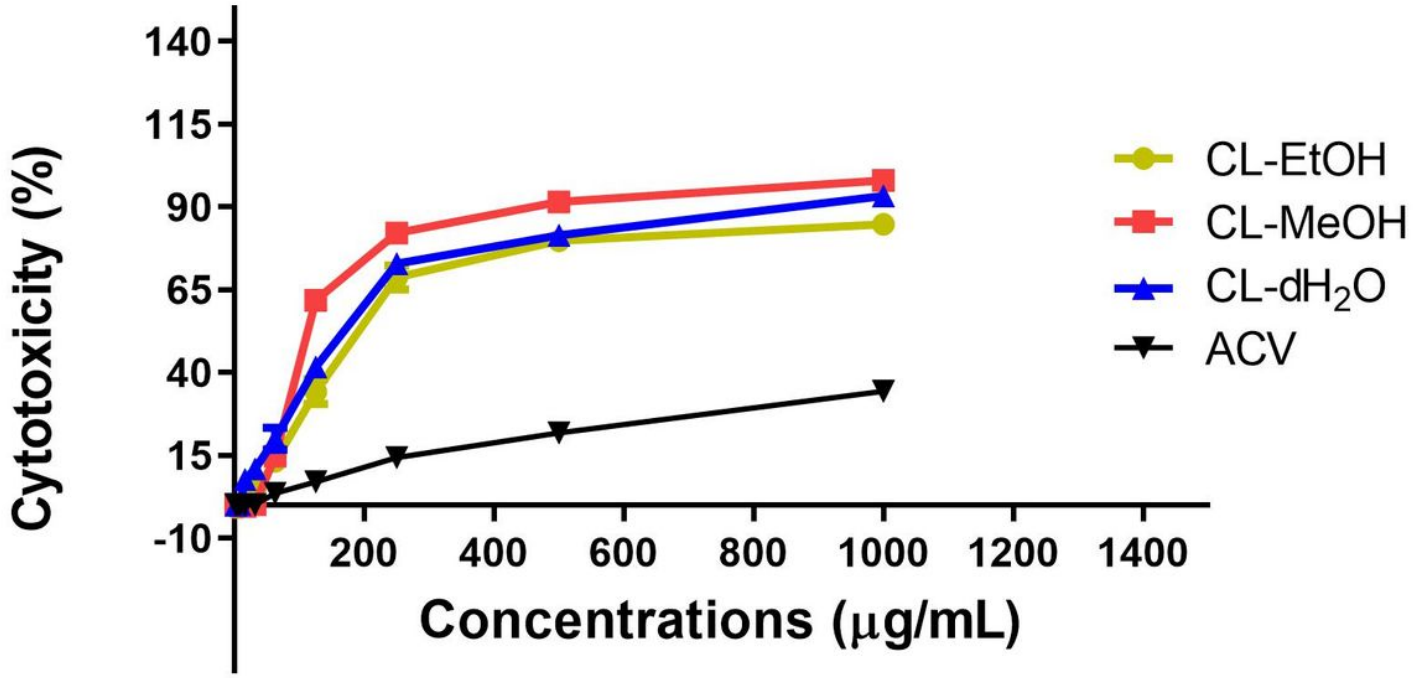

Figure 2

Cytotoxicity of crude extracts of *C. laurifolius*and ACV on Vero cell.

