

UDC 616.31-002:616.311.2-002:616.314.17-008.1:616-08

IROKO EMAMUZO MATTHEW, D.S. ZHURENKO, O.P. STRILETS, N.V. KHOKHLENKOVA
/National University of Pharmacy, Kharkov/

Study of antimicrobial activity of «Aloedental» gel for treatment of periodontal diseases

Summary

This study was conducted to evaluate antimicrobial effect of gel containing plant extracts for treatment of periodontal disease.

Antimicrobial activity was determined by evaluating antibiotic sensitivity on the following test microorganism strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885/653.

According to the data, «Aloedental» gel has a broad spectrum of antimicrobial activity and is characterized by the activity against gram-positive (*Staphylococcus aureus* ATCC 25293, *Bacillus subtilis* ATCC 6633), gram-negative (*Escherichia coli* ATCC 25922) bacterial cultures microorganisms and shows high activity against fungus *Candida albicans* ATCC 885-653.

Key words: antimicrobial activity, diffusion method, gel, periodontal diseases, extract of Aloe vera, Extract of Oak bark

Annotation

Introduction. Oral diseases are one of the biggest general health problems of life and are expensive to treat. In the treatment of inflammatory periodontal disease and oral mucosa it is very important to establish the agent and use antibacterial drugs, considering sensitivity to microorganisms.

The activity of each component of the drug should be directed to a particular factor in the inflammatory process. Local medical treatment is carried out in order to influence the microflora of pathological gum pockets, and preferred mostly herbal medicines. These requirements meet the Oak (*Quercus robur* L.), which has long been used in dental practice for the treatment of inflammatory periodontal disease. Also, Aloe vera is extremely helpful in the treatment of gum diseases like gingivitis, periodontitis.

Composition and technology of «Aloedental» gel based on plant extracts was developed at the Department of Drug Technology of National University of Pharmacy.

The purpose of this work is to study the antimicrobial activity of «Aloedental» gel for the treatment of periodontal diseases.

Materials and methods. The antimicrobial activity was investigated using cup diffusion method. The results of studies characterize the antimicrobial activity of the drug as well as the release of a substance from the base, as stunted growth of microorganisms zone formed by diffusion of substances in dense nutrient medium.

Results & discussion. The level of antimicrobial activity of the sample gel «Aloedental» is not inferior to the comparator. It exceeded antibacterial action of gel «Kamistad» and «Metrogyl Dent» to the culture of *S. aureus* and *E. coli*. Antifungal activity is most showed against yeast fungus (*C. albicans*).

Conclusion. According to the data «Aloedental» gel has a broad spectrum of antimicrobial activity and is characterized by the activity against gram-positive, gram negative bacterial cultures microorganisms and shows high activity against fungus *Candida albicans*.

Introduction

Oral diseases are one of the biggest general health problems of life and are expensive to treat. Dental caries, gingivitis and periodontal disease in children and adults are among the most important preventable global infectious diseases in Ukraine, Nigeria and the world in general. According to latest WHO report, more than 90 % of the adult population of the Earth are prone to periodontal diseases, which lead to loss of teeth, chronic oral diseases, significantly impairs the health and quality of life [1, 2].

Local treatment is an important part of a comprehensive treatment of periodontal tissue which is aimed at pain relief, reduction of microbial contamination of periodontal pockets, eliminating inflammation, restore normal homeostasis, stimulation of regeneration processes, recovery disturbed functions periodontal tissues.

One promising avenue is considered the development of long-acting medications that provide local and uniform release of the active ingredient from the dosage form, creating its high therapeutic concentrations at the site of application without significant impact on the level of the drug in the systemic circulation.

The gel is the most balanced medicinal form for the local therapy of periodontal diseases, which well distributes and absorbs on the mucosa and it stipulates the high bioavailability of active substances. The gel has the properties of solids and liquids, so that it is effective at drawing application. The gels are well absorbed and distributed in the mucous that cause high bioavailability of active substances and

prolongation of action depending on the type and nature of the base. Into the structure of the gels hydrophilic and lipophilic medicinal substances can be administered that affect on their release and bioavailability, the efficacy and harmlessness of the finished product. Taking into account the above factors, dosage form soft gel was chosen in our work [3].

