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Study of antimicrobial activity of *Plantago major* and *Acorus calamus* carbon dioxide extracts.

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ABSTRACT

Given article deals with the results of the study antimicrobial activity and microbiological purity of dioxide carbon extracts of *Plantago major* and *Acorus calamus*. It has been found that both extracts exhibit antimicrobial activity. Thus *Plantago major* extracts have a broad spectrum of activity, and all cultures of microorganisms are sensitive to this extract. *Acorus calamus* extract has activity against gram-pozitive bacteria cultures as well as antifungal activity against *Candida albicans*. Both samples meet the Pharmacopeia of Ukraine in terms of "microbiological purity of non- sterile drugs." These extracts can be used to develop formulations with antimicrobial properties.

Keywords: Antimicrobial activity, dioxide carbon extracts, *Plantago major*, *Acorus calamus*, microbiological purity.

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INTRODUCTION

Currently proposed and actively developed the technology of extraction of vegetable raw materials, compressed and liquefied gases. BAS are in carbon dioxide - extracts in a natural environment consisting of resins, oils, waxes. Therefore, they are more active, and their therapeutic efficiency is much higher.

Obtained using the proposed technology extracts are completely natural, as evidenced by their chemical analysis. In addition, attracting and environmental friendliness of the process, because carbon dioxide is not toxic, and extract from it almost completely removed in the final stages of the technological cycle, and it does not require of any additional measures. Another interesting fact is that the process parameters provides a unique microbiological purity of the products obtained, satisfying the most stringent current requirements on the use of plant extracts in food, perfume and cosmetic and pharmaceutical industries. In the course of our research were obtained carbon dioxide - extracts sweet flag marsh and plantain. Antimicrobial activity was studied, as well as microbial purity of the resulting products.

MATERIALS AND METHODS

Studies of antimicrobial activity of the extracts was performed at the Department of Biotechnology of the National University of Pharmacy. For the analysis of samples obtained extracts (Department of Industrial Pharmacy NFaU):

№1. carbon dioxide - extract Plantago major;
№2. carbon dioxide - extract Acorus calamus.

The antimicrobial activity of the extracts of the samples was studied in vitro by the method of diffusion in agar (a "well") which is based on the ability of active substances diffuse into the agar previously seeded cultures of microorganisms. All studies were conducted in strict aseptic conditions, using a laminar box (biological safety cabinet AS2-4E1 "Esco", Indonesia).

As a test cultures using microorganisms from the American Type Culture Collection (ATCC - American Type Culture Collection): 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 fungi candida - Candida albicans ATCC 885-653[2].

Antimicrobial activity index is the size of the zone of delay of growth of test microorganisms that is formed agarized medium on Petri dishes. The diameter of the zones of growth inhibition considering the wells was measured with a diameter up to 1 mm, while guided by the complete absence of visible growth.

In conducting research using DSA suspension of bacterial microorganisms in saline, and a two-day culture of yeasts. Microbial load was 1×10^7 microbial colony forming units in 1 ml culture medium (CFU / ml).

In petri dishes mounted on a horizontal plane were 10 ml 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 layer agar surface thereof at an equal distance from each other and from the edge of the cup was placed a sterile steel cylinders (height 10,0 ± 0,1 mm, an outer diameter of 8,0 ± 0,1 mm) and filled in an upper layer of melted and chilled to 45- 48 ° C from agar cultures of microorganisms in an amount of 15 ml (13.5 ml melted agar and 1.5 ml of a microbial suspension with a microorganism load 1×10^7 CFU / ml). After cooling and solidification of the upper layer of the culture medium was removed with sterile forceps cylinders and formed in the wells were studied extracts samples (0.25-0.3 ml) until their completion.

Petri dishes with crops placed in an incubator - bacterial cultures at 32,5 ± 2,5°C for 18-24 Godin, culture yeasts at 22,5 ± 2,5°C for 48 Godin. The diameters of the zones of inhibition of microbial growth characterize the antimicrobial activity of the samples.

When studying microbiological purity of samples was used method of extracts the State Pharmacopeia of Ukraine (1.4, p. 5.1.4- microbiological purity of non – sterile medicines, p.171), which allows

objectively evaluate the quality characteristics of the samples on the basis of experimentally obtained statistically processed results [2,3]. Estimation of microbiological contamination degree of the drug include the identification the total number of aerobic mesophilic bacteria (TAMC) and total yeasts and molds (TYMC) 1 g extracts, establishing the absence of bacteria Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa. In 1 g of non –aqueous medicinal products for oral and rectal administration the total number of aerobic microorganisms (TAMC) may be not more than 103 CFU (colony forming units); the Total Combined Yeast and Mould Count (TYMC) not more than 102 CFU [3]. To check the suitability of determination methods of total viable aerobic microorganisms as test –strains were used the following bacteria from the American Type Culture Collection (ATCC): Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Bacillus subtilis ATCC 6633, Candida albicans ATCC 10231, Aspergillus brasiliensis ATCC 16404 [3].

According with the requirements of the Ukrainian Pharmacopoeia was used following dense and liquid nutrient medium: Casein soy agar (to determine the number of live bacteria), Sabouraud dextrose agar (to determine the number of fungi), soybean casein broth (preincubation for in determining the presence of certain microorganisms), manitn – saline agar (for identification of bacteria Staphylococcus aureus), cetrimide agar (to identify Pseudomonas aeruginosa), Mac Conkey agar (for bacteria detection of Escherichia coli).

