SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

# Biocompatbility, *in vitro* and *in vivo* testing of pharmaceutical excipients and external dosage forms

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# 1. Introduction

Nowadays, natural products are getting more and more popular among patients due to their efficacy and few side effects. Pharmacies have a growing range of herbal supplements and medicines. However, natural does not necessarily mean better or safer. Growing practice of the herbs must happen carefully, the processing is a challenging task and in case of many herbs we only have limited information in scientific literature, mostly the experiences of folk medicine are available. Beside the extraction from the herb, the identification of active components and the standardisation of the active ingredient content are essential.

Beside natural products, the investigation of new pharmacons and drug candidates is also an important and rapidly developing area, as there are many diseases and indications for which no suitable treatment is available or the current therapy is not satisfactory and a more effective, and well-tolerated medicine with less side effect is needed.

Transdermal drug delivery is an interesting and challenging area of pharmaceutical technology. Rapid advances in modern technologies are leading to an increasing proportion of drugs being delivered transdermally, including drugs that can be well tolerated by conventional therapeutic methods. Transdermal drug delivery has several advantages, bypassing the gastrointestinal tract, thereby avoiding the liver's first-pass effect and both local and systemic effects can be achieved. It enables local treatment of certain conditions with fewer side effects and is a non-invasive, painless treatment, patients are able to self-administer the drug, thus transdermal administration is one of the most preferred routes. This translates into increased patient compliance, especially for long-term treatment.

Ointments and transdermal therapeutic systems are the most commonly used pharmaceutical dosage forms for dermal drug delivery. With ointments, the aim is to achieve a local effect by treating the skin surface or deeper skin layers, whereas with transdermal therapeutic systems the aim is to deliver the active substance into the systemic circulation. Based on their effects, there are covering or protective ointments, wound ointments, penetration ointments and resorptive ointments. The main purpose of covering ointments is to protect the skin. Wound ointments are used to treat damaged skin. For these two groups, as well as for penetration ointments, the aim is to penetrate into the upper layers of the skin tissue. Resorption ointments are a special group, since the aim of these preparations is to ensure that the active substance is absorbed into the systemic circulation (e.g. in the treatment of rheumatic arthritis, bronchitis). Transdermal administration involves the use of therapeutic systems that allow the continuous

release of various active substances through the skin into the systemic circulation according to a predetermined programme. The most common transdermal indications include pain relief, hypertension treatment, hormone replacement, hormonal contraception, smoking cessation. The main limitation of these therapeutic systems is the short list active substances that can be administered due to the barrier function of the skin. A transdermally administered active substance should fulfil the following criteria: low molecular weight (<400 Da), adequate lipophilicity (log P o/v~1-4), half-life max. 10 hours, effective at low doses (about 20 mg/day), low melting point, non-irritating to the skin. Due to these limiting factors, transdermal drug delivery is an area of intensive research.

Knowledge of the anatomical structure of the skin is crucial, as it greatly determines our ability to deliver drugs through the skin. The skin can be divided into 3 layers: epidermis, dermis and subcutis. The epidermis has several layers, the outermost is stratum corneum (SC) where the cells are closely connected. Due to the high keratin content of the cells, substances with apolar character can penetrate the SC, but only substances with a certain hydrophilic property can pass through the SC, which enables them to penetrate the deeper, water-containing layers of the epidermis. This is crucial for drug delivery, the above-mentioned properties of SC make dermal drug delivery a challenging task.

The absorption of active components through the skin can be enhanced by the addition of penetration enhancing excipients. Another important key to improve the penetration of active ingredients is the selection of an appropriate vehicle system.

**BGP-15**

BGP-15 is a nicotinic amidoxim derivative, a Hungarian-developed drug candidate, which was originally intended to alleviate neuro-, nephro- and myelotoxic effect of different cytostatic preparations. In the past few years, several other beneficial effects of the drug have been revealed, including the insulin sensitizing effect and some scientific publications also mention its potential anti-inflammatory effect. In addition, its cardioprotective properties are under intense research, with promising results in the treatment of heart failure, atrial fibrillation and atrial cardiac enlargement. Its effects in promoting skeletal muscle regeneration and preventing muscle damage have been investigated in several areas, with considerable attention paid to the preservation of diaphragm function. It may be a useful and promising adjuvant in the treatment of Duchenne muscular dystrophy and its efficacy has been demonstrated in animal studies in mice with muscular dystrophy.

BGP-15 has entered to clinical phase II. in the indication of diabetes, but the determination of the proper indication is still ongoing, as we still have little knowledge about the drug. As for the matter, the exact mechanism of the effect is still unknown, though many research groups are studying BGP-15. As mentioned above, potential anti-inflammatory effect is attributed to the drug but it's an under-investigated field. Topical application of the drug is also a less investigated research area, as well as the external anti-inflammatory effect of BGP-15. Farkas et al. have formulated creams of BGP-15, though the indication was slightly different. They investigated the photoprotective effect of the ointments, by pretreating mice with the preparations, then exposed the animals to direct UV radiation. They have found that the creams reduced the number of sunburn cells significantly and they observed the down-regulation of epidermal cytokines IL-10 and TNF-α. Considering the above mentioned properties, investigating the possible anti-inflammatory effect of BGP-15 in external dosage forms could complete our current knowledge and provide further useful information about the drug.

***Philadelphus coronarius***

*Philadelphus coronarius (P. coronarius)* belongs to Hydrangeaceae family, in the order of Cornales. *P. coronarius* is a less studied plant, but it is commonly used in folk medicine for a number of diseases, although the relevant scientific literature is very limited; only a few articles are available about its chemical elements and pharmacological characteristics. Previous chemical studies have revealed the flavonoid, triterpene, coumarin and phenolic-acid content of the herb. *P. coronarius* is said to exhibit antibacterial effects, which is confirmed by the experience of folk medicine using it in the treatment of different gynaecological illnesses. The external application of the plant has never been investigated, even though it could be beneficial based on the above-mentioned facts, especially utilizing both the flower and the leaf.

As the subject of my PhD thesis a drug candidate pharmacon, BGP-15 and a herb, *P. coronarius* was selected. Utilizing them, external dosage forms were formulated, highlighting the differences in the steps of formulation due to the different character of the active ingredients. My main focus during formulation was to increase bioavailability and biocompatibility. Testing the potency of the selected active ingredients was also an important part of my research.

# 2. Aims

The aim of my experimental work was to develop an external dosage form incorporating a synthetic and natural active ingredients to highlight the similarities and differences in the steps of formulation due to the different character of the active ingredients. Increasing bioavailability of the active ingredients and achieving adequate biocompatibility of the formulations were also in the focus of the research.