It was established, that in Ukraine there are recorded 10 names of gels used in dentistry practice [4]. The majority of Ukrainian consumers need gels produced abroad, reducing the economic and physical accessibility. Pharmacological activity caused by synthetic active ingredients that restricts their use, particularly in children and geriatric practice. Only 30 % of dental gels contain a combination of active substances of natural origin (chamomile flowers) and synthetic substances. No one gel with substances is of natural origin.

In the treatment of inflammatory periodontal disease and oral mucosa it is very important to establish the agent and use antibacterial drugs, considering sensitivity to microorganisms [5]. The continuous growth of antibiotic resistance in microorganisms makes it necessary to update the arsenal of antibiotics and the efficiency of existing antibacterial drugs. Dentists in the treatment of periodontal disease prefer drug, which along with high therapeutic effect does not have negative side-activities - drugs of plant origin.

The activity of each component of the drug should be directed to a particular factor in the inflammatory process. Local medical treatment is carried out in order to influence the microflora of pathological gum pockets, and preferred mostly herbal medicines that have anti-inflammatory, antimicrobial, reparative action. These requirements meet the Oak (*Quercus robur* L.), which has long been used in dental practice for the treatment of inflammatory periodontal disease and oral [6, 7]. At the Department of Drug Technology of National University of Pharmacy thick Oak bark extract (TOBE) with antimicrobial, anti-inflammatory, haemostatic activity are developed and put into industrial production.

Aloe vera is one such product exhibiting multiple benefits and has gained considerable importance in clinical research. Dental uses of Aloe vera are multiple. It is extremely helpful in the treatment of gum diseases like gingivitis, periodontitis. It reduces bleeding, inflammation and swelling of the gums. It is a powerful antiseptic in pockets where normal cleaning is difficult, and its antifungal properties help greatly in the problem of stomatitis, aphthous ulcers of the mouth [8, 9].

Composition and technology of «Aloedental» gel based on plant extracts (Aloe Vera and Oak bark) was developed at the Department of Drug Technology of National University of Pharmacy. The combination of TOBE with extract of Aloe Vera in developed dental gel provides comprehensive complex therapeutic effect (antimicrobial, anti-inflammatory, reparative, haemostatic).

The purpose of this work is to study the antimicrobial activity of «Aloedental» gel for the treatment of periodontal diseases.

Materials and methods

The antimicrobial activity was investigated using cup diffusion method [10]. This method is based on the ability of active substances to diffuse into the agar medium that matters preliminary cultures inoculated microorganisms. The results of studies characterize the antimicrobial activity of the drug as well as the release of a substance from the base, as stunted growth of microorganisms zone formed by diffusion of substances in dense nutrient medium. Prepared samples were stored under conditions of gels refrigerator (5-3 ° C).

All studies were conducted in strict aseptic conditions, using a laminar box (cabinet of biological safety AS2-4E1 «Esco», Indonesia).

As a test cultures pure cultures using: Gram-positive bacteria *Staphylococcus aureus* ATCC 25293, *Bacillus subtilis* spore culture ATCC 6633, a Gram-negative *Escherichia coli* culture ATCC 25922. Antifungal activity was determined with respect to the yeast-like fungus - *Candida albicans* ATCC 885-653. In the experiments used 1 daily suspension of bacterial microorganisms in saline and 2 daily culture of yeast-like fungus.

Antimicrobial activity test samples were compared to the registered in Ukraine dental gels with such action: gel for gums «Metrogyl Dent» (Unic Pharmaceutical Laboratories, India) - classical drug for the treatment of inflammatory periodontal diseases, and gel for gums «Kamistad» (Stada, Germany).

In Petri dishes mounted on a horizontal plane were placed 10 ml of uninfected «hungry» AGV agar (for the upper layer when using bacterial cultures used the meat-peptone agar (MPA), working with yeast like fungi - agar Saburo). After solidification of this agar layer on its surface at an equal distance from each other and from the edge of the cup were placed a sterile steel cylinders (height 6.0 ± 0.1 mm, outer diameter 10 ± 0.1 mm) and was poured melted and chilled to $45-48^\circ\text{C}$ upper agar layer with cultures of microorganisms in an amount of 14ml.

