AMERICAN JOURNAL OF APPLIED MEDICAL SCIENCE
ISSN: 2996-5101 (online) | ResearchBib (IF) = 10.81 IMPACT FACTOR
Volume-4| Issue-3| 2026 Published: |30-03-2026

ОЦЕНКА АНТИМИКРОБНОЙ АКТИВНОСТИ ФОТОСЕНСИБИЛИЗАТОРОВ FICUS CARICA L..

https://doi.org/10.5281/zenodo.18976760

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Abstract

The potential of photodynamic therapy (PDT), a 21st-century technology, is far from exhausted**.** The mechanisms of cytotoxic action of plant-derived photosensitizers remain largely unstudied. Addressing current issues and expanding the scope of PDT applications in medicine is possible through collaborative research between chemists, biophysicists, physiologists, pathophysiologists, and physicians. The mechanism of action of PDT is quite complex and not fully understood. Variations in photosensitizer types, different laser radiation sources, and the diversity of tissue pathologies are all significant. Understanding the patterns and mechanisms of action of PDT will allow us to introduce a modern treatment method in our country, as well as begin research into the development and production of more effective and affordable domestic photosensitizers..

Key words

photosensitizer, psoralen, psoriasis, photodynamic therapy, antimicrobial activity, Ficus carica L., isopsoralen

Introduction. Photodynamic therapy (PDT) is a promising area of modern photobiology, and has experienced rapid development over the past 10-15 years due to new advances in the diagnosis, prevention, and treatment of human

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diseases. The PDT method is based on the introduction of chemical drugs into the body – photosensitizers (PS), which have increased affinity for target cells (cancer cells, inflammatory tissues, microbes and viruses) [1; 2; 10; 11; 14; 15]. When exposed to light of a certain wavelength and energy, FS begin to produce atomic (singlet) oxygen, as well as the generation of other reactive oxygen species (ROS), which cause oxidative damage to various molecules (proteins, unsaturated fatty acids, nucleic acids) and cellular structures (membranes, enzyme systems, genetic apparatus, etc.), which leads to the inactivation of pathogens. Currently, an active search is underway for new photosensitizers that could be effectively used in PDT. Among these, drugs specifically synthesized for PDT (chlorin e6, photolon, photosens), as well as traditional drugs that are phototoxic (antiseptics, antibiotics, etc.) have found application [4; 8; 13]. They are classified based on their chemical properties; however, there is no unified classification of photosensitizers used for PDT [5].

The potential of PDT, a 21st-century technology, is far from exhausted. The mechanisms of cytotoxic action of plant-derived photosensitizers remain largely unexplored. Addressing current issues and expanding the scope of PDT's medical applications is possible through collaborative research by chemists, biophysicists, physiologists, pathophysiologists, and physicians. Thus, the mechanism of action of PDT appears to be quite complex and not fully understood. Variations in photosensitizer types, different laser radiation sources, and the diversity of tissue pathologies are all significant. Understanding the patterns and mechanisms of PDT action will enable the introduction of a modern treatment method in our country, as well as initiate research into the development and production of more effective and affordable domestic photosensitizers. Phytochemical research at the Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan has established that plants native to Uzbekistan are sources of natural compounds with photodynamic properties. One such class of compounds with photosensitizing activity are furocoumarins. Furocoumarins are produced by plants of the Umbelliferae and Legume families in the flora of Uzbekistan (Fig. 1).

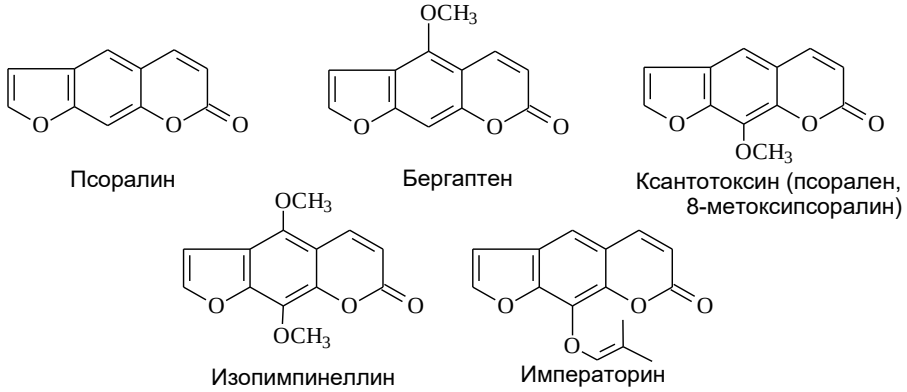

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Fig. 1. Structural formulas of furocoumarins produced by plants of the flora of Uzbekistan.

