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Influence of The *Chlorophytum Comosum* Extract on Some Microorganisms.

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ABSTRACT

The present study was conducted to evaluate the effect of C. comosum extract on some probiont microorganisms. The effect of the extract was evaluated by adding it to culture medium at a concentration (0.1%; 1.0%; 10.0% of the volume of a nutrient medium), The controls were the same medium but without the addition of the extract. Microorganisms of Escherichia coli, Candida albicans, Lactobacillus plantarum, Bifidobacterium bifidum. Bacillus subtilis was plated on this medium, later was taken into account the number of grown colonies. C. comosum extract had no impact on Lactobacillus plantarum, Bifidobacterium bifidum, showed stimulating effect on Escherichia coli, Bacillus subtilis, and antifungal effect on Candida albicans.
Keywords: Chlorophytum comosum, extract, Escherichia coli, Candida albicans, Lactobacillus plantarum, Bifido bacterium bifidum. Bacillus subtilis.

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INTRODUCTION

Chlorophytum comosum (Ribbon plant, Spider plant) is a plant from the genus Chlorophytum, that is known for their therapeutic potential with a vast range of pharmacologically important substance, such as alkaloids, vitamins, minerals, proteins, carbohydrates, saponins, polysaccharides, steroids and flavonoids [5,9,12]. It is a medicinal plant which has got maximum demand and commercial value today as one of the fast growing ever green plants of Chlorophytum species traditionally known to be used against bronchitis, cough, fracture, burns, it also shows hepatoprotective, anti-tumor properties and cytotoxicity against cancerous cell line [3,8]. In the literature there are some reports about antibacterial and antifungal activity of extracts of this plant against certain microorganisms [4,7,10], however scientifically proven that even antibiotics have been shown to possess biological activities other than inhibition [2,6]. This fact prompted us to examine the possibility that Chlorophytum extracts in certain doses are able to modulate the growth of certain types of bacteria. As objects of research we chose some representatives of the most common view probiont microorganisms, which constitute strains of endogenous microflora (bifidobacteria, lactobacilli, E.coli etc.), that restores the normal flora in microorganism, or strains of transient microorganisms (eg, nonpathogenic species of the genus Bacillus), inhibiting pathogenic and opportunistic microbes. Besides also interesting the extract influence on Candida albicans, which is currently etiologically important in the development of microecological disorders associated with different pathologies.

MATERIALS AND METHODS

Chlorophytum comosum extract preparation: C. comosum was grown in a Scientific research laboratory of immunopathology, immune morphology and immune biotechnology of North-Caucasus Federal University. Leaves were collected and washed under water and placed in a cooling chamber at a temperature +4°C for 5 days. Then shredded to a size of 1-2 cm, put into a low temperature refrigeration chamber (Tefcold se10-45, Denmark) at the temperature of -40° C for 48 hours, then it was dried by freeze-drying (P.I.T., LC-500, Russia) to a moisture level 8-10% at the operating pressure of sublimator 70-80 Pa, the condenser temperature was -45°C, and the total drying cycle of 35 hours. After the drying C. comosum leaves was filled with 70% ethyl alcohol in a ratio of 1:25 and placed in a shaker-incubator (ES-20/60, Latvia) at temperature +70°C and stirring rate of 125 rpm for 1 hour, then removed from the incubator and left at room temperature for 24 hours. The first extract was filtered. The processed feedstock was extracted two more times under the same scheme. Three of the resulting extracts were combined and concentrated to a volume of 50 ml (for removing alcohol) on a rotary evaporator (IKA RV 10, Germany) at a bath temperature of +50° C and 7 mbar vacuum. The concentrated extract was autoclaved at a temperature of +121°C for 10 min. The extract prepared according to the proposed technology was used for the further experiment.

Bacteriological examination: The study of the influence of the extract on the growth of microorganisms was made by applying the Koch plate method. In the experiment were used test strains of microorganisms: Bifidobacterium bifidum N1, Lactobacillus plantarum 8P-A3, Escherichia coli M-17, Bacillus subtilis 26-D. Candida albicans.

C. comosum resulting extract was added to the culture medium in the concentrations recommended in microbiological practice (0.1%; 1.0%; 10,0% of the volume of a nutrient medium), with the subsequent bringing of pH of environment to the desired value. Agar for bifidobacteria (HiMedia, India), MRS agar (HiMedia, India), Endo (HiMedia, India), Hottinger agar (Medgamal, Russia), Sabourauds peptone agar (NICF, Russia) were used. The controls were the same medium but without the addition of the extract. Study was carried out in threefold repetition.