After cooling and solidification of the upper layer of the culture medium cylinders was removed with sterile forceps and in the formed wells were poured studied gel samples until their holes made filling investigated.

Petri dishes were kept for 30-40 minutes at room temperature and placed in an incubator – bacterial cultures at $32.5 \pm 2.5^\circ\text{C}$ for 18-24 h, yeast-like culture at $22.5 \pm 2.5^\circ\text{C}$ for 48 h.

The culture zone at 5 to 48 hours of records of the results was performed by measuring microbial growth inhibition zones including the diameter of the holes. The measurements were performed with an accuracy of 1 mm, while the complete lack of visible growth.

The diameter zone of inhibition of growth of microorganisms described the antimicrobial activity of the experimental samples:

- absence of zones of inhibition of growth of microorganisms around the hole, and zone of inhibition of growth a diameter not more 10 mm, evaluated as insensitivity to microorganisms introduced into the hole sample;
- diameter zone of inhibition of growth of microorganisms is 11-15 mm was assessed as weak growth of cultural sensitivity to the concentration of the studied active antimicrobial substances;
- diameter zone of inhibition of growth of microorganisms is 15-25mm was assessed as sensitivity strain microorganisms to the sample;
- diameter zone of inhibition of growth of microorganisms more than 25mm was assessed as a high sensitivity of microorganisms to the sample.

Statistical processing of results was carried out according to the State Pharmacopoeia of Ukraine at a confidence level $P = 0.95$, and the number of repeats $n=6$ [11].

Results & discussion

The results of the study of antimicrobial activity of «Aloedental» gel for the treatment of periodontal diseases in relation to different cultures microorganisms are listed in table 1.

The data obtained experimentally and are presented in table 1 indicates that all gels have antimicrobial activity.

The level of antimicrobial activity of the sample gel «Aloedental» is not inferior to the comparator. It exceeded antibacterial action of gel «Kamistad» and «Metrogil Dent» to the culture of *S. aureus* and *E. coli*. Antifungal activity is most against yeast fungus (*C. albicans*)

Table 1. Results of antimicrobial activity of samples gels (n= 5)

Name of gel	Culture of microorganisms			
	Staphylococcus aureus ATCC 25923	B. Subtilis ATCC 6633	Escherichia coli ATCC 25922	Candida albicans ATCC 653/885
	Diameter of Inhibition Zone, mm			
«Aloedental»	19.8 ± 0.4	21.4 ± 0.5	200 ± 07	29.4 ± 0.5
«Metrogil Denta»	15.1 ± 0.3	25.1 ± 0.7	14.7 ± 0.6	22.0 ± 0.1
«Kamistad»	17.5 ± 0.2	16.6 ± 0.2	16.2 ± 0.5	15.8 ± 0.3

It should be noted that the most antimicrobial activity of the «Aloedental» gel shown against the bacterial test strains: *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 885-653. The diameters of the zones stunted growth of test microorganisms were respectively 21.4 ± 0.5 mm and 29.4 ± 0.5 mm.

Conclusion

According to the data, «Aloedental» gel has a broad spectrum of antimicrobial activity and is characterized by the activity against gram-positive (*Staphylococcus aureus* ATCC 25293, *Bacillus subtilis*

ATCC 6633), gram negative (*Escherichia coli* ATCC 25922) bacterial cultures microorganisms and shows high activity against fungus *Candida albicans* ATCC 885-653.

