

## К ВОПРОСУ РАЗРАБОТКИ КОМПЛЕКСНОЙ ДИФТЕРИЙНОЙ ВАКЦИНЫ С БАКТЕРИАЛЬНЫМ АДЪЮВАНТОМ Елисеєва І. В., Бабіч Е. М., Ждамарова Л. А., Белозерський В. І., Колпак С. А.

**Резюме.** Стаття присвячена вивченню впливу нативних антигенних комплексів збудителя дифтерії, отриманих за допомогою ультразвукової дезинтеграції мікробних кліток в нейтральній і слабокислій середі, на їх фагоцитоз-стимулюючу активність і адгезивні властивості тест-штамма *C. diphtheriae* для подальшого удосконалення технології виробництва комплексної дифтерійної вакцини проти маніфестного проявлення і персистируючої форми дифтерійної інфекції.

**Ключевые слова:** дифтерійна вакцина, бактеріальний адьювант, адгезія, фагоцитоз.

## TO THE DEVELOPMENT OF THE COMPLEX DIPHTHERIA VACCINE WITH BACTERIAL ADJUVANT Yeliseyeva I. V., Babich E. M., Zhdamarova L. A., Belozersky V. I., Kolpak S. A.

**Abstract.** The lessons of the great diphtheria epidemic in Eastern Europe in the 1990s and the increasing trend of diphtheria in the world over the past few years have forced the medical community not to forget about the threat of a diphtheria outbreak. Sporadic cases of disease in Ukraine over the last 10 years are just the tip of the iceberg, as the transmission of infection through bacterial carriers and latent forms of diphtheria unrestrained continues. An analysis of the number of cases of diphtheria registered in Ukraine since the late 1980s and the corresponding rates of vaccination coverage against diphtheria reveals a paradoxical phenomenon: a significant increase in the incidence since 1990 was accompanied by an annual increase in the percentage of population coverage of DTP3. The maximum number of diphtheria cases in 1994-1996 was accompanied by the highest – almost 100 % – rates of vaccination. Thus, the epidemic has not been stopped by increasing the number of vaccinated persons. For a number of years, in our laboratory are conducted the research on the development of a complex diphtheria vaccine with a bacterial component. The vaccine has not only a protective effect against diphtheria disease, but is also directed against the colonization of the respiratory tract by the pathogen and the sanitation of *C. diphtheriae* bacterial carriers. The development of bacterial adjuvant is carried out using ultrasonic disintegration of bacteria in line with two modern vaccine design strategies, namely: an anti-adhesive strategy that develops drugs that prevent colonization by the pathogen of the mucous membranes of the macroorganism and its subsequent invasion, as well as strategies for potentiation of the trained innate immunity, and to promote the elimination of the pathogen from the body in immunodeficiency states, which are associated with prolonged bacterial activity, and to enhance the immune protection of the body after vaccination. The development of bacterial adjuvant is in line with two modern vaccine design strategies, namely: (1) anti-adhesive strategy that implements drugs that prevent the pathogen colonization of the mucous membranes of the macroorganism and its subsequent invasion; (2) strategies for potentiation of trained innate immunity, which can protect against infection and promote the elimination of the pathogen in immunodeficiency states that are associated with prolonged bacterial activity, as well as the treatment of inverse immunotolerant states to enhance immune response. The experimental candidate vaccine has been preclinically tested in an enterprise setting. However, the study of the effect of experimental antigenic preparations on cell-mediated immunity is being continued and the technological process of manufacturing the experimental candidate vaccine is being worked out. It was established that the tested samples of *C. diphtheriae* antigenic preparations increased the adhesiveness of the *C. diphtheriae* test strain at previous exposure with formalinized human red blood cells, and also demonstrated phagocytosis-stimulating effect ( $t > 2$ ;  $p = 0.05$ ). The decrease in the indices of adhesion and phagocytosis of the test strain at pH=5.5, apparently indicates that even a weakly acidic environment damages the molecular structures – PAMS of erythrocytes and adhesines of corynebacteria, respectively – which partially lose their specificity and ability to stimulate mechanisms of innate immunity. The obtained data indicate the importance of determining the optimum pH value in the technological process of obtaining a bacterial antigenic preparation in the design of combined diphtheria vaccines.