These compounds, while relatively inert in the dark, become biologically active, often very highly, when illuminated by ultraviolet or sunlight. Under these conditions, furanocoumarins become cytotoxins; some are toxic to human leukocytes at concentrations as low as 10^{-10} M/L under sufficient light.

The photodynamic properties of xanthotoxin, bergapten and some other psoralen derivatives are used in practice to treat skin diseases such as vitiligo, alopecia areata, psoriasis, and fungal infections [3; 6; 7; 12]. Methods are being developed for the use of light-activated 8-methoxypsoralin for the treatment of malignant blood diseases [9].

The photodynamic properties of furanocoumarins are explained by the fact that by absorbing ultraviolet radiation quanta, their molecules enter an excited state and, in this form, are capable of reacting with biologically important cellular components containing a double bond. Estrogen-like activity, as well as antitumor and antibacterial activity, have been established for psoralen and isopsoralen [16].

Study of antimicrobial activity of photosensitizers.

The antimicrobial activity of psoralen substances was studied using the well method (Egorov. Guide to practical classes in microbiology. Moscow, 1983).

Pseudomonas aeruginosa 003841/114, Staphylococcus aureus 60, Candida albicans 003592/723, Citrobacter freundii 002801/27, Serratia marcescens 367, Proteus mirabilis 002810/399, Escherichia coli NC101, Enterococcus faecalis OGIFR1, Klebsiella pneumonia B1823 were used as indicator strains. The indicator strains were restored by double subculture in CM broth (broth with brain heart infusion) and plating on MPA. The experiment was carried out at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.

Results of the study of antimicrobial activity of photosensitizers.

As is known in medical practice, photosensitizers based on furocoumarins are used not only internally, but also externally.

In this regard, we conducted studies on the antimicrobial activity of photosensitizers. To prepare the inoculum, we directly suspended colonies of a pure 18-24-hour bacterial culture grown on MPA medium in a sterile isotonic solution. Several morphologically similar colonies were collected with a sterile inoculating loop. The bacterial suspension was adjusted to a density of 0.5 according to the McFarland turbidity standard, which approximately corresponded to a load of 1-2 x 108 CFU/ml (for *Escherichia coli*) by adding microbial mass to the suspension or diluting it with sterile isotonic solution.

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The bacterial suspension was inoculated onto Miller-Hinton agar for 15 minutes (for *Candida albicans 003592/723*, Sabouraud agar was used). A sterile cotton swab was dipped into the suspension, and excess suspension was removed by squeezing the swab against the sides of the tube. To obtain a continuous lawn, the inoculum was applied evenly over the entire agar surface using streaking motions in three directions, rotating the Petri dish 60°. Wells with a 7 mm diameter hole punch were made on plates inoculated with indicator strains. Dilutions of the substances were prepared. When studying the antimicrobial activity of psoralen solutions in isopropanol, no activity was observed against *Pseudomonas aeruginosa 003841/114, Candida albicans 003592/723, Citrobacter freundii 002801/27, Serratia marcescens 367, Enterococcus faecalis OGIFR1, Klebsiella pneumoniae B-1823.* The clinical isolate *Proteus mirabilis 9* showed sensitivity to all psoralen solutions, including the solvent, the diameter of the growth inhibition zone was 12 mm (Fig. 2).

Fig. 2 – Suppression of growth of clinical isolate *Proteus mirabilis 9*, strains *Staphylococcus aureus 60 and Escherichia coli NC101* by different concentrations of psoralen.

