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APPLICATION OF EXTRACT OF *GERANIUM PALUSTRE* HERB AS A DENTAL FILM IN THE TREATMENT OF ORAL MUCOSA DISEASES

Marta Khavrona [*1], Igor Benzel [2], Roman Fedin [3] and Oleg Pinyazhko [1]

Department of Pharmacology [1], Department of Pharmacognosy and Botany [2], Department of Drug Technology and Biopharmaceutics [3], Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

Keywords:

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Correspondence to Author:
Marta Khavrona

Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine.

E-mail: o.khavrona@gmail.com

ABSTRACT: Investigation of antioxidant, anti-inflammatory and antimicrobial activity of a dental film, which easily reabsorbs with applied phytocomplex of *Geranium palustre*, has been performed to use it for the treatment of oral mucosa diseases. To get the extract, the dried material was grounded, extracted and lyophilized. Quantitative determination of biologically active substances in lyophilized phytocomplex was performed on spectrophotometer and high performance liquid chromatograph. For investigation of antioxidant properties of the extract, the method, based on the interaction with stable chromogen-radical DPPH, was used. Investigation of anti-inflammatory activity of a dental film with extract of *Geranium palustre* herb was carried out based on carrageenin model of inflammatory. The study of antimicrobial properties of extract was performed using laboratory strains of microorganisms as a test-culture. The obtained qualitative indices of biologically active substances of the investigated phytocomplex showed high content of tannins, polyphenol compounds, polysaccharides, phenolcarboxylic acids, organic acids, ascorbic acid, free amino acids and other compounds, due to which lyophilized extract possesses a wide range of properties, important for treatment: antioxidant, anti-inflammatory, antimicrobial and antiviral. The obtained dental film firmly adheres to the mucosa surface, has pH of the oral cavity and quickly reabsorbs, providing regular release of plant extract and convenient application. Moderate antioxidant, anti-inflammatory and antimicrobial activities as well as marked fungistatic action of lyophilized extract were detected. Fast reabsorbing dental film with applied *Geranium palustre* phytocomplex, possessing necessary properties for the treatment of oral mucosa diseases, is a safe and perspective remedy for implementation into dental practice.

INTRODUCTION: Nowadays, various lesions and inflammatory processes of oral mucosa are among the most common pathological conditions in dentists' practice [1]. According to WHO data, such diseases occur in 80% of children and 90% of adult population.

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Despite a wide choice of highly active anti-inflammatory and antibacterial medications, traditional treatment of this pathology not always gives a desirable result. Besides, there is a significant increase in complications, which appear after the use of the aforementioned medications [2]. Thus, the use of new remedies, which are able to have a long-lasting effect on basic areas of pathogenesis of inflammatory diseases of oral mucosa without causing side effects, is a topical issue.

Modern look at this problem implies complex influence on all areas of pathologic process. Thus,

medications with multiple mechanisms of action are used [3]. These medicines, applied on dental film, provide local and regular release of active substance from the drug, causing its high therapeutic concentration locally without a considerable elevation of medicine level in the circulatory system. In fact, film form of medicine is ideal for the use in dentistry [4].

Nowadays, despite a wide range of medicines, application of medicinal plants and plant-based remedies significantly increases. Advantage of phytomedications is mild therapeutic action, capability of complex influence on various areas of pathologic process, low toxicity and possibility of long-lasting application in different age groups. Rational combination of biologically active components in phytomedicines results in their clinical efficacy, even when used in small doses [5].

We decided to apply phytocompex from herb of *Geranium palustre*, which has been widely used in folk medicine, on the dental film. It is known from literature data that plants of *Geranium* species possess anti-inflammatory, hemostatic, anti-microbial and antioxidant properties [6 - 10].