In the first phase of the experiments, external dosage forms were formulated and tested utilizing BGP-15 drug candidate. For this purpose, different ointment formulations were determined and appropriate excipients were selected and biocompatibility testing was performed. The ointments were studied from several aspects, Franz diffusion study and texture analysis. The anti-inflammatory activity of the active ingredient was tested *in vitro* and *in vivo*, and its antioxidant properties were also investigated.

In the second phase of the experiments, extracts of the leaves and flowers of the herb *P. coronarius* were prepared and characterized by identification of bioactive components, determination of drug content and antioxidant capacity. From the extracts, lyophilized extracts were prepared and their activity was investigated from several aspects. Ointments were formulated from the lyophilized extracts, texture analysis and studied the in vitro release was studied as well.

# 3. Materials and methods

## 3.1. Materials

SP50, SP70 and PS750 sucrose esters were kindly gifted by Sisterna (Roosendaalc, The Netherlands). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT paint), Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffered saline (PBS), Trypsin-EDTA, heat inactivated fetal bovine serum (FBS), L-glutamine, non-essential amino acids solution, penicillin-streptromycin, allyl-isothiocyanate (AITC), LPS were purchased from Sigma Aldrich. 96 well plates, culturing flasks were purchased from Corning (Corning, New York, USA). Cetostearyl alcohol, propylene glycol, stearic acid, isopropyl myristate, conservant solution were obtained from Hungaropharma Ltd. (Budapest, Hungary). HaCaT cells were supplied from Cell Lines Service (CLS, Heidelberg, Germany). BGP-15 was purchased from SONEAS Chemicals Ltd. (Formerly known as Ubichem Pharma Services) Illatos street 33, Budapest, H-1097, Hungary. Cremophor A6, A25 was purchased from BASF (Ludwigshafen, Germany). Transcutol was a kind gift from Gattefossé (Lyon, France). Firefly luciferase substrate was purchased from Promega.

## 3.2. Módszerek

### *3.2.1. Biocompatibility investigations*

*MTT assay*

To evaluate cytotoxicity of the selected excipients and the formulated ointments, MTT assay was performed. The experiments were carried out on HaCaT cell line. HaCaT cells are human immortalized keratinocytes, thus they perfectly represent human skin. The cells were maintained by weekly passages in Dulbecco's DMEM culture media. For MTT assay, the cells were seeded on a 96-well plate in the density of 10.000 cells/well. When the cells fully grow over the well's membrane, the experiment is ready to perform. First, we removed the culture media, then we applied the test solutions and incubated the cells with them for 30 min. After 30 min we removed the test substance and added MTT paint solution at 5 mg/mL concentration to the cells (tetrazolium bromide). Then, we let it incubate with the cells for 3 h. The viable cells will transform the water-soluble tetrazolium bromide into formazan precipitate. When the incubation is done, formazan precipitate was dissolved with the isopropanol:hydrochloric acid = 25:1 ratio. Then, the absorbance of these solutions was measured by spectrophotometer (Fluostar Optima) and is directly proportional to the number of viable cells.

### *3.2.2.Ointment formulation and dosage form studies*

*Ointment formulation*

Different emulsifiers were incorporated into the formulations: sucrose esters (SP50, SP70, PS750) Tefose 63, Sedefos 75, Labrasol, Cremophor A6:A25. The ointments were produced by melting stearic acid, cetostearyl alcohol, isopropyl myristate and mixed to prepare the oily phase of the formulation. The aqueous phase contained propylene glycol, emulgent, glycerol, and purified water, and was heated to the same temperature as the oil phase (~60 °C), mixed together and cooled down to room temperature. After that, the active substance and conservant solution was added to the preparation. The active substance was previously dissolved in a 1:1 mixture of Transcutol and purified water.

*Texture analysis*

Resistance of the creams was evaluated with the help of CT3 Texture Analyzer (Brookfield, Middleboro, MA, USA). During the investigation, a compression test was performed in normal mode with the following settings: target value (5 mm), target load (4 g), target speed (0.5 mm/s)

*In vitro release with Franz diffusion chamber*

*In vitro* release test was performed with the help of a Franz diffusion-chamber apparatus. During the test, a membrane is placed between the donor and the acceptor phase. The concentration profile of the test substance is obtained by taking samples at predetermined times. Samples weighing 300 mg were placed on artificial cellulose-acetate membrane (0.5 μm pore size) as the donor phase, and as the receptor phase, pH = 5 buffer was chosen in order to imitate the pH of the skin. The membrane was pretreated with isopropyl myristate to characterize the lipophilic property of the skin. The rotation of the magnetic stirrer was 450 rpm. To imitate the temperature of the skin, the receptor phase was held at 32 °C. BGP-15 content was measured with mass spectrometry, while *P. coronarius* content was measured with spectrophotometry.

### *3.2.3.Investigation of efficacy*

*Luciferase assay*

Raw 264.7 cells stably transfected with the pNFκB-Luc/neo. reporter construct were plated at $6 \times 10^4$/well on luminescent assay plates in 200 μL of DMEM/F12 medium supplemented with 10% heat-inactivated FBS. After one-day culturing, the cells were treated with 100, 10 and 1 μM BGP-15 (dissolved with different emulsifiers (3 *w*/*w*%): SP50, SP70, PS750 in PBS) with or without LPS (100 ng/mL). After 6-h incubation (37 °C; 5% $CO_2$), media was removed; cells were washed with 200 μL PBS/well and lysed with 20 μL Cell Culture Lysis Reagent/well for

10 min. After adding the firefly luciferase substrate (20 μL/well), luciferase activity was measured with Luminoscan Ascent Scanning Luminometer. Cell viability was routinely determined using trypan blue exclusion test during the assays to make sure that assays were always carried out on viable cells.

*Inflammation Model Induced by Allyl-Isothiocyanate (AITC)*

Animals were premedicated with BGP-15 before inducing inflammation. BGP-15 treatment happened by applying either ointments or solutions to mice ears. Both the solution and the ointments contained 10 *w*/*w*% BGP-15.

