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IDENTIFICATION OF EFFECTIVE DILUTIONS OF DENTAL HERBAL REMEDY WITH ANTIMICROBIAL ACTIVITY

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The antimicrobial activity of tincture under the conditional name Casdent through a traditional method of twofold serial dilution in respect to museum and clinical strains of gram-positive and gram-negative of microorganisms and candida fungi has been examined. The effective dilutions of Casdent herbal agent having the bacterial static and bactericide effects have been identified. The findings of microbiological studies that we have obtained testify to prospectivity of applying the developed Casdent tincture in therapeutic dentistry.
Key words: tincture, antimicrobial effect, effective dilutions, therapeutic dentistry.

Many researchers deal with the problem of resistance of microorganisms against antibiotics. Drug up-titration is one of the methods to solve the problem [13]. On the one hand, a therapeutic effect is achieved, on the other – such arrangements may cause an emergence of adverse reaction and negative impact on the organism as a whole.

As the World Health Organization reviews (May 18, 2015) the global program to counteract resistance to antimicrobial drugs, the enhancing resistance to antimicrobial drugs threatening an ability of public health care to respond to many infectious diseases is stated. An urgent necessity to develop the new antimicrobial agents is also emphasized.

For some decades, the medical and pharmacy professionals have been consistently focused on studying the medicinal plants and traditional use thereof [1,3,4,7]. The drugs produced in the developed countries have a share of herb preparations constituting 25% [5]. Numerous researches have proved the antimicrobial activity of medicinal plant raw materials and biological medicines as well historically used in the comprehensive drug therapy of infectious and inflammatory dental diseases [2,8,12,16].

The persistent growth of number of inflammatory periodontal diseases justifies the need of developing the new efficient drugs, including the herb preparations [9,10,11,15].

Our prior studies show the results of developing the tincture conventionally called Casdent. A possibility of using three types of pharmacopeia medicinal plant raw materials has been substantiated: licorice roots, sedge cane rootstocks, burnet rootstocks with its roots as components of the complex tincture for curing the periodontal inflammation. Following the findings of microbiological surveys, the optimal «raw materials to extracting medium» ratio has been proven [15].

Also the antimicrobial activity of the developed extraction agent through diffusion to agar in the «wells» modification in respect to the gram-positive and gram-negative of microorganisms and candida fungi has been identified, availability of fatty acids in the tincture is confirmed and they are identified, whereby the biological value of the herbal product is supplemented since fatty acids deal with the antimicrobial properties along with the flavonoids and volatile compounds [14].

Some aspects of clinical use of natural drugs in dentistry require profound surveys. The common view assumes that a vital role, among the requirements made to antimicrobial drugs, besides the efficiency and safety, is given towards the entrance of adequate concentrations of biologically active substances into the insulted area.

Method to prepare dilutions and further application thereof for the liquid drugs deployed in dentistry is shown in the insert, which method for some herb preparations is displayed in the table below.

It is essential to take into account that upon external use of liquid dosage forms in dentistry there is an additional several-fold dilution thereof with saliva, crevicular fluids, and if any abnormal focus – with exudates [6]. The aforesaid, in spite of the good state of knowledge of the antimicrobial and antifungal activity of Casdent dental tincture, serves as a basis for the tincture's dilution antimicrobial peculiarities.

Table - Recommendations to apply liquid dental agents

Herbal remedy	Auxiliary substance	Dosage form	How to use and recommended period of use
Stomatophyt, «Phytopharm Klenka S.A.», Poland; gargle of 45 ml, 120 ml in flasks No. 1	60-70 % ethanol	10 ml per ¼ glass of water (15 % liquor)	mouth wash with the liquor 3–4 times a day
Phytodent, PJSC «Red Star», Ukraine; tincture of 100 ml in flasks, cups	40 % ethanol	1 share per 1 or 2 shares of water	gargling, rinsing, spraying with the drug solution, 3–5 times a day for 5 minutes; stomatic bathing, 3–5 times a day for 2–3 minutes
		1 teaspoonful (5 ml) per ¼ glass of water	

		1 share per 1 share of water	plugging of the drug saturated sponges into gingival pockets and dental gaps, 1–2 times a day for 15–20 minutes applications with the diluted drug solution (2–3 times a day for 15–20 minutes)
Stomatoklin, preventive agent by Pharmaceutical company «Vertex», Ltd in cooperation with GNCLS Research Plant, Ltd, Ukraine; bottle 100 ml in a pack-age 40 % ethanol	1 teaspoonful (5 ml) per 1 glass of water	applications (15–20 minutes), stomatic bathing (1–2 minutes) 2–3 times a day, rinsing with the drug solution, 2–3 times a day 2–3 teaspoonfuls (10–15 ml) per glass of water	
Rotocan, OJSC «Lubnypharm», Ukraine; liquid in flasks of 55 ml or 110 ml	40 % ethanol	1 teaspoonful (5 ml) per 1 glass of warm water	every other day plugging of the drug saturated sponges into for 20 minutes (4–6 times); applications within 15–20 minutes or stomatic bathing (1–2 minutes) 2–3 times a day during 2–5 days

The aim is to study various dilutions of Casdent tincture and identify the most effective ones for substantiating the reasonable application of the developed liquid dosage form in therapeutic dentistry.