**Key words:** diphtheria vaccine, bacterial adjuvant, adhesion, phagocytosis.

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## ANTIMICROBIAL, ANTIOXIDANT AND SOME BIOCHEMICAL PROPERTIES OF *ARNICA MONTANA* L.

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**Publication relation to planned scientific research projects.** The present study is a fragment of the research project at the Department of Genetics, Plant Physiology and Microbiology of the Uzhhorod National University «Research of genetic, physiological and biochemical mechanisms of the various organization level biological

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**Introduction.** In recent years, many researchers have focused on medicinal plants derived from natural products due to their wide range of pharmacological significance [1]. Moreover, natural resources of vegetable origin represent an important source of drugs in

the process of developing new pharmacologically active compounds [2]. The World Health Organization established that, in many developing countries, traditional medicine plays an important role in meeting the primary health care needs of the population, and highlights specific types of this medicine (WHO, 2014).

Plants are prospective source of antimicrobial agents in different countries [3]. About 60 to 90% of populations in the developing countries use plant-derived medicine. Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases [3-6]. Plants are rich in a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial properties [6-7]. Although the mechanism of action and efficacy of these herbal extracts in most cases is still needed to be validated scientifically, these preparations mediate important host responses [8-9].

Global prevalence of infectious diseases caused by bacteria is a major public health problem [5,10]. The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections [11,12]. Recent emergence of antibiotic resistance and related toxicity issues limit the use of antimicrobial agents [13] and is prompting a revival in research of the antimicrobial role of plants against resistant strains due to comparable safety and efficacy [3].

**The purpose of the study** was to explore the antimicrobial, antioxidant and some biochemical properties of alcoholic extracts of *Arnica montana* L.

**Object and methods.** The plant materials were collected in the Mizhhiria rayon, Zakarpatska oblast (Transcarpathia), dried at the temperature of 30-35°C in shadow, then ground and placed in tightly closed containers.

**Extracts manufacturing techniques.** We made ethyl and methyl extracts of *Arnica montana* L. A 10 g batch of dry plant material was pulverized to powdery mass. In an Erlenmeyer flask, 10 g of plant material was blended with 200 ml of 97% ethyl (methyl) alcohol (Sigma, Germany). The opening was closed with a food wrap to avoid evaporation. Following a 30-minute-long incubation in the ultrasonic bath (Kraintek) at 35°C, the blend was filtered through Whatman No. 1 filter paper. The clear solution was placed in an evaporative device (16-17/32" x 34-59/64" G5B, Coated Dry Ice Condenser Rotary Evaporator) to obtain pure alcoholic extract at 50°C, 82 rpm. When the alcohol evaporated, 10 ml of ethyl (methyl) alcohol were added to the pure extract left on the bottom of the flask. As a result, the following pure extracts were obtained: ethyl extract of *Arnica montana* L. – 0,81 g; methyl extract of *Arnica montana* L. – 2,295 g.

As test cultures, the following bacteria and yeasts from the American Type Culture Collection were used: *Candida albicans* ATCC 885-653; *Staphylococcus aureus* ATCC 25923; *Escherichia coli* ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Streptococcus pyogenes* ATCC 19615. We also used clinical strains of bacteria and yeasts (*S. aureus*, *E. coli*, *S. pyogenes*, *C. albicans*) isolated from the oral cavities of patients suffering from inflammatory periodontium. From the oral cavities of 155 patients were isolated microorganisms characterised by resistance to at least 10 antibiotic preparations belonging to two and more classes: *S. aureus* (28 isolates), *E. coli* (11 isolates), *S. pyogenes* (25 isolates), *C. albicans*

(17 isolates). We chose the clinical strains with multiple resistance to antibiotics [8].

The isolates that caused periodontium inflammatory processes were isolated on the basis of the Dental Polyclinic, Uzhhorod National University; the extracts were manufactured and their antioxidative activity and contents of tannins and flavonoids were determined on the basis of the Department of Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; the antimicrobial activity of plant extracts was studied at the Microbiological Laboratory of the Department of Genetics, Plant Physiology and Microbiology, Uzhhorod National University, and Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice.

