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ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM ECUADORIAN LICHENS

Antimicrobial activity of the ethanolic, isopropanolic, acetone, DMSO and aqueous extracts of the two lichen species from Ecuadorian highland, Usnea sp. and Stereocaulon sp. were explored in vitro against bacteria Bacillus subtilis, Escherichia coli and Staphylococcus aureus by the disc-diffusion method. Also the minimal inhibitory concentration (MIC) was determined.

The strongest antimicrobial activity was found in DMSO extract of Usnea sp. compared to antibacterial activity of ciprfloxacin and cefazolin antibiotics. The inhibition zone was 28 mm, 30 mm, 31mm (DMSO extract, ciprfloxacin and cefazolin respectively) in case of B. subtilis usage as the test bacteria. MIC value for Usnea sp. and Stereocaulon sp. DMSO extracts was 0.4 mg/ml. E. coli was resistant to all kinds of extracts. The S. aureus sensitivity to lichen DMSO extracts was comparable to sensitivity of these microorganisms to tetracycline and vancomycin. Thereby, most kinds of extracts (ethanol, isopropanol, hexane, DMSO and acetone solvents) from Ecuadorian lichens Usnea sp. and Stereocaulon sp. with the exception of aqueous Stereocaulon sp. extracts possessed antibacterial activity against B. subtilis. DMSO lichen extracts had also antimicrobial activity against S. aureus. At the same time the extracts studied didn't demonstrate antibacterial activity against the representatives of the most common and harmful phytopathogenic bacteria tested.

Further investigations of Ecuadorian lichens especially study of plants collected from extremal highland biotops can be very important in study of possibility of treatment of numerous diseases caused by pathogenic microorganisms.

Key words: Usnea sp., Stereocaulon sp., Antimicrobial activity, Ecuador

Ecuador is the country located on Equator in South America. The country has unique geographical location and includes four main regions: low-lying the Pacific coastline, high-altitude Andes mountain belt, Amazon rainforest areas and Galapagos Islands region. In this country there is a great variety in the climate largely determined by altitude. The great biodiversity which is determined by a variety of climate is the main feature of the Ecuador flora. There are many lichen species in Ecuador. Lichens are symbiothic organisms consisting of fungi and an alga or a cyanobacterium. These plants are found in the south and north regions of the Earth, on mountains and plains and grow on rocks, ground, on the trees. The chemical constituents of lichens have been shown to have biological activity including antimicrobial activity [1, 4-6]. Although the biological activity of lichens studied for a long time, the problem still remains urgent. The study of plants collected from equatorial highland regions which is difficult of access is particularly interesting and promising. This is primarily due to climatic conditions of these extreme regions. Plants in the highlands are exposed to a number of extreme stress factors (for example UV-radiation) which leads to the formation of resistance mechanisms, including the increase of antimicrobial activity.

Studying of the antimicrobial activity of extracts of two lichens from Ecuadorian highland, *Usnea* sp. and *Stereocaulon* sp., was the aim of this work.

Materials and methods. The plants were collected from Papallacta, Ecuador (4000 m) in October 2013 and were identified as *Usnea* sp. and *Stereocaulon* sp. (fig. 1) (Pabón Garcés Galo Jacinto, Northern Technical University, Ibarra, Ecuador and Gagarina L. V., Stepanchikova I. S. Botanical Institute RAS, Saint-Petersburg, Russia). For extraction lichen sampls were pounded, then 0,1 g portions were taken and added to 0,5 ml of ethanol, isopropanol, distilled water, hexane, DMSO or acetone solvents. The mixtures were sonicated, then left at room temperature for 3 or 24 hours for extraction.

The lichen crude extracts were tested for antibacterial activity using *Bacillus subtilis*, *Escherichia coli* B906 gram positive bacteria and gram negative bacteria *Staphylococcus aureus* B918. The bacteria were obtained from the culture collection maintained at the Institute of Microbiology and Virology NAS of Ukraine. The inhibitory effect was determined according to the Kirby and Bauer disk diffusion method (National Committee for Clinical Laboratory Standards, 1993). 0.04 ml of lichen extracts were added to filter paper disks (6 mm in diameter), allowing the solvent to evaporate (leaving the lichen extracts on disks without the solvent). Disks with pure solvents (0.04 ml) were used as negative control. Discs with antibiotics tetracycline, rifampicin, ampicillin, vancomycin, erythromycin, ciprofloxacin, cefazolin were used as positive control substances. The bacteria were incubated in Petri dished for 24 h at 28 °C (*B. subtilis*) or 37 °C (*E. coli* and *S. aureus*). The inhibition zones for bacteria growth were measured after 24 h. Minimal inhibitory concentration (MIC) was determined using the broth dilution method [7] with some modifications.