RESULTS AND DISCUSSIONS

The diameter of the microorganism growth characterizes the antimicrobial activity of the experimental samples as follows:

- no growth inhibition zones around the wells of the microorganisms, as well as growth inhibition zone diameter of 10 mm was evaluated as the sensitivity of microorganisms to not extract the samples introduced into the wells;
- the zone of growth inhibition diameter of 11-15 mm were evaluated as weak sensitivity to the culture of the active ingredients of the samples extracts;
- the zone of growth inhibition diameter 15-25 mm - sensitive strain to the sample;
- the zone of growth inhibition, with a diameter greater than 25 mm, testified to the high sensitivity of microorganisms to the sample extracts.

As a result of studies on the antimicrobial activity of extracts of samples for the various cultures of microorganisms obtained the following results, shown in table 1.

Table 1: Antimicrobial activity of extracts

Samples	Cultures of microorganisms			
	S.aureus	B. subtilis	E. coli	C. albicans
	Zoni delay diameter growth of microorganisms, mm			
№1. carbon dioxide - extract Plantago major;	18-19	18-19	16-17	21-22
№2. carbon dioxide - extract Acorus calamus.	18-19	17-18	-	19-20

" – " – no zone of growth inhibition of microorganisms.

The experimentally obtained data showed that the that the sample extract №1 (carbon dioxide extract *Plantago major*) exhibits antimicrobial activity against all the strains of the used microorganisms (Gram-positive and Gram-negative bacteria), and also has activity against yeasts Candida . Thus, the extract №1 has a broad spectrum of action and all cultures of microorganisms are sensitive to this extract (diameter of the zones of microbial growth delay from 16 to 22 mm). Sample extract №2 (carbon dioxide extract Acorus calamus) exhibits activity against gram-positive bacterial cultures: Staphylococcus aureus - 18-19 mm, Bacillus subtilis - 17-18. Regarding Escherichia coli gram-negative culture activity was observed. Culture yeasts Candida albicans is sensitive to the action sweet flag extract, which is manifested in the antifungal activity.

Thus, the sample extracts №1 (carbon dioxide extract Plantago major) and №2 (carbon dioxide extract Acorus calamus) are promising for further developing formulations with antimicrobial properties.

When determining the microbiological purity of the analyzed extracts in orderbto prevent errors in the evaluation of results preliminary studies it was found that all the samples of the extracts have antimicrobial activity. For neutralization of the antimicrobial action have been prepared diluted extracts (1:10) antimicrobial activity in all of the samples was not observed.

For analysis were taken 2.0 g of the extracts test sample was added to the buffer solution of sodium chloride and peptone pH 7.0 to a final volume 20 ml (1:10 dilution). In a Petri dish 9 cm in diameter was added 15 ml of casein –soya agar or Sabouraud-dextrose agar at a temperature from 45 to 50^0 S, culture media was allowed to cool.

One ml of the test dilutions (1:10) were added into tubes containing 4 ml of melted and cooled to a temperature not more then 45^0 S agar medium. The tube contents were rapidly mixed and transferred into a Petri dish with the prepared first leyer of the nutrient medium. By a quick shake of the Petri dishes evenly distributed top layer of the medium. For eash dilution were prepared there Petri dishes for eash culture medium. Dishes with casein –soya agar were incubated at 30-35^0 S days dishes with Sabour and dextrose agar were incubated at 20-25^0 S for 7 days. For each nutrient medium was calculated arithmetic mean value the number of colonies, and was determined the number of CFU per gram of medicament. Incubation of prepared extracts samples (1:10 dilution) for manitno – salt agar (temperature 30-35^0 S -72 hours), cetrimid agar (temperature 30-35^0 S -72 hours) and Mac Conkey agar (temperature 30-35^0 S -72 hours) showed the absence of colonies, which corresponds to the results "no bacteria *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* in 1 g of the test samples of extracts samples studies are shown in the table 2.

Table 2: The results of the microbial purity control of extracts

Sample	Amount of sample	Dilution	The total number of microorganisms in 1 g of the extract		Microorganisms		
			Bacteria (TAMC)CFU/g	Fungi (TYMC)CFU/g	E.coli	Staph.aureus	Ps.aeruginosa
№1. carbon dioxide - extract Plantago major	2.0 g	1:10	<10	<10	No growth	No growth	No growth
№2. carbon dioxide - extract Acorus calamus	2.0 g	1:10	10	<10	No growth	No growth	No growth

Thus, it is experimentally proved that the samples of extracts №1 and №2 did not reveal the presence of bacteria *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*.

Found that the total number of fungi (TYMC) is less than 10 CFUs/ g in all extract №1 (carbon dioxide extract *Plantago major*) and №2 (carbon dioxide extract Acorus calamus).

The number of bacteria (TAMC) in 1 g of the test sample №2 carbon dioxide extract of *Acorus calamus*.

For sample №1 (*Plantago major* carbon dioxide extract) number of bacteria (TAMC) in 1 g of the test samples of extracts is less than 10 CFUs/ g.

The results show that extracts samples №1 and №2 meet the requirements of the Pharmacopeia of Ukraine in terms of "microbiological purity of non –sterile drugs".

CONCLUSIONS

Based on these studies we can conclude that the resulting carbon dioxide extracts of plantain and Calamus exhibit antimicrobial activity. Thus plantain extract have a broad spectrum of activity, and all cultures of microorganisms are sensitive to this extract (growth inhibition zone ranges from 16 to 22 mm). A sweet flag extract has activity against gram-positive bacterial cultures: Staphylococcus aureus - 18-19 mm, Bacillus subtilis

- 17-18, as well as antifungal activity against Candida albicans. These extracts can be used to develop formulations with antimicrobial properties.

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