Anesthesia was induced by isoflurane (3–5%) with the help of a desktop anesthesia instrument, which made the process safe for the animals. Before each treatment and measurement mice were anesthetized. Inner and outer surface of mice's left ear was smeared with 1% AITC dissolved in paraffin oil. This treatment happened 30 min later than the premedication with the ointments. After the ointments permeated into the skin (~30 min), AITC was applied and ear thickness was measured every 10 min

*Antioxidant Assay-Superoxide Dismutase (SOD) Assay*

The antioxidant activity of BGP-15 was evaluated on HaCaT cell line, in different concentrations: 1; 5; 10; 50; 100 mg/mL. The solutions were prepared with PBS. As a negative control, PBS was selected. Cells were seeded on 24well plate with the cell density of 50,000/well and grown in $CO_2$ incubator for 7 days at 37 °C. During the experiment, culture media was removed and the cells were incubated with the sample solutions for 1 h. For the experiment, artificial UVB radiation for 10 min was used to cause oxidative stress, induce free radical production after the treatment with BGP-15. Then, cells were collected with the help of a rubber policeman and centrifuged for 10 min, 1000 rpm, 4 °C. Cell pellet was homogenized in HEPES buffer and centrifuged again for 15 min, 10,000 rpm, 4 °C. Antioxidant activity of the supernatant was investigated with Cayman assay kit based on the instructions of the manufacturer. The experiments were performed in triplicate.

*Total Antioxidant Capacity (TAC)*

Twenty-weeks-old rats were assigned to the following three groups CMP3, CMP4, CMP5. First, rats were shaved, on the second day animals were treated with different ointments containing BGP-15 for 24 h. On the third day, the animals were treated on another skin region with ointments for one hour. After the treatment, animals were sacrificed with ketamine–xylazine overdose. Three skin samples were obtained from each animal, one from the area

treated for 24 h, one from area treated for one hour and one from untreated area, which served as control. Total antioxidant capacity measurements were carried out with antioxidant assay kit according to the manufacturer information. Briefly, approximately 100 mg of samples were homogenized with assay buffer, and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatants were used for the assay. The absorbance was measured at 405 nm using a MutiscanGo microplate spectrophotometer. Values are expressed as a percentage of the value of the untreated region.

### *3.2.4. Preparation of extracts and physico-chemical characterization*

*Preparation of Lyophilized Products*

Fresh leaves and flowers of *P. coronarius* were collected from the garden of University of Oradea, Faculty of Medicine and Pharmacy. A specimen of the *Philadelphus coronarius* L. species, young stem, leaves and flowers were kept in the Herbarium of the Faculty of Medicine and Pharmacy Oradea, Romania, registered in NYBG Steere Herbarium, UOP code 05313. With the purpose of obtaining the fluid extract, the maceration–extraction process at 20 °C was performed. The collected parts of the plant were dried in a dry room and protected from sunlight at room temperature. The product was fragmented and sieved with a No. III. pharmaceutical sieve. For the extraction, a mixture of ethanol and distilled water was chosen as a solvent (30 $^{v}/_{v}$%). The mass ratio of the product to the solvent was 1:10 ($^{m}/_{m}$%). The extract was cleared from ethanol with the help of a rotary evaporator, Hei VAP Precision-Platinum 3, at 40 °C temperature, 80 rpm and 200 mBars. The remaining aqueous phase was frozen to −80 °C and then lyophilized (ALPHA 2-4 LSC plus lyophilizer) to gain a solid, water-soluble product

*Physicochemical Characterisation by HPLC-PDA Method*

Identification and quantification of the bioactive compounds from *P. coronarius* extract were performed by using a Shimadzu Nexera-i LC–2040C 3D plus liquid-chromatograph system equipped with a photodiode array detector (PDA). A Phenomenex C18 (2) 100 A, 150 mm × 4.6 mm × 5 μm column was selected, and it was kept at 30 °C temperature. The mobile phases used for elution contained methanol (A) and formic acid 0.1% (B). The gradient program used was: 5% A and 95% B from 0 to 3 min, 25% A and 75% B from 3 to 6 min, 37% A and 63% B from 9 to 13 min, 54% A and 46% B from 18 to 22 min, 95% A and 5% B from 26 to 29 min and 5% A and 95% B from 30 to 36 min. The flow rate was 0.5 mL/min, and the injection volume was 10 μL. The detection was performed at multiple wavelengths: 254, 270, 275, 326, 337 and 360 nm. The polyphenols from the extract were identified by comparing the retention times from the extract chromatograms with the ones from the standard-solution chromatograms.

*Phytochemical Investigation of the Lyophilized Extract*

Bioactive-compound content of *P. coronarius* extracts (respectively, the total polyphenol and total flavonoid contents) were assessed. In order to calculate the total polyphenol content, the Folin–Ciocâlteu method was selected. It is based on the electron-transfer reaction, measuring the reductive capacity of an antioxidant. The outcomes of the Folin–Ciocâlteu method were very well correlated with the results attained from other antioxidant studies, such as ABTS and DPPH. Total polyphenol content was calculated as the gallic-acid equivalent (GAE/100 g) of dried plant based on the calibration line of gallic acid (5–500 mg/L, $Y = 0.0027 \times -0.0055$, $R^2 = 0.9999$). All determinations were performed in triplicate. For the assessment of total flavonoid content, the aluminium-chloride colorimetric method was selected. Quercetin was used for the standard calibration curve. The stock quercetin solution was made by dissolving 5 mg quercetin in 1 mL methanol, and afterwards a standard quercetin solution preparation with serial dilutions using methanol (5–200 μg/mL). Absorbance was measured at a wavelength of 420 nm with an UV–Vis Varia spectrophotometer. Total flavonoid concentration was calculated according to the calibration line ($Y = 0.0162\times + 0.0044$, $R^2 = 0.999$) and expressed as the quercetin equivalent (QE) mg/100 g in dried herb. All determinations were performed in triplicate.

*Evaluation of Antioxidant Activity by DPPH*

The assay is based on the ability of the free radical DPPH to change its color in the presence of antioxidants. Antioxidant capacity of P. coronarius leaf and flower was tested. Samples were reacted with DPPH free radical in ethanol (96%). Reaction mixture contained 100 μL of P. coronarius leaf or flower test solution, 900 μL of ethanol and 2 mL of DPPH solution (0.06 mM). The reaction mixtures were incubated for half an hour. When DPPH reacts with an antioxidant, it changes its color from dark violet to yellow. Measurement of the remaining DPPH quantity was completed with UV spectrophotometer (Shimadzu Spectrophotometer, Tokyo, Japan) at the wavelength 517 nm. Purified water and absolute ethanol acted as background in 1:2 ratio.

*Evaluation of Antioxidant Activity by FRAP Method (Ferric-Reducing Antioxidant Power)*

FRAP method is a simple spectrophotometric technique that evaluates the antioxidant power of the test substance, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reducer at an acidic pH. The stock solutions consisted of: 300 mM acetate buffer; 270 mg $FeCl_3 \cdot 6\ H_2O$ dissolved in 50 mL distilled water; 150 mg TPTZ and 150 μL HCl, dissolved in 50 mL distilled water. FRAP solution was prepared by mixing 50 mL acetate

buffer, 5 mL $FeCl_3 \cdot 6\ H_2O$ solution and 5 mL TPTZ solution. Trolox was selected as a standard solution and absorbance was detected at 595 nm.