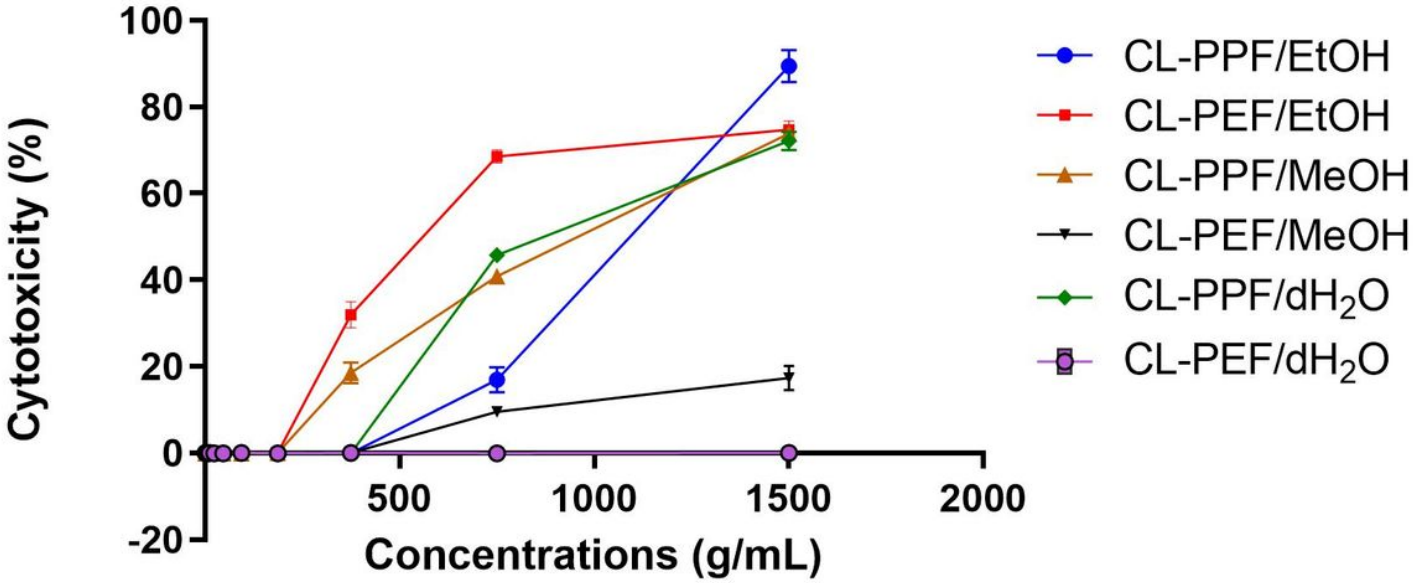

Figure 3

Cytotoxicity of polyphenol-poor fraction and a polyphenol-enriched fraction of *C. laurifolius*.

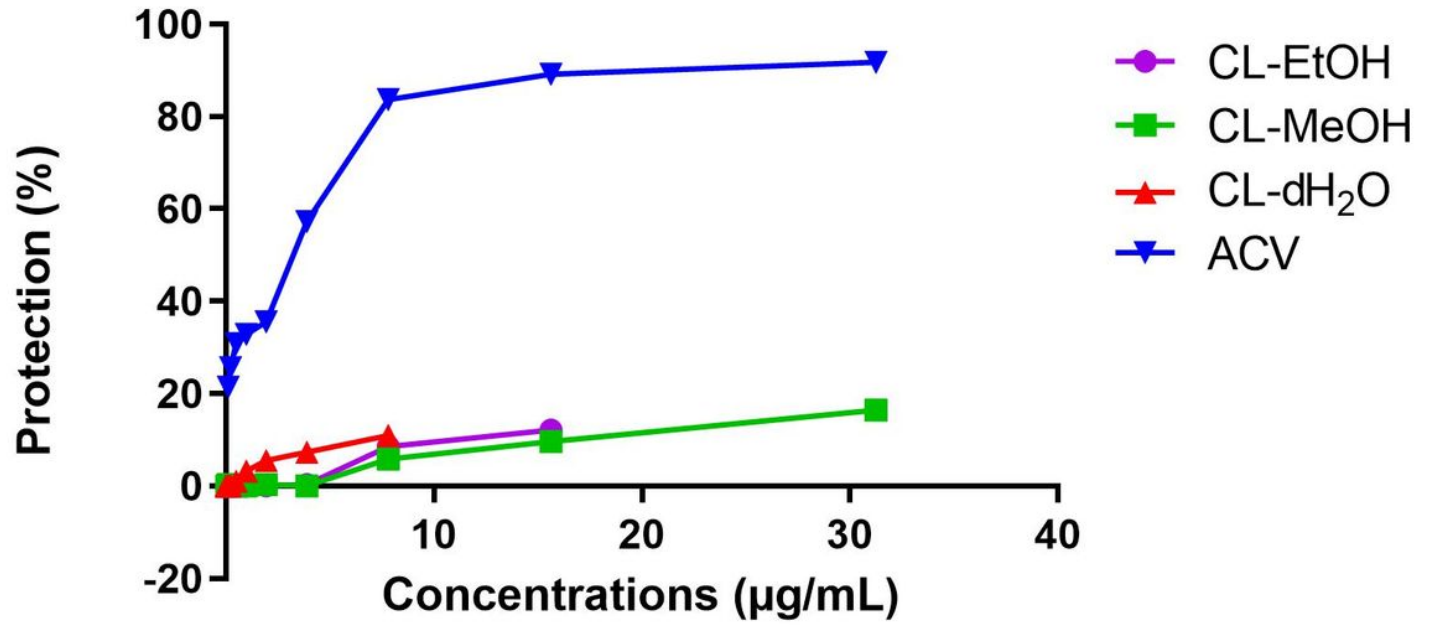

Figure 4

Antiviral activity of crude extracts of *C. laurifolius* and ACV.