Materials and methods

Targets of research include the Casdent tincture as developed by employees of the Department of General Pharmacy and Drug Safety of the Institute of Pharmacy Professionals Qualification Improvement of the National University of Pharmacy and the comprehensive herb preparation as the liquid extract Stomatophyt («Phytopharm Klenka S.A.», Poland) chosen as the comparative drug, which herb medicine is widely used in dentist's practice against seropurulent gingivitis, periodontitis, fungus inflammation of tunica mucosa of mouth caused by yeast-like fungi.

Examination has been held by the twofold series dilution method common for microbiological practice in the Microorganisms and Mediums Biochemistry Laboratory (the Laboratory for Biochemistry and Biotechnology since April 2015) of the State Establishment «Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine», guided by Ph. D. in biol., senior scientist T. P. Osolodchenko.

The museum and clinical strains of the following microorganisms: *S. aureus* ATCC 25923, *S. epidermidis* No. 16589, *S. haemolyticus* No. 16595, *S. pneumonia* ATCC 49619, *S. mutans** clinical strain No. 45, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* K-7 NCTC 9127, *B. subtilis* ATCC 6633, *C. albicans* ATCC 885-653, *C. catenulata* (*C. rugosa*) Sclar C-27, *C. albicans* clinical strain No. 23, have been used for estimating the antimicrobial effect.

In the course of researches, the daily microorganism cultures, grown at respective digest media and fit with the State Pharmacopoeia of Ukraine, have been used.

Upon studying the antimicrobial effect of tinctures, the solvents with ethanol concentration identical to the targeted items have been prepared as controls.

In addition, the model samples with preparations in 10 ml of digest media have been prepared. The optical density value is 0.5 points by McFarland scale according to the order No. 167 of Ministry of Health of Ukraine issued on 05.04.2007. The obtained data of survey are statistically processed according to Student.

Results and discussion

The conducted examination of antimicrobial activity of Casdent tincture has demonstrated an antimicrobial effect in respect to the taken strains of gram-positive, gram-negative microorganisms and candida fungi to a various extent.

Reviewing the findings, one should note an inhibiting concentration of Casdent tincture in dilution (1:32) in respect to the following microorganisms: *S. epidermidis, S. pneumoniae, B. subtilis* and candida fungi: *C. albicans, C. catenulata* (*C. rugosa*) Sclar C-27*, C. albicans* clinical strain No. 23.

It is identified that minimal inhibiting concentration (MIC) values for the strains: *S. aureus, S. haemolyticus, S. mutans* clinical strain No. 45, *E. coli, P. aeruginosa* correspond to dilution of (1:16), with the only exception of antimicrobial activity of the test-strain *K. pneumoniae*, where MIC is at the rate of (1:8).

Comparing the results of antimicrobial effect of Stomatophyt and the developed Casdent tincture, the said trend of antimicrobial activity has been ascertained. However, Stomatophyt is valid in dilution of (1:32) only in respect to *S. aureus* and *C. albicans,* but in respect to other reviewed microorganisms: *S. epidermidis, S. haemolyticus, S. pneumoniae, S. mutans* clinical strain No. 45, *E. coli, P. aeruginosa, K. pneumoniae, B. subtilis, C. catenulata* (*C. rugosa*) Sclar C-27*, C. albicans* clinical strain No. 23, Stomatophyt is active in dilution of (1:16). It is quite difficult to identify the efficient dilution of Casdent tincture under review, based only on the obtained findings.

Therefore, for purpose of identifying the use rate Casdent tincture and the control agent: Stomatophyt have been respectively recalculated as per 10 ml of the nutrient broth. Besides, there is an intention to have additional researches on antimicrobial activity of dilutions (1:10) and (1:20) of Casdent tincture and of the control: herbal remedy Stomatophyt.

Application of the above-mentioned dilutions of the studied items has enabled to make a more precise designation of concentrations' transfer as required for identifying MIC and the minimal bactericide concentration (MB_cC) due to availability of interim values. Results of determining the inhibiting and bactericide concentration of Casdent and Stomatophyt are presented in the Fig. 1 and Fig. 2.