Escherichia coli NC101 was also sensitive to all solutions, with an inhibition zone diameter of 10 to 14 mm. *Staphylococcus aureus 60* was inhibited by a solution containing 12.5 µg/ml psoralen, with an inhibition zone diameter of 18 mm. Thus, psoralen exhibits selective antimicrobial activity and is active against *Proteus mirabilis 9, Staphylococcus aureus 60* strains and *Escherichia coli NC101* at concentrations of 12.5 µg/ml and higher.

To study the photosensitizing activity of new compounds, to study the mechanisms of their action at the micro- and macro-level, we defined an algorithm of actions, selected methods. Preparations of natural plant origin were taken: alcohol- and water-soluble fractions of an individual compound of the furanocoumarin series - psoralen (95% purity), the preparation psoberan - a mixture of two furocoumarins (psoralen and bergapten), isolated from the leaves of *Ficus carica L.* of the *Moraceae family*, as well as a furanocoumarin - *anglecin* (isopsoralen), isolated from the leaves of *Psoralea drupacea Bunge* at the Institute of Plant Chemistry. The drugs psoberan and psoralen are white crystalline powders

with a specific aromatic odor, some of the fractions of these furanocoumarins are insoluble in water, but soluble in ethanol, methanol, acetone and fatty oils, some of the fractions of psoralen and anglecin are soluble in water.

When studying the antimicrobial activity of psoralen solutions in isopropanol, no activity was observed against *Pseudomonas aeruginosa 003841/114, Candida albicans 003592/723, Citrobacter freundii 002801/27, Serratia marcescens 367, Enterococcus faecalis OGIFR1, Klebsiella pneumoniae B-1823.* The clinical isolate *Proteus mirabilis 9* demonstrated sensitivity to all psoralen solutions, including the solvent, with a growth inhibition zone diameter of 12 mm. *Escherichia coli NC101* also demonstrated sensitivity to all solutions, with a growth inhibition zone diameter of 10 to 14 mm. *Staphylococcus aureus 60* was inhibited by a solution containing 12.5 µg/ml psoralen, with a growth inhibition zone diameter of 18 mm..

Thus, psoralen exhibits selective antimicrobial activity and is active against *Proteus mirabilis 9, Staphylococcus aureus 60 strains and Escherichia coli NC101* at concentrations of 12.5 µg/ml and higher. Due to the antimicrobial activity of psoralen and its antioxidant properties under UV irradiation, it was of interest to study the effects of this PS in collagen films on the ability to accelerate the regeneration of purulent wounds in experimental models. The results of these studies showed that wound dressings based on collagen and furanocoumarins, and in particular psoralen isolated from Central Asian varieties of *Ficus carica L.*, stimulated wound healing activity, accelerating the healing of seeded full-thickness skin wounds, which is undoubtedly ensured by both collagen itself - the main protein of the extracellular matrix, as an ideal substrate for the proliferation of fibroblast skin cells, and psoralen, as evidenced by a slight increase in the regenerative effect with the combined use of these films and PDT (UV rays).

Discussion.

To study the photosensitizing activity of new compounds and the mechanisms of their action at the micro- and macro-levels, we defined an algorithm of actions and selected methods. Preparations of natural plant origin were taken: alcohol- and water-soluble fractions of an individual compound of the furanocoumarin series - psoralen (95% purity), the preparation psoberan - a mixture of two furocoumarins (psoralen and bergapten), isolated from the leaves of *Ficus carica L.* of the *Moraceae family*, as well as a furanocoumarin - anglecin (isopsoralen), isolated from the leaves of *Psoralea drupacea Bunge* at the Institute of Plant Chemistry.

The drugs psoralen, anglecin and psoralen are white crystalline powders with a specific aromatic odor, some of the fractions of these furanocoumarins are insoluble in water, but soluble in ethanol, methanol, acetone and fatty oils, some of the fractions of psoralen and anglecin are soluble in water.

Conclusions.

Psoralen exhibits selective antimicrobial activity and is active against *Proteus mirabilis 9, Staphylococcus aureus 60, and Escherichia coli NC101* at concentrations of 12.5 µg/ml and above.

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