*Evaluation of Antioxidant Activity by CUPRAC Assay*

We added 0.25 mL $CuCl_2$ solution (0.01 M), 0.25 mL ethanolic neocuproine solution ($7.5 \times 10^{-3}$ M) and 0.25 mL $CH_3COONH_4$ buffer solution (1 M) and mixed the samples. Then, we adjusted the total volume to 2 mL with distilled water and mixed thoroughly, sealed the tubes, and kept them at room temperature. Absorbance was measured at 450 nm against a reagent blank 30 min later. Increased absorbance of the reaction mixture indicated increased reduction capability.

*Evaluation of Antioxidant Activity by ABTS Assay*

ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) was used to determine antioxidant activity of different compounds. ABTS assay is able to measure the ability of antioxidants to scavenge ABTS, compared to Trolox standard. ABTS was dissolved in water at a 7 mM concentration. ABTS radical cation was produced by reacting the stock solution of ABTS with potassium persulfate and letting the mixture stand in a dark room overnight. $ABTS^+$ and the samples were diluted with ethanol. A total of 10 µL of diluted sample (or Trolox standard) was added to 1 mL of $ABTS^+$. Absorbance values were measured with a spectrophotometer (Fluostar Optima) at a wavelength of 734 nm.

### *3.2.5. Investigation of the effects of the herb*

*Antimicrobial testing of the lyophilized extracts by microdilution method*

Antifungal- and antimicrobial-tendency testing was carried out by using microdilution method against *C. albicans, P. aeruginosa, E. coli* and *S. aureus*. The experiment was performed by using 96-well standard microtiter plates and the concentrations of the tested compounds were prepared in RPMI-1640 and Mueller–Hinton medium for fungal species and bacteria. All test samples were prepared 10 min before starting the incubation. The final volume of each well contained 100 µL of the test compound and 100 µL of the fungal or bacterial inoculum. Plates were incubated for 24 h at 37 °C. After incubation, absorbance was measured at 492 nm and 600 nm. Inhibition was determined based on turbidity, in terms of at least a 50% growth reduction compared with the test-compound-free control.

*In Vitro Time-Kill Antimicrobial Test*

During antimicrobial testing, *E. coli*, *S. aureus* and *C. albicans* were chosen as reference strains. Activity of *P. coronarius* leaves and flower (dissolved in the relevant broth) was determined

against *C. albicans* and bacteria strains in RPMI-1640 and Mueller–Hinton broth at 5 $^{w}/_{w}$ % concentration with a starting inoculum of $10^5$ cells/mL, in a final volume of 5 mL. In the case of *C. albicans,* 100 µL aliquots were removed at 0, 4, 8, 12, 24 h of incubation, ten-fold dilution series were prepared, and sample dilutions (4 × 30 µL) were plated onto Sabouraud dextrose agar plate and incubated for 48 h at 35 °C. In the case of *E. coli* and *S. aureus,* 100 µL aliquots were removed at 0, 2, 4, 6, 8, 10, 12, 24 h of incubation, ten-fold dilution series were prepared, and sample dilutions (4 × 30 µL) were plated onto Mueller–Hinton plates and incubated for 48 h at 35 °C.

*Investigation of IL-4, Enzyme-Linked Immunosorbent Assay (ELISA)*

To investigate if *P. coronarius* flower or leaves have any effect on inflammation, ELISA was performed on HaCaT cells. Cells were seeded on 96-well plates in the density of 10.000 cells/well. When the cells fully grow over the wells' membrane, the experiment is ready to perform. Culture media was removed, then the cells were incubated with the samples for 1 h. Samples were made of lyophilized *P. coronarius* leaves or blooms dissolved in PBS, then filtrated (0.2 µm). Samples were prepared in different concentrations: 1 $^{w}/_{w}$%, 3 $^{w}/_{w}$%, 5 $^{w}/_{w}$%. After samples were removed, 50 µL of TNFα (20 ng/mL) and 50 µL of IL-1β (25 ng/mL) were added to the cells and incubated with them over night. The next day the supernatant was removed, and a human IL-4 ELISA kit was performed according to the manufacturer's instructions.

# 4. Results

### 4.1. Biocompatibility testing of excipients

Cytotoxicity of the selected excipients was tested on HaCaT cell line. Cell viability was compared to the negative control (PBS). A positive control, Triton-X 100 was also used in order to make sure that the test works correctly.

**Sucrose esters** were dissolved in PBS and they were tested in 1% and 3% concentrations. According to the results, SP50 in 1% concentration is the safest of all sucrose esters, but all of them proved to be safe and non-toxic in the investigated concentration range because the cell viability values are above 70% in each case.

To test the cytotoxicity of **Transcutol**, solutions of different concentrations were prepared with PBS. The samples were non-toxic, cell viability was above 70% in all cases, however a decrease in cell viability values was observed with increasing concentration.

In case of **Labrasol**, cell viability values were above 70%, 72% at 0,10% concentration. For the experiment, a dilution series of the surfactant was prepared with PBS. The cytotoxicity of the substance shows an increasing tendency with increasing concentration.

For the MTT test of **Cremophor A6:A25** the excipient was tested in different concentrations, based on the results cytotoxicity increases with increasing concentration. The 3% concentration was used to formulate the ointment, in this concentration cell viability was above 70%, but when used at 5%, cell viability is under 70%.

In the case of **Tefose 63** surfactant, the toxicity was lower than in the previous case. In the tested concentration range, the excipient is safe to use, as cell viability was above 70% in each case, even at 5% concentration the cell survival rate was 85%.

In the MTT test of **Sedefos 75**, the excipient was safe in all concentrations, with cell survival rates above 70%.

### 4.2. Ointment formulation

A total of 11 formulations were prepared during the formulation of the ointments, of which 5 contained BGP-15, 3 contained lyophilized P. coronarius leaf extract and 3 contained lyophilized P. coronarius flower extract. Additional compositions were created by varying the emulsifiers.

### 4.3. Texture analysis of BGP-15 ointments

Based on the compression force values, those formulations, which contain sucrose esters (CMP 3, 4, 5) have harder consistency. The formulation made of Labrasol (CMP 1) has the softest consistency, while the hardest is prepared with SP50 (CMP 3). Lower resistance values are preferable because of the easier applicability and better liberation of the active pharmaceutical ingredient.