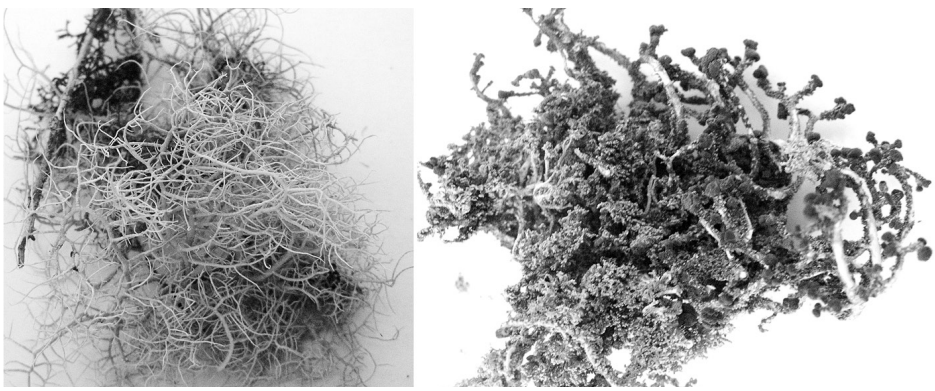

Fig. 1 – Plant material: lichens *Usnea* sp. (a) and *Stereocaulon* sp. (b)

Determination of the sensitivity of phytopathogenic bacteria to plant extracts was carried out by limiting dilution on a potato agar (PA) solid nutrient medium. 0.1 ml of the bacterial suspension in concentration 1×10^9 colony forming units/ml were inoculated in Petri dishes with PA medium. 0.1 ml of plant extracts in two-, five- and tenfold dilution were added in the middle of each dishes. Growth inhibition zones were measured after 24-48 h incubation of bacteria at 28°C. The experiments were repeated three times.

Results and discussions. Previously antimicrobial activity of different lichen species collected in India, Serbia, Brasilia and other countries was studied but Ecuadorian lichens were not studied. For example acetone, methanol and aqueous extracts of the lichens *Lecanora atra*, *L. muralis*, *Parmelia saxatilis*, *P. sulcata* and *Parmeliopsis ambigua* were tested [2] and in this investigation *E. coli* was resistant to all lichen extracts. In another investigation the difference in sensitivity of *E. coli*, *S. aureus* and *B.subtilis* to lichen extracts was shown [3].

In our investigations the crude extract from Ecuadorian lichens demonstrated antibacterial effect against *B. subtilis* and *S. aureus*. Antimicrobial activity of the extracts depended on the solvent used. The aqueous extracts of the lichens tested didn't have antimicrobial activity on any of the test microorganisms except Usnea aqueous extract, whereas the other extracts showed an activityes related to the organisms tested.

The strongest antimicrobial activity was found when we used DMSO extract of *Usnea* sp. It was compared to antibacterial activity of ciprfloxacin and cefazolin antibiotics. The inhibition zone was 28 mm, 30 mm, 31mm (DMSO extract, ciprfloxacin and cefazolin respectively) in case of *B. subtilis* usage as the test bacteria.

E. coli was resistant to all kinds of extracts. It should be noted that the microorganisms were resistant to most of commercial antibiotics tested. *S. aureus* was sensitive to DMSO extracts of *Usnea* sp. and *Stereocaulon* sp. and the inhibition zone was 14 and 10 mm respectively. The *S. aureus* sensitivity to lichen extracts was comparable to sensitivity of

these microorganisms to antibiotics tetracycline and vancomycin. So, *B. subtilis* were more sensitive to lichen extracts compared to the other bacteria compared sensitivity. MIC value for *Usnea* sp. and *Stereocaulon* sp. DMSO extracts was 0.4 mg/ml. Only aqueous *Stereocaulon* sp. extract have any inhibition effect on *B. subtilis* growth. The results are summarized in table 1.