For plating on nutrient media in Petri dishes were used the bacterial suspension at volume of 0,1 ml from concentration of 1000 CFU in 1 ml, derived from tenfold dilutions from culture inoculum prepared by Tarasevich turbidity standard (using sterile phosphate buffer saline solution pH 7.2). Counting colonies of bacteria after the incubation time of 48 hours at 37 °C was carried out. Cultivation of bifido bacteria and lactobacilli was performed under microaerophilic conditions using the anaerobic system for the cultivation (INFORS MT Multitron, Switzerland). Calculation of colonies was made by use of the counter of colonies of microorganisms Scan 100 (Inter science, France). Microbiological smears prepared from the grown colonies were subjected to Gram's staining. Visualization of the image of micro preparations was made with use of the specialized photo camera Axio Cam MRc5 and the software Zena 2012 Pro (Carl Zeiss Microscopy, Germany).

Statistical analysis

To facilitate the statistical presentation of data on the number of colony forming units of bacteria logarithms were used (base 10). All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 11.0 packed program. Data was presented as mean ± standard deviation. The difference between the control and experimental groups was analyzed using Mann-Whitney U test. $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results obtained during the experiment are shown in the Table 1. It was first detected that adding of C. comosum extract to the medium for lactobacilli and bifidobacteria in any of the selected concentrations did not affect significantly on the number of the grown colonies. Adding of C. comosum extract to the medium for E. coli at the concentration 0.1% and 1% allowed increasing the quantity E. coli on 24% and 17% respectively compared with the control, and its reduction on 19% at the concentration of 10%. Obtained results are partially in agreement with the findings of Valya et.al. (2009) [11] and Ahmad et.al. (2014) [1] reported about the antibacterial activity of Chlorophytum borivilianum to E. coli, however stimulating affect of the extract may be explained with low concentrations of the main antibacterial agents. C. comosum extract showed strong stimulating activity in all concentrations in relation to Bacillus subtilis. Its application at the concentration of 0.1% it increased Bacillus subtilis number on 23% compared with the control, at the concentrations of 1% and 10% it increased Bacillus subtilis number on 46% and 41%. These results are in contrast with the findings of Sundaram et.al. (2011) [10], Valya et.al. (2009) [11] who studied the influence of tubers and crude extracts of Chlorophytum, and with Ahmad et.al (2014) [1] studied leave and stem extract all by agar disc cup diffusion method. Obtained results can be explained whit application of leave extract with lower concentration of active agents as well as the selection of various methods. Furthermore, it should be noted that Bacillus subtilis is normally a representative of plant cenoses, for growth of which substances contained in the plant leaves may be necessary. Application of an extract had significant depressing effect on Candida albicans which is manifested in the decrease of the number of grown colonies on 42% in the concentration of 1%, and the complete suppression of growth the concentration of 10%. Obtained results are in agreement with the findings of researchers above.

Table 1: Quantity of colonies of the studied microorganisms grown under the influence of C. comosum resulting extract

Studied microorganism strain, CFU	Quantity of the test extract in a nutrient medium, %			
	Control, 0%	0.1%	1%	10%
Lactobacillus plantarum 8P-A3	55.55±1.5	49.22±1.4	51.22±1.6	53.33±1.7
Bifidobacterium bifidum N1	68.00±1.7	66.33±1.8	60.78±1.4	63.55±1.4
Escherichia coli M-17	65.60±1.4	81.67±1.6*	76.89±1.5*	52.58±1,6*
Bacillus subtilis 26-D	62.22±1.5	76.44±1.4*	91.22±1.4*	88.11±1.5*
Candida albicans	57.33±2.4	48.66±1.6	35.33±1.6*	0.00

*P≤0.05 (in comparison with parameters of control)

CONCLUSION

Therefore, for the first time studied the effect of the extract of C. comosum as a culture medium component on a number of microorganisms. The above results showed that bifidobacteria and lactobacilli can grow on a media, contained the extract of C. comosum, but it doesn't show any promoting affect on it. The potential antifungal effect on Candida albicans was proved. In comparison with other authors C. comosum extract showed mixed effect to E. coli and Bacillus subtilis. Interesting is the research the underlying mechanisms of its effects in relation to these microorganisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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