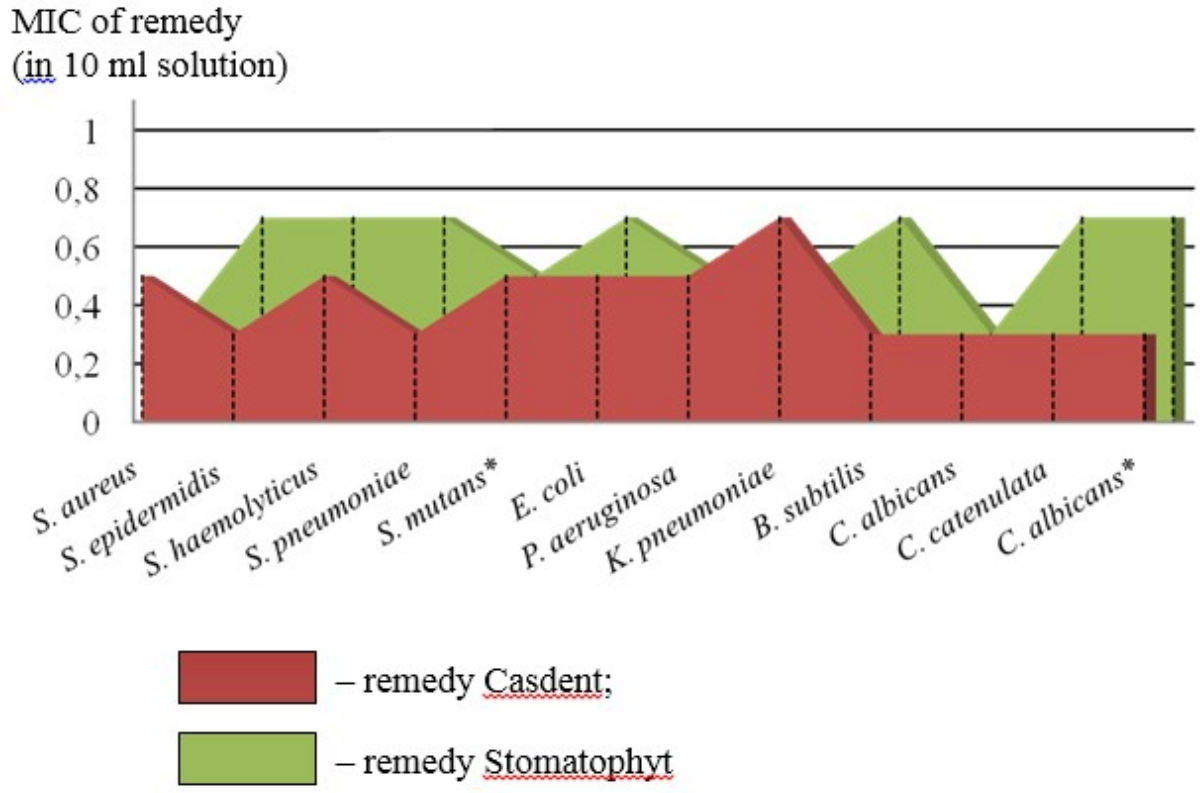

Fig. 1. Profile of the inhibiting concentrations of herbal remedies dilutions in respect to the museum and clinical* strains of microorganisms

Interpretation of MIC values on dilution of the targeted item and control has made it possible to assess the Casdent tincture antimicrobial activity degree, which is no worse than the one of the imported herbal medicine Stomatophyt.

Considering over the values of bactericide concentration of Casdent solutions (1:20), we have noted that the MB_cC values for *S. aureus, S. pneumoniae, B. subtilis* are higher than those of similar dilutions Stomatophyt.

Conclusion

1. The presented approach has enabled to identify the effective doses of Casdent preparation under examination, which doses make it possible to calculate its effective concentrations for applying it in dentistry with a purpose of treatment and prevention.
2. In order to achieve bacterial static effect, one should use the solution of 1 teaspoonful (5 ml) of Casdent tincture per 100 ml of water. Bactericide effect is ensured through application of the following solution: 2 teaspoonfuls (10 ml) of Casdent herbal remedy in the same volume of water.
3. Subject to the results of microbiological investigations, there are prospects for the developed Casdent tincture, its dilutions are substantiated, which fact will be accounted for upon issuing the recommended application of the domestic liquid dosage form in therapeutic dentistry.

