HERBAL MEDICINES ANTIMICROBIAL EFFECT

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Abstract.

The aim. To study and compare the sensitivity of museum strains of microorganisms to the herbal medicines.

Materials and Methods. Standard strains of *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 were used for the microbiological examination. Antimicrobial activity of the herbal medicines was studied by the disk diffusion method and serial dilutions method according to European Committee on Antimicrobial Susceptibility Testing.

Results. According to the obtained results *E. coli* standard strain is maximally sensitive to tymsal. *Enterococci* were statistically significant high sensitive to panavir only. Museum strain of *S.epidermidis* was 2.17 more sensitive to tymsal than to chlorhexidine control ($p<0.05$). Yeast like fungi standard strain was the most sensitive to proteflazid. Growth inhibition zone was showing the sensitiveness of *C.albicans* to this herbal medicine, the fungicidal activity was statistically significantly higher than chlorhexidine effect in 1.2 times ($p<0.05$).

Conclusions. Analysis of microorganism's sensitivity to the herbal medicines action had showed the best antimicrobial activity of herbal medicines tymsal, panavir and proteflazid compared to traditional drugs.

Key words. Herbal medicines, bacteria sensitivity, antimicrobial effect, oropharyngeal diseases.

Introduction.

Herbal medicines are widely used in medicine due to their natural composition, complementary action, high safety, possibility of using in children under 5 years old and in women during pregnancy and lactation. They show an antiseptic and anti-inflammatory action and have a great difference with synthetics medicines such as lack of antigenic features and side effects. In this regard, plant natural products may offer a great potential and hope in oropharyngeal diseases treatment [1]. There are numerous examples of medicinal plants species which are currently the most often used in folk and official medicine in Ukraine and other countries for the treatment of oral candidiasis, periodontal and mucous membrane diseases [1-3]. Herbal medicines have a regulatory and normalization effect. They are easy to digest, nontoxic, do not cause side effects and allergic reactions. Biologically active substances contained in herbal extract are cause a pronounced therapeutic and preventive effect, stimulate regeneration processes, modulate immune reactivity of the human organism, and support the natural body colonization resistance [4-6].

Some of the herbal remedies are widely used in otolaryngology, it is assumed that they can be effective in the dental practice too [7-9]. Even «in the antimicrobial therapy of denture stomatitis, it is desirable to inhibit the growth of not only the primary causative organism, *Candida albicans*, but also other oral bacteria closely associated with the condition» [10].

In turn, the extensive use of antibiotics has resulted in antibiotic resistance that has been reported to be particularly pronounced among opportunistic species. Through the last decades facing the challenge of growing antibiotic resistance, scientists are seeking new options of antimicrobial agents, and thereupon the use of plant sources seems to be promising. This directs the attention toward the medicines, whose active ingredients approaches in developing new drugs to treat and prevent oral infectious and inflammatory diseases [7].

Thus, it is great significance to study sensitivity of microorganism's stains that can colonize the oral and respiratory tract mucosa in cases of infectious and inflammatory diseases and can cause opportunistic infections under certain conditions, to the medicine mentioned above in terms of searching for new antibacterial agents.

Some antimicrobial plants' compounds are possible therapeutic alternatives to antibiotics with unavoidable side effects.

So, the possibilities of use of medicinal plants and groups of compounds with antibacterial, antifungal, and antiviral activity in the treatment of oropharyngeal diseases have been discussed. In this regard, new formulations of antimicrobials, combination therapies and development of new bioactive compounds might be useful for a better therapeutic outcome. This work is an attempt to create a modern system of information about antimicrobial activity of plants' medicines.

Aim.

This study was aimed to compare the sensitivity of museum strains of microorganisms to the medicines that are produced from medicinal plants extracts base for the best choice of oropharyngeal diseases treatment.

Materials and Methods.

We have chosen for our study the herbal medicines that are traditionally used for oropharyngeal diseases treatment: tymsal, chlorophyllipt and compare their antimicrobial action with herbal antiviral pharmacological agents: panavir and proteflazid.