MATERIALS AND METHODS:

Plant Material: Herb of *Geranium palustre* L. was collected from Lviv in the Western region of Ukraine in July 2016. The plant was authenticated by Associate Professor Leonid Benzel, Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University (DHLNMU), Lviv, Ukraine. Voucher specimens (LNMU/PhB/ 2016/056) have been deposited at the herbarium of the Department of Pharmacognosy and Botany, Faculty of Pharmacy, DHLNMU.

To get the extract, the dried *Geranium palustre* herb was grounded, extracted and lyophilized. Determining quantitative content of the main groups of active substances is necessary for standardization of plant phytosubstances. Quantitative determination of biologically active substances in lyophilized phytocomplexes was performed on spectrophotometer Cari 50 Scan and high performance liquid chromatograph AGILENT 1200 3D LC System with following computer processing of the investigation results. The technology of dental film manufacturing implies the following: certain amount of dry lyophilized extract of *Geranium palustre* herb is grounded, and then mixed, adding propylene glycol and tween 80. Saccharine and polivinol are dissolved in half of purified boiling water. Sodium-carboxymethyl cellulose is infused for 3 - 4 h at room temperature with the rest of purified water to complete dissolution and formation of gel. Grounded powder of *Geranium palustre* extract with propylene glycol and tween 80, cooled to room temperature saccharine and polivinol solution are added to the gel. The mixture is triturated for 3-5 min to form homogenous viscous solution, which is poured into metal mold with the size 60.0 × 10.0 × 0.5 mm and dried at temperature 45 ºC for 6 - 8 h.

Investigation of anti-inflammatory activity of dental film with extract of *Geranium palustre* herb was carried out based on carrageenin model of inflammatory swelling of white rats' legs [11]. Ethical Committee or Institutional Animal Care and Use Committee Approval: 16/11/2015 No. 9. For the experiment, the animals were divided into two groups (10 white mature male rats weighing 180-220 g in each group). Acute inflammatory process and exudative swelling were provoked with injection in aseptic conditions of 0.1 ml 20% carrageenin solution under the plantar aponeurosis of the rats' hind leg.

The presence of inflammatory reaction was established detecting alteration of the leg circumference by oncometric method at the start of the experiment and during 4 h after injection of fluorogenic agent. The first, control group of animals did not undergo any treatment; animals of the second group were injected dissolved dental film with *Geranium palustre* extract in the form of viscous aqueous solution 40 min before the injection of carrageenin solution.

Antioxidant activity was detected using the method based on correlation with a stable chromogen-radical 2.2 diphenyl-1-picrylhydrazyl (DPPH) [12]. Optic density of the solutions was measured by wavelength 517 nm on spectrophotometer. Based on the data, received as a result of threefold repetition of estimation for each concentration, IC_{50} value was calculated by linear regression equation of dependence of inhibition percentage on extract concentration. IC_{50} value, which was the measure

in samples for the content of substances that possess antiradical activity, corresponds to a concentration of the investigated extract, necessary for binding 50% DPPH. Quercetin was used as an etalon substance.

Investigation of antimicrobial properties of lyophilized aqueous extract of *Geranium palustre* was performed in bacteriological laboratory of Lviv regional pediatric specialized clinical hospital. Laboratory strains of *Klebsiella pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter aerogenes, Alcaligenes faecalis, Esherichia coli* and *Candida albicans* were used as test-cultures for estimation of antibacterial activity. Screening investigation of antimicrobial activity was performed by diffusion method in agar. Magnifier with eyepiece micrometer was used for estimation of the diameters of the areas of microorganism growth delay around pits in one day.

Data are represented as mean ± standard error of the mean (SEM). For statistical analysis, we calculated differences between the experimental groups using a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls post-hoc test. We defined $p<0.05$ as the threshold for statistical significance.

RESULTS: The obtained *Geranium palustre* extract is a concentrated complex of biologically active substances in the form of dry, hygroscopic, amorphous powder of light-brown color, odorless with astringent taste.