List of references

1. WHO Global Oral Health. Available: <http://www.who.int/mediacentre/factsheets/fs318/en/>
2. Poul Erik Petersen The global burden of oral diseases and risks to oral health / Denis Bourgeois, Hiroshi Ogawa, Saskia Estupinan-Day // Bulletin of the World Health Organization. – 2005. – Vol. 83. – P. 661–669.
3. Стоматологічні гелі як сучасна лікарська форма для лікування й профілактики інфекційно-запальних захворювань порожнини рота / Н. М. Дев'яткіна, О. М. Важнича, Т. О. Петрушанко [та ін.] // Український стоматологічний альманах. – 2010. – № 3. – С. 3–7.
4. Iroko Emamuzo Matthew, Khokhlenkova Natalya Research assortment of gels in pharmaceutical market of Ukraine // Ukrainskyi visnyk psikhonevrolohii. – 2015. – Vol. 23, 3(84). – P. 40–43.
5. Paddmanabhan P. Antimicrobials in treatment of periodontal disease – A review [Text] / P. Paddmanabhan // Journal of Dental and Medical Sciences. – 2013. – Vol. 4, Issue 5. – P. 19–23.
6. Ярних Т.Г. Дуб звичайний. *Quercus robur* L. Аналітичний огляд / Т.Г. Ярних, Н.В. Хохленкова, В.М. Чушенко, М.В. Бурак // Провізор. – 2008. №8. – С. 3638.
7. Bhattacharjee S. K. Handbook of Medicinal Plants / S.K. Bhattacharjee. – India: Pointer Publishers, 2001. – 540 p.
8. Ajmera N. Aloe vera: It's effect on gingivitis / N. Ajmera, A. Chatterjee, V. Goyal // J. Indian. Soc. Periodontol. 2013. – Vol. 17. – P. 435–438.
9. Bhat G. Aloe vera: Nature's soothing healer to periodontal disease / G. Bhat, P. Kudva, V. Dodwad // J. Indian Soc. Periodontol. 2011. – Vol. 15. – P. 205–209.
10. The study of the specific activity of antimicrobial drugs: a method. recommendations [Text] / Yu. L. Volyanskiy, I. S. Gritsenko, V. P. Shyrokovikov [et al.] – Kiev: SiEntScPhC Ministry of Helthcare of Ukraine, 2004. – 38 p.
11. State Pharmacopoeia of Ukraine [Text] / State Enterprise «Scientific and Expert Pharmacopoeias Centre». – 1 st Ed. – H.: RIREH, 2001. – Appendix 1. – 2004. – 520 p. 11.

Резюме

Исследования антимикробной активности геля «Алоэдентал» для лечения заболевания пародонта

Ироко Имамзоз Метью, Д.С. Журенко, О.П. Стрилец, Н.В. Хохленкова
Национальный фармацевтический университет, Харьков

Данное исследование было проведено для оценки антимикробного эффекта геля, содержащего экстракты растений для лечения заболеваний пародонта.

Антимикробную активность изучали *in vitro* методом диффузии в агар путем оценки чувствительности к антибиотикам на следующих штаммах тест-микроорганизмов: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885/653.

В результате исследований установлено, что «Алоэдентал» гель обладает широким спектром антимикробной активности и характеризуется активностью в отношении как грамположительных (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), так и грамотрицательных (*Escherichia coli* ATCC 25922) бактериальных культур микроорганизмов. Доказана высокая активность геля в отношении дрожжеподобного гриба рода *Candida Albicans* ATCC 885-653.

Ключевые слова: антимикробная активность, метод диффузии в агар, гель, заболевания пародонта, экстракт Алоэ вера, экстракт Дуба коры.

Резюме

Дослідження антимікробної активності гелю «Алоедентал» для лікування захворювань пародонта

Ироко Имамзоз Метью, Д.С. Журенко, О.П. Стрилец, Н.В. Хохленкова
Національний фармацевтичний університет, Харків

Дане дослідження було проведено для оцінки антимікробної ефекту гелю, що містить екстракти рослин для лікування захворювань пародонту.

Антимікробну активність вивчали *in vitro* методом дифузії в агар шляхом оцінки чутливості до антибіотиків на наступних штаммах тест-мікроорганізмів: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885/653.

В результаті досліджень встановлено, що «Алоедентал» гель володіє широким спектром антимікробної активності і характеризується активністю у відношенні як грампозитивних (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), так і грамнегативних (*Escherichia coli* ATCC 25922) бактеріальних культур мікроорганізмів. Доведено високу активність гелю відносно дріжджоподібного гриба роду *Candida Albicans* ATCC 885-653.

Ключові слова: антимікробна активність, метод дифузії в агар, гель, захворювання пародонта, екстракт Алоє вера, екстракт Дуба коры.