






Rationale for antimicrobial activity of phytocomplexes recommended for use in oncological pathology

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The modulation of the composition of the oral microbiome in cancer patients is a significant approach to preventing and managing complications from chemotherapy and radiation. The use of mouthwashes is the first treatment option to rehabilitate these patients. **Aim:** This study evaluated the characteristics of the impact of phytocomplexes on the dynamics of the formation of a mixed microbial consortium comprising potential agents of infectious complications of oncological processes. **Methods:** The work carried out an experimental assessment of the antimicrobial properties of the phytocomplexes 'Mukosept' and 'Aphrodite', by analyzing their influence on the consortium microbial group *S. sanguis* and *F. nucleatum*, using the infrastructure of the unique scientific installation "Transgenebank". **Results:** Based on the results of the experiments, using the automatic programmable cultivation technique, differences in the development of the bacterial population were identified, considering the addition of the studied samples of phytocomplexes at different concentrations. It has been revealed that the phytocomplex 'Mukosept' exhibits a more evident antibacterial effect in comparison to the phytocomplex 'Aphrodite'. **Conclusion:** Thus, the influence of phytocomplexes on the dynamics of the development of a mixed microbial consortium of potential pathogens of infectious complications of oncological processes has been determined.

Keywords: Mouthwashes. Phytotherapy. Palatal neoplasms. Gastrointestinal microbiome. Mucositis. Microbial interactions.



Introduction

The treatment and rehabilitation of cancer patients is an intricate and crucial process that necessitates close collaboration across diverse disciplines^{1,2}. A special category of patients consists of cancer patients with postoperative defects of the upper jaw. Their rehabilitation is carried out through the work of a polymer removable denture with an obturator, which ensures the delimitation of the oral and nasal cavities, which is important for the restoration of chewing and speech functions, as well as facial aesthetics³. Nonetheless, a removable denture is not capable of preventing the mutual infiltration of the microflora of the oral and nasal cavity, thereby resulting in an imbalance of microflora and the emergence of complications⁴. At the same time, the microflora disorders are also aggravated by oncological therapy (chemo- and radiotherapy)⁵. Furthermore, the microbiota content may be influenced by the construction materials from which the removable denture-obturator is made, which may contribute to increased values of microbial adhesion and their colonization⁶⁻⁸. Meanwhile, the microbiota content plays an important role in establishing favorable conditions for tissue regeneration, as well as the possibility of developing local and general diseases⁹⁻¹¹.

All of the aforementioned factors indicate the necessity to enhance the procedures for preventing and treating dysbiotic complications in cancer patients, as well as potential approaches to rectify the microbiotic component. In this connection, the objective of this study was to evaluate the characteristics of the impact of phytocomplexes on the dynamics of the formation of a mixed microbial consortium comprising potential agents of infectious complications of oncological processes.

Materials and Methods

In order to experimentally evaluate the antimicrobial properties of the studied samples (the innovative mixture 'Mukosept' and the well-known one 'Aphrodite'), successive series of dilutions of phytocomplexes were carried out in order to assess their effect in relation to the consortium microbial group *S. sanguinis* + *F. nucleatum* during automatic programmable cultivation of the bacterial population. Cultivation of the mixed microbial consortium was carried out using Wilkins Chalgren Anaerobic Broth liquid nutrient (Himedia, India). In order to cultivate microorganisms in a bioreactor, 50 ml conical centrifuge round-bottom flasks equipped with a membrane filter (TTP, Switzerland) were used. The anaerobiosis condition was achieved by replacing atmospheric air with a control gas mixture SSS CGM 10700-2018 (Linde Gas Rus JSC, Russia) by means of a vacuum pump. Composition of the control gas mixture comprises of H₂ (10%); CO₂ (10%); NO (residual). To control the maintained conditions of anaerobiosis, an exhaustion indicator was used. The bioreactor settings are as follows: temperature of 37.1°C; rotor speed of 50 min⁻¹; indicator sampling rate of 2 h⁻¹; reverse period of 0 sec; volume of substrate in a flask is 20 ml; wavelength is 850 nm. The cultivation time is 48 hours. The infrastructure of the distinctive scientific installation "Transgenebank" was utilized in the execution of the task. The primary solution of the phytocomplex was prepared by utilizing sterile distilled water that was heated to 80°C, followed

by the addition of the contents of the filter bags and their subsequent storage in a sealed flask for a duration of 10 minutes. The tested concentration was 150 and 300 µg/ml. The obtained results were analyzed by changes in optical density (OD) under batch cultivation conditions, with interpretation of changes in key points of the process in McFarland standards (Umcf). For each type of microorganism separately, as well as for the formation of a consortium suspension, a pure growth suspension was prepared, bringing the concentration to 1.5×10^8 CFU, which corresponded to an optical density of 0.5 Umcf.