Tymsal (Herbapol Krakow, Poland). Extract of *Thymus vulgaris* (thyme) and sage is used as a medicine for the local therapy of otorhinolaryngological diseases, it have comprehensive, anti-inflammatory, and antiseptic action and consists of 0,7 mg/ml thymol, extracted from *Thymus vulgaris* (thyme) and 0,03 mg/ml of *Salvia officinalis* in 40 % alcohol. Thyme has a beneficial effect on airways. Sage contributes to soothing irritation of a throat, larynx and vocal cords and supports the natural body immunity. This extract contains 0,73 mg/ml terpenoids as an active substance.

Chlorophyllipt (Arterium, Ukraine) is a medicine by natural origin, which belongs to the group of antiseptics, widely used in otolaryngology. 10 mg/ml phytoncide extracted from the eucalyptus tree leaves in 96% alcohol is the main component of the medicines.

Panavir (Zelenaya Dubrava, Russia) is the original antiviral medicines of plant origin with a wide spectrum of antiviral action. It is used in the treatment of herpetic lesions of the oral mucous membrane and red border of lips. The substance contains 0,2 mg/ml of biologically active hexose glycoside, extracted from the *Solanum tuberosum*.

Proteflazid (Ecopharm, Ukraine) extract obtained from wild *Calamagrostis epigeios L.* and *Deschampsia caespitosa L.* by extracting of 0,3 mg/ml stable molecular complexes of tricine, apigenin, luteolin, and quercetin in 96% alcohol. This extract possesses specific antiviral properties against numerous viruses, and its direct action consists of the inhibition of DNA-virus synthesis in infected cells by reducing the activity of virus-specific enzymes of DNA polymerase and thymidine kinase. Proteflazid extract is known for its bactericidal action against some gram-positive and gram-negative microorganisms and fungi [7].

It was studied antimicrobial activity of the antiviral preparations proteflazid and panavir in comparison with similar effect of traditional plant antiseptics tymsal and chlorophyllipt. We have chosen 0,05% chlorhexidine bigluconate water solution as a basic antiseptic, which is used usually in dentistry as a control.

Standard strains of gram-positive bacteria *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *E. faecalis* ATCC 29212, gram negative bacteria *E. coli* ATCC 25922 and yeast-like fungi *C. albicans* ATCC 10231 were used for the microbiological examination. Museum strains of the golden and epidermal staphylococci, enterococci, colon bacilli and yeast-like fungi were obtained from the Institute of epidemiology and infectious diseases named by L. V. Gromashevskiy, Ukraine.

The sensitivity assessment of the museum strains to herbal medicines and 0,05% chlorhexidine bigluconate (Red star, Ukraine) was done by double serial dilutions according to the standard procedure approved by CLSI. ISO/TC 212 Clinical laboratory testing and in vitro diagnostic test systems [11].

Each extract studied was diluted in the volume of 1 cm^3 with final microorganism culture concentration of approximately 5 x 10^5 CFU (colony forming unit)/sm^3. For inoculation we used microbial suspension equivalent to 1.0 by McFarland Equivalence Standards, diluted 1/100 in saline, and then the concentration of microorganisms in this suspension was 3 x 10^6 CFU / cm^3. Inoculum taken in a dose of 0.1 cm^3 was brought into each test tube containing 1,0 cm^3 of appropriate dilutions of process solution, and into a test tube with 1.0 sm^3 of the broth medium without the herbal extract, to have so-called "negative control". Inoculum was brought in the test tubes with dilutions immediately after having been prepared.