Phytopathogenic bacteria *Pseudomonas*, *Xanthomonas*, *Pectobacterium*, *Agrobacterium*, *Clavibacter* genera, as well as fungi, viruses and mycoplasma are known for their ability to reduce the productivity of plants and to a damage to agriculture. The agents of bacterial diseases affect all types of crops and weeds, causing necrosis and wilting of part or of whole plant and cause also rot of fruit and berries. Phytopathogens harmfulness exhibits in reducing of seed germination, in seedling and plants death or in significant inhibition of their growth. The one also results in early leaves desiccation, spotting and necrosis of the leaf surface. Plants metabolism delay, decrease in commercial quality and quantity of the crop are the effects of the pathogenic bacteria plants infection.

Table 1

Antibacterial activities of lichen *Stereocaulon* and *Usnea* extracts to *E. coli*, *S. aureus* and *B. subtilis*

Bacteria	Inhibition zone, mm																		
	Stereocaulon extracts						*Usnea* extracts						Antibiotics						
	Ethanol	Isopropanol	Hexane	Water	Acetone	DMSO	Ethanol	Isopropanol	Hexane	Water	Acetone	DMSO	Tetracycline	Rifampicin	Ampicillin	Vancomycin	Erythromycin	Ciprofloxacin	Cefazolin
E. coli	0	0	0	0	0	0	0	0	0	0	0	0	11	7	0	8	0	30	0
S. aureus	0	0	0	0	0	10	0	0	0	0	9	14	14	24	24	16	20	25	28
B.subtilis	15	11	13	0	13	19	16	16	16	12	17	28	22	22	18	20	23	30	31

Phytopathogenic bacteria can persist for a long time in a latent form in plants, plant debris and seeds [8]. Unfortunately there are no effective chemical or biological plant protection substances against the agents of bacterial diseases both in Ukraine and in the world.

Native and diluted lichen extracts studied have no antagonistic effect against phytopatogenic bacteria *Pectobacterium carotovorum* subsp. *carotovorum* 8982 (polyphage, a wide range of agricultural and flowering plants rot causative agent), *Agrobacterium tumefasiens* 8628 (plants tumor and necrosis agent), *Clavibacter michiganensis* subsp. *michiganensis* 10_2 (bacterial cancer of tomato and other Solanaceae, pepper brown spot agent), *Pseudomonas syringae* pv. *syringae* 8511 (polyphage, agent of fire blight and leaf spots of crops and flowers), *Xanthomonas campestris* pv. *campestris* 8003b (pathogen caused vascular bacteriosis), *Pseudomonas fluorescens* 8573 (pathogen caused spotting and soft rot), *Pseudomonas syringae* pv. *atrofaciens* 9400 (cereals basal bacteriosis agent), *Pseudomonas syringae* pv. *coronafaciens* 9030 (agent of oat halo bacteriosis), *Erwinia rhapontici* YA7 (pink grain pathogen of cereals), *Xanthomonas oryrae* pv. *oryzae* 122 (agent of bacterial blight of rice), *Xanthomonas axonopodis* pv. *glycines* (agent of soybean pustule bacteriosis), *Xanthomonas axonopodis* pv. *phaseoli* (agent of brown bacteriosis of beans).

Thereby, most kinds of extracts (ethanol, isopropanol, hexane, DMSO and acetone solvents) from Ecuadorian lichens *Usnea* and *Stereocaulon* with the exception of the aqueous *Stereocaulon* sp. extracts have possessed antibacterial activity against *B. subtilis*. DMSO lichen extracts also characterized by antimicrobial activity against *S. aureus*. However, the extracts from Ecuadorian lichens did not demonstrate an inhibitory effect against the representatives of the most common and harmful plant pathogenic bacteria.

Further investigations of Ecuadorian lichens especially study of plants collected from extremal highland biotops can be very important in study of possibility of treatment of

numerous diseases caused by pathogenic microorganisms and for introducing of the results in the Ecuadorian pharmacology.

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АНТИМИКРОБНАЯ АКТИВНОСТЬ ЭКСТРАКТОВ ИЗ ЛИШАЙНИКОВ ЭКВАДОРА

Р е з ю м е

Изучена антимикробная активность этанольного, изопропанольного, ацетонового, гексанового, ДМСО и водного экстрактов из лишайников двух видов, *Usnea* sp. и *Stereocaulon* sp., собранных в высокогорье Эквадора, по отношению к *Bacillus subtilis*, *Escherichia coli and Staphylococcus aureus* с использованием диск-диффузного метода. Определены минимальные ингибирующие концентрации (МИК) экстрактов.