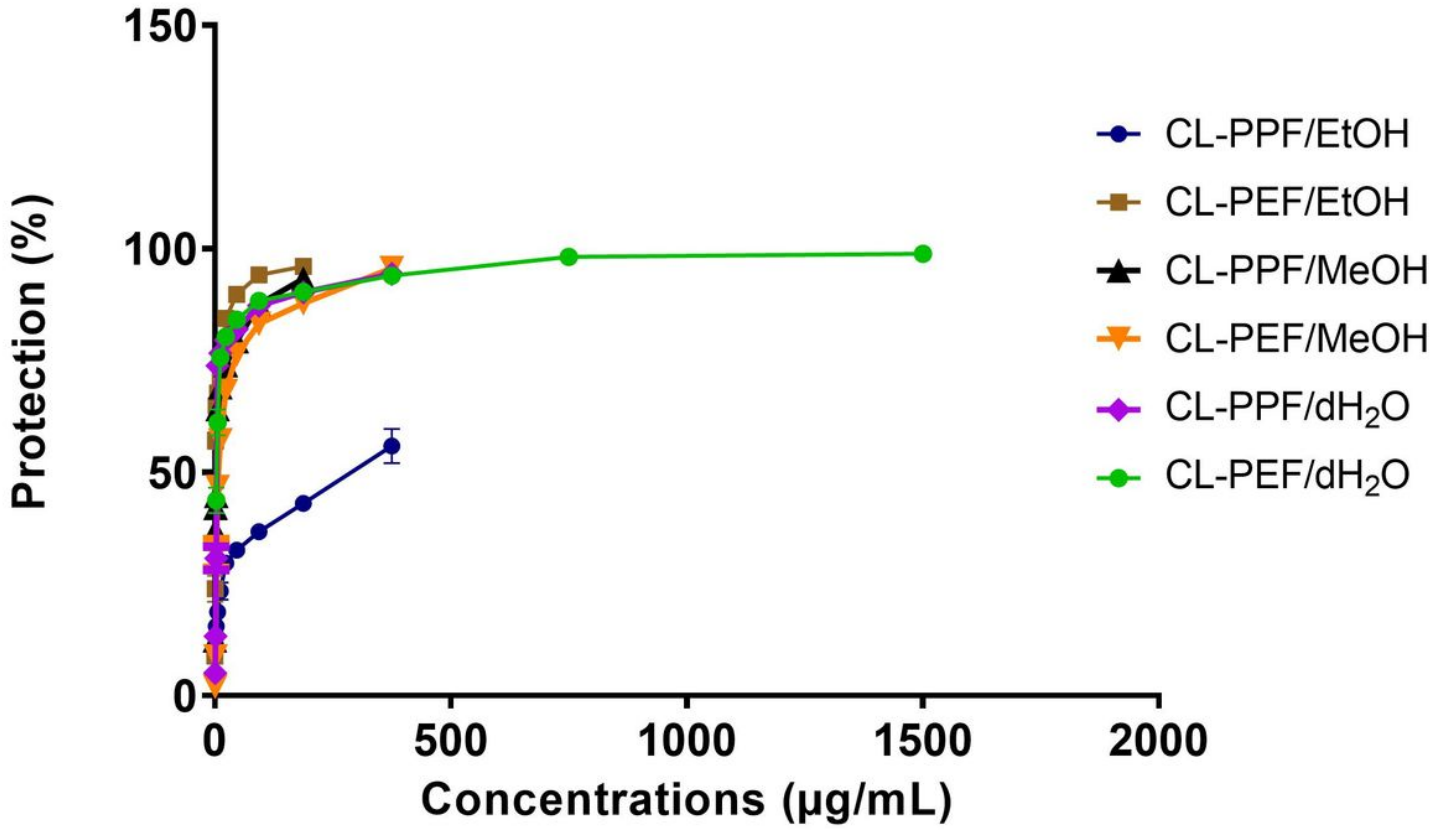

Figure 5

Antiviral activity of polyphenol-poor fraction and a polyphenol-enriched fraction of *C. laurifolius*.