Biological material samples from the mucous membrane of the nidus of the inflammatory process were taken with the help of a sterile transport system (a test-tube with a gel and applicator for biological liquids, made by FLmedical, Italy). The material was plated on the following nutrient media: Sabouraud Dextrose Agar, and HiCrome™ Candida Differential Agar (Himedia) for cultivation of microscopic fungi; blood agar for haemolytic microflora, in particular *Streptococcus* and *Neisseria* genera microorganisms; Endo and Ploskorev agar (Farmaktiv, Ukraine) for *Enterobacteriaceae*; Mannitol Salt Agar (Biolif-Italia) for *Staphylococcus* genus bacteria, Bile esculin agar (Biolif-Italia) for *Enterococci*. The pure culture of microorganisms was obtained by sector inoculation according to Gold. The bacteria and yeasts were identified based on macromorphological, micromorphological, physiological and biochemical tests with the use of ENTERO-test, STREPTO-test, and STAPHYLO-test, made by Erba Lachema.

**Antibiotic susceptibility testing.** The antibiotic sensitivity of bacteria and microscopic fungi was identified by the disc diffusion method according to (Order No. 167 of the Ministry of Public Health of Ukraine of 05/04/2007; EUCAST (European Committee on Antimicrobial Susceptibility Testing).

The sensitivity of microorganisms to plant extracts was determined by the agar diffusion test [9]. The bacterium inocula 100 µL in the physiological solution were adjusted to the equivalent of 0.5 McFarland standard, and evenly spread on the surface of Muller-Hinton agar (incubated at 37±2°C for 24 hours); yeasts – on SDA agar (incubated at 35±2°C for 48 hours). The extracts 20µL were introduced into wells 6 mm in diameter. The diameters of the inhibition zones were measured in millimetres including the diameter of the well. The antimicrobial effect was assessed by presence of growth inhibition zone. Each antimicrobial assay was performed at least three times.

**Antioxidant activity.** Detection of free radical scavenging activity of the samples was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) [10] (Medini). A sample of 0.1mL was mixed with 1.9 mL of DPPH solution in methanol (0.06 mmol l<sup>-1</sup>). The absorbance of the reaction mixture was detected with a spectrophotometer Beckman Coulter DU 530. Following incubation in dark for 30 min., the absorbance of each solution was measured at 515 nm (A). The antioxidant activity was expressed as percentage (%) of the scavenging activity. The percentage of DPPH radical scavenging activity was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs (control)} - \text{Abs(sample)}}{\text{Abs (control)}} \times 100$$

where Abs (control): Absorbance of DPPH radical + methanol; Abs (sample): Absorbance of DPPH radical + extract.

**Determination of Total Tannins (TT).** The content of tannins was determined using Folin-Ciocalteus method [12]. The absorbance was measured as the absorbance at 750 nm (A), with the use of water as the compensation liquid. The percentage of tannins expressed as pyrogallol was calculated based on the following expression:

$$\text{Tannins (\%)} = \frac{3,125 \cdot A}{0,316 \cdot m}$$

where m – mass of the sample to be examined, in grams; A – absorbance

The absorbance of the reaction mixture was detected with a spectrophotometer Beckman Coulter DU 530v.

**Determination of Total Flavonoids (TF).** The flavonoid content was determined by a colorimetric assay as described by aluminium chloride colorimetric method [13] Djeridane. The absorbance of the test solution was measured at 425 nm. with a spectrophotometer Beckman Coulter DU 530.

$$X = \frac{A \cdot 1.25}{m}$$

where A – absorbance at 425 nm; m – mass of the herbal drug to be examined in grams.

**Statistical analysis.** Data obtained were expressed as mean ± standard deviation (SD) of three measurements. The Tukey's test was applied for comparisons of means; differences were considered significant if p < 0.05. Statistical analysis and comparisons among means were carried out using Microsoft Excel. The parameters calculated alongside with the basic variation were: average and standard deviation; minimum and maximum coefficients of variation; and frequency of the size of the inhibition zones.