Results

As per the findings obtained from the cultivation of the bacterial consortium comprising *S. sanguinis* and *F. nucleatum* (Fig. 1), all the classical stages of the individual cultivation process were observed: lag phase, log phase (exponential), stationary phase, and period of cell death. The initial indications of the growth of the bacterial population were observed during the duration of 4 to 8 hours of the experiment, resulting in the subsequent transition of the development trend of the curve into a pronounced exponential ascent. The key indicator of optical density at the end of the P-2 period (α indicator) is 3.22 ± 0.3 Umcf (14 hours). Further formation of the development curve according to a linear trend made it possible to compare the period from 18 hours to 24 hours with the period of stationary culture (P-4), where there were no signs of an increase in the OD value. The average optical density in this interval is 3.66 ± 0.3 Umcf.

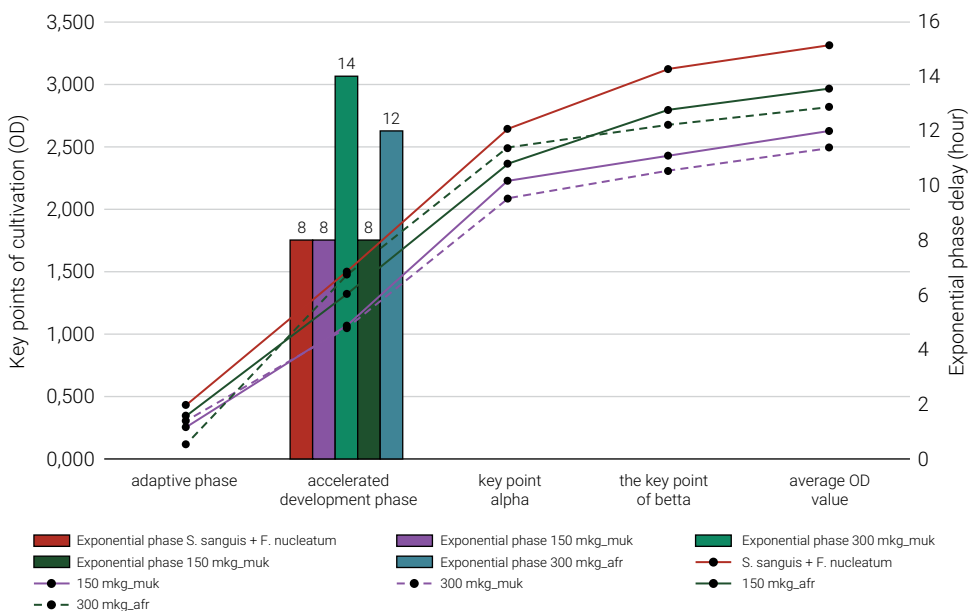


Figure 1. The key points of optical density and prolongation of log phase during cultivation *S. sanguinis* + *F. nucleatum* with phytocomplexes (Compiled by the authors)

A similar principle of population development was observed when analyzing the studied samples of 'Mucosept' and 'Aphrodite' at a concentration of 150 g/ml. The time of generative activity of cells in the log phase was decreased in comparison with the control sample. This resulted in lower values of optical density. For the 'Mucosept' sample, there was also an additional delay in reaching the key indicator α . Furthermore, in contrast to the control sample, a more pronounced decrease in the intensity of bacterial growth was observed in the 'Mucosept' sample. The maximum OD value at the end of the P-2 period is as following: 2.21 ± 0.3 Umcf (14 hours) for the sample named 'Aphrodite', 3.1 ± 0.3 Umcf (16 hours) for the sample named Mukosept. Without a significant increase in optical density, the cells reached the period of stationary equilibrium (P-4) simultaneously. The difference in optical density between samples in this period was 8.7%, with a lower value for the Mucosept sample. When analyzing the studied samples at an increased concentration of $300 \mu\text{g/ml}$, in addition to statistically significant differences in the cells reaching key OD values, there was also a delay in the exponential period of cell development, which was more pronounced for the Mucosept sample (16 hours). During the experiment designed to evaluate the efficacy of the analyzed samples by their delayed addition to the microbial suspension (at a concentration of $300 \mu\text{g/ml}$) (Fig. 2), a distinction was observed in the decrease in the time of generative activity in both samples, with a decrease of 23% for the phytocomposition Aphrodite and 38% for the Mucosept phytocomposition.