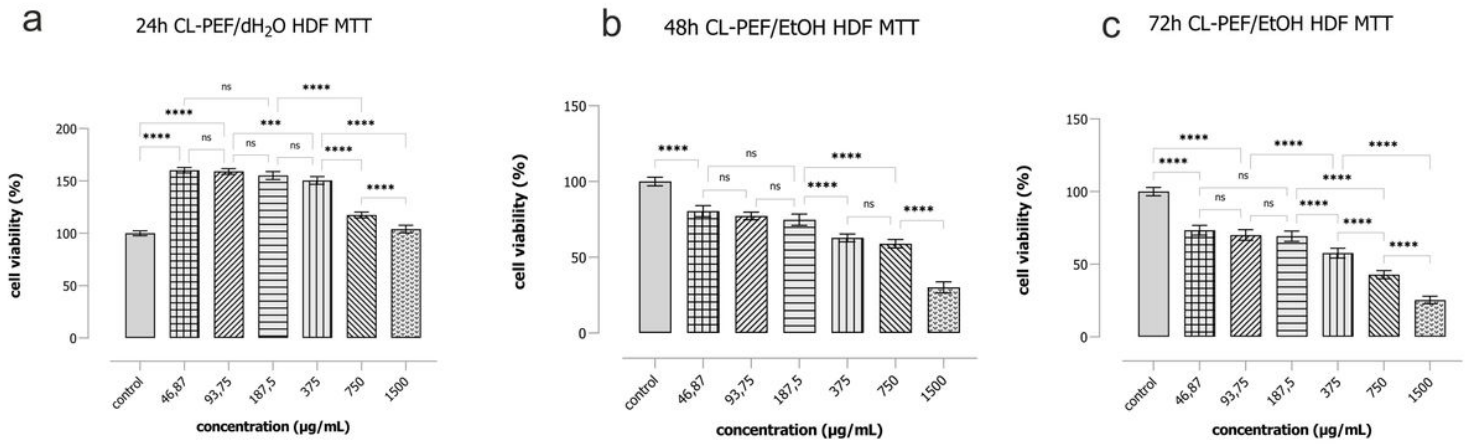

Figure 6

Cytotoxicity of CL-PEF/EtOH fraction on HDFa cells for 24 (a), 48 (b), 72 (c) h.

* P <0.005, ** P <0.01, *** P <0.001, **** P <0.0001.

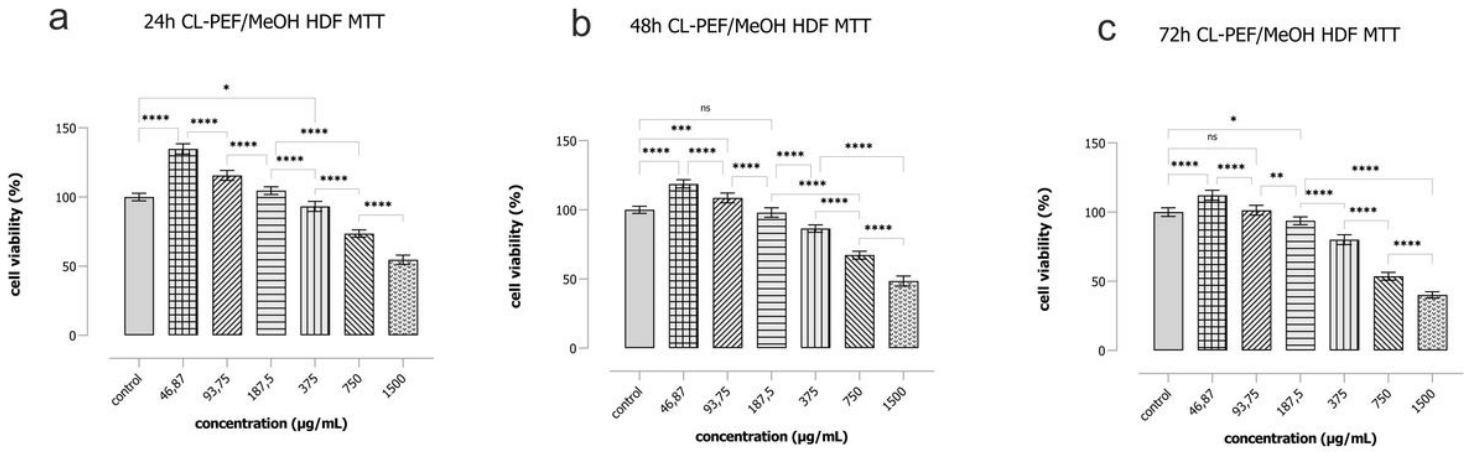

Figure 7

Cytotoxicity of CL-PEF/MeOH fraction on HDFa cells for 24 (a), 48 (b), 72 (c) h.