The test tubes were incubated in a normal atmosphere at the temperature 37° C for 20-24 hours. Bactericidal minimum concentration was identified by inoculating of the microorganisms from the broth medium into the agar medium (State Research Institute of Food Resources, National Academy of Agricultural Sciences of Ukraine) and Saboraud agar (Pharmactiv, Ukraine) for *C. albicans* cultivation. Cultures of the microbial species were incubated in a normal atmosphere at a temperature 37° C for 20-24 hours. To determine the growth of the microorganisms, the test tubes with the cultures were not evaluated in transmitted light, unlike the "negative" control, because the mixing of broth medium and the solution containing some plant extracts, for example, flavonoids causes the turbidity. By taking this into the account, we only determined minimum bactericidal concentration (MBC) of herbal medicines by inoculating the microorganisms from broth medium into the agar medium.

Undiluted solutions of each herbal substance in a quantity of 1000 µg/disc were put on the standard paper discs (6 mm in diameter) to determine the sensitivity of the museum strains of microorganisms by disk-diffusing method [12]. 0.05% aqueous solution of antiseptic chlorhexidine bigluconate was applied to the standard paper discs in quantities of 25 mcg/disk. Cultures of microorganisms were inoculated onto the surface of the solid nutrient medium and indicator discs were put after drying. The Petri plates were incubated in the thermostat during 24 hours at a temperature of 37ºC. Growth inhibition zone of microorganisms was measured by using calipers, which are clearly contrasted against the background of microbial growth around the disks. These studies were repeated five times.

Statistical data processing was performed using Microsoft Excel 2010. Statistical significance was determined by Student`s t-criterion. Data were statistically significant at $p<0.05$.

Results and Discussion.

The results of the comparative analysis of antimicrobial activity of herbal medicines by the serial dilution method are shown in table I.

Tymsal did produce the best antibacterial effect to the museum strain of *E. coli*, *E.faecalis* and both of standard strains of staphylococci. Antibacterial activity of tymsal was 14.9 times higher ($p<0.001$) to colon bacilli, ($p<0.001$), 14.3 times more ($p<0.001$) to golden staphylococci and 10.7 times higher ($p<0.001$) to epidermal staphylococci compared with the chlorhexidine control. Antifungal effect of this herbal substance was 2.8 times higher ($p<0.01$) than control.

Other herbal medicines were show the same action to *C.albicans* in 2.5 times more ($p<0.05$) than to chlorhexidine exclude chlorophyllipt. Chlorophyllipt substance had the less effect to the all-museum microorganism strains compared with all other herbal medicines and the chlorhexidine control.

Enterococci were the most sensitive to panavir (MGID is 7.4±3.1 mcg/ml) and it was 5.9 more ($p<0.01$) than to chlorhexidine control (MGID is 43.6±16.9 mcg/ml).

Antimicrobial activity of herbal preparation panavir to *E. coli* was revealed in 12.5% solution. It was 3.2 times more ($p<0.05$) than to chlorhexidine and less in 4.6 times than tymsal effect on colon bacilli.

The results of the antimicrobial action comparative analysis of undiluted herbal preparations were studied with use of disc-diffusion method are presented in the table II.

According to the obtained results *E. coli* standard strain was maximally sensitive to tymsal. Growth inhibition zone was showing the high sensitiveness of colon bacilli and was

Table 1. Comparative characteristic of the herbal medicines antimicrobial activity, the serial dilution method, minimal growth inhibition dose (MGID), mcg/ml.

Medicines	Microorganism strain minimal growth inhibition dose, mcg/ml n =5				
	E. coli ATCC 25922	*E.faecalis* ATCC 29212	*S.aureus* ATCC 25923	*S.epidermidis ATCC 14990*	*C.albicans* ATCC 10231
Tymsal thymol	2.5±0.9*	19.8±8.9*	3.5±1.4*	2.9±1.1*	39.5±10.9*
Chlorophyllipt phytoncide	350±135	400±137	175±60.2	225.0±55.9	450±112
Proteflasid complex	75±20.3	135±33.5	105±41.1	60.2±20.1	45.4±16.5*
Panavir hexose glycoside	11.6±3.8*	7.4±3.1*	80±27.4	90±22.4	45±20.2*
Chlorhexidine 0,05% solution	37.2±13.9	43.6±16.9	49.9±17.0	31.0±10.1	112.5±28.2

Note: – significant difference in performance between herbal medicines antimicrobial effect and chlorhexidine control (p<0.05).*

Table 2. Comparative characteristic of antimicrobial activity of the herbal medicines, the disc diffusion method, M±m.