### 4.4. *In vitro* release of BGP-15 ointments

Those compositions, which contained sucrose esters produced better results. The best diffusion was achieved by CMP 3 (contains SP50). The second best result was shown by CMP 5 (contains PS750) and the lowest release rate was achieved by CMP 2 (contains Cremophor A6:A25). CMP 1 (contains Labrasol) showed a slowly increasing tendency.Our results showed that the best release rate was achieved by those formulations that contained sucrose esters. Release profiles of ointment compositions were compared to each other. Difference factors were also calculated. Two formulations are considered to be different if their difference factor (*f1*) is between 0–15. CMP3 and CMP5 are different according to the calculation.

### 4.5. Cytotoxicity of BGP-15 ointments

An MTT assay of the ointments was performed with samples prepared by Franz diffusion chamber apparatus using pH = 5 buffer as the receptor phase. A total of 1 mL of samples were taken out of the receptor phase after 6 h of incubation. CMP 3 showed the best results, but all preparations were non-toxic according to these results. Cell viability is over 70% in every case.

### 4.6. Luciferase assay

The in vitro anti-inflammatory effect of BGP-15 was measured in mice macrophages through the response of NF-κB. During the experiment, the selected Raw264.7 cells were stably transfected with the pNFκB-Luc/neo. reporter construct. The effect of BGP-15 on the NF-κB response of macrophages and the NF-κB of LPS-activated macrophages was investigated. With the experiment, we tried to verify if BGP-15 treatment could reduce the macrophage's inflammatory factor (e.g., NF-κB) production. BGP-15 was applied in different concentrations (1, 10 and 100 μM). The measured luminescence was proportional to the activity of the transcription factor NF-κB. According to the results, it appears that BGP-15 treatment was unable to reduce the LPS-activated macrophages' NF-κB response, nor was it able to result in significant decrease in macrophage activity.

### 4.7.AITC induced inflammation model with BGP-15 ointments

All three ointments that contained BGP-15 significantly decreased ear thickness compared to the positive control (AITC by itself). CMP 4 (the ointment prepared with SP70 and BGP-15) shows the largest decrease of ear edema. Both CMP 3 and 5 (the ointments made of SP50 or PS750+ BGP-15) show similarly good results. Those preparations that did not contain the active ingredient (BGP-15) were not able to significantly reduce ear thickness, nor did the aqueous solution containing only BGP-15. This leads us to the conclusion, that BGP-15 has an anti-inflammatory effect but the aqueous solution could not penetrate into skin, while the ointments with the penetration enhancers were able to penetrate and prevent the inflammation.

### 4.8. Antioxidant activity of BGP-15

The potential antioxidant effect of BGP-15 was tested by two methods, *in vitro* SOD activity and *in vivo* total antioxidant capacity (TAC).

The function of an SOD enzyme is to protect cells from superoxide toxicity, which is one of the main reactive oxygen species (ROS) produced if cells are exposed to oxidative stress (e.g., UVB radiation). BGP-15 solutions, prepared with PBS in different concentrations, were selected for *in vivo* antioxidant assays. The SOD enzyme activity of the group that was not exposed to UVB radiation was taken as 100%. The SOD activity of the treated groups were compared to the enzyme activity of the group that was not exposed to UVB radiation. The groups where the cells were previously treated with BGP-15 solution, SOD enzyme activity was decreased, similarly to the group that was treated with PBS only. The decreased level of SOD enzyme activity may be the consequence of the intense oxidative stress caused by UV B radiation, which resulted in severe cell damage. Pretreatment of the cells with any concentration of BGP-15 was not able to prevent the decrease in the SOD enzyme level.

The result of TAC measurement showed no significant alteration with CMP 3 (SP50) and CMP 4 (SP70); however, it decreased with CMP 5 (PS750). Since no alterations were seen with the other two ointments, we speculate that it is probably due to the surfactant of the ointment.

### 4.9. Bioactive compound content of *P. coronarius* extracts

During the investigation many phytochemicals were identified by HPLC method. The leaf of *P. coronarius* contains a high amount of delphinidin 3-rutinoside chloride (0.3354 mg/100 mg), as well as luteolin 7-glucoside (0.2528 mg/100 mg) and 7-methoxycoumarin (0.2061 mg/100 mg) compared to the other components. Out of all the identified components, chlorogenic acid was detected in the smallest amount compared to the other compounds.

The *P. coronarius* flower contains bergapten in a high amount (2.8370 mg/100 mg), as well as caffeic acid (1.8407 mg/100 mg), delphinidin 3-rutinoside chloride (1.7928 mg/100 mg), 7-methoxycoumarin (1.6725 mg/100 mg). The flower contains delphinidin 3-rutinoside chloride and 7-methoxycoumarin in a much higher amount than the leaf.

### 4.10. Antioxidant Activity of *P. coronarius*

The antioxidant capacity of *P. coronarius* leaf and flower extracts was evaluated with different methods. The flower exhibited significantly better antioxidant activity with ABTS ($p \leq 0.0241$) and Cuprac methods ($p \leq 0.0093$); however, with the DPPH and FRAP methods no significant differences were observed. The experiments were carried out in triplicate.

### 4.11. Antimicrobial testing of lyophilized *P. coronarius* extracts

To investigate the antimicrobial potential of *P. coronarius* leaves and flowers, two antimicrobial assays were performed, microdilution and time-kill test.

The lyophilized extract of the flower and the leaf was dissolved in the relevant broth and the experiment was performed. The flower was able to reduce the viability of *E. coli*, since cell viability was decreased to 46.4%. In the case of the leaf, it decreased cell viability of *S. aureus* to 68.6% and *E. coli* to 41.5%. Neither the *P. coronarius* flower nor the leaf inhibited the viability of *C. albicans* or *P. aeruginosa* in these experiments.

The time-kill test was performed to study the antimicrobial effect of the *P. coronarius* leaf and flower over time. Samples were prepared by dissolving the lyophilized product in the relevant broth (RPMI 1640 or Mueller-Hinton). At predetermined times, 100 μL of aliquots were plated on agar plates and counted. Killing activity was determined by the threshold of 99.9% ($\log_{10}$ CFU = 2.24) killing of initial CFUs. As Figure 2 demonstrates, *E. coli* was not affected by either the flower or the leaf, while only the latter was able to delay the growth of the *C. albicans* and *S. aureus* species. As a conclusion, none of the samples demonstrated any killing activity, and only a limited reduction in viable cell count was observed.