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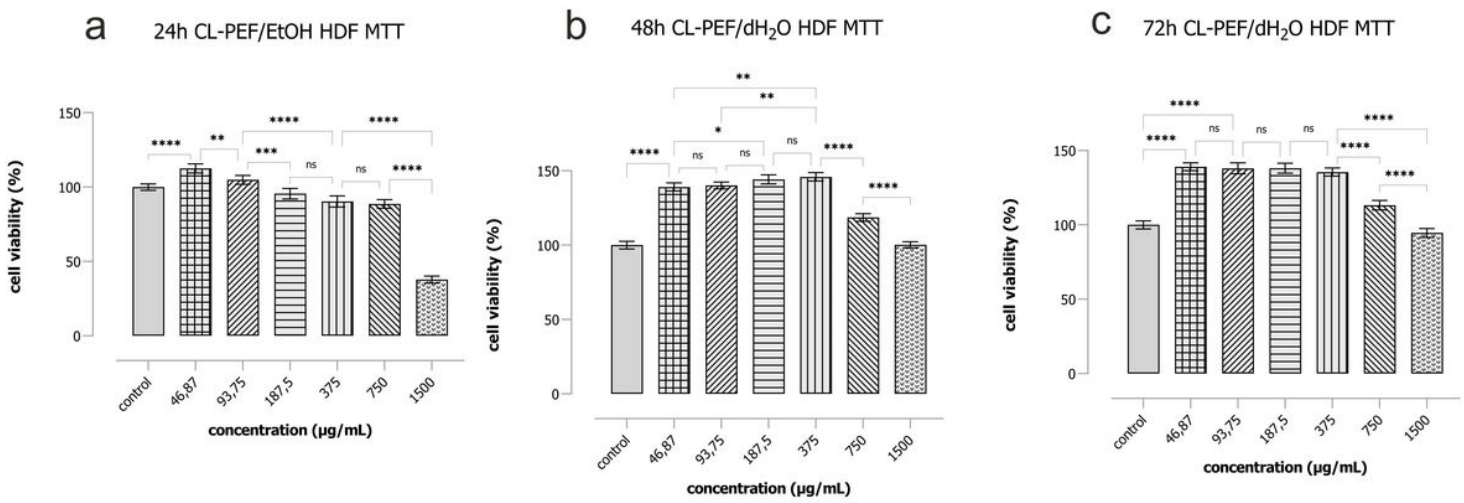

Figure 8

Cytotoxicity of CL-PEF/dH_2O fraction on HDFa cells for 24 (a), 48 (b), 72 (c) h.

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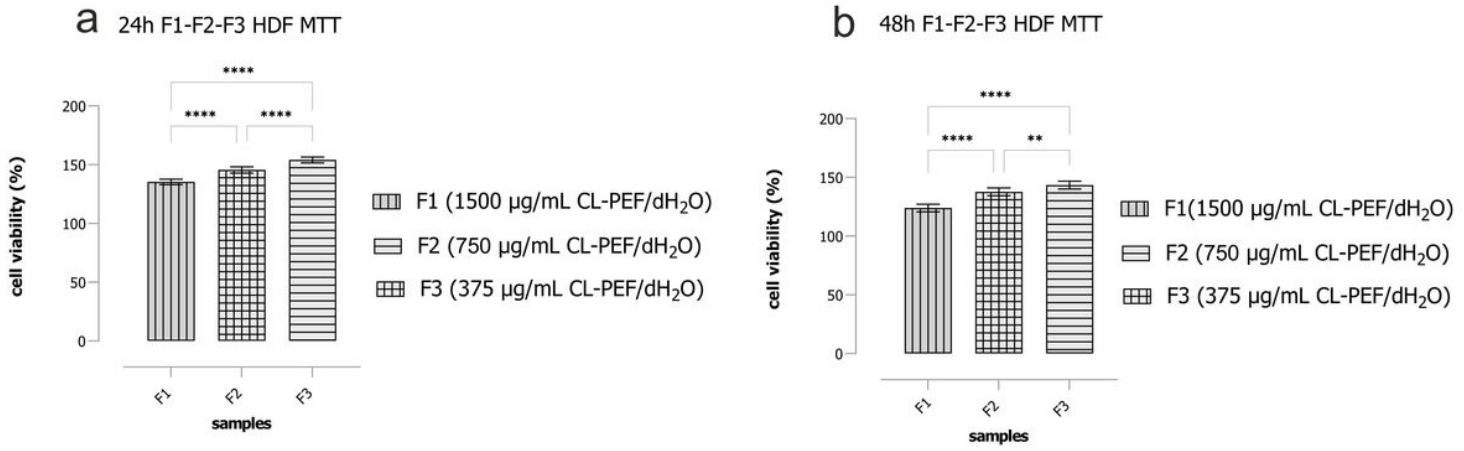

Figure 9

Biocompatibility of herbal lip balm formulation

** P <0.01, *** P <0.001, **** P <0.0001

Supplementary Files

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