**Results and discussion.** Test cultures differently reacted to extracts of *A. montana*: of the tested strains of bacteria the most sensitive found *S. aureus* and *S. pyogenes* (table 1). Thus, the highest antibacterial activity was displayed by the ethyl extract of *Arnica montana L.* It is worth noting that the extracts displayed antimicrobial activity against both typical and clinical isolates of *S. aureus*, including methicillin-resistant ones. The ethyl and methanol extracts were established to show antimicrobial effect against *S. pyogenes*. The ethanol fruit and leaf extracts and methanol fruit extract were observed to show weak antimycotic activity. The fruit extracts were characterized by low antimycotic activity.

Full absence of growth inhibition was registered in case of the use of ethyl and methyl extracts to *Candida albicans*, and low level of antimicrobial activity – in case of the use of ethyl extracts to *Enterococcus faecalis*. The obtained results pointed to the ability of extracts to inhibit the growth of antibiotic-resistant strains of staphylococci and streptococci.

The extracts were characterized by high antioxidant activity; the highest activity was displayed by ethyl extracts, somewhat lower – by methyl extracts (table 2).

Out of all extracts under review, it was the ethyl extract that showed the highest level of tannins

**Table 1 – Antimicrobial activity of *Arnica montana L.* extracts, zones inhibition in millimeters including diameter of well, mm (n=3, x ± SD)**

Test culture	ethyl extract	methyl extract
<i>S. aureus</i> ATCC 25923	20.2±0.3	19.3±0.3
<i>S. aureus</i> clinic	20.5±0.5	20.2±0.3
<i>S. aureus</i> MRSA clinic	18.6±0.6	19.5±0.5
<i>E. coli</i> ATCC 25922	10.3±0.6	10.6±0.3
<i>E. coli</i> Clinic	9.5±0.5	10±0.5
<i>E. faecalis</i> ATCC 29212	9.6±0.3	9.3±0.6
<i>E. faecalis</i> clinic	8.5±0.5	11.2±0.3
<i>S. pyogenes</i> ATCC 19615	14.2±0.3	13.3±0.3
<i>S. pyogenes</i> clinic	13.3±0.6	12.3±0.3
<i>C. albicans</i> ATCC 885-653	-	-

Note. «-» – no inhibition.

**Table 2 – Antioxidant activity of *Arnica montana L.* extracts**

ethyl extract		methyl extract	
Absorbance (nm)	%	Absorbance (nm)	%
0.072	85.4	0.087	82.4

**Table 3 – Level of tannins and flavonoids in ethyl and methyl extracts of *Arnica montana L.***

ethyl extract		methyl extract	
Absorbance (nm)	%	Absorbance (nm)	%
2.281	2.8	0.087	0.860
0.040	0.05	0.052	0.065

(table 3). A low level of flavonoids was also registered in the methyl and ethyl extracts. The tannic contents of ethanol and methanol extracts equaled to 2.8 % and 0,860 %, respectively.

*Arnica montana L.* is a homeopathic medicine specifically used for the treatment of trauma [14-15]. It is a member of the *Asteracea* family, previously known as *Compositae*, first described by Linnaeus in 1753 in his book *Species plantarum*. It is native to the mountainous regions of central Europe (Alps and Pyrenees), south of Scandinavia extending through the states of the former Soviet Union. The name *Arnica* derives from the Latin “*Parnica*” which means “sneeze-making”. The popular names of *Arnica* are sneezing tree, holy herb and falling herb. The active constituents of this plant are mainly flavonoids (including quercetin and its derivatives like quercetine-3-mono-glucosideo and quercetine-3-glycogalacturonic), sesquiterpene lactones (arnicolide, helenaline and dihydro-helenaline), alcohols (arnidiol, arnilenediol, isoarnilenediol), carotenoids, essential oil, inuline, and tannins among other constituents [16].

*Arnica montana* is used for treating wounds and injuries on account of its supposed abilities to control bruising, reduce swelling, and promote recovery [17]. It is one of the widely used homeopathic preparations and is popular with patients undergoing surgery. *Arnica* has effectively reduced the pain and stiffness due to arthritis of the knee [18]. It also significantly decreased the bleeding time in another randomised, placebo-controlled, crossover study [19].

The sensitivity of *S. aureus* to the extracts was shown to vary from 20.2±0.3 mm (a typical strain) to 18.6±0.6 mm (a clinical methicillin-resistant strain).