### 4.12. Anti-inflammatory testing of lyophilized *P. coronarius* extracts

The anti-inflammatory effect of *P. coronarius* leaf or flower was studied using the ELISA test on HaCaT cells. Test solutions were prepared of lyophilized product dissolved in PBS (phosphate-buffered saline). During the study, PBS was selected as a negative control. It was taken as 100% during the evaluation and the values were compared and expressed as the percentage of the control. Pretreatment with the leaf (5%) and flower (3 and 5%) did not significantly reduce IL-4 production in the cells.

### 4.13. *In vitro* release of *P. coronarius* ointments

CMP 1, 2, 4 and 5 (compositions that contained Tefose 63 or SP70) showed the best results. The lowest release rate was achieved by CMP 3 and 6 (compositions, which contained Sedefos 75). Release profiles of the preparations were compared to each other. Two formulations are different if their difference factor (*f1*) is between 0–15. Based on the calculated values, a difference is confirmed between CMP 1 and CMP 2. CMP 1 and 4, CMP 1 and 5, CMP 2 and 4, CMP 3 and 6, CMP 4 and 5, and CMP 5 and 6 are different as well.

### 4.14. Texture analysis of *P. coronarius* ointments

Maximum compression-force values express the firmness of the preparations. Based on the results, CMP 2 and CMP 5 (which contain Tefose 63 as an emulsifier) had the hardest consistency (152; 153 N) compared to the other preparations. CMP 3 and 6 (which contain Sedefos 75 as an emulsifier) had the softest consistency of all (112, 106 N).

### 4.15. Cytotoxicity of *P. coronarius* ointments

The experiment was carried out with samples taken from the Franz diffusion-chamber apparatus, after 6 h of incubation, using pH = 5 buffer as the acceptor phase. PBS was chosen as the negative control and Triton-X 100 as the positive control. Cell-viability values have been compared to the negative control (PBS) and expressed as the percentage of it. According to our results, CMP 2 produced the best results, but all test samples were safe and non-toxic, because cell-viability values were above 70% in each case.

# 5. Discussion

### 5.1. Discussion of the experiments with BGP-15

In the first phase of my experimental work, different O/W emulsion ointments with different surfactants were formulated of BGP-15, and these compositions were investigated from different aspects, such as texture analysis and in vitro release. Texture analysis revealed that the compositions have adequate consistency; those formulations that contain sucrose esters have a slightly harder consistency. Based on the in vitro release tests, those three ointments (CMP 3, 4 and 5) that showed the best release profile were selected for further testing on animals. In parallel with these experiments, the biocompatibility studies of the ointments and the selected excipients were carried out on a HaCaT cell line. According to the MTT test results, all of the ointments and the excipients proved to be safe and non-toxic. The anti-inflammatory effect of BGP-15 ointments was investigated on C57BL/6J mice with an ear edema test. During this experiment, a local inflammation was induced on mice ears with AITC solution, and ear thickness was screened throughout the whole experiment. Mice ears were premedicated with the previously formulated ointments, or the BGP-15 aqueous solution. In the animal experiment, we found that those ointments containing the active ingredient significantly reduced or prevented the inflammation, while in those cases where mice were treated with those compositions that did not contain BGP-15, inflammation was induced almost to the same extent as when the ear was treated with the positive control (inflammatory agent (AITC)) alone.

Antioxidant properties, as a possible explanation of the anti-inflammatory effect of BGP-15, were evaluated with an SOD assay on a HaCaT cell line. Our results demonstrate that the drug was unable to protect the cells from UV radiation-caused oxidative stress; SOD enzyme levels decreased in exactly the same way as when the cells were only treated with PBS. TAC assay was also performed, but it showed no significant alteration with CMP 3 and CMP 4; however, it decreased with CMP 5, probably because of the surfactant's chemical structure. Our findings are similar to the results of Sümegi et al., who tried to investigate the possible antioxidant effect of the drug by other methods, but they also came to the conclusion that the drug, although it inhibits mitochondrial ROS production, has no antioxidant effect.

Luciferase assay was performed as another possible way to explain the experienced anti-inflammatory effect of BGP-15. In the experiment, the in vitro anti-inflammatory effect of BGP-15 was measured in mice macrophages. Based on the results, it appears that BGP-15

treatment did not have any effect on LPS-activated macrophages' NF-κB response, nor was it able to result a significant change in macrophage activity.

Our results can confirm the suggestions that BGP-15 has a protective effect against inflammation if applied topically, however, the exact mechanism of the effect remains unclear despite our attempts to identify it: we could only exclude a few possible explanations. In the scientific literature, a potential anti-inflammatory property is attributed to the PARP-inhibiting property of BGP-15. PARP inhibitors are able to protect cells from ROS-induced injuries. ROS production induces inflammatory processes. Moreover, BGP-15 is able to protect mitochondria from ROS-induced damages by reducing mitochondrial ROS production.

The composition of the ointments and the choice of appropriate penetration enhancers are important factors, not only in terms of pharmaceutical technology but also in terms of drug penetration and bioavailability. When the ointment compositions were determined, we tried to create formulations that contained the proper penetration enhancers and excipients to achieve the best possible bioavailability of BGP-15. Based on the scientific literature, the combination of sucrose esters (SP50, SP70, PS750) together with an active substance can enhance the effect, the same applies to Transcutol. However, Cremophor and its derivatives do not always prove to be the best choice, Malingré et al. studied paclitaxel and they found that using Cremophor limited the absorption of the drug. Our experience was similar, in the in vitro release test, CMP 2 (which contains Cremophor) showed the poorest results. Labrasol and IPM can be an advantageous combination as Okur et al. found that it can increase the permeation rate of naproxen in dermal drug delivery. However, Labrasol does not always prove to be the right surfactant to choose either. Shafiq et al. investigated ramipril in their study and they came to the conclusion that Labrasol was not a suitable surfactant to enhance drug absorption. In our case, the combination of sucrose esters and Transcutol proved to be the best choice according to the results of Franz diffusion, and these compositions achieved remarkable results in the animal experiment as well.

### 5.2. Discussion of the experiments with *P. coronarius*

In the secon phase of my experimental work, *Philadelphus coronarius* L. was characterized and investigated, because this plant was less studied in the literature. However, many beneficial pharmaceutical effects such as anti-inflammatory and antimicrobial activities were noticed during traditional applications. As a novelty, both the leaf and the flower of the plant were utilized, and a lyophilized product was developed from the extracts. Additionally, antioxidant

capacity was evaluated by different methods and a topical dosage form was formulated with the herb.

In the case of natural preparations, well-characterized active-substance content, stability, and easy processability are crucial points. For these purposes, a lyophilized product was prepared. In this form, the active ingredients are stable and easy to process. The preparation of the lyophilized products is unique according to the scientific literature, since most studies have only investigated the extracts of *P. coronarius*, which still contains the organic solvent, e.g., ethanol and methanol, which may affect the safety and can lead to false conclusions.

As part of our study, the antioxidant activity of *P. coronarius* leaf and flower was evaluated, as well as the total polyphenol and flavonoid content. Polyphenols are important metabolites and strong antioxidants that are able to protect against UV radiation and reduce inflammation. Flavonoids act as antimicrobial agents and antioxidants, and they have anti-inflammatory effect as well. The flower exhibited significantly better antioxidant capacity with the ABTS and Cuprac assays; however, no significant differences were observed with the DPPH and FRAP methods. Only the results of the ABTS and Cuprac assays were significantly different for the leaf and the flower, the main reason of which is presumably that more active components were detectable from the flower than from the leaf. Gallic acid and quercetin are two well-known, potent antioxidant compounds; they provide protection from oxidative stress. These two compounds were only identified in the flower, which explains the better antioxidant capacity. Different antioxidant-activity-determining methods are based on different mechanisms and have varying sensitivities, which explains why no significant difference was detected between the antioxidant capacity of the leaf and flower by any method. This is why it is recommended to test antioxidant capacity by different methods.

According to the scientific literature, *P. coronarius* possesses antimicrobial properties. It has been previously studied by the microdilution method for Gram-positive and negative bacteria as well. It was confirmed that the extract had a strong antibacterial effect compared to the other herbal extracts in the study. In our series of experiments, the antibacterial properties of the lyophilized flower and leaf were studied, followed by antifungal investigations. First, microdilution was carried out. In this investigation, the *P. coronarius* flower reduced the viability of *E. coli* and the leaf decreased the cell viability of *S. aureus* and *E. coli*. For further information in this topic, antimicrobial testing was continued with the in vitro time-kill test, whereby the effect of *P. coronarius* was studied on the growth of microbes over time. The growth of *S. aureus* and *C. albicans* was delayed by the leaf, while the flower did not elicit any

changes. However, these results are not consistent with the results of the microdilution, since it is obvious that the leaf has antimicrobial potential. The time-kill test is considered to be the more accurate of the two methods, because it includes time as an important factor in the experiment.

The extracts from the *P. coronarius* leaf and flower were tested separately on previously inflamed HaCaT cells. The results show that premedication with the flower did not significantly reduce IL-4 levels, even though the anti-inflammatory effect of Hydrangeaceae species has been evaluated in the past. Dilshara et al. have investigated if *H. macrophylla* leaf extract had any anti-inflammatory effect. It was observed that the extract suppressed the expression of some pro-inflammatory cytokines, e.g., NO, $PGE_2$, and TNFα. Moreover, Nakamura et al. learned that *H. macrophylla* reduced the mRNS expression of IL-6.

Carefully selected excipients may influence many properties of topical formulations, and in general they can also support the pharmaceutical effects by increasing the bioavailability. When the ointment compositions were determined, excipients were chosen in order to achieve the best potential bioavailability of the active ingredients. According to Csizmazia et al., sucrose esters such as SP70 are able to enhance the penetration and the effect of an active substance, e.g., ibuprofen. The same applies to Tefose 63; in the study of Abd-Elsalam et al., Tefose 63 improved the bioavailability and the effect of voriconazole. It was also confirmed by our in vitro release studies. CMP 2 produced the best results, closely followed by CMP 1, 4, and 5. The lowest release-rate values belonged to CMP 3 and 6. According to these results, those formulations, which contained either Tefose 63 or SP70 as a surfactant, ended up having better release profiles, which may lead to better bioavailability of the active pharmaceutical ingredients.

However, the application of surfactants and penetration enhancers may extremely influence the biocompatibility of different topical preparations. Cytocompatibility is a minimum requirement. It must be compulsory to check the safety of the preparation in each case. In our experiment, ISO-standard MTT tests were performed. Based on the results, the *P. coronarius* leaf and flower, as well as the ointment compositions were safe and non-toxic. The value of cell viability was over 70% in every case, which complies with ISO 10993-5 recommendations.

In the case of both pharmaceutical and cosmetic products, patient compliance is an important factor. The preparation must be as patient friendly as possible and little inconveniences must be reduced or avoided. For ointments, texture may be part of the investigation of this

compliance, therefore a texture analysis was performed. For this purpose, an O/W emulsion-type ointment was selected as the dosage form, since it is popular and preferred among patients due to its lighter consistency. The texture analysis revealed that CMP 2 and 5 had the hardest consistency (both contained Tefose 63), while CMP 3 and 6 was the softest (both contained Sedefos 75), but all compositions were easy to spread and apply.

### 5.3. Summary of new scientific results

New scientific results from my experimental work:

- O/W emulsion type ointments from BGP-15 were formulated, those formulations, which contained sucrose esters were found to be the best in terms of drug delivery, supported by Franz diffusion study and *in vivo* animal experiment
- Cytotoxicity testing of excipients selected for the formulations were safe and complied with the recommendations of ISO 10993-5
- The anti-inflammatory activity of BGP-15 ointments confirmed in *in vivo* studies
- Lyophilized extracts of the leaves and flowers of the herb *Philadelphus coronarius* was prepared to improve processability and stability, which is unique in scientific literature of the plant
- The components of the leaves and flowers of the plant were identified and their active components and the quantities were determined
- The antioxidant capacity of the herb was tested, the flower had better antioxidant properties than the leaf, it was confirmed by CUPRAC and ABTS antioxidant test
- In antimicrobial testing of the lyophilized extracts, the leaf delayed the growth of *C. albicans* and *S. aureus*
- O/W type emulsion ointments were formulated from the lyophilized extracts, those formulations which contained either sucrose ester SP70 or Tefose 63 were the best in terms of drug delivery, confirmed by a Franz diffusion study
- The selected excipients and the formulations were safe in cytotoxicity testing, complied with the recommendations of ISO 10993-5

# 6. Summary

In my experimental work two active substances were selected, a drug candidate pharmacon, BGP-15, currently under clinical trial and *Philadelphus coronarius L.*, a popular and widespread, but scientifically less-investigated herb.

In my research, O/W emulsion ointments were formulated from both active ingredients and different emulsifiers and penetration enhancer excipients were added to the formulations to achieve proper bioavailability. The ointment compositions were formulated to assess biocompatibility, excipients and safe administration of the formulations, which is an important aspect of adequate patient compliance.

The effects of the active ingredients were in the focus as well as they have a high novelty value. The effects of BGP-15 are currently the subject of intensive researches. Although empirical information about *P. coronarius* is available based on folk medicine, the scientific base is often incomplete.

In my research with BGP-15, ointments were formulated from the pharmacon and subjected these ointments to various tests: texture analysis, Franz diffusion study. Several versions of the ointments were prepared by changing the emulsifiers. In the texture analysis, the formulation containing SP70 had the hardest consistency, while the one containing Labrasol had the softest consistency. In Franz diffusion study, the formulations containing sucrose esters (SP50, SP70, PS750) had the best drug release profile, so the research was continued with these formulations. The biocompatibility studies showed that the excipients and the ointment compositions were safe and well-tolerated. Anti-inflammatory effect of BGP-15 was tested *in vivo* in animal studies using mice in ear oedema test. In this study, those ointments, which contained BGP-15 significantly reduced ear thickness, thus inhibited the development of local inflammation. Luciferase assay was performed to investigate the mechanism of action of the pharmacon, the results suggested that BGP-15 does not inhibit the NF-κB pathway. In antioxidant assays, BGP-15 did not show antioxidant properties.

In my experiments with *Philadelphus coronarius*, an extract of the leaves and flowers of the plant was prepared, followed by a lyophilization to gain a solid, stable, water soluble product. The constituents of the extracts were identified and the bioactive compound content was determined. The antioxidant capacity was tested by several assays, demonstrating that the flower of the herb has better antioxidant activity than the leaf. In the antimicrobial assay, it was demonstrated that the lyophilized leaf extract inhibited the growth of *C. albicans* and *S. aureus*.

In the ELISA test, neither the leaf nor the flower reduced IL-4 expression in inflamed cells, so an anti-inflammatory effect of the herb was excluded.

Using the lyophilized extracts, different O/V type emulsion ointments were formulated and dosage form studies were performed. In the texture analysis, those formulations, which contained Tefose 63 had the hardest consistency and those ones, which contained Sedefos 75 had the softest texture. In the Franz diffusion study, the formulations containing SP70 and Tefose 63 had the best drug release profile. In biocompatibility studies, the excipients and the ointment compositions were safe and well-tolerated by the cells.

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## List of publications related to the dissertation

1. **Pető, Á.**, Kósa, D., Haimhoffer, Á., Nemes, D., Fehér, P., Ujhelyi, Z., Vecsernyés, M., Váradi, J., Fenyvesi, F., Frum, A., Gligor, F. G., Vicaș, L. G., Marian, E., Jurca, T., Pallag, A., Muresan, M., Tóth, Z., Bácskay, I.: Topical Dosage Formulation of Lyophilized Philadelphus coronarius L. Leaf and Flower: antimicrobial, Antioxidant and Anti-Inflammatory Assessment of the Plant.
*Molecules. 27* (9), 1-18, 2022.
DOI: http://dx.doi.org/10.3390/molecules27092652
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2. **Pető, Á.**, Kósa, D., Haimhoffer, Á., Fehér, P., Ujhelyi, Z., Sinka, D. Z., Fenyvesi, F., Váradi, J., Vecsernyés, M., Gyöngyösi, A., Lekli, I., Szentesi, P., Marton, A., Gombos, I., Dukic, B., Vígh, L., Bácskay, I.: Nicotinic Amidoxime Derivate BGP-15, Topical Dosage Formulation and Anti-Inflammatory Effect.
*Pharmaceutics. 13* (12), 2037, 2021.
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## List of other publications

3. Sinka, D. Z., Doma, E., Szendi, N., Páll, J., Kósa, D., **Pető, Á.**, Fehér, P., Ujhelyi, Z., Fenyvesi, F., Váradi, J., Vecsernyés, M., Szűcs, Z., Gonda, S., Cziáky, Z., Kiss-Szikszai, A., Vasas, G., Bácskay, I.: Formulation, Characterization and Permeability Studies of Fenugreek (Trigonella foenum-graecum) Containing Self-Emulsifying Drug Delivery System (SEDDS).
*Molecules. 27* (9), 2846-, 2022.
DOI: http://dx.doi.org/10.3390/molecules27092846
IF: 4.927 (2021)

4. Kósa, D., **Pető, Á.**, Fenyvesi, F., Váradi, J., Vecsernyés, M., Budai, I., Németh, J., Fehér, P., Bácskay, I., Ujhelyi, Z.: Oral Bioavailability Enhancement of Melanin Concentrating Hormone, Development and In Vitro Pharmaceutical Assessment of Novel Delivery Systems.
*Pharmaceutics. 14* (1), 1-15, 2022.
DOI: http://dx.doi.org/10.3390/pharmaceutics14010009
IF: 6.525 (2021)

5. Kósa, D., **Pető, Á.**, Fenyvesi, F., Váradi, J., Vecsernyés, M., Gonda, S., Vasas, G., Fehér, P., Bácskay, I., Ujhelyi, Z.: Formulation of Novel Liquid Crystal (LC) Formulations with Skin-Permeation-Enhancing Abilities of Plantago lanceolata (PL) Extract and Their Assessment on HaCaT Cells.
*Molecules. 26* (4), 1-15, 2021.
DOI: http://dx.doi.org/10.3390/molecules26041023
IF: 4.927

6. **Pető, Á.**, Kósa, D., Fehér, P., Ujhelyi, Z., Sinka, D. Z., Vecsernyés, M., Szilvássy, Z., Juhász, B., Csanádi, Z., Vígh, L., Bácskay, I.: Pharmacological Overview of the BGP-15 Chemical Agent as a New Drug Candidate for the Treatment of Symptoms of Metabolic Syndrome.
*Molecules. 25* (2), 429-441, 2020.
DOI: http://dx.doi.org/10.3390/molecules25020429
IF: 4.411

7. Nemes, D., Ujhelyi, Z., Arany, P., **Pető, Á.**, Fehér, P., Váradi, J., Fenyvesi, F., Vecsernyés, M., Bácskay, I.: Biocompatibility investigation of different pharmaceutical excipients used in liquid dosage forms.
*Pharmazie. 73* (1), 16-18, 2018.
DOI: http://dx.doi.org/10.1691/ph.2018.7098
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8. Nemes, D., Kovács, R. L., Nagy, F., Mező, M., Poczok, N., Ujhelyi, Z., **Pető, Á.**, Fehér, P., Fenyvesi, F., Váradi, J., Vecsernyés, M., Bácskay, I.: Interaction between Different Pharmaceutical Excipients in Liquid Dosage Forms: assessment of Cytotoxicity and Antimicrobial Activity.
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