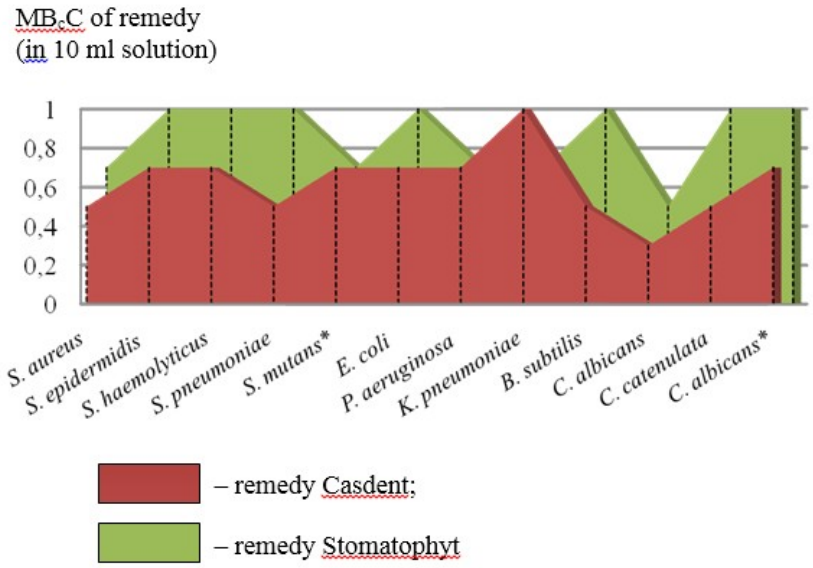

Fig. 2. Profile of the bactericide concentrations of herbal remedies dilutions in respect to the museum and clinical* strains of microorganisms

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Introduction. There are different ways to solve the problem of resistance of microorganisms. One of them includes an increase of the drug dose, thereby arising adverse reaction, and the other includes a development of the new antimicrobial agents, where no less focus is given to drugs on basis of medicinal plant raw materials owning to the antimicrobial activity confirmed by scientific researches. Herbal remedies are included into treatment

codes against infectious and inflammatory dental diseases, however while there is a persistent growth of mouth inflammatory diseases we have signals for expediency of new ones to be produced. Our prior studies deal with developing the tincture conventionally called Casdent, substantiation of using such three types of pharmacopeia medicinal plant raw materials as: licorice roots, sedge cane rootstocks, burnet rootstocks with its roots. The previously determined antimicrobial and antifungal activity of Casdent tincture is insufficient for recommending the use thereof in the therapeutic dentistry, as the external use provides for an additional dilution thereof with saliva, crevicular fluids, exudates.

The aim is to identify the most efficient dilutions of Casdent tincture to substantiate its reasonable application in dentistry.

Materials and methods. Target of examination is Casdent tincture as developed by employees of the Department of General Pharmacy and Drug Safety of the Institute of Pharmacy Professionals Qualification Improvement of the National University of Pharmacy. Control is the herb preparation: Stomatophyt («Phytopharm Klenka S.A.», Poland). The work is performed at the State Establishment «Mechnikov Institute of Microbiology and Immunology of the National Academy of Medical Sciences of Ukraine» through use of museum and clinical strains of microorganisms, which daily cultures have been grown on the respective digest media according to the requirements of the State Pharmacopoeia of Ukraine. The twofold serial dilution method (microbial load has been determined at 0.5 unit as per McFarland scale) has been used. The obtained data of survey are statistically processed according to Student.

Results and discussion. Inhibiting concentration of Casdent tincture diluted in (1:32) has been empathized in respect to: *S. epidermidis, S. pneumoniae, B. subtilis* and candida fungi: *C. albicans, C. catenulata* (*C. rugosa*) Sclar C-27*, C. albicans* clinical strain No. 23. For strains: *S. aureus, S. haemolyticus, S. mutans* clinical strain No. 45, *E. coli, P. aeruginosa,* the minimal inhibiting concentration value corresponds to dilution of (1:16). Although a similar trend of antimicrobial activity is ascertained at comparing the antimicrobial effect's values of Casdent tincture and those of the control: Stomatophyt, the compared preparation is valid as diluted in (1:32) only in respect to *S. aureus* and *C. albicans,* and in respect to other cultures – as diluted in (1:16). Additional researches of antimicrobial properties of solutions Casdent and Stomatophyt: (1:10), (1:20) have made it possible to identity the minimal inhibiting and bactericide concentration thereof. Based on the level of antimicrobial activity of Casdent tincture it is determined that it is highly competitive with the level of the imported preparation: Stomatophyt, and upon examination of values of bactericide concentration of Casdent dilutions: (1:20) for *S. aureus, S. pneumoniae, B. subtilis,* somehow excels similar dilutions of Stomatophyt.

Conclusion. The effective solutions of Casdent tincture are identified: 1 teaspoonful (5 ml) of Casdent tincture per 100 ml of water – for bacterial static effect and 2 teaspoonfuls (10 ml) in the same volume of water – for bactericide effect. The results of microbiological examinations testify to prospectivity of the developed tincture and will be taken into account when issuing the recommended application thereof in therapeutic dentistry.

Key words: tincture, antimicrobial effect, effective dilutions, therapeutic dentistry.