Most plants contain several compounds with antimicrobial properties for protection against aggressor

agents, especially microorganisms. Plant secondary metabolites are mostly responsible for their antimicrobial activity. Major groups of phytochemicals that possess antimicrobial properties are phenolics and polyphenols (flavonoids, quinones, tannins, coumarins), terpenoids, alkaloids, lectins and polypeptides [20-24].

Iauk (2003) reported a study designed to evaluate the antibacterial activity of i.e [25]. Arnica against anaerobic and facultative aerobic periodontal bacteria (18 overall) [25]. As a positive control a macrolid antibiotic, spiramycin was used. A methanol extract and a decoction were assayed, each 10% from 10 g powder for decoction and 15 g of the drug for extraction. The methanol extract showed an inhibiting activity against many of the species tested (MIC=2048 mg/l). The flowers of Arnica species contain especially sesquiterpene lactones which have a pseudoguaionolide structure, which often may occur as ester derivatives. Beside essential oil compounds other constituents are flavonoids, hydroxycoumarines and phenyl acrylic acids. The medicinal usage of Arnica has stimulated extensive research on constituents. Different sesquiterpenes were isolated already in the 50ies and 60ies of the 20th century [26]. The most relevant constituents so far are helenalin and 11,13-dihydrohelenanin and their derivatives. The content is varying with respect to the geographical origin. More recent investigations led to the detection of methylated flavonoids and further sesquiterpene lactones [27]. The natural variability of sesquiterpene lactones in the herbal substance is 0.3 to 1.0%. Other natural constituents of Arnica montana are flavonoids (0.4 to 0.6%), essential oil (0.2 to 0.35%), mono- and sesquiterpenes.

In our preceding works, we showed the antimicrobial activity of materials obtained from vegetable matter, phytoextracts and essential oils that showed the antimicrobial activity against isolates from the oral cavity, pharynx, and respiratory tract, including antibiotic-resistant ones.

The antimicrobial and high antioxidant activity of the ethanol extract of *Arnica montana* L., and absence of the antimicrobial effect against the probiotic strain of *Bacillus subtilis*, characterized by the antagonistic activity against opportunistic microorganisms, cause the advisability of their complex application as the basis for phytotherapeutics. Both recent works and our previous studies have shown that generalised periodontitis is complicated by persistence of associations of opportunistic antibiotic-resistant microorganisms [23]. A positive impact of the application of probiotics, including based on lactobacilli, for inflammatory diseases of the oral cavity including generalised periodontitis, has also been described [22]. This is why, it remains promising to explore a possibility of complex use of compositions based on extracts of *Arnica montana* L. and probiotic reduction of persistence of opportunistic microorganisms due to additive effect.

**Conclusion.** Thus, we have shown the antimicrobial activity of *Arnica montana* L. extracts against typical and clinical isolates of *S. aureus*, including methicillin-resistant ones, and against *S. pyogenes*. The absence of antimicrobial activity of ethanolic and methanolic extracts of arnica is revealed, and high antioxidant activity of their ethyl extracts is shown. The antimicrobial activity of the extracts to a greater extent correlated with the contents of antioxidant activity, which was the highest. The established regularities cause good prospects for further studies of the use of *Arnica montana* L. as a source of substances with antimicrobial activity against antibiotic-resistant representatives of opportunistic microbiota. The antimicrobial and high antioxidant activity of ethanol extract of *Arnica montana* L. cause the advisability of their application as the basis for phytotherapeutics.

**Perspectives of further developments.** The obtained results indicated to good prospects for further research in order to create Arnica based preparations as mouth cavity care and hygienic products.

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## АНТИМІКРОБНІ, АНТИОКСИДАНТНІ ТА ДЕЯКІ БІОХІМІЧНІ ВЛАСТИВОСТІ *ARNICA MONTANA* L. Кривцова М. В., Труш К. І., Кошова Я., Ефтімова Я.

