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DEVELOPMENT OF EYE OINMENTS COMPOSITION FROM SAFFLOWER EXTRACT

Abstract. In recent years, the growth of self-medication with the help of antibiotics, water and air pollution has led to increasing in the number of allergic and fungal diseases. Synthetic drugs exacerbate pathological processes, in addition, the side effects of these drugs exceed the expected therapeutic effect. Therefore, in recent years, there has been a significant increase in demand for herbal products. The results of the search for promising sources of antimycotic activity among representatives of plant flora also confirm the real possibilities of creating on their basis the effective antifungal agents in ophthalmic practice.

Key words: fungicidal and antibacterial actions, safflower, eye ointments.

At present, the main attention in pharmaceutical activity in the Republic of Kazakhstan is aimed at ensuring an annual increase in the nomenclature and volumes of production of high-quality pharmaceutical products by domestic producers. The drug policy of the Republic of Kazakhstan is based on the principles of providing medical institutions and the population of the republic with effective, high-quality, safe and affordable medicines.

Today, plants are one of the most important sources of medicines (LP) used in various areas of medical practice. This is explained by the fact that herbal preparations have a wide range of pharmacological activity and, as a rule, do not cause side effects with rational application. The most optimal dosage form for the treatment of eye diseases is ointment, since the effect of eye drops and solutions is short-lived, and the ointment forms a film on the eye mucosa and gives a long-term therapeutic effect [2, 6, 24]. The purpose of this study was to study the antibacterial and antifungal activity of the extracts provided and to determine their minimum bactericidal and fungicidal concentrations against museum strains of microorganisms, and to determine the optimal composition of ocular ointment bases.

Cathamus tinctoris (Safflower *C. tinctorius*) belongs to Asteraceae family in the order of Asterales which contains about 22,750 genera and more than 1,620 species. The *Carthamus* species probably originated from Southern Asia and is known to have been cultivated in China, India, Iran and Egypt, almost from prehistoric times. During the Middle Ages it was cultivated in Italy, France, and Spain, and was introduced into the United States in 1925 from the Mediterranean region. *C. tinctorius* has been known as ‘Golrang’ in Iran. It is grown for the red/orange pigment in the flower petals which is used for colouring rice and bread, and for dyeing cloth. The flowers of *C. tinctorius* are an important medicinal material in prescriptions used for cardiovascular, cerebrovascular and gynecological diseases. In China, the water extract of *C. tinctorius* has been developed as an intravenous injection, which is extensively applied to treat cardiovascular diseases clinically [5]. Its dye is mainly used as a colouring agent [6].

The objects of the study were samples of CO_2 -extracts obtained from flowers of safflower (*Carthamus tinctorius L.),* collected during the flowering phase.

Determination of antimicrobial and fungicidal activity of extracts was carried out by disco-diffusion method (DDM). This method was realized by applying the discs processed with the test drug to Petri dish, using sterile tweezers at a distance of 15-20 mm both from the edge of the cup and from each other. The results of DDM were taken into account by counting the diameter of the delay / growth inhibition zones with an accuracy of 1 mm.

The test samples were CO_2-extracts No 1 and No 2. Sample No 1 was an oily liquid of limpid color. Sample No 2 was a bright orange liquid. Due to the lack of data concerning the concentration of the active substance, the stock solution was the initial concentration. The method of serial dilution was carried out.

As test microorganisms, the following groups of strains were used: 1. for the research of antibacterial activity: *Staphylococcus aureus* ATCC 6538-P (museum sensitive strain); *Staphylococcus aureus* ATCC BAA-39; *Staphylococcus epidermidis* ATCC 12228; *Escherichia coli* ATCC BAA-196. *2.* for the research of antifungal activity: *Candida albicans* ATCC 10231 (museum sensitive strain).

Testing was carried out on liquid nutrient media: Mueller-Hinton broth (standard antibiotic sensitivity medium) and RPMI 1640 medium (medium recommended by CLSI M27-A2: «Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast» for testing fungicides).

To determine the antimicrobial activity, 10 tubes of the Eppendorf type were used. In all test tubes, except for the 1st (from 2 to 10), the nutritious broth of MHB was dispensed in the amount of 0.5 ml. The working solution (sample No 1 and sample No 2) was added in pure form (in a volume of 0.5 ml) to the first tube, with the MXB (0.5 ml) already present in it. Next, serial dilutions were made, which were carried out by taking the mixture (MXB (0.5 ml) + test preparation (0.5 ml)) from the 2nd tube in the amount of 0.5 ml into the 3rd tube already containing 0,5 ml of broth. 0.5 ml of the test sample in the broth was thoroughly mixed and transferred from the 3rd tube to the 4th also containing initially 0.5 ml of broth. This procedure has being repeated until the required amount of dilution was achieved. 0.5 ml of the mixture from the last tube was removed. Thus, the following dilutions were obtained: 1: 1; 1: 2; 1: 4; 1: 8; 1:16; 1:32; 1:64; 1: 128; 1: 256, which corresponds to the tubes from the 1st to the 10th. After conducting a series of dilutions, 0.05 ml of test strains of microorganisms at a concentration of 1.5 × 106 COE / ml were added to all the tubes. The procedure was repeated for all test samples.

All samples have being incubated for 18-24 hours at 37°C. On the expiry of incubation the sowings were carried out onto Petri dishes to determine the living cells. After seeding, the dishes were placed in a thermostat for 18-24 hours at 37° C for bacteria.

The results were taken into account by the presence of visible growth of microorganisms on the surface of a dense nutrient medium. The minimum bactericidal concentration (MBC) was the smallest dilution in a test tube that suppressed the growth of microorganisms.

The results of the research of antibacterial and fungicidal activity of extracts in respect of five strains of pathogenic microorganisms *S. aureus* ATCC 6538-P, *S. aureus* ATCC BAA-39, *S. epidermidis* ATCC 12228, *E. coli* ATCC BAA-196 and *C. albicans* ATCC 10231 are presented in tables 1, 2 and figures 1, 2.

From the data presented in Table 1 for the serial dilution method, it can be seen that the test sample No 1 exhibits an expected bactericidal activity against the susceptible strain of *S. aureus* ATCC 6538-P at 1: 128 dilution, as well as fungicidal activity against C. *albicans* ATCC 10231 in 1:1 dilution (MBC at this dilution).

From the data presented in Table 5 for the serial dilution method, it can be seen that the test sample No 2 exhibits bactericidal activity against sensitive and resistant strains. For instance, for multidrug resistant *S. aureus* strain ATCC BAA-39, sample No 2 is active at a dilution of 1: 4, for *S. aureus* sensitive *S. aureus* ATCC 6538-P in a ratio of 1:128, for epidermal staphylococcus *S. epidermidis* ATCC 12228 - in the dilution 1: 2, and for E. coli ATCC BAA-196 - 1: 4. Sample No 2 was not active against fungi of the genus *Candida.*

To make ointments, you need to go to marketing analysis. In the next study, the data obtained from plant substances for ophthalmic ointments from the State Register of medicines of 2018 are indicated. The collected results were included in computer forecasts and market share analysis models.

Tables 1 – The results of the research of antibacterial and fungicidal activity

Test sample	*S. aureus* ATCC 6538-P	*S. aureus* ATCC BAA-39	*S. epidermidis* ATCC 12228	*E. coli* ATCC BAA-196	*C. albicans* ATCC 10231
Sample No 1	1:128	Not active	Not active	Not active	1:1