Самая высокая антимикробная активность при тестировании на *B. subtilis*, сравнимая с антибактериальной активностью антибиотиков ципрфлоксацина и цефазолина, была обнаружена при использовании ДМСО экстракта из лишайника *Usnea*, причем зона отсутствия роста составила 28 мм, 30 мм, 31 мм для ДМСО экстракта, ципрфлоксацина и цефазолина, соответственно. МИК ДМСО экстрактов из *Usnea* sp. и *Stereocaulon* sp. по отношению к B. subtilis составила 0,4 мг/мл. Бактерии *E. coli* были устойчивы ко всем типам экстрактов. Чувствительность *S. aureus* к ДМСО экстрактам из лишайников была сравнима с чувствительностью этих микроорганизмов к тетрациклину и ванкомицину. Выявлено, что большинство растительных экстрактов, полученных при использовании этанола, изопропанола, гексана, ДМСО, воды и ацетона в качестве экстрагирующих веществ, за исключением водного экстракта из *Stereocaulon*, обладали антибактериальной активностью в отношении *B. subtilis*. ДМСО экстракты из лишайников обладали также противомикробной активностью в отношении золотистого стафилококка.

Вместе с тем, у исследованных экстрактов не выявлено антибактериальной активности ко всем тестированным представителям наиболее распространенных и вредоносных фитопатогенных бактерий.

Дальнейшие исследования эквадорских лишайников, особенно изучение растений, собранных в экстремальных горных биотопах, может быть очень важным в изучении возможности применения растений для лечения заболеваний, вызванных патогенными микроорганизмами, а также в использовании в национальной фармакологической промышленности в Эквадоре.

Ключевые слова: *Usnea* sp., *Stereocaulon* sp., антимикробная активность, Эквадор.

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АНТИМІКРОБНА АКТИВНІСТЬ ЕКСТРАКТІВ З ЛИШАЙНИКІВ ЕКВАДОРУ

Р е з ю м е

Вивчено антимікробну активність етанольного, ізопропанольного, ацетонового, гексанового, ДМСО та водного екстрактів із лишайників двох видів, *Usnea* sp. та *Stereocaulon* sp., які були зібрані у високогір'ї Еквадору, щодо *Bacillus subtilis*, *Escherichia coli* та *Staphylococcus aureus* диск-дифузним методом. Визначено мінімальні інгібуючі концентрації (МІК) екстрактів.

Найвища антимікробна активність при тестуванні на *B. subtilis*, порівняна з антибактеріальною активністю антибіотиків ципрфлоксацину та цефазоліну, була визначена при

використанні ДМСО екстракту з лишайника *Usnea*, при цьому зона відсутності росту становила відповідно 28 мм, 30 мм, 31 мм для ДМСО екстракту, ципрфлоксацину та цефазоліну. МІК ДМСО екстрактів з *Usnea* sp. та *Stereocaulon* sp. щодо *B. subtilis* становила 0,4 мг / мл. Бактерії *E. coli* були стійкі до усіх типів екстрактів. Чутливість *S. aureus* до ДМСО екстрактів з лишайників була порівняна з чутливістю цих мікроорганізмів до тетрацикліну та ванкоміцину. Виявлено, що більша частина рослинних екстрактів, отриманих при використанні етанолу, ізопропанолу, гексану, ДМСО, води та ацетону у якості екстрагуючих сполук, за виключенням водного екстракту зі *Stereocaulon*, мали антибактеріальну активність проти *B. subtilis*. ДМСО екстракти з лишайників мали також антимікробну активність щодо золотистого стафілококу. Однак, досліджені екстракти не виявили токсичної дії до усіх досліджених представників найбільш поширених та шкодочинних фітопатогенних бактерій.

Подальші дослідження еквадорських лишайників та особливо рослин, зібраних у екстремальних високогірних біотопах, можуть бути дуже важливими у вивченні можливості використання рослин для лікування захворювань, викликаних патогенними мікроорганізмами, а також у використані у національній фармакологічній промисловості в Еквадорі.

Ключові слова: *Usnea* sp., *Stereocaulon* sp., антимікробна активність, Еквадор.

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