**Резюме.** Протягом останніх років зростає інтерес до рослинної сировини з точки зору її потенційної протимікробної активності. Рослини багаті найрізноманітнішими вторинними метаболітами, такими як дубильні речовини, терпеноїди, алкалоїди та флавоноїди, для яких *in vitro* було виявлено антимікробні властивості. Як відомо, рослини роду *Arnica* містять цілий спектр біологічно активних речовин із протизапальними, генопротекторними, антидіабетичними, антиоксидантними та антимікробними властивостями. *Метою роботи* було вивчення антимікробних, антиоксидантних та деяких біохімічних властивостей спиртових екстрактів надземних частин Арніки гірської зібраної в Українських Карпатах. *Методи.* Рослини для дослідження були зібрані у Міжгірському районі, Закарпатської області. З надземних частин були створені етилові та метилові екстракти. Об'єктом дослідження були їх антиоксидантна активність (методом DPPH), загальна кількість танінів та флавоноїдів (спектрометричним методом) та антимікробна (диско-дифузійним методом). Для досліджень були використані типові та клінічні ізоляти, виділені із ротової порожнини людей із запальними захворюваннями пародонту, що характеризуються широким спектром резистентності до антибіотиків. *Результати.* Тест культури мали різну чутливість щодо екстрактів Арніки гірської: із випробуваних штамів бактерій найбільш чутливі виявились *S. aureus* та *S. pyogenes*. Виявлена відсутність протигрибкової активності етилових та метилових екстрактів. Екстракти характеризувались високою антиоксидантною активністю, при цьому найвище її значення було характерне для етилових екстрактів, дещо нижчу – метилових. Найвищий рівень танінів із всіх досліджених екстрактів був характерний для етилового екстракту. *Висновки.* Отримані результати вказують на перспективу подальших досліджень для створення препаратів на основі *Arnica* для догляду та гігієною ротової порожнини.

**Ключові слова:** антимікробні властивості, *Arnica montana* L., антиоксидантна активність, флавоноїди, таніни.

## АНТИМІКРОБНЫЕ, АНТИОКСИДАНТНЫЕ И НЕКОТОРЫЕ БИОХИМИЧЕСКИЕ СВОЙСТВА *ARNICA MONTANA* L.

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**Резюме.** В последние годы наблюдается растущий интерес к растительному сырью с точки зрения его потенциальной антимикробной активности. Растения богаты самыми разнообразными вторичными метаболитами, такими как дубильные вещества, терпеноиды, алкалоиды и флавоноиды, для которых *in vitro* было обнаружено антимикробные свойства. Как известно, растения рода *Arnica* содержат целый спектр биологически активных веществ с противовоспалительными, генопротекторными, антидиабетическими, антиоксидантными и антимикробными свойствами. *Целью работы* было изучение антимикробных, антиоксидантных и некоторых биохимических свойств спиртовых экстрактов надземных частей Арники горной собранной в Украинских Карпатах. *Методы.* Растения для исследования были собраны в Межгорском районе, Закарпатской области. Из надземных частей были созданы этиловые и метиловые экстракты. Объектом исследования были их антиоксидантная активность (методом DPPH), общее количество танинов и флавоноидов (спектрометрическим методом) и антимикробная активность (диско-диффузным методом). Для исследований были использованы типичные и клинические изоляты, выделенные из ротовой полости людей с воспалительными заболеваниями пародонта, характеризуются широким спектром резистентности к антибиотикам. *Результаты.* Тест культуры имели разную чувствительность к экстрактам Арники горной: из испытанных штаммов бактерий наиболее чувствительны оказались *S. aureus* и *S. pyogenes*. Обнаружено отсутствие противогрибковой активности этилового и метиловых экстрактов. Экстракты характеризовались высокой антиоксидантной активностью, при этом высокое ее значение было характерно для этилового

экстрактов, несколько ниже – метиловых. Самый высокий уровень танинов из всех исследованных экстрактов был характерен для этилового экстракта. *Выводы.* Полученные результаты указывают на перспективность дальнейших исследований по созданию препаратов на основе *Arnica* для ухода и гигиены за ротовой полостью.

**Ключевые слова:** антимикробные свойства, *Arnica montana* L., антиоксидантная активность, флавоноиды, танины.

## ANTIMICROBIAL, ANTIOXIDANT AND SOME BIOCHEMICAL PROPERTIES OF *ARNICA MONTANA* L.

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**Abstract.** Over the past years there has been a growing interest to plant raw materials evaluated from the viewpoint of their potential antimicrobial activity. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. *Arnica* genus plants are known to contain a whole spectrum of biologically active substances with anti-inflammatory, gene-protective, antidiabetic, antioxidant and antimicrobial properties.