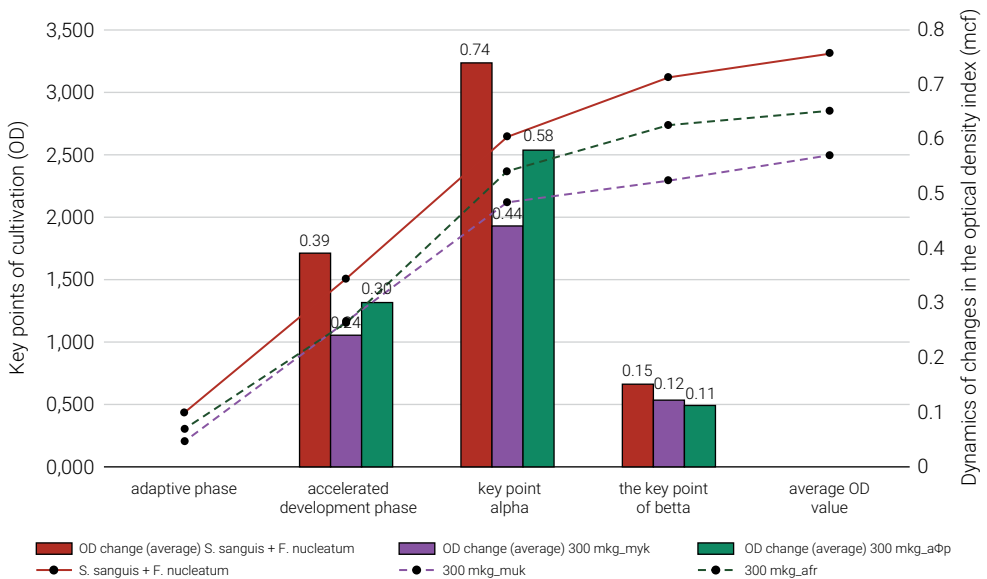


Figure 2. The key points and dynamics of changes in optical density during cultivation of a microbial consortium *S. sanguis + F. nucleatum*. (Compiled by the authors)

Discussion

Within the confines of the aforementioned study, and taking into account its limitations, it can be inferred that the phytocompositions 'Mukosept' and 'Aphrodite' have a significant impact on the dynamics of the formation of a mixed microbial consortium of potential causative agents of infectious complications of oncological processes. Considering the potential correlations between the processes of formation and adhesion of bacterial biofilms on the surface of dental prostheses utilized in the rehabilitation of patients, and the emergence of infectious complications of mucositis, the applied methods for modeling mixed microbial consortia *in vitro* and methods for their inhibition contribute to the detailed development of a strategy for preventing the inflammatory process. Complications of mucositis induced by mixed bacterial biofilm are reversible at the level of stabilization of the colonization activity of primary bacterial agents (planktonic form), causing the bacterial growth-inhibitory activity of therapeutic and prophylactic agents¹². In the experimental study, the main critical points for assessing the effectiveness of the phytocompositions were the comparison of the nature of the development of the cultivation curve with the control sample. At the same time, incorporating the varying influence of the precipitating external factor, the tendency to attain crucial optical density values and alter the boundaries of time intervals during various phases of the consortium development was analyzed. Also, during dysbiotic disruption of the oral microbiota during mucositis, there is a risk of the onset of an infectious candidiasis process, as a result of a violation of the nonspecific and specific resistance of the body, both at the local and general level¹³. An advisable continuation of this study would be to assess the effect of the studied phytocomplexes on fungi of the genus *Candida spp.*, since such a task was not implemented in this study. The data gathered during the experiments serves as the foundation for analyzing the impact of phytocompositions on intricate polyspecies microbial biofilms. Furthermore, the implementation of hygienic preventive measures in patients with postoperative defects of the upper jaw is a crucial factor in preventing infectious complications of mucositis.

In conclusion, based on the outcomes of the experiments, utilizing the automatic programmable cultivation technique, distinct variations in the growth of the bacterial population were identified, taking into account the incorporation of the studied phytocompositions in varying concentrations. In general, it can be inferred that the examined specimen, namely 'Mukosept', exhibits a more prominent antibacterial effect, owing to its potential ability to delay the rapid growth of bacterial cells, as well as lower values of optical density indicators at crucial cultivation points, in contrast to the phytocomposition 'Aphrodite' and the reference control sample.

Competing Interests

The authors declare no competing interests.

Acknowledgments

Not applicable.

Data availability

Datasets related to this article will be available upon request to the corresponding author.

Author Contribution

All authors have equally contributed to the findings of the manuscript and have revised and approved the final version of the manuscript.

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