The obtained qualitative indices of biologically active substances of the investigated phytocomplex showed high content of tannins, polyphenol compounds, polysaccharides, phenol-carboxylic acids, organic acids, ascorbic acid, free amino acids and other compounds, percentage ratio of which is presented in **Table 1.**

TABLE 1: QUANTITATIVE CONTENT OF THE MAIN GROUPS OF ACTIVE SUBSTANCES IN LYOPHILIZED EXTRACT

Quantitative content	
Biologically active substances %	**Lyophilized extract of *Geranium palustre***
Polyphenol substances	47.79
Tannins	34.12
Flavonoids	2.36
Hydroxycinnamic acids	6.07
Free organic acids	14.20
Ascorbic acid	0.136
Free amino acids	12.03
Polysaccharides	9.21

Ready dental films have the following characteristics: films of dark-brown color with aroma of *Geranium palustre* extract, sweet taste, average mass constitutes 223.0 ± 3.6 mg, solubility not more than 12 min, tensile testing 64.4 ± 3.4 kg/cm^2, percentage elongation 56.2 ± 4.3%, osmotic activity 68.4 ± 6.5%, adhesion 80.4 ± 3.7%, pH of the medium 6.0.

The obtained results of inflammatory process development indicate gradual enlargement of a leg circumference in experimental animals due to swelling in both groups, however, it was significantly less marked in the use of dental film with *Geranium palustre* extract **Table 2**.

TABLE 2: LEG CIRCUMFERENCE IN EXPERIMENTAL ANIMALS IN CARRAGEENIN SWELLING (M±m, n=10)

Object of investigation	Results of experiment %			
	at the beginning	**1 h**	**2 h**	**4 h**
Control	10.05 ± 1.23	36.54 ± 2.21	91.24 ± 2.42	126.50 ± 2.30
Dental film with *Geranium palustre* extract	9.35 ± 0.27	27.26 ± 1.84*	68.56 ± 1.54*	102.60 ± 2.80*

*****$p<0.05$ compared with the control

The investigation of antioxidant activity showed that *Geranium palustre* extract possesses high antioxidant action, which is only twice lower than antiradical activity of the most potent natural antioxidant – quercetin. The results of investigation of antioxidant activity are presented in **Table 3**.

TABLE 3: RESULTS OF INVESTIGATION OF ANTI-OXIDANT ACTIVITY OF *GERANIUM PALUSTRE* PHYTOCOMPLEX

Sample	IC_{50} mcg\ml
Aqueous lyophilized extract of *Geranium palustre*	5.80
Quercetin	2.30

Moderate antimicrobial and marked fungistatic action of lyophilized extract was observed after microbiological investigation of aqueous extract of *Geranium palustre* herb **Table 4**.

TABLE 4: INVESTIGATION RESULTS OF ANTI-MICROBIAL PROPERTIES OF *GERANIUM PALUSTRE* PHYTOCOMPLEXES

Strains of microorganisms	Diameter of the area of growth inhibition (mm)
	Aqueous lyophilized extract of *Geranium palustre*
Klebsiella pneumoniae	15±0.75
Staphylococcus epidermidis	12±0.67
Staphylococcus aureus	14±0.62
Proteus mirabilis	18±0.84
Pseudomonas aeruginosa	18±0.76
Enterobacter aerogenes	16±0.86
Alcaligenes faecalis	22±0.94
Esherichia coli	12±0.64
Candida albicans	-