*The purpose of the work* was to study the antimicrobial, antioxidant and some biochemical properties of alcoholic extracts of aboveground parts of *Arnica montana* L., gathered in the Ukrainian Carpathians. *Methods.* The plants for the study were gathered in Mizhhiria rayon, Zakarpatska oblast (Transcarpathia). From aboveground parts, ethyl and methyl extracts were produced. The subjects for study were their antioxidant activity (by DPPH method), total tannin and flavonoids (by spectrophotometric method), and antimicrobial activity (by diffusion-into-agar method). For the purpose of study, typical and clinical isolates were used from the oral cavity of human patients suffering from inflammatory diseases of periodontium, characterized by wide resistance spectrum to antibiotics. *Results.* Test cultures differently reacted to extracts of *A. montana*: of the tested strains of bacteria the most sensitive found *S. aureus* and *S. pyogenes*. The ethanol and methanol extracts were observed to show weak antimycotic activity. The extracts were characterized by high antioxidant activity, with the highest effect peculiar for ethyl extracts, and somewhat lower one for methyl extracts. The ethanol extract was characterized by the highest tannin level out of all extracts under review. *Conclusions.* The results obtained indicate the prospect of using extracts from *A. montana* as antistatic and antimycological drugs. The obtained results indicated to good prospects for further research in order to create *Arnica* based preparations as mouth cavity care and hygienic products.

**Key words:** antimicrobial properties, *Arnica montana* L., antioxidant activity, flavonoids, tannins.

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## БІОЛОГІЧНІ ОСОБЛИВОСТІ СТАФІЛОКОКІВ ТА СТРЕПТОКОКІВ ВИДІЛЕНИХ ПРИ ЗАПАЛЬНИХ ЗАХВОРЮВАННЯХ ДИХАЛЬНИХ ШЛЯХІВ

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**Зв'язок публікації з плановими науково-дослідними роботами.** Дослідження виконано у межах науково-дослідної теми: «Перспективні для використання людиною біологічні властивості мікроорганізмів – компонентів природних і штучних біоценозів» (№ державної реєстрації 0118U003277), що виконується на кафедрі мікробіології, вірусології та біотехнології Дніпровського національного університету імені Олеся Гончара.

**Вступ.** Інфекції дихальних шляхів є однією з основних глобальних проблем охорони здоров'я. За офіційними даними МОЗ, на захворювання респіраторного тракту, щороку захворює від 10 до 15 млн. осіб, становить близько 75-90% інфекційної захворюваності в країні [1].

На склад мікробних спільнот слизових оболонок верхніх дихальних шляхів впливають найрізноманітніші чинники, в тому числі чистота повітря, наявність в ньому пилу, хімічних і бактеріальних забруднень. Оскільки носоглотка займає проміжне місце між носом, синусами, вухами, гортанню та нижнім відділом респіраторного тракту, резидентна мікрофлора може бути джерелом захворювань як верхніх, так і нижніх відділів дихальних шляхів. Найбільш поши-

реними бактеріальними етіологічними факторами запалення респіраторного тракту є грам-позитивні коки – стафілококи, стрептококи. Відомо, що хронічний тонзиліт є одним з найпоширеніших захворювань верхніх дихальних шляхів, так як зустрічається у 4-10% працездатного населення і 12-15% у дітей [2,3].

Інфекції, викликані резистентними штамми, відрізняються довготривалим процесом, частіше вимагають госпіталізації і підсилюють тривалість перебування в стаціонарі, погіршують прогноз для пацієнтів. Все це підвищує прямі і непрямі фінансові витрати, а також збільшує ризик поширення резистентних штамів в оточенні [4].

Актуальність цієї теми спонукає до вивчення стафілококів, та стрептококів, оскільки вони найчастіше викликають різні захворювання, як верхніх так і нижніх дихальних шляхів людини. Проте проблема полягає не тільки в поширеності та виділення кокової мікрофлори, але і в зростанні антибіотикорезистентних штамів мікроорганізмів, які грають важливу роль запальних захворювання дихальних шляхів людини.

**Метою роботи** було виділити і вивчити біологічні властивості стрептококів та стафілококів та їх чутли-