Medicines	The zone of microorganism strain growth inhibition, mm n=5				
	E. coli ATCC 25922	*E.faecalis* ATCC 29212	*S.aureus* ATCC 25923	*S.epidermidis ATCC 14990*	*C.albicans* ATCC 10231
Tymsal thymol	28.3±3.3*	16.2±0.4	25.8±2,9	26.7±3.4*	19.8±2.1*
Chlorophyllipt phytoncide	7.8±0.2	7.3±0.8	9.7±0.9	8.3±0.7	6.9±0.1
Proteflasid complex	12.0±0.71	11.6±0.5	14.2±2.5	14.1±2.1	18.2±1.3*
Panavir hexose glycoside	22.2±2.1	30.5±3.4*	7.3±0.8	10.3±0.5	15.2±2.9
Chlorhexidine 0,05% solution	20.6±3.2	14.6±2.1	23.3±2.9	12.3±2.1	14.2±2.5

Note: – significant difference in performance between herbal medicines antimicrobial effect and chlorhexidine control (p<0.05).*

statistically significant in compare with chlorhexidine control (p<0.05). *Enterococci* were statistically significant highly sensitive to panavir only. Growth inhibition zone was more than chlorhexidine control in 2.1 times (p<0.05). Museum strain of *S.epidermidis* was 2.2 more sensitive to tymsal than to chlorhexidine control (p<0.05). Yeast like fungi standard strain was the most sensitive to proteflazid. The fungicidal activity of this herbal medicine was statistically significantly higher than chlorhexidine effect in 1.2 times (p<0.05).

So, undiluted tymsal also was the most active against *E. coli* and epidermal staphylococci in comparison to other plant medication and chlorhexidine control. Panavir was the most effective medication against the museum strain of *Enterococcus*, antibacterial effect was 2,1 times exceeded the activity of chlorhexidine control (p<0.05). Proteflasid and tymsal have the best antifungal effect among all herbal remedies and did show the fungicidal activity which was higher than chlorhexidine effect in 1.2 times and 1.4 times (p<0.05).

These results obtained with help of disc diffusion method were confirmed the data when the serial dilution method was used. According to the obtained data, the choice of the method does not affect the test result in terms of "antimicrobial activity" and it only depends on the characteristics of the sample. So, the results of the evaluation by the two methods did not significantly differ among themselves. As the serial dilution method is the most sensitive for minimal microbial growth inhibition concentration detection as the disc diffusion method is the most effective for the best choice of medicine for oropharyngeal diseases treatment.

Conclusions.

1. Tymsal can produce the maximal antimicrobial effect to the strains of gram-negative bacteria *E. coli* ATCC 25922, gram positive cocci *E.faecalis* ATCC 29212, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 14990.This herbal medicine antimicrobial effect is statistically significantly higher than the controls chlorhexidine index and other herbal medicines (p<0.05).
2. Panavir is the most effective herbal medicine against the museum strain of *E. faecalis* ATCC 29212. This medicine antimicrobial effect is statistically significantly higher than the controls chlorhexidine and other herbal medicines (p<0.05).
3. Tymsal, panavir and proteflasid have the same antifungal effect to standard strain *C.albicans* ATCC 10231 which was statistically significantly higher than the chlorhexidine (p<0.05).
4. Both of standard methods for antimicrobial activity study are effective for the choice of the best medicines during infectious and inflammatory diseases treatment.
5. So, tymsal, panavir and proteflasid can be useful for the oropharyngeal diseases treatment if they are caused by the same species of microorganisms as the standard strains were used.

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