DISCUSSION: Obtained phytocomplexes were standardized based on the content of tannins (34.12%), flavonoids (2.36%), hydroxycinnamic acids (6.07%), total free organic acids (14.2%), ascorbic acid (0.136%), total free amino acids (12.03%) and polysaccharides (9.21%). According to literature data, *Geranium palustre* phytocomplexes contain gallic acid, 2,5-digalloyl glucose, bergenin; ellagic acid; pyrocatechin; flavonoids: catechin, quercetine, kaempferol, luteolin, myricetin; flavonoid glycosides: rutin, hyperoside, quercitrin, trifoline, kaempferol-3-O-β-D-glucopyranoside and apigenin-7-O-β-D-glucopyranoside; cinnamic acid and its derivatives: caffeic, chlorogenic, neochlorogenic, ferulic and p-coumaric acids; 7 free organic acids (malic, citric, oxalic, succinic, tartaric, pyruvic and ascorbic) and 15 amino acids, among which 8 essential (isoleucine, leucin, lysine, methionine, phenylalanine, threonine, histidine, and valine) [13]. Due to the aforementioned active substances, lyophilized extract possesses a wide spectrum of properties important for treatment: astringent, antioxidant, anti-inflammatory, anti-microbial and antiviral.

A basic active pharmaceutical ingredient of a dental film is a dry extract of *Geranium palustre*, however, the film also contains additional substances, which provide appropriate organoleptic, physical, chemical and technological properties. In particular, saccharine serves as a coregent of bitter taste of dry lyophilized *Geranium palustre* extract. Polivinol and sodium-carboxymethyl cellulose possess necessary binding properties, aqueous solutions of which after drying form a strong film and provide long-lasting pharmacological effect of active pharmaceutical ingredients due to occurrence of hydrogen connections between adjacent branches of macromolecules. Optimal amount of propylene glycol and tween 80 give the film appropriate elasticity, hydrophilic property, and capability of active pharmaceutical ingredient to penetrate through the mucous membrane.

All diseases of the oral mucosa are accompanied by the appearance of a typical inflammatory reaction with its classic manifestations: development of swelling, redness, appearance of pain syndrome and function impairment. Thus, the primary aim at this stage is the use of medicines with anti-inflammatory action. It was established on carrageenin model of inflammation that *Geranium palustre* phytocomplex possesses anti-inflammatory properties that is manifested by a 1.3 time reduction of the swelling of a rat's leg during each hour of measuring in the development of inflammatory process as compared with the animals of control group.

Taking into consideration literature data that activation of peroxide lipid oxidation plays one of the leading roles in the development of inflammatory process, its correction with antioxidant medicines enables to optimize the intensity of inflammation, decrease a period of catabolic phase and accelerate regeneration start[14]. Revealed high antioxidant activity of *Geranium palustre* phytocomplex enables to assume a decrease in the processes of free radical oxidation after application of a dental film with an extract of the investigated plant in various diseases of the oral mucosa, but, considering the absence of such investigations, it is necessary to prove such assumption experimentally.

According to literature data, organic acids and phenol substances, as well as alkaloids and glycosides, primarily, are referred to biologically active substances that possess antimicrobial and antifungal properties [15]. It is obvious from quantitative analysis that these substances are present in phytocomplex of *Geranium palustre* in large amounts as biologically active substances, thus explaining antimicrobial action of the investigated phytocomplex, which is particularly potent against *Staphylococcus epidermidis* and *Esherichia coli* and significantly marked antimicrobial action against *Candida albicans*, which completely confirms literature data [16]. Positive effect was also observed on all other microorganisms. Among the investigated microorganisms, *Geranium palustre* extract had the least effect on *Alcaligenes faecalis* inhibition. Antimicrobial activity is of utmost importance, since one of the most significant tasks of conservative treatment for different oral mucosa lesions is combating pathogenic microflora.

CONCLUSION: The obtained dental film firmly adheres to the surface of mucous membrane, has pH of the oral cavity and rapidly reabsorbs, providing regular release of plant extract and convenient application. The investigated phytocomplex of *Geranium palustre* shows anti-inflammatory, antioxidant and antimicrobial properties. *Geranium palustre* shows the highest antimicrobial activity to *Candida albicans, Staphylococcus epidermidis* and *Esherichia coli.* Fast reabsorbing dental film with applied phytocomplex of *Geranium palustre* has all the necessary properties for the treatment of oral mucosa diseases; it is a safe and perspective remedy for